Core Concepts in Parenchymal Kidney Disease

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Editors

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To my daughter Sophia, whose smile and love inspire me every day.

—Fernando C. Fervenza

For my parents, Lung-Nan and Cheng-Hue Lin, for their many years of love and support.

—Julie Lin

To my parents, Saroj Sethi and Indrash Chandra Sethi, for their love and wisdom.

—Sanjeev Sethi

To my brother, Sanjay, with love.

—Ajay K. Singh

Foreword

"Think of that, - a man of my kidney, - think of that, - that am as subject to heat as butter; a man of continual dissolution and thaw:it was a miracle to scape suffocation"
'The Merry Wives of Windsor'

play by William Shakespeare

"Core Concepts in Parenchymal Kidney Disease" is a timely, comprehensive, and informative book, written by an international group of outstanding experts in the field. The book's chapters are enough detailed to be of value to the renal community and to nephrology fellows and trainees in internal medicine and can appeal to a broad medical audience with an interest in how to diagnose and treat the most common glomerulopathies. The book is timely in that classical paradigms in parenchymal renal disease have been recently challenged by real advances and true discoveries that just 10 years ago one could barely imagine. Progress in molecular biology and the formidable chance offered by deciphering the genome have impacted nephrology to a major extent.

In last 10 years numerous abnormalities in genes involved in renal structure and function have been identified, but the molecular basis for the most common renal parenchymal disease is either unknown or incomplete, with a few notable exceptions. All the recent studies have generated more questions than offered answers but have so far contributed to clarify at least some of the mechanisms initiating and driving disease progression of complex disease (that up to now we have only been able to recognise as diverse by different clinical and pathologic features) and find novel and effective treatment in others. Thanks to these advances we start to appreciate what we can expect for the future.

Will focal and segmental glomerulosclerosis (FSGS) for example remain a "diverse group of clinical disorders" as Barua and Pollak are suggesting in their chapter? They point to dysfunction of the podocyte as central to the pathogenesis of the disease. My prediction is that in the future FSGS will be redefined and split into many disease entities that genomic studies are now helping to recognise.

Membranous nephropathy (MN) today is not any longer the disease we were used to think about in terms of pathophysiology and treatment. After more than 50 years of experimental studies—devoted to finding podocyte antigens as target of circulating antibodies for in situ formation of immunocomplexes—at least two relevant antigens have been identified in humans that account for over 70 % of cases of human MN. More than that, genome-wide association studies have contributed to clarify the association between HLA-DQ and PLA2RI loci and the disease in Caucasian patients. An antibody that targets B cell has revolutionised the treatment of this disease and we can easily foresee that in the future novel biological tools will help not simply to treat patient but to unravel the pathophysiology of immunocomplex formation and subsequent injury. The authors of the chapter on MN give an account of these new developments in the treatment of the diseases, which point (their words) to "a direct effect on the pathophysiology of the disease process". They underline that the future is a more specific targeting therapy for MN.

Membranoproliferative glomerulonephritis (MPGN) cannot be considered a single disease today: immunocomplex-mediated MPGN and complement-mediated MPGN due to dysregulation

of alternative pathway of complement are at least distinct disease entity that will likely require different treatment not to mention a number of additional MPGN-associated conditions. These changes of perspective in the classical way of looking at these groups of diseases are well documented in the pages devoted to MPGN and the attempt to underline pitfalls in the current classification and propose a new one building on three decades of past studies and research is of special value. Of great interest, the book devotes a chapter to IgG4-related disease, a newly recognised condition of systemic inflammation and lymphoplasmacytic infiltrates that are rich in IgG4-positive plasma cells and are found virtually in every organ, including the biliary tract, salivary glands, lungs, thyroids, pericardium, and skin. Kidney involvement mainly includes manifestations of tubulo-interstitial nephritis, as it is accurately described in the pages devoted to this disease. Our knowledge about this disease is evolving while other renal phenotypes are emerging that include glomerular changes and lesions of membranous nephropathy that impose significant therapeutic challenges to clinicians. Chapters dealing anti-GBM disease and systemic lupus are less novel but still provide a scholarly overview of pathophysiology, clinical manifestation, and treatment of these diseases that can appeal nephrologists and internists with an interest in renal medicine.

The chapter dealing with thrombotic microangiopathies deserves a special mention. Ten years ago our understanding of the pathophysiology of this group of diseases was very limited. A tremendous research effort has allowed us to reach an extreme grade of sophistication in identifying genetic and acquired initiators and modifiers of atypical HUS, type I MPGNI, Dense deposit diseases (DDD), and C3 nephropathy (C3GN); in developing technology for the diagnosis; and in selecting biomarkers of response to therapy. Finally, we have now better choice to treat these conditions and to prevent post-transplant disease recurrence. All of these can be found at least in part in the book.

The chapter on HUS and TTP also alludes to the similarities rather than to differences in these disease entities in particular as for the pathophysiology of microvascular thrombosis and the role of complement. It is nice to see that this concept, which was very much debated until recently, is now appreciated to the point to find room in book chapter. In a not distant future we will be able to personalise our approach to treatment of monogenic diseases by a limitless potential to modify damaged tissues and organs by genetic engineering and cell transplantation. HUS and TTP, as it emerges from the chapter in the book, will be most amenable to this.

One of the merits of "Core Concepts in Glomerular and Tubulointerstitial Diseases" is to include topics usually not covered by conventional publications. The chapter "Tropical infections diseases of the kidney" offers a comprehensive review of conditions such as tuberculosis, leptospirosis, and malaria and CMV, BK, and other viral infections and some viral hemorrhagic fever. Those living in rich countries tend to forget about such devastating diseases. In several instances, infection may remain silent for years while irreversible kidney destruction takes place.

The chapter on Collagen IV Nephropathies is probably the best chapter in the book. New concepts in genetics and pathophysiology of these diseases are highlighted in a comprehensive, scholarly, and sophisticated way and effectively integrate the even enlarging knowledge on phenotype–genotype correlation and pathophysiology of basement membrane lesions with less recent and very novel related conditions, such as the basement membrane nephropathy and Hanac Syndrome. As it is the chapter dealing with primary disease with systemic features worth reading are the sections on C1q nephropathy, idiopathic nodular glomerulosclerosis, and fibrillary and immunotactoid diseases. The section on diagnosis and treatment in the chapter on amyloidosis and related disorders is of great interest and of particular value to clinicians.

In summary, "Core Concepts in Parenchymal Kidney Disease" is going to become an essential reference book to be found in every renal department library. It will help training fellows in focusing their learning path, but also the experienced nephrologist will not fail to find it an important ally with its refreshing and up-to-date information.

Bergamo, Italy Giuseppe Remuzzi M.D., F.R.C.P.

Preface

We are pleased to offer the 1st edition of *Core Concepts in Parenchymal Kidney Disease*.

Since the original descriptions of parenchymal kidney disease by Richard Bright in 1927, much has changed about our knowledge of glomerular and tubulointerstitial disease. Newer information on the diagnosis and classification of glomerulonephritis has emerged, and there has been much recent progress in pathogenesis. Our book encapsulates some of these advances and provides an updated review. We hope that this book, either in its paper or electronic forms, is a valuable resource to our target audience—practicing nephrologists and nephrology trainees.

We would like to acknowledge the hard work and commitment of our administrative colleagues and Barbara Lopez-Lucio at Springer, who have been magnificent in their support. We hope that you find the book useful and enriching.

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General Approach to the Diagnosis and Management of Glomerular Diseases

1

David Philibert and Daniel C. Cattran

The diversity in clinical presentation, diagnosis, natural history, and treatment of glomerular diseases is a significant challenge for the practicing physician. Part of the diversity is related to the focal and segmental nature of many of these disorders, and this variation should perhaps be expected, given the approximately 800,000 glomeruli per kidney in normal individuals [1]. This chapter will review the important generic issues that relate to all patients with these disorders. This will include an approach to the main clinical syndromes, with an emphasis on the assessment of the risk factors for progression of the underlying glomerular disease. In addition, we will review the challenges of interpreting older studies, given our current knowledge about these diseases as well as the major principles guiding current treatment with a focus on immunotherapy risks, benefits, and limitations. Our objective is to give an overview to the "in common" problems related to management of this patient population.

Clinical Presentation

Patients affected with glomerular diseases present in a variety of ways. It is the clinician's role to recognize these various syndromes and proceed in an organized manner that will lead the physician to the correct diagnosis, a rational assessment of the patient's risk of progression, and a tailored approach to the patient's management that balances the risk and benefits of treatment.

Hematuria

Hematuria is a cardinal manifestation of almost all renal diseases. This applies regardless of whether it is macroscopic or microscopic in form and whether it is glomerular or non-glomerular in origin. The first challenge in the evaluation of hematuria is establishing whether it is pathological or is within normal limits. Indeed, we do not know with certainty the numerical upper limit of red cells in normal urine, but we do know that they are present in large numbers. In addition, the sensitivity of the dipstick testing is high and close to the normal range [2]. This dictates that if the dipstick shows only trace hematuria, repeat testing must be done before considering it of pathological significance; however, when present on repeat testing even in small amounts, the physician is obliged to investigate further including ruling out benign or unrelated conditions such as its occurrence postexercise or due to contamination related to the menstrual cycle.

Once the presence of hematuria is determined to be abnormal, the next important step is to rule out non-glomerular etiologies. There are a wide variety of potential causes including bleeding disorders, stones, infections, and even genitourinary cancer. It is not the purpose of this chapter to review them all, but the treating physician must be aware of their existence and explore these and other causations if indicated on clinical grounds. These clinical indicators for further investigation vary widely from the working environment of the patient to their age at presentation. The likelihood, for instance, of finding a urological cancer before the age of 40 is very low [3], but this risk rises with increasing age, smoking history, and certain environmental exposures such as hair dyes [4] and various industrial chemical carcinogens. The general screening after a complete history and physical examination (including urinalysis and microscopic examination of the urinary sediment) for non-glomerular causes should include a coagulation screen and a renal ultrasound. If the patient is older (>40), cystoscopy should be considered if an explanation of the hematuria is not found on these initial assessments.

There are specific clues that point towards glomerular hematuria. Attention must be paid to the family history, since the presence of isolated hematuria in other family members is commonly found in patients with thin basement membrane disease and in patients with Alport's syndrome.

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The urine sediment may reveal the presence of granular or red cell casts or >5 % acanthocytes (erythrocytes with blebs protruding from the cell body) [5]. The presence of dysmorphic erythrocytes may be more difficult to interpret, although studies suggest that if they constitute more than 80 % of the erythrocytes in the urine, glomerular disease is likely present [6]. Any of these findings strongly suggests a glomerular cause of the hematuria. These findings in the urine sediment will be specific (healthy people will not show them), but not sensitive (patients with glomerular disease will not always show them).

The following should be considered once benign causes for the hematuria and non-glomerular causes have been ruled out. If the hematuria is microscopic and isolated, i.e., there is no proteinuria, and normal blood pressure and no other features on history or urine sediment examination point towards glomerular disease, further investigation including a kidney biopsy is rarely indicated. Exceptions to this rule may include investigation required for insurance or employment purposes to rule out Alport's syndrome in women of childbearing age or to further assess the kidney's suitability for live donor transplantation. If there is microscopic hematuria and only low-grade proteinuria (<0.5 g/day), then close follow-up is recommended, with further investigation including renal biopsy held in reserve if any other signs indicative of worsening glomerular disease subsequently develop.

Further investigation, including a kidney biopsy, should be considered if the differential diagnoses that remain have a significantly different prognosis and/or the therapeutic options available are likely to alter the patient's management and/or outcome. If other signs and symptoms suggestive of more significant disease, such as proteinuria >1 g/day, systemic disease features, abnormal GFR, hypertension, or red cell casts, are found in the urine, further investigation is warranted straightaway.

Asymptomatic Proteinuria

Proteinuria is another frequent manifestation of renal glomerular disease and similar to hematuria has a broad differential diagnosis. The kidney normally excretes a maximum of 150 mg/day of protein, with most of the healthy population ranging between 40 and 80 mg/day. When the albuminuria is between 30 and 300 mg/day, it is considered pathological and designated microalbuminuria and when >300 mg/day, overt albuminuria (or clinical proteinuria), even though most patients remain asymptomatic up to the 3 g/day level. The urinary loss of albumin, a protein with low molecular weight (69 kDa), generally occurs earlier than the larger weight proteins such as IgG (150 kDa) or IgM (950 kDa), and it is therefore not surprising that albumin excretion of more than 30 mg/day in diabetic patients is one of the first signs indicative of nephropathy. Even without diabetes, microalbuminuria has been associated with increased cardiovascular risk [7, 8], with several lines of evidence suggesting that these low levels of albuminuria reflect glomerular capillary (and perhaps systemic) endothelial dysfunction.

Proteinuria (presumably mostly albumin) is not always of pathological significance, and transient low-level proteinuria can be seen during fever and after vigorous exercise. In the United Kingdom Prospective Diabetes Study (UKPDS), albuminuria >50 mg/dL was present in 4 % of apparently healthy individuals [9]. Similarly, in the Third National Health and Nutrition Examination Survey in the US (NHANES III) population, the authors found the prevalence of microalbuminuria (defined as albumin/creatinine ratio 30–299 mg/g regardless of sex) in the general population without comorbid conditions was 5.1 % [10]. When even these levels of albuminuria or proteinuria are persistent and therefore considered pathological, they should be properly quantitated. There are two different approaches to this assessment. The first is the albumin/creatinine ratio (or the protein/creatinine ratio if albumin/creatinine ≥500 mg/g) on a morning sample. This is the method of choice advocated by the NKF/KDOQI because of its convenience and low risk of error [11]. This ratio is most useful when using traditional units of measurement (because the ratio closely approximates the total protein excreted in 24 h) but is somewhat more complicated when SI units are the standard (where mmoles are used for the creatinine estimate compared to the traditional grams), although the precision of the measurement remains the same. Microalbuminuria is said to be present if the albumin/creatinine ratio is 17–255 mg/g in women and 25–355 mg/g in men. This approach is valuable and is recommended, although the second and more traditional method using 24-h urine collection has some unique values. It is not influenced by the time of day, and it allows the clinician to gather other useful information such as the measured creatinine clearance and sodium excretion. The risk of under- or over-collection is real but can be assessed by examining the urine creatinine in the completed collection and comparing it to the expected value based on the muscle mass of the patient. This method is particularly helpful in clinical trials where repeated samples collected over time in each patient are compared. A compromise is a ratio where the collection period for the aliquot is significantly longer, e.g., 6–8 h.

In young people (especially young males) with isolated dipstick proteinuria, normal GFR, and normal urine sediment, benign orthostatic proteinuria should be ruled out. The results from two separate 12-h collections, one in daytime

when the patient is predominantly in the upright position and the other while the patient is in the recumbent position, most conveniently performed during the overnight period are compared. The total proteinuria is almost always <1 g/day in this condition, and the diagnosis is made when the 12-h recumbent value is virtually zero and the abnormal proteinuria confined to the upright position time period.

After proteinuria quantification, its quality may need to be evaluated by electrophoresis; this is especially important in older patients where additional signs and symptoms might suggest a systemic condition such as multiple myeloma or amyloidosis.

Evaluation of the patient with significant proteinuria should include measurement of serum albumin and a lipid profile. Blood pressure assessment and GFR measurement should also be determined. Screening for secondary causes should be considered in most cases, including tests for antinuclear antibodies (ANA), antineutrophil cytoplasmic antibodies (ANCA), complement profile (C3 and C4 levels), and renal anatomy evaluation by ultrasound. If any of these are abnormal and/or if there are systemic symptoms such as unexplained fever or weight loss, a kidney biopsy is usually performed. If the proteinuria is isolated and in the subnephrotic range, and there are no additional systemic features, then the need to perform a kidney biopsy is more controversial. Primary FSGS or membranous nephropathy can sometimes present with isolated low-grade proteinuria, but immunosuppression is not currently indicated at these levels (<3.0 g/day) of proteinuria particularly if the GFR and blood pressure are normal. On the other hand, even though rare, some systemic diseases, such as amyloidosis, can present as isolated proteinuria, so the decision in regard to a biopsy is often dependent on the nephrologist judgment. A conservative approach of watching and waiting is a viable option, provided regular assessment of GFR, blood pressure, and proteinuria is maintained, with the renal biopsy performed only if the clinical setting changes and becomes more suggestive of worsening glomerular or systemic disease.

The Nephrotic Syndrome

If the proteinuria worsens, other features of the nephrotic syndrome may develop such as hypoalbuminemia and peripheral edema. Lipid metabolism is also altered, leading to hyperlipidemia [12]. Patients with this syndrome are more prone to have thromboembolic events [13, 14], especially when it is associated with certain histological subtypes such as membranous nephropathy or focal segmental glomerulosclerosis [15]. In a large review of 898 patients with biopsy-proven membranous nephropathy, for instance, the prevalence of clinically apparent venous thromboembolism was 7.2 %, with hypoalbuminemia (<2.8 g/dL) being the most important risk factor [16]. The reasons for this increased risk have not been fully elucidated, but hypovolemia plus disturbances in the complex balance of thrombogenic/antithrombogenic proteins undoubtedly plays a role [17]. Increased susceptibility to infections can also be seen in nephrotic patients, particularly in children. Physical factors such as fluid collections secondary to the lowered oncotic pressure in the pleural, peritoneal, or other spaces have been implicated in these infections, but additional factors including depressed lymphocyte function, low serum immunoglobulins (especially IgG), and decreased complement levels secondary to urinary losses of these components likely contribute to this risk. Finally, urinary loss of binding proteins and hormones [18], like vitamin D, thyroid-binding globulin, and erythropoietin, occurs in patients with the nephrotic syndrome, although their clinical relevance is often small or difficult to estimate.

The causes of the nephrotic syndrome are numerous. A kidney biopsy should generally be performed in order to determine diagnosis, estimate prognosis, and guide treatment. Antinuclear antibodies (ANA), anti-dsDNA antibodies, levels of complement (both C3 and C4), hepatitis B and C serology, serum protein electrophoresis, and cryoglobulins are reasonable screening tests to rule out secondary causes. A VDRL or equivalent may be needed to exclude syphilis, although this is rare today as causation in developed countries. Anti-GBM antibodies are not usually needed, unless there is associated renal failure or clinical features suggestive of pulmonary involvement. Antineutrophil cytoplasmic antibody (ANCA) tests should be ordered if there are significant systemic features suggestive of an underlying vasculitis such as unexplained fever; malaise; weight loss; upper airway lesions of the ear, nose, or throat; or pulmonary involvement. Although renal-limited ANCA vasculitis with pure nephrotic syndrome is possible, it is uncommon.

A workup for an underlying malignancy should be considered particularly in patients older than 60 and with membranous nephropathy [19]. Examination of the prostate and measurement of prostate-specific antigen (PSA) in males and mammography in female, as well as, in both sexes, a chest X-ray (consider chest CT in high-risk patients) and GI evaluation including an abdominal ultrasound or CT, will cover most primary origins for tumor-associated disease.

Renal Vein Thrombosis

Patients presenting with the nephrotic syndrome particularly with high-grade proteinuria and hypoalbuminemia (<2.8 g/dL) have a higher risk of renal vein thrombosis (RVT) [20]. Previous studies using renal venography noted RVT in 22 % of 151 patients that presented with the nephrotic syndrome regardless of the cause [14]. A more recent review showed

that prevalence of RVT was highest in patients with membranous nephropathy (37 %) [13]. RVT can present with acute loin pain, hematuria, kidney enlargement, rapid increase in lower limb edema, and with unexpected deterioration in renal function. In some cases, the presentation can be quite asymptomatic except for a slow decline in renal function. Its diagnosis therefore requires a high index of suspicion, although routine and repeated screening of every patient with nephrotic range proteinuria is not currently recommended. Pulmonary embolism can accompany RVT and was found in 20 % of the chronic RVT patients who underwent a ventilation/perfusion lung scan in one series [14]. This complication should be considered when RVT is diagnosed even in the absence of acute pulmonary signs and symptoms.

Rapidly Progressive Glomerulonephritis

The most dramatic presentation of glomerular disease is the somewhat misnamed rapidly progressive glomerulonephritis (RPGN). This is a clinical scenario, not a type of glomerulonephritis, characterized by rapid deterioration in renal function over weeks or months, associated with glomerular hematuria and proteinuria (usually not in the nephrotic range) and often, but not always, systemic symptoms and hypertension. When this scenario occurs, immediate investigation is warranted since more renal parenchyma is likely to be preserved the earlier appropriate treatment is begun. The progression of the renal disease can be dramatic, leading in some cases to end-stage renal disease in a matter of days (e.g., anti-GBM disease). The classic histological correlation is the presence of crescents on renal pathology. Crescentic glomerulonephritis can be divided into four variants according to the immunofluorescence (IF) findings:

- Type I: Anti-GBM disease, characterized on IF by linear deposits of IgG along the glomerular basement membrane.
- Type II: A heterogeneous group of diseases characterized by prominent granular deposits usually along the capillary loops but occasionally confined to the mesangium associated most commonly with lupus nephritis, cryoglobulinemia or an underlying primary glomerulonephritis such as IgA nephropathy, membranoproliferative glomerulonephritis (MPGN), membranous nephropathy, or post-infectious proliferative glomerulonephritis.
- Type III: Pauci-immune glomerulonephritis, a condition where the glomerular deposits by immunofluorescence are rare or absent. The ANCA-associated variants are found within this class.
- Type IV: They have features of both types 1 and 3, being double-antibody diseases.

Today the ANCA-associated glomerular diseases dominate the clinical scenario of RPGN. They are responsible for more than 80 % of the cases in patients that present over the age of 60. Systemic signs and symptoms are often associated with these diseases and should be diligently sought since these can be the important clues to this underlying condition.

When a patient presents with a clinical picture compatible with RPGN, determining the specific renal pathology should be considered an emergency. In addition to a renal biopsy, serologic testing, including anti-GBM, ANCA, and ANA (and anti-dsDNA), complement, and cryoglobulins should be assessed on presentation to help in determining the most likely cause of the scenario. Treatment is often started on clinical suspicion of the diagnosis (see individual chapters for treatment of particular diseases), often before the histology or serologic screening test results are available, because with active crescent formation, irreversible kidney tissue damage can occur within days.

Some patients with a known glomerular disease, such as diffuse proliferative lupus nephritis, IgA nephropathy, or Henoch-Schönlein purpura, can develop crescents at any time in the course of their disease and present with an acute deterioration in their previously stable chronic renal disease. It is important to establish the correct cause of this deterioration since it will often alter management. For instance, an episode of acute kidney injury in a patient with IgA nephropathy may be associated with an intercurrent illness and only findings of acute tubular necrosis on biopsy or, at the other extreme, the development of crescentic glomerulonephritis.

There can be a clinical scenario of RPGN secondary to diffuse proliferative glomerulonephritis. They can present clinically as an acute nephritic syndrome (acute renal failure, elevated blood pressure, glomerular hematuria) but with pathology showing only a pattern of endocapillary proliferation and no crescent formation [21]. This variant of RPGN illustrates another reason for rapid histological diagnosis, since recovery with only symptomatic management is a common outcome with this histology (e.g., complete recovery following a streptococcal-related glomerulonephritis).

The definition of rapidly progressive glomerulonephritis implies that a rapid deterioration in renal function has been documented. In clinical practice, patients will not always have previous creatinine measurement to compare with their presenting value. If a clinical setting is compatible with RPGN, i.e., if there is no other obvious cause of renal failure (e.g., prerenal, obstruction, infection, etc.), a renal biopsy should not be delayed but should be done in concert with seeking this other information.

Factors That Bear on Progression of Glomerular Diseases

Progression of both diabetic and nondiabetic glomerular disease is influenced by both comorbid conditions and factors directly related to the disease process. A full discussion

on cardiovascular management of the patient with chronic kidney disease is beyond the scope of this chapter. Instead, we will review the specific impact of proteinuria, arterial hypertension, and hyperlipidemia, which are factors considered to influence the rate of kidney disease progression.

Proteinuria

Proteinuria is one of the cardinal manifestations of glomerular injury. In many of the glomerular diseases, its severity is closely linked to prognosis. It has been used as a surrogate endpoint of renal survival in many studies because of this relationship. Moreover, in recent years, studies have suggested that proteinuria not only reflects the degree of glomerular damage but also may have a direct nephrotoxic effect at the post-glomerular level [22, 23]. There is strong experimental data that indicates proteinuria per se can cause tubular epithelial cell dysfunction and interstitial inflammation and fibrosis. Many mechanisms have been implicated in this process including upregulation of the transcription factors that are felt to mediate inflammatory, vasoactive, and fibrogenic genes, as well as those that are involved in activation of the complement cascade. In addition, studies have indicated that tubular cell activation by protein-bound circulating molecules can result in an apoptotic response to the presence of protein in the tubule. The net effect is tubular and interstitial toxicity. The extent of the toxicity is probably dependent on both the duration of exposure and the specific composition of the proteinuria: albumin, for example, is likely to be less toxic than oxidized lipoproteins. These activation pathways have largely been derived from in vitro experimental models, but many clinical studies have indicated a close association between the quantity of proteinuria and progression of the underlying renal disease. In the REIN study, which included a variety of renal diseases (but excluded diabetics), the reduction in proteinuria was inversely correlated with the rate of decrease in GFR. This correlation was seen despite almost identical BP control [24]. Similar findings have been found in diabetic nephropathy. In the RENAAL study [25, 26], for example, the severity of albuminuria was almost linearly related to renal outcome. Formal proof of causation between proteinuria and clinical progression has not been established, but these studies and others strongly support the experimental evidence of the direct nephrotoxicity of proteinuria.

It is clear, however, that all proteinuria is not the same. Its nephrotoxic potential should be considered both in terms of quantity and quality. Small increases in proteinuria even in the sub-nephrotic range in patients with diabetes mellitus or IgA nephropathy [27] are associated with a worse renal survival. However, this is not true in every glomerular disease; for instance, in membranous nephropathy, the long-term outcome of proteinuria in the 1–2 g/day range remains excellent. The nature of the proteinuria currently evaluated by a variety of techniques including immunoelectrophoresis and, in the future, by more sophisticated analyses of the urinary proteome is likely to provide us with much more detail about the variations in tubular/interstitial damage caused by individual protein moieties. We are already aware of other significant variations in the quality of the proteinuria. One example is in myeloma nephropathy, where there are clear differences in renal damage observed when lambda versus kappa light chains are produced. We also know that little tubular interstitial damage occurs in minimal change disease despite heavy proteinuria. It is likely that in part this is related to the composition (quality) of the proteinuria, i.e., almost entirely albumin, and also to the limited duration of exposure of the tubules to protein, given the usual rapid reversal of this condition with treatment.

Hypertension

Arterial hypertension also directly damages the kidney. It is a strong independent risk factor for end-stage renal disease, even without underlying glomerular disease [28]. The presence of hypertension, however, in association with glomerular disease remains an important and independent risk factor for progression. This was recently confirmed in a study of 298 patients with IgA nephropathy [29]. The authors found that the level of blood pressure and proteinuria over time (but not at presentation) were the only independent factors related to progression. Certainly, it has been demonstrated repeatedly that controlling the blood pressure in patients with diabetic nephropathy, predominantly a glomerular process, significantly slows progression rate [30]. Similar results have also been found in studies of patients with nondiabetic nephropathy, such as the MDRD trial, where BP reduction was associated with both a decrease in proteinuria and disease progression rate [31, 32].

Renin-Angiotensin System Inhibition

Renin-angiotensin system (RAS) inhibition is now a central part of the treatment of both diabetic nephropathy and nondiabetic proteinuric nephropathy. Both angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II receptor blockers (ARB) protect the kidney by their antihypertensive effect and by inducing efferent arteriole dilation, which in turn leads to a fall in intraglomerular pressure and a reduction of proteinuria. These agents are well known to decrease proteinuria and slow progression to end-stage renal disease (ESRD) in diabetic nephropathy [26, 33, 34]. A benefit of RAS inhibition also seems present in nondiabetic proteinuric disease and has been mostly studied with ACE

inhibitors, where various meta-analyses have suggested a reduction in the rate of progression to ESRD and doubling of serum creatinine [35, 36], although it is not clear if this effect is independent of blood pressure control. Patients without significant proteinuria (<500 mg/day) do not seem to benefit from the same protection, although duration of the follow-up may be the limiting factor in these studies [36]. ARB may well have a similar effect, but the data are more limited [37]. Despite suggestion that patients with early (stage 1–3) chronic kidney disease may not benefit as much from RAS blockade [38], ACEI and ARB remain effective and well-tolerated antihypertensive agents, hence their widespread use in clinical practice. However, combination therapy with agents must be done with great care and is not generally recommended, even though it may lead to greater proteinuria reduction. This is based on the large ONTARGET trial that showed a worsening of renal endpoints with a combination of ramipril and telmisartan compared to single-agent therapy [39].

Lipids

In many animal models of renal damage, hyperlipidemia has been shown to accelerate renal dysfunction [40, 41]. It has also been associated with accelerated progression of kidney disease in some studies in humans [42]. However, the beneficial effect of statins to directly slow progression of glomerular-based kidney disease has not been proven in humans. Although many studies and meta-analyses [43] support this contention, this effect is not consistently observed. More recently, the SHARP trial [44] showed no significant difference in the progression to end-stage renal disease in a population of CKD patients, although many in the cohort did not suffer specifically from a glomerular disease and were already at an advanced stage of renal failure prior to study entry. Despite this, in the broader context of cardiovascular disease prevention, the efficacy of statins has been well established, and since many patients with glomerular disease have both hyperlipidemia and accelerated vascular disease, the use of such agents is now a critically important element in their management.

Interpretation of Studies in Glomerular Diseases

Evidence-based medicine is a well-established paradigm in clinical medicine, but it has limitations. When dealing with diseases with a low incidence and often-slow progression rate over many years, it is difficult and expensive to carry out the appropriate randomized controlled trials that would provide grade A evidence for different treatment modalities. On review of the current literature, the lack of such trials is evident. Currently, much of the evidence on which we rely for support of our treatment comes from less high-quality trials and retrospective or observational studies.

Many other issues are relevant to the interpretation of studies in glomerular diseases. Studies based on renal pathology illustrate one particular type of limitation. A kidney biopsy, by definition, is a cross-sectional assessment, and although usually sufficient to make a diagnosis, a clear estimate of the prognosis, especially in the setting of a slowly progressive glomerular disease, is unlikely. Pathology does allow us to establish the amount of damage that has occurred as reflected by the severity of tubular and interstitial injury, but the rate of disease progression and the determination of the acuity of ongoing injury are often difficult to quantitate using our current techniques.

Another confounding issue in the interpretation of evidence relates to the temporal sequence in the development of therapeutic strategies. A classic example is the use of corticosteroids in IgA nephropathy. In the best RCT studying this approach [45], a 6-month course of steroids was shown to reduce proteinuria and to protect against subsequent deterioration in renal function. However, only a fraction of the patients received angiotensin-converting enzyme inhibition, whose efficacy was being tested in IgA nephropathy during the same time frame of the corticosteroid study. The use of agents to blockade the renin-angiotensin system is now considered first-line treatment and is currently recommended to be given prior to the introduction of immunosuppressive agents. This makes the assessment of the additive value of corticosteroid treatment in IgA nephropathy more problematic, given that trial did not have a uniform baseline renin-angiotensin system blockade in place.

In addition, many factors previously unknown or unrecognized that impact on the prognosis of the disease complicate the interpretation of earlier studies. Part of this problem is because although the histological label of the disorder has remained unchanged, the number of causative agents that will produce the identical lesions has grown dramatically. In recent years, for instance, the discovery of genetic mutations in some of these glomerular diseases (such as IgAN and FSGS) has confirmed new factors that impact on both susceptibility and progression rates of these classical patterns of injury. Additionally, new external factors have been identified as causative agents through the development of new technology. This has allowed us to recognize, for instance, a new virus designated hepatitis C, which was subsequently found responsible for many of the previously designated idiopathic cases of membranoproliferative glomerulonephritis. This finding has cast considerable doubt on previous therapeutic studies in this disease completed prior to this association being recognized. Other examples include the recent discovery of antibodies directed against phospholipase

A2 receptor as the main culprit in the so-called primary (idiopathic) membranous nephropathy [46]. Another development has been the changing incidence and age at presentation of many of the major progressive GN variants. The prevalence of membranoproliferative glomerulonephritis, for instance, has steadily decreased over the past 20 years in the developed world [47], whereas the frequency of FSGS on biopsy has tripled over the same time frame.

The use of surrogate endpoints in the study of most glomerular diseases is almost unavoidable, given the most commonly observed temporal pattern is slow progression over years. The most important criterion for the validity of a surrogate endpoint is its ability to predict the effect of an intervention on the most definitive endpoint, renal survival [48]. The most frequently used surrogate endpoints in nephrology are changes in GFR, serum creatinine, or proteinuria. Although the majority of nephrologists believe these surrogate markers meet this fundamental criterion, a number of challenges persist in regard to their assessment. The measurement of GFR, often considered as the best surrogate endpoint, is most useful in studying patients who have a rapid rate of deterioration in renal function. This is because early in the course of most renal disease, functional changes compensate for organic damage and true estimate of change in GFR may only be seen after this compensation is exhausted. In addition, GFR is influenced by acute hemodynamic changes, such as volume contraction that can be induced by diuretic therapy, high-grade proteinuria especially when associated with lowered oncotic pressure, or with certain drug treatments such as the calcineurin inhibitors. In some studies, GFR is estimated by measurement of creatinine clearance. In these cases, the assumption is that creatinine generation and nonrenal excretion remain constant throughout the study. This is not always the case. Protein restriction, for example, can have an impact on creatinine generation and secretion, as illustrated in the MDRD study [49]. An alternate approach often used in studies consists of comparing slopes of GFR decline between groups. This approach is complicated by the imprecision in slopes associated with patients with shorter follow-up. Although this can be partially accounted for by giving more weight to patients with longer follow-up, this could introduce a different bias since subjects with shorter follow-up may have the most clinically significant endpoints like death or end-stage kidney failure.

Another common approach uses the "time-to-event" endpoints, such as time to doubling of serum creatinine. In comparison with slope-based analysis, time to event is more sensitive for fast progressors and, because by definition it requires a large change in GFR, is a closer approximation of the definitive endpoint of ESRD. However, in patients with early stage CKD or with slow progression, the low event rate makes time-to-event endpoint analysis almost unachievable or requires a very large sample size. Moreover, the possibility of competing events, such as cardiovascular disease, may lead to informative censoring. Including death as part of the composite can account for this problem, but then it must be recognized that the data may actually be confounded since all causes of death are not necessarily related to renal disease progression.

Changes in proteinuria have both biological plausibility and a strong correlation with end-stage renal disease in most glomerular disease. This has led to its use as a surrogate endpoint, although it is not per se a required element in the deterioration of renal function. Its utilization as a surrogate endpoint therefore requires rigorous analysis. The well-recognized relation between proteinuria, as both an effect and cause of progression, does not necessarily mean that a treatment that decreases proteinuria will slow progression, since the treatment may affect progression through an entirely independent pathway. In some diseases like diabetes and IgA nephropathy, we have sufficient data to confirm that reduction in proteinuria is associated with reduction in ESRD, whereas this is not the case in all glomerular diseases. Proteinuria may impact on renal parenchymal tissue damage by both its quantity and quality differently, and therefore, its reduction does not necessarily translate into similar benefit (see section "Proteinuria" above). Reducing proteinuria from 3 g/day to <1 g/day, for example, does not have the same prognostic significance in membranous nephropathy that it does in IgA nephropathy.

Principles of Immunotherapy

Specific treatment protocols of immunosuppression in the different histological variants of glomerular disease will be reviewed in the following chapters. We will discuss immunosuppression only from the perspective of assessing risk of such therapy in a patient with progressive glomerular disease. Risk is particularly difficult to assess when the adverse effects of therapy have a different timeline than the benefit, appearing either early in treatment or long after therapy has been discontinued. Benefit of treatment, on the other hand, at least its definitive effect on slowing or preventing ESRD, often falls between these two extremes of time. This difficulty in including the risk of treatment also applies to most predictive algorithms of progression that have been developed. They rarely, if ever, include patient morbidity issues related to treatment. Part of the difficulty in adding this element is the inability to generalize or semi-quantitate the potential adverse effects since they often are very dependent on the specific characteristics of the sample population. The patient's chronologic age, for instance, is easy to determine, but adding risks for any and all comorbid conditions is virtually impossible. Therefore, although all decision about treatment should be based on best available evidence, there must be clinical judgment brought to bear in every case.

There are generic ways of reducing the likelihood of morbidity: specifically lower doses of the treatment, shorter duration of therapy, alternative administration route, or the choice of other agents. In addition, there are specific preventive strategies that can be used to combat potential adverse events that impact on morbidity. Many of these approaches have been applied in glomerular diseases and will be discussed in subsequent chapters. We will only review the fundamental principles about the commonly used immunosuppressive agents in glomerular disease, their risks, and management strategies to minimize these effects.

Glucocorticoids

Corticosteroids, probably the best known and certainly one of the oldest immunosuppressive agents, have been used for many years in the treatment of glomerular diseases. Their biological effects are broad [50], but in large part they act by binding to specific intracellular receptors, leading to conformational change in DNA, and subsequent modulation of gene transcription. This results in the production of anti-inflammatory products and, through inhibition of nuclear factor kappa B (NF-κB), to the inhibition of synthesis of many inflammatory cytokines. The end result is a broad effect not only on immune function of B and T lymphocytes but on functional elements of circulating neutrophils and monocytes.

Multiple studies have confirmed the clinical efficacy of steroids in the glomerular diseases, e.g., minimal change disease. Steroids are also commonly used in conjunction with other immunosuppressive agents in other glomerular diseases such as lupus nephritis and membranous nephropathy.

The side effects of steroids are well known. They are related to both the total dose and the duration of use, but even doses as low as 5 mg/day are thought to carry increased risk of adverse effects [51]. The most important ones include accelerated atherosclerosis, alterations of glucose metabolism, psychiatric issues including psychosis, increased susceptibility to infection, development of cushingoid features, and musculoskeletal complications such as osteoporosis, myopathy, and avascular necrosis. Steroids have one distinct positive feature compared to all other immunosuppressive agents in that they do not increase the risk of cancer. This is a substantial advantage in the glomerular disorders that evolve over years and that often require more than one course of treatment.

There are strategies for minimizing adverse events. These include prophylaxis for osteoporosis and advising physical exercise for prevention of myopathy. In addition, alternate day administration can be used. It is always important that the duration of treatment should be kept to a minimum. Another approach consists of alternating this therapy with another agent such as in membranous nephropathy [52, 53], where the routine is a 6-month course of treatment that consists of corticosteroids alternating monthly with a cytotoxic agent.

Pulse treatment with methylprednisolone is another strategy. Bolus steroids are felt to have a more rapid onset of action, a longer effect, and higher bioavailability than oral corticosteroids [54]. This approach has been used in the treatment of rapidly progressive glomerulonephritis and in the treatment of vasculitis.

Special attention should be paid to bone-sparing strategies. It is now recognized that most of the corticosteroid damage to bones occurs in the first few weeks of utilization [55], and hence prophylaxis with calcium supplements, vitamin D, and bisphosphonates is advised [56] when therapy beyond weeks is considered. Bisphosphonates are quite safe even in the mild to moderate renal failure category that commonly accompanies the glomerular diseases [57, 58]. When GFR is lower than 30 mL/min, they should be used with caution [59] because of the concern that other nonresponsive bone disorders, such as adynamic bone disease, may be present at that level of renal function.

Cyclophosphamide

Cyclophosphamide is an alkylating agent thought to act largely through the alkylation of purine bases producing its cytotoxicity effect. Induction of DNA damage leads to cell death or altered cell function [60]. Both T- and B-cells are affected, making cyclophosphamide a potent immunosuppressive agent commonly used in a variety of the progressive glomerular diseases including crescentic glomerulonephritis, vasculitis, and lupus nephritis.

Cyclophosphamide is toxic. It increases the long-term risk of leukemia, skin and bladder cancers, as well as other malignancies. In a retrospective study of its use over 20 years in a cohort of rheumatoid arthritis patients, relative risk for malignancies was 1.5 [61]. Hematological long-term toxicity includes an increased risk of the development of myelodysplastic syndrome [62]. In a review of 293 patients with Wegener's granulomatosis [63], a cumulative dose of more than 36 g was associated with a significantly higher risk of malignancies, especially acute myeloid leukemia, bladder cancer, and nonmelanoma skin cancer (standardized incidence ratio of 59, 9.5, and 4.7). Many of these malignancies occurred years after initiation of therapy (between 6.9 and 18.5 years for leukemia and bladder), thus emphasizing the importance of long-term follow-up.

Gonadal toxicity and infertility constitute the second most concerning adverse effect of this class of agents. In females,

risk factors are mainly the total cumulative dose, irrespective of the route of administration [64], and the age at which therapy is initiated. Women whose therapy was given before the age of 25 are at lower risk of this complication than those started after the age of 30 [65]. In men, long-term gonadal toxicity can occur at a cumulative dose as low as 100 mg/kg [66].

Bone marrow depression is common with this agent and is manifested most commonly by leucopenia. White blood cell count must be monitored frequently, and intravenous doses should be adjusted to target absolute neutrophil count above 1,500 per μL. For patients on oral cyclophosphamide, drug should be stopped if total white cell count falls below 4,000 per μL; medication can be restarted at 75 % of previous dose once total white cell count increases to >5,000 per μL. Increased susceptibility to infections also occurs and is a worrisome consequence of treatment. Prophylaxis against Pneumocystis pneumonia with an agent such as trimethoprim-sulfamethoxazole is recommended. Infections can evolve rapidly and can be fatal in the immunocompromised host, and cyclophosphamide therapy should be discontinued if significant infection develops.

Other effects although less serious to life but important to the patient include alopecia which usually recovers once the medication is stopped, gastrointestinal intolerance limited to the duration of treatment, and hemorrhagic cystitis especially when bolus IV therapy is used. To lower this, mesna (which binds to acrolein, a toxic metabolic of cyclophosphamide) may be prescribed [67]. A suggested protocol is 300 mg/m^2 at the beginning of infusion and then 150 mg/m^2 4 h later.

Total cumulative dose plays a major role in long-term toxicity of cyclophosphamide, and therefore, reduction in total drug exposure is a major prevention strategy. The CYCAZAREM trial [68] determined that it was both safe and effective to substitute azathioprine for cyclophosphamide as maintenance therapy in patients with ANCA-associated vasculitis. This substantially reduced the total exposure to cyclophosphamide and subsequent adverse events. A similar strategy in lupus nephritis of substituting mycophenolate mofetil or azathioprine was associated with lower rates of infection and less amenorrhea compared to ongoing cyclophosphamide therapy [69]. An alternate and equally effective strategy has been the substitution of intravenous bolus treatment for oral cyclophosphamide in the treatment of lupus nephritis and ANCA vasculitis [70, 71].

In summary, cyclophosphamide is a potent immunosuppressive medication that has proven efficacy in a variety of glomerular diseases. Its high toxicity, however, has limited its use, and the physician should carefully consider the alternatives and balance its benefits versus risks whenever it is being considered as a treatment option.

Mycophenolate Mofetil

A more recently developed antiproliferative agent is mycophenolate mofetil (MMF). The drug acts through the inhibition of inosine monophosphate dehydrogenase, an enzyme implicated in the de novo synthesis of purine bases. This leads to both a decrease in T- and B-cell proliferation and a decrease in antibody formation.

There have been a number of clinical treatment studies published since its introduction. The highest-quality glomerular-based disease studies have been in lupus nephritis where it has proven to have equivalent efficacy but less toxicity than cyclophosphamide as both induction and maintenance therapy [72, 73].

Gastrointestinal intolerance, including nausea, diarrhea, and abdominal pain, is very common with this agent, but its frequency and severity are reduced with time [74]. Dose reduction or splitting the daily dose into smaller but more frequent ones can help manage these side effects. Anemia or leucopenia is seen in 10–20 % of patients secondary to bone marrow suppression. Regular assessment of the complete blood count with MMF dose adjusted if necessary is mandatory. Other adverse effects, in common to all immunosuppressive agents, include increased susceptibility to infection and increased risk of malignancy. Most of the latter data comes from transplant studies, where MMF has been used in combination with other potent immunosuppressive drugs and information regarding risk as monotherapy is scarce.

In summary, MMF is less cytotoxic than cyclophosphamide, and in addition, it has many advantages over the other common class of immunosuppressive agents, the calcineurin inhibitors (CNI). This class of agent has, for instance, no effect on blood pressure or renal function and no alteration in the lipid profiles or on glucose metabolism, common side effects with CNI's treatment.

Azathioprine

This drug, one of the oldest immunosuppressive agents, is a purine analogue that has an antiproliferative effect. Although it has been largely replaced by MMF in transplant patients, it is still used in glomerular diseases, most commonly as maintenance therapy replacing cyclophosphamide after induction [68]. Azathioprine is usually well tolerated and has a lower teratogenicity potential than cyclophosphamide in pregnancy. Bone marrow toxicity occurs and is most commonly limited to the leukocyte lineage. It is also significantly less expensive than MMF and showed similar tolerability to this agent and possibly even better efficacy in ANCA vasculitis maintenance therapy [75]. However, in lupus, MMF has recently demonstrated significantly better efficacy than azathioprine for maintenance therapy [76].

Calcineurin Inhibitors

Cyclosporine

Cyclosporine is a potent immunosuppressive drug first used in the field of solid organ transplantation. Upon entry into the cell, it binds to the immunophilins, and this complex inhibits calcineurin. Calcineurin is normally involved in dephosphorylation of nuclear factor of activated T-cells (NFAT). Following the dephosphorylation, it normally enters the nucleus and activates the transcription of various cytokines, including interleukin-2. Inhibition of calcineurin blocks this process, thus inhibiting T-cell activation.

Cyclosporine has proven to be effective, either alone or in combination with steroids, in the treatment of minimal change disease, FSGS, and membranous nephropathy.

Cyclosporine has many adverse effects. The most worrisome and feared side effect is nephrotoxicity that can be severe and has been documented to progress to renal failure when prolonged high doses of the drug have been given. There appear to be two separate mechanisms involved: an acute renal hemodynamic effect that can be managed by decreasing the dose and a second more worrisome one associated with chronic tubulointerstitial nephrotoxicity that may progress without a matching decline in GFR until late in the process. This seems linked to both dose and duration of treatment and is aggravated by the narrow therapeutic window between efficacy and toxicity of this agent. Most feel cyclosporine is safe [77] even long term, provided the daily dose is kept under 3–4 mg/kg with 12-h trough level maintained <150 ng/mL. It has been this concern about nephrotoxicity that has limited the use of cyclosporine to refractory cases or to situation where alternatives are not available or have been less effective. Cyclosporine, as with all immunosuppressive agent, is associated with an increased risk of cancer and infection. Most of the toxicity data we have come from transplant studies where it is most commonly used in combination with other immunosuppressive agents. Toxicity of cyclosporine in patients with glomerular disease, when used as a single agent and in the low-dose regimen outlined above, has substantially less toxicity, although this risk should be constantly monitored. Some additional effects are more cosmetic in nature, like hypertrichosis and gum hypertrophy. However, both can be severe and may lead to nonadherence or the need for alternate treatments. Other side effects include arterial hypertension, induction of hyperkalemia, and hypomagnesemia. These can be managed medically.

Tacrolimus

Tacrolimus is a newer calcineurin inhibitor that has a similar biological effect as cyclosporine. Its use has been largely limited to the field of transplantation, although data on its use in glomerular disease is increasing, notably in membranous nephropathy [78]. Its adverse effect profile is similar to cyclosporine, with the exception that it has less hypertrichosis and does not have the potent "skunky" smell associated with the former. On the other hand, it does appear to have a greater effect on glucose metabolism, and new onset diabetes is higher with its use.

mTOR Inhibitors

This class includes sirolimus and a similar agent, everolimus. mTOR inhibitors are relatively new agents currently restricted to the solid organ transplantation field. They are known to have both an immunosuppressive and antiproliferative effect. They act by inhibition of the mammalian target of rapamycin (mTOR) protein, which is involved in cell-cycle regulation via the signal transmission of cytokines such as IL-2. The clinical studies of these drugs in glomerular disease are very limited. In rats with Adriamycin nephropathy, some data suggest a reduction in renal scarring [79]. An open-label trial of patients with steroid-resistant FSGS has shown a substantial benefit in terms of reducing proteinuria with supporting concurrent physiological studies that indicated it reduced glomerular pore size and improved the ultrafiltration coefficient [80]. In contrast, a safety study of sirolimus in FSGS [81] was prematurely stopped because of serious adverse events in five out of six patients including acute renal failure, increasing proteinuria, and dramatic elevation in triglyceride values. Presently, it is not recommended in the treatment of glomerular diseases.

Rituximab

Rituximab is a chimeric monoclonal antibody directed against the CD20 molecule found on pre-B-cells and mature B-cells. It prevents proliferation of B-cells, which then undergo apoptosis. It was originally designed for the treatment of non-Hodgkin's lymphoma, but because many autoimmune diseases, including some types of glomerulonephritis, have an important humoral component, interest has been high in studying the drug's effects in these conditions. In ANCA vasculitis, two randomized trials have shown it was as effective as cyclophosphamide for the induction phase of treatment [82, 83]. Adverse event rates were similar. In membranous nephropathy, pilot studies have suggested rituximab reduces proteinuria and can induce remission even in patients who have failed alternate immunosuppressive regimens [84, 85]. Other potential uses include refractory lupus nephritis, where the data is more limited, and hepatitis C-related mixed cryoglobulinemia, where studies have suggested it could increase the rate of remission [86, 87]. Main

adverse effects of rituximab are usually mild and related to infusion reactions such as fever and chills. More serious adverse effects have been described but, in general, are more commonly seen in the original indication for the agent, i.e., non-Hodgkin's lymphoma, and are related to the tumor burden rather than to the drug itself.

Plasma Exchange

Plasma exchange has been used in various types of autoimmune diseases for more than 30 years. It has proven efficacy in antiglomerular basement membrane disease [88]. The results in the ANCA-associated vasculitis trial in patients with poor renal function (serum creatinine >500 μ[mu]mol/L), the MEPEX trial [89], suggested that the addition of plasma exchange to standard therapy increased both the percent of patients and the speed of renal recovery compared to IV methylprednisolone. Other suggested indications have included thrombotic microangiopathy and lupus erythematosus [90]. In lupus erythematosus, it may be used in certain patients with life-threatening or therapy-resistant manifestations, but the evidence base is weak, and its major advantage seems to be its safety. Although generally felt to be a benign therapy (but expensive), adverse events are not negligible, and a recent report from the World Apheresis Registry indicated such effects in 11 % of 388 procedures; many of them were related to citrate anticoagulation. No deaths were reported [91]. A specific chapter is devoted in this textbook to the use of plasma exchange in patients with kidney diseases.

Intravenous Immunoglobulins

Initially introduced in the 1950s as replacement therapy for patients with congenital antibody deficiencies, intravenous immunoglobulins (IVIG) are now used in a wide range of immunological/autoimmune conditions. Their mechanism of action, although incompletely understood, seems to depend largely on antigen binding and modification of effector functions [92], including the modulation of T- and B-cell activation. They may control the disease process without inducing severe immunosuppression in contrast to cytotoxic agents [93]. Pilot studies in both lupus nephritis and ANCA-associated glomerulonephritis have been done, but these studies are small and the benefits hard to assess [94, 95]. Its appeal is its safety profile. Adverse effects, although common, are usually mild. Nephrotoxicity is a potential complication affecting up to 6.7 % of patients in one series [96], and this adverse effect may not always be reversible.

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Minimal Change Disease in Adults

2

Jonathan Hogan and Jai Radhakrishnan

Introduction

Minimal change disease (MCD) is defined when nephrotic syndrome occurs without any glomerular lesions on light microscopy (or only minimal mesangial prominence), negative staining on immunofluorescence microscopy (or low-level staining for C3 and IgM), and foot process effacement but no electron dense deposits on electron microscopy (Fig. 2.1) [1].

Most children with the nephrotic syndrome are not biopsied; instead, they are typically treated empirically with steroids. However, most adult patients with the nephrotic syndrome are biopsied. Hence this chapter will deal with biopsy-proven, adult-onset MCD.

Pathophysiology

The pathophysiology of minimal change disease is not well understood. The animal model which most closely resembles MCD is puromycin aminonucleoside nephrosis (PAN) in rats. Administration of puromycin aminonucleoside to rats causes the production of reactive oxygen species which leads to direct DNA damage. This alters the podocyte actin cytoskeleton, resulting in foot process effacement, detachment from the glomerular basement membrane, and proteinuria. This effect is dose-dependent, and the podocyte changes and proteinuria spontaneously reverse if doses are limited [2].

Regulatory T-Cell Dysfunction

Shalhoub was the first to propose that "lipoid nephrosis is produced by a systemic abnormality of T-cell function resulting in the secretion of a circulating chemical mediator toxic to an immunologically innocent glomerular basement membrane." This theory was based on several observations: lack of a humoral antibody response, remission induced by measles and steroids/cyclophosphamide (which modify cell-mediated immunity), and the occurrence in Hodgkin's lymphoma [3].

Many cases of idiopathic nephrotic syndrome remit spontaneously, and significant transient albuminuria may occur during viral and febrile illnesses. This implies that there is different or additional pathogenesis of persistent, non-remitting, and relapsing nephrotic syndrome and MCD. Recent research has focused on the role of regulatory T-cell (Treg) dysfunction in MCD.

Garin et al. [4] examined urine soluble CTLA-4 levels in patients with MCD in relapse and remission, other glomerular disease, and control subjects. CTLA-4 is a protein secreted by Treg cells which binds to CD80 and therefore blocks the costimulatory activation of T cells. Although there was not a significant difference in urine sCTLA-4 levels between these groups, there was a significant decrease in the urinary ratio of sCD80/sCTLA-4 in patients with MCD in remission, pointing to a role of Treg dysfunction and relative CTLA-4 deficiency in suppression the continued activation of CD80 in MCD.

Araya et al. studied T cells in a small group of patients with MCD in relapse, MCD in remission, control patients, and MPGN [5]. They found that Treg suppression of T-effector cells was decreased in MCD patients in relapse versus MCD patients in remission and control patients. This was the first direct evidence of impaired Treg function in MCD. More recently, investigators have reported that nuclear factor-related kappa B, which is involved in chromatin remodeling, is upregulated in CD4+ T cells and B cells in relapsing MCD, suggesting that alterations in transcription factors of immunity may also play an important role [6].

LeBerre et al. used Buffalo/Mna rats, a model for idiopathic nephrotic syndrome with histological focal segmental

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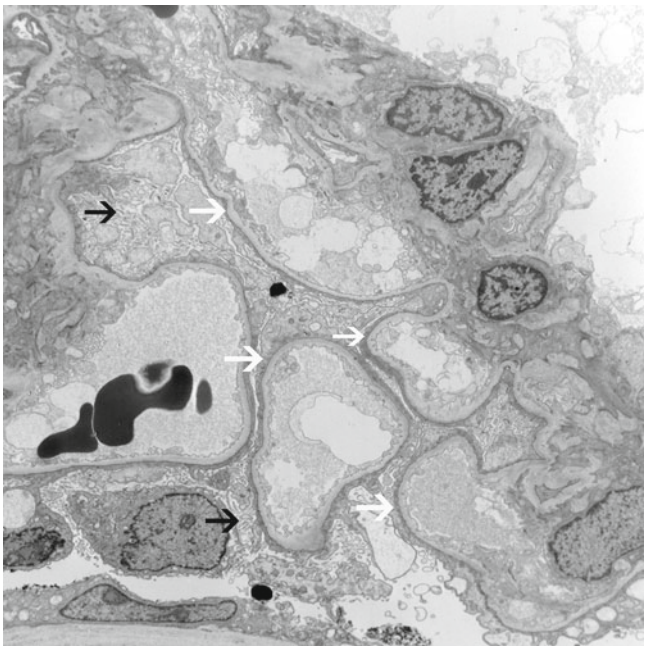

Fig. 2.1 Electron microscopy of a glomerulus from a renal biopsy of a patient with minimal change disease, showing diffuse foot process effacement (*white arrows*) and microvillous degenerative changes (*black arrows*) of the visceral epithelial cells (×2,900). Courtesy of Sanjeev Sethi, MD

glomerular sclerosis (FSGS) lesions, to explore the activity of T cells and the effects of different treatments [7]. Buffalo/Mna rats develop nephrotic syndrome with FSGS lesions that recurs after transplant with a normal kidney, implying the presence of a circulating permeability factor. The deoxyspergualin derivative LF15–0195 induced remission of proteinuria and improved the pathological features of Buffalo/Mna rats compared to untreated rats. There was a similar but less profound effect of this treatment on recurrent disease after transplant. A series of experiments showed that LF15–0195 decreases the quantity of renal monocytes and T cells, decreased levels of IL-10 and IL-13, significantly increased Treg cell quantity, and was associated with increased expression of Treg transcripts, including CTLA-4. Moreover, untreated Buffalo/Mna rats that received Treg cells from treated Buffalo/Mna rats demonstrated decreased proteinuria, although these results were not significant. This study demonstrated that augmentation or supplementation of Treg cell function, including an increase in CTLA-4 expression, induces remission of disease in the Buffalo/Mna model of idiopathic nephrotic syndrome.

Barrat et al. showed that a combination of vitamin D3 and dexamethasone led to in vitro differentiation of mouse naïve CD4 T cells into Treg cells [8], which provides another potential mechanism of the effect of glucocorticoids in SSNS.

Induction of Podocyte Proteins

Recent studies have investigated the role of podocyte proteins CD80 and angiopoietin-like protein 4 in response to damage from exogenous injury such as puromycin and endogenous circulating factors that result in the development of MCD-like phenotypes. CD80 (also known as B7–1) is a transmembrane protein that is present on antigen presenting cells (APCs). It is a costimulatory signal for T-cell activation. In 2004, Reiser et al. first showed that CD80 is also expressed in mouse podocytes after injection with lipopolysaccharide (LPS). LPS injection resulted in proteinuria which was CD80 *dependent* (the proteinuria *did not* occur in CD80 knockout mice injected with LPS) and T and B cell *independent* (the proteinuria *did* occur in SCID mice injected with LPS) [9]. The same study found that LPS interacts with cultured podocytes via Toll-like receptor 4 (TLR-4) and results in podocyte actin cytoskeleton reorganization. Lai et al. then demonstrated that IL-13 overexpression in rats produced a MCD-like phenotype (albuminuria, hypoalbuminemia, hypercholesterolemia, normal histology on light microscopy, and podocyte foot process effacement on EM) which also resulted in increased glomerular expression of CD80 [10]. In 2011, Shimada et al. demonstrated that polyIC (a TLR-3 ligand) induced human podocyte CD80 expression and podocyte injury [11], as well as increased NFκ(kappa)B and type 1 and 2 IFN activity. Both CD80 mRNA silencing and NFκ(kappa)B inhibition attenuated CD80 expression in the presence of polyIC, which also resulted in decreased evidence of podocyte injury. This model may explain the propensity of viral infections to induce MCD. This study also showed that incubation with dexamethasone attenuated the increase in CD80 activity and podocyte injury in the presence of polyIC, suggesting a mechanism of the beneficial effects of corticosteroid treatment in MCD.

The clinical utility of CD80 measurement in humans with MCD has been explored in two studies. In 2009, Garin et al. showed that there was an increased urinary excretion of soluble CD80 (sCD80) in children and adolescents with MCD in relapse compared to those with MCD in remission, patients with other glomerular disease, and control subjects [12]. Levels of serum sCD80 were not elevated in MCD compared to the other patient groups. The same group then measured urinary CD80 levels in 17 patients with MCD compared to 22 patients with FSGS and found an increased urinary CD80/creatinine ratio in MCD in relapse compared to MCD in remission or FSGS [12]. Thus, CD80 may be a useful biomarker in MCD.

A second protein which has been shown to play a role in steroid-sensitive nephrotic syndrome is angiopoietin-like-4 (Angptl4), a secreted glycoprotein which is a member of the angiopoietin-like protein family. Angptl4 is highly expressed

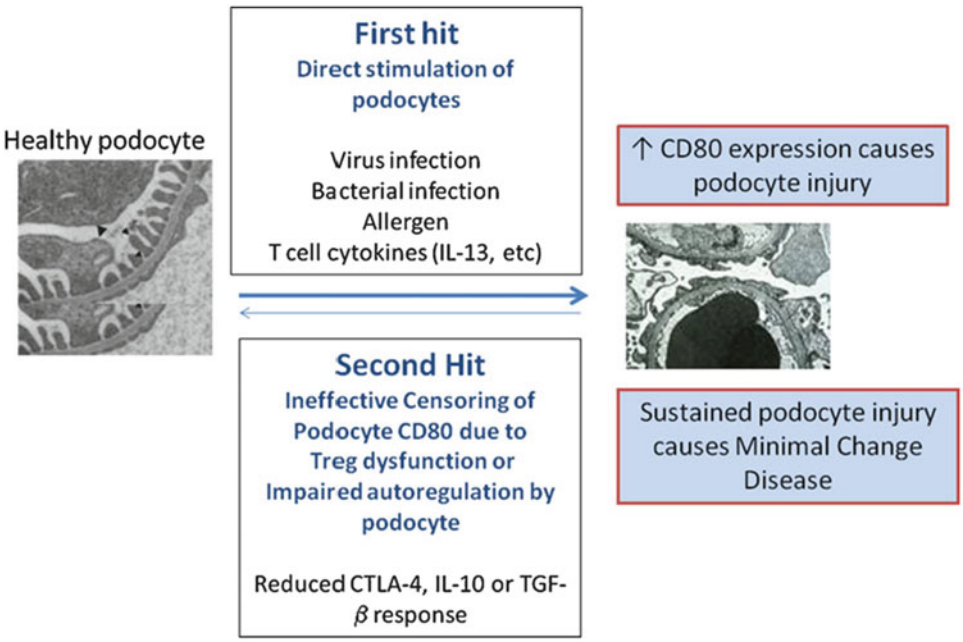

Fig. 2.2 Minimal change disease: a *two-hit* podocyte immune disorder. The first hit consists of the induction of CD80 in podocytes by microbial products, allergen, or T-cell cytokines such as interleukin (IL)-13. In normal settings, CD80 expression on podocytes is terminated by regulatory cytokines from T regulatory cells (Treg) and/or cytotoxic T lymphocyte-associated (CTLA)-4, and interleukin (IL)-10 by podocytes, and as a consequence, proteinuria is transient and mild. However, we propose a second hit occurs in MCD and consists of abnormal censoring of podocyte CD80 expression due to a defective autoregulatory response by Tregs or by the podocyte itself. As a consequence, CD80 expression becomes persistent and nephrotic syndrome results. From Shimada M, Araya C, Rivard C, Ishimoto T, Johnson RJ, and Garin EH. Minimal change disease: a "two-hit" podocyte immune disorder? Pediatr Nephrol. 2011;26(4): 645–49. With kind permission from Springer Science and Business Media

in the liver and adipose tissue and previously had only been shown to have low-level renal expression as shown by Northern blot of whole kidney specimens [13]. Clement et al. demonstrated that Angptl4 expression is upregulated in the glomeruli of rats exposed to several models of podocyte injury, including puromycin [14]. This upregulation was specific to models of SSNS compared to those of membranous nephropathy (Passive Heymann Nephritis), mesangial injury (Thy1.1 nephritis), and collapsing FSGS (injection of rats with serum from patients with collapsing FSGS). A transgenic mouse model showed that upregulation of podocyte Angptl4 resulted in proteinuria, normal light microscopy, foot process effacement, and loss of glomerular basement membrane charge, similar to changes seen in MCD. Increased levels of Angptl4 produced by adipose tissue did not lead to proteinuria or glomerular changes, suggesting that Angptl4 does not act as circulating permeability factor. This study also highlighted important implications for treatment of SSNS. Firstly, glucocorticoids attenuated proteinuria and decreased Angptl4 expression in rats exposed to puromycin, implying a target of steroid therapy in this disease. Secondly, the glomerular Angptl4 was shown to be hyposialylated, and the addition of sialic acid precursors to the diet of NPHS2-Angptl4 transgenic rats improved proteinuria, leading to the consideration of a steroid-sparing therapy for SSNS.

Taken together, these studies show that circulating factors (PA, LPS, IL-13, and polyIC) induce increased expression of proteins such as CD80 and Angptl4 in podocytes, resulting a MCD-like disease in vitro and in vivo. The effects of glucocorticoids and sialic acid precursors on these molecular pathways and subsequent disease states point to a link between these therapies and the pathophysiology of SSNS.

In summary, the prevailing theory on the pathogenesis of MCD suggests that an initial insult in the form of exogenous or endogenous circulating factors induces podocyte expression of proteins such as CD80 and Angptl4, which in turn leads to podocyte actin cytoskeleton disorganization, foot process effacement, detachment from the GBM, and the nephrotic syndrome. Many cases spontaneously resolve. Those that do not resolve may have abnormalities in Treg cell function, and immunosuppressive therapy with glucocorticoids may mitigate disease activity by affecting these pathways (Fig. 2.2).

Clinical Presentation

MCD classically presents with nephrotic syndrome characterized by heavy albuminuria, marked edema, and hypoalbuminemia with an accompanying elevation in circulating lipids. Spontaneous remission have been reported in

MCD [15, 16], but because of significant morbidity including thromboembolism and infections in untreated patients [17, 18], most practitioners will elect to treat MCD patients. MCD in children is usually corticosteroid responsive with remissions occurring within a few weeks of starting in most cases. However, adults tend to respond less promptly and it may take up to 3–4 months after initiating steroid therapy for remission to occur. Also in contrast to children, up to 20–30 % of adults may fail to respond to steroid therapy, which implies different pathophysiologic mechanisms between the two age groups. A significant proportion of non-responders will show fibrosis on a subsequent biopsy with lesions of FSGS. Unlike in the pediatric age group, there is a paucity of well-designed randomized controlled trials (RCTs) in adult MCD.

MCD patients may present with acute kidney injury (seen in up to 20–25 % of adults) [19, 20]. Chronic kidney disease or end-stage kidney disease is not typically seen in adult MCD and should suggest other etiologies. MCD patients will typically experience relapses, and up to a third of patients may become frequent relapsers or corticosteroid-dependent [20–23]. Relapses are also seen in 40 % of adults who as children experienced MCD [24].

Secondary etiologies associated with MCD are uncommon but should be elucidated, especially in adults. They include malignancies (typically Hodgkin's disease and thymoma), lithium therapy, and non-steroidal anti-inflammatory drugs [25].

From a risk perspective, severe nephrotic syndrome is associated with significant morbidity from dyslipidemia [26], infections [18, 27], and thromboembolic events [28]. These risks, along with the relative ease and tolerance of a short course of corticosteroids, prompt most physicians to treat patients with severe nephrotic syndrome. Drug-related adverse effects, however, are common with prolonged/repeated steroid courses in steroid-dependent (SD) or frequently relapsing (FR) patients.

Immunosuppressive/Immunomodulatory Treatment of MCD

Treatment of the Initial Episode

Corticosteroids

There are few controlled studies of immunosuppressive therapy in adults (Table 2.1) [15, 16, 29]. Experience with corticosteroids are mainly derived from large prospective RCTs in children [30, 31] and observational studies in children and adults [19, 20, 22, 23]. A multicenter controlled study of corticosteroids in 125 adult patients (including 31 MCD patients), which used at least 20 mg/day of prednisone for at least 6 months, showed an early and rapid decrease in proteinuria compared to the control group. However, by 2½ years, a significant number of patients in the control group underwent a spontaneous remission, leading ultimately to similar outcomes with respect to proteinuria or serum albumin in the two groups [15]. Similarly, in another randomized controlled trial which compared prednisone 125 mg every other day for 2 months with placebo in 28 adult MCD patients, there was no difference in overall remission rates over 77 months of follow-up. Similar to the previous study, patients remitted more rapidly when treated with prednisone, with 12 of 14 treated patients in complete remission before 2 months compared to 6 of 14 controls [16, 32].

Although there are no controlled trials comparing daily versus alternate day corticosteroids in adults, observational studies have not shown any difference in response rates [20]. Corticosteroid therapy leads to complete remission in over 80 % of adults with MCD. The time course to a complete remission is prolonged, with 50 % responding by 4 weeks and 10–25 % requiring 12–16 weeks of therapy. However, unlike in children where every other day therapy may have a more favorable effect on growth rates, the advantages of this regimen in adults is not known [19, 20].

The optimal duration of corticosteroid therapy is unknown in adults. In children, 6 months of corticosteroid treatment was associated with less relapse rates compared to 3 months of therapy [30]. Furthermore, the optimal corticosteroid taper prescription in adults is not known, but they are commonly tapered by 5–10 mg/week after achieving remission for a total period of corticosteroid exposure of at least 24 weeks [18–20].

Alternative Regimens for the Initial Episode

In patients who have relative contraindications or who are unwilling to take steroids, there are little data on alternate regimes. Cyclophosphamide [23, 33, 34] and cyclosporine [35] have been used with response rates comparable to corticosteroids (in the 75 % range) with this limited experience. Doses are listed in Table 2.2.

Treatment of Infrequent Relapses

For patients who relapse infrequently, a repeat course of corticosteroids may be used as in the first episode of MCD. However, there are no data to guide the therapy of relapse in MCD.

Frequently Relapsing and Corticosteroids-Dependent Patients

In patients who relapse frequently (defined as ≥2 relapses/year) or who are steroid dependent (defined as recurrence of proteinuria during or within 2 weeks of completing steroid therapy), alkylating agents such as cyclophosphamide and chlorambucil were the first class of drugs used and generally

Table 2.1 Controlled studies of MCD in adults

Reference	Study	*N*	Treatment protocol	Results
Black et al. [15]	Multicenter controlled trial	125 (31 with MCD)	Prednisone 20–30 mg/day × >6 months (>10 mg/12 months)	Earlier response in treated patients
Coggins [16]	Multicenter controlled trial	28	Prednisone 120 mg every other day for 2 months	Earlier response in treated patients. No difference at 2 years
Imbasciati et al. [29]	Multicenter (adults + children). Pulse IV steroids versus high dose oral prednisone	22 adults (67 children)	Methylprednisolone 20 mg/kg/day × 3 days	Earlier response in children, no difference in adults. Tendency to earlier/frequent relapses in pulse. More side effects in oral group

Table 2.2 Dosage regimens for adult minimal change disease

Initial treatment
Prednisone
Daily single dose of 1 mg/kg (maximum 80 mg) or alternate day single dose of 2 mg/kg (maximum 120 mg)
– Until complete remission (minimum 4 weeks, maximum period of 16 weeks)
– After complete remission, taper slowly over a total period of 6 months
Frequently relapsing or steroid dependent
(1) Cyclophosphamide (oral) single course
2.0–2.5 mg/kg/day as tolerated for 8 weeks
(2) Relapsed despite cyclophosphamide, or patients of childbearing age
(a) Cyclosporine: starting dose 3–5 mg/kg/day
(b) Tacrolimus 0.05–0.1 mg/kg/day in divided doses
Following 3 months of remission, taper to reach the minimum dosage that maintains remission for 1–2 years
(3) Intolerant to corticosteroids and/or calcineurin inhibitor
Mycophenolate mofetil 750–1,000 mg twice daily for 1–2 years

achieve a remission of at least 1 year in 70 % of patients with 12 weeks of therapy. Because of the toxicity associated with the alkylating agents, second courses are generally not used.

Antimetabolites such as azathioprine and mycophenolate mofetil are also useful (but not as effective compared to alkylating agents) but they have less toxicity and can be administered for an extended period of time.

A third option is the calcineurin inhibitors such as cyclosporine and tacrolimus. These agents can induce a prolonged remission in nearly 80–90 % of patients while on the drug (doses are listed in Table 2.2).

Cyclophosphamide

In observational studies, cyclophosphamide led to remission in a significant number of adults who were frequent relapsers (FR) or steroid-dependent (SD) [19, 20, 23]. The relapse-free interval appears to be longer than with cyclosporine (see below). In the study by Mak et al., the initial response rates with cyclophosphamide in FR/SD adults appeared excellent with all nine patients being able to be weaned off steroids [19]; however, five of these patients subsequently relapsed. In this study FR MCD patients appeared to fare better than SD MCD, with 80 % of FR patients showing sustained remission at a mean follow-up of 9.1 years compared to 45 % of SD MCD patients. Similarly, SD children may be less responsive than FR to cyclophosphamide [36]. In the study by Nolasco, 21 of 36 patients (58 %) with FR/SD MCD went into remission within 8 weeks and 25 patients (69 %) within 16 weeks. The addition of prednisone to cyclophosphamide did not appear to be of added benefit. Remissions appeared to be more durable, with 40 % of patients relapsing at 5 years with cyclophosphamide compared to 75 % with steroids [23]. In a retrospective study by Waldman et al., 55 % of 20 patients treated with cyclophosphamide (for any indication) had a complete or partial remission [20]. There is one report on the efficacy of IV cyclophosphamide in adults [37].

Calcineurin Inhibitors (Cyclosporine and Tacrolimus)

Cyclosporine has been reported to be effective in FR/SD patients with remission rates in the 70–90 % range [20, 38]. In an RCT of 73 adults and children with FR/SD nephrotic syndrome (31 patients with MCD; 42 patients with FSGS), cyclophosphamide (2.5 mg/kg/day) for 8 weeks was compared with cyclosporine (5 mg/kg/day) for 9 months, followed by a 3-month taper to withdrawal. At 9 months, 64 % (18/28) of patients on cyclophosphamide and 74 % (26/35) of patients on cyclosporine maintained remission (P = NS). However, at 2 years, 25 % of patients assigned to cyclosporine versus 63 % of patients assigned to cyclophosphamide

were still in remission [39]. In another RCT of 52 patients, remission was achieved sooner in patients treated with cyclosporine (AUC 1,700–2,000 ng/ml) with 0.8 mg/kg/day prednisolone compared with patients receiving only prednisolone monotherapy at 1 mg/kg/day, with the possible additional benefit of lower exposure to steroids [40].

The optimal dose and duration of therapy with cyclosporine is unknown. In a trial by Ponticelli, cyclosporine was dosed at 5 mg/kg/day for 9 months followed by a taper over 3 months [39]. Abrupt cessation of cyclosporine after attaining remission is associated with relapse and the possibility of cyclosporine dependency. However, prolonged treatment in 36 adult patients for a mean of 26 months followed by slow withdrawal led to sustained remissions without steroids in 11 of 14 patients and with low doses of corticosteroids in three patients. In 20 % of patients who remained cyclosporine-dependent, doses of <3 mg/kg/day were sufficient to maintain remission. The cumulative rate of remissions peaked at 6 months [41, 42].

Tacrolimus, administered for 24 weeks, was compared to IV cyclophosphamide in a small RCT in steroid-dependent patients with similar response rates to cyclosporine. All patients in this study were able to discontinue corticosteroids [37].

Mycophenolate

In children with MCD, mycophenolate (MMF) has been used as a steroid-sparing agent. The experience with MMF in adults has been limited to case reports [43–45].

Rituximab

Rituximab has been used in small uncontrolled case series in both adults and children with immunosuppression-dependent MCD with varying degrees of success [46, 47].

Corticosteroids-Resistant MCD

An estimated 10 % of adult MCD patients are steroid-resistant, defined as failing 16 weeks of daily or alternate day corticosteroids as outlined previously. A repeat biopsy may show focal segmental glomerulosclerosis (FSGS) which is associated with a worse prognosis, and treatment regimens should follow the recommendations as for steroid-resistant FSGS.

Other Treatments

Treatment of Minimal Change Disease with Acute Kidney Injury

Acute kidney injury (AKI) can occur in patients with MCD and may be severe enough to require dialysis. Risk factors for AKI in MCD include older age, hypertension, severe nephrotic syndrome, and arteriosclerosis on the kidney biopsy [20, 48]. Kidney function typically recovers even in the most severely affected patients, although patients who have experienced AKI may have residual chronic renal impairment [20]. Careful attention to patients' volume status, supportive therapy for AKI, and continued therapy with corticosteroids are suggested.

Supportive Therapy

Proteinuria in adult MCD typically remits with corticosteroid treatment, as does the accompanying hyperlipidemia. This usually negates the need for inhibitors of the renin-angiotensin-aldosterone (RAAS) system or statin therapy, respectively.

One study of 40 adults who had relapsing NS as children did not show a higher incidence of cardiovascular disease, implying that long-term cardiovascular risk was not increased by intermittent hyperlipidemia during nephrotic relapses in childhood, although this study was small and no firm conclusion can be derived from the data [49]. The use of antihyperlipidemic agents and RAAS blockers may be considered on a case-by-case basis in FR/SD MCD adults.

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Childhood Onset Nephrotic Syndrome

3

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Introduction

Nephrotic syndrome in children presents with the clinical constellation of nephrotic-range proteinuria, hypoalbuminemia, edema, and hyperlipidemia [1]. Childhood nephrotic syndrome may be primary or secondary to medications, infection, neoplasia, or other toxic exposures. Of primary nephrotic syndromes, idiopathic nephrotic syndrome is the most common form in childhood, representing 90 % of all cases [2]. Histological lesions on renal biopsy of idiopathic nephrotic syndrome include minimal change nephrotic syndrome (MCNS), diffuse mesangial proliferation, and focal segmental glomerulosclerosis (FSGS). It is uncertain whether these histological types represent distinct disease entities or are part of a continuum of disease that ranges from MCNS to FSGS. Membranous nephropathy is a separate histological entity that is rarely found in children [2].

Idiopathic nephrotic syndrome is further categorized by the child's response to corticosteroid therapy. Children who demonstrate remission of proteinuria with corticosteroids are labeled as having steroid-sensitive nephrotic syndrome (SSNS).

Overall approximately 80 % of children with incident nephrotic syndrome are steroid responsive at presentation. Over 90 % of MCNS is responsive to steroids, whereas only 30 % of children with FSGS will respond to therapy [3]. Children with SSNS have a favorable kidney function prognosis. On the contrary, children with steroid-resistant nephrotic syndrome (SRNS) portend a poorer prognosis with 36–50 % of children reaching end-stage kidney disease over 10 years [4, 5].

Nephrotic syndrome manifesting before 1 year of age may be primary or secondary. Secondary cases are usually due to infection. Congenital nephrotic syndrome presents before 3 months of age and is most commonly associated with the nephrin mutation (Finnish type). Infantile nephrotic syndrome presents between 3 and 12 months of age. Genetic mutations are found in the majority of cases that occur in the first year of life [6–8]. In adolescents with primary nephrotic syndrome, the causes of disease are similar to adults with a higher frequency of FSGS and membranous nephropathy and a correspondingly lower rate of MCNS [9, 10].

Epidemiology

The incidence of idiopathic nephrotic syndrome in the USA is 2–7 per 100,000 with a prevalence of 16 per 100,000 [11]. The incidence of nephrotic syndrome varies with age, race, and geography. The median age of onset of MCNS is 3 years and 6 years for FSGS [2]. There appears to be a 2:1 male preponderance for idiopathic nephrotic syndrome [2]. Nephrotic syndrome is also associated with atopy. One study reported three times more allergic rhinitis and ten times more atopic dermatitis in children with nephrotic syndrome than the general population [12].

MCNS is the most common lesion found in primary nephrotic syndrome. The International Study of Kidney Disease in Children (ISKDC) prospectively followed 521 children with nephrotic syndrome in the 1970s. The distribution of pathology included MCNS (77 %), FSGS (8 %), membranoproliferative glomerulonephritis (6 %), mesangial proliferation (2 %), and membranous nephropathy (2 %) [2]. Eighty percent of children with MCNS and 50 % of children with FSGS present before 6 years of age. In recent years, studies have demonstrated that the incidence of FSGS appears to be rising without a change in the incidence of primary nephrotic syndrome [13–16].

Pathophysiology

Reversible effacement of podocyte foot processes is a hallmark of MCNS. It may be mediated by various intra- and extracellular signaling pathways such as the Rho family of

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GTPases and integrins [17]. It has been demonstrated that markers of focal adhesion complex-mediated Crk-dependent signaling are enhanced in MCNS but not FSGS [18]. There is increased production of proteins such as CD80 and angiopoietin-like protein 4 by podocytes in models of MCNS [19, 20], which supports the central role of podocyte damage in the pathogenesis of MCNS. In addition, there are occasional cases of MCNS that have been linked to genetic abnormalities in podocyte proteins (see below). Traditionally, defective glomerular barrier function has been considered the sole cause of proteinuria. However, recent studies have implicated abnormal reabsorption of filtered protein in the proximal tubule as a key contributor to the pathogenesis of proteinuria in nephrotic syndrome [21, 22].

MCNS is unique clinical entity in that it reflects predominantly a decrease in the negative charge in endothelial cells, the glomerular basement membrane (GBM), and podocytes causing selective proteinuria, rather than an increase in effective pore size. As a consequence, the reduction in negative charge is a diffuse abnormality that is manifest in capillaries throughout the body with leakage of albumin in the peripheral circulation and accumulation of interstitial fluid. The diminished negative charge density in the renal microvasculature results from immune-mediated defects that inhibit the incorporation of moieties such as sulfate into the GBM. In addition, immunoeffector cells may elaborate soluble molecules such as vascular endothelial growth factor that directly increase GBM permeability to protein. Other candidate molecules include hemopexin and cardiotrophin-like cytokine-1 (CLC-1) [23]. The nature and molecular identity of these circulating permeability factors that cause proteinuria are likely to differ in patients with MCNS and FSGS. This is supported by the recent report that circulating levels of soluble urokinase-type plasminogen activator receptor (suPAR) are elevated in nearly 70 % of patients with FSGS but undetectable in patients with MCNS or MN [24].

A link between abnormal T-cell function and MCNS was first proposed over 30 years ago by Shalhoub [25]. Since then, many studies have documented altered subtype distribution and activity of lymphocytes in children with MCNS. For example, patients with MCNS have a higher Th17/Treg cell ratio in their circulation [26]. Albuminuria and podocyte foot process effacement can be induced by injection of CD34+ stem cells isolated from patients with MCNS or FSGS into immunodeficient NOD/SCID mice. This underscores the pivotal role of the immune system in the pathogenesis of MCNS [27].

The pathogenesis of all of the cardinal features of the nephrotic syndrome is still not fully explained despite over 30 years of extensive investigations in animal models and in patients. For example, the cause of edema in nephrotic syndrome is controversial. The underfill theory proposes that the sodium and water retention are due to decreased oncotic pressure from hypoalbuminemia, whereas proponents of the overfill theory suggest that edema is due to resistance to atrial natriuretic peptide and primary sodium retention [28]. Hypoalbuminemia has been attributed to the urinary losses; however, there is no uniform inverse correlation between proteinuria and serum albumin concentration, suggesting that altered distribution or catabolism of protein contributes to the development of hypoalbuminemia in children with MCNS. Hyperlipidemia is thought to be due to increased hepatic lipoprotein synthesis possibly in response to low oncotic pressure and a decreased catabolism of lipoproteins; however, the exact pathogenesis remains uncertain.

Podocyte dysfunction is considered the central part of the pathogenesis of NS. Broad etiologic classifications of this dysfunction include injury due to immune system factors (thought to be the major etiologic factor in SSNS) [29] or decreased numbers of functioning nephrons or hyperfiltration (associated with forms of SRNS/FSGS) [30, 31]. Human genetics studies over the past 15 years have also demonstrated that rare mutations of genes, mostly localized to the podocyte, can also underlie the pathogenesis of SRNS/FSGS [32].

Upon discovery of these genes, functional analysis of their gene products occurred, revealing that they perform a variety of functions contributing to the maintenance of the glomerular filtration barrier. This includes maintenance of the slit diaphragm (*NPHS1*, *NPHS2*, *PLCE1*, *CD2AP*), calcium-based signaling (*TRPC6*), attachment of the podocyte to the glomerular basement membrane (*LAMB2*), and actin-based cytoskeletal dynamics (*ACTN4*, *INF2*) [33]. Rare, syndromic forms of NS associated with extrarenal features are associated with non-podocyte-specific genes, such as those that encode mitochondrial (*COQ2*, *COQ6*), lysosomal (*SCARB2*), and basement membrane (*LAMB2*, *ITGB4*, *ITGA3*) proteins, as well as transcription factors (*LMX1B*) [32, 34, 35]. Mutations in the transcription factor *WT1* are an important monogenic cause of isolated SRNS while also resulting in syndromic forms of SRNS such as Denys-Drash or Frasier syndrome [36].

Reviewing the results of studies from around the world that sequence NS-associated genes in subjects with NS illustrates the genetic complexity underlying this disease. NS displays both significant genetic and phenotypic heterogeneity. Mutations in *NPHS2*, *NPHS1*, *WT1*, and *PLCE1* have all been shown to cause SRNS/FSGS [37]. Conversely, mutations in *NPHS2* have been shown to cause congenital nephrotic syndrome (CNS), childhood onset NS, and adult onset NS [38]. SRNS can also be caused by compound heterozygosity of NS genes [39–41] in which two different mutations in an NS gene such as *NPHS1* or *NPHS2* are present (one on each allele) or by bigenic heterozygosity in which two different NS genes harbor a single pathogenic mutation (e.g., *WT1* and *NPHS1*) [42]. Specific variants within a given gene and the combination of mutant alleles possessed by a subject

influence the clinical presentation and outcomes of SRNS, such as age of onset and risk for progression to end-stage renal disease [37, 43].

The worldwide scope of these studies also illustrates, not surprisingly, that a child's ethnicity and the degree of their genetic isolation due to geography and/or cultural preference also influences the frequency of mutations observed in specific NS genes. The effect of ethnicity on the frequency of SRNS-causing variants has been summarized by Chernin et al. in regard to *NPHS2* [44]. They note that the prevalence of pathogenic *NPHS2* mutations in SRNS ranges from 55 % in Israeli-Arabs to 13–27 % in a Turkish cohort, to 4–13 % in a Chinese cohort, and to 0 % seen in Israeli Jewish, Japanese, South Korean, and their 18-subject African-American cohort [44]. Patients from isolated communities have enrichment of deleterious founder mutations in the population. This has been seen with the "Fin-major" and "Fin-minor" mutations of nephrin (*NPHS1*) seen in over 90 % of Finnish patients with congenital nephrotic syndrome [45], the R1160X variant in *NPHS1* seen in CNS patient in Malta [46], and the R138X and A1023G variants of podocin (*NPHS2*) seen in Israeli-Arabs with SRNS [47].

Despite the challenges posed by the impact of ethnicity and clinical and genetic variability, there are a number of well-recognized genotype-phenotype correlations that have been elucidated in NS. Infants with CNS or NS diagnosed at less than 1 year of age are likely to have a genetic cause of their NS. In addition to the Finnish study of CNS noted above, a recent study reported that 80 % of non-Finnish infants with CNS had a genetic cause of their disease, with 60 % having rare, causal variants in *NPHS1*, 15 % in *NPHS2*, and the rest in *WT1*, *PLCE1*, and *LAMB2* [8]. A study of infants of non-Scandinavian European and Turkish ancestry demonstrated that 66 % of cases of NS occurring in the first year of life are caused by rare variants in *NPHS1*, *NPHS2*, *WT1*, or *LAMB2* [6, 32]. Across ethnicities, the most commonly found rare variants in children with nonsyndromic SRNS are in *NPHS1*, *NPHS2*, and WT1 [36, 41, 47–52], while those in *PLCE1* [53], *LAMB2* [54], *INF2* [55], and *TRPC6* [56] have been found much less frequently. Causal variants in *CD2AP* and *ACTN4* are rarely found in children [57]. Children with SRNS found to have a genetic cause of their condition are less likely to respond to immunosuppressive therapy [58, 59] but have a lower risk of recurrence of their disease after renal transplantation when compared with individuals with SRNS/FSGS who were not found to have known mutations [60].

The attempt to discover single gene causes of familial SSNS using positional cloning has been unsuccessful thus far. With rare exception, the monogenic causes of SRNS/FSGS have not been replicated in children with SSNS. *NPHS2* and *WT1* mutations were not found in cohorts of children with SSNS [49, 61], and *NPHS2* variants were not observed in a small group of children with late SRNS [62]. There are rare reports of children with known pathogenic mutations in NS genes that are nonetheless steroid sensitive [63, 64] or even unaffected [53]. These findings require additional study to be generalizable. Of note, there is a recent report of the discovery of a causal variant in cubulin found in a consanguineous kindred with intermittent nephrotic-range proteinuria [65].

Advances in genomic sequencing technology and decreases in their costs are allowing an increasing number of patients with NS and their families to undergo genome-wide testing that can both screen for mutations in known NS genes and discover novel single gene causes of NS [34, 66, 67]. However, monogenic causes will never explain a certain proportion of genetic susceptibility in NS cases, particularly SSNS and sporadic cases of SRNS from outbred populations. Alternative methods to define genetic susceptibility in these cases are desirable. One complementary approach that has found success is the identification of genetic variants that, while present in unaffected members of the general population, nonetheless increases the risk of developing NS in those individuals that possess this variant.

The R229Q variant of *NPHS2* has a minor allele frequency of 2–7 % in healthy individuals of European descent [68]. Individuals with one or two copies of this allele do not seem to have increased risk of SRNS [69]. However, when R229Q is found in concert with a second NPHS2 variant of known pathogenicity, there is an increased risk of adult onset SRNS in Europeans [39, 70]. More recently, variant frequencies were compared across the genome between African-Americans with FSGS and controls. This population-genetics-based approach identified variants in linkage with *MYH9* and *APOL1* that, while present in control populations, are associated with a 5 or 10× increased risk of disease, respectively [71, 72]. It remains to be seen whether the increased risk of FSGS is conferred by these risk allele effects on the *APOL1* or *MYH9* gene alone or, through interaction of these risk alleles with another risk factor, whether it is a second genetic variant (similar to the R229Q story) or a nongenetic factor such as obesity, nephron mass, or certain infections. Similar methodologies are currently being pursued in other cohorts of adults and children with SSNS to identify other risk loci associated with this disease.

Clinical Features

The initial episode and subsequent relapses of childhood nephrotic syndrome are typically preceded by an upper respiratory tract infection or other inciting trigger. The most common presenting symptom is edema, although asymptomatic proteinuria may be found especially in children with SRNS. The edema, which is usually symmetrical in distribution and painless, often begins in the periorbital region. At the onset of

nephrotic syndrome, the swelling is frequently misclassified as allergic in nature. Anasarca may develop with ascites, labial/scrotal edema, and pleural and pericardial effusions. Ascites may be associated with respiratory compromise, umbilical or inguinal hernias, early satiety, and peritonitis.

Patients may also present with nonspecific symptoms of poor appetite, irritability, colicky abdominal pain, malaise, and fatigue. Urine output is often decreased, and urine is described as foamy. Gross hematuria is not associated with MCNS, but there are scattered reports of hematuria occurring with FSGS. Hypertension prior to corticosteroid therapy is unusual in MCNS but is present in 50 % of children with FSGS [2].

Laboratory Investigations

Recommendations from the Children's Nephrotic Syndrome Consensus Conference for the initial evaluation of nephrotic syndrome include obtaining a urinalysis, first-morning urine protein:creatinine ratio, serum albumin, serum electrolytes, serum urea nitrogen, creatinine, cholesterol level, complement C3 level, antinuclear antibody level for children greater than 10 years of age, hepatitis B and C, and HIV in high-risk populations [73]. Although the classic definition of nephrotic syndrome includes (1) nephrotic-range proteinuria, (2) hypoalbuminemia, (3) edema, and (4) hyperlipidemia, only the first criterion is essential for diagnosis, especially in clinical trials, because edema, hypoalbuminemia, and hyperlipidemia are not present in all cases of idiopathic nephrotic syndrome.

Nephrotic-range proteinuria in children is defined as >40 mg/m^2/h. However, it is often difficult to obtain an accurate 24 h urine collection in children; therefore, the protein:creatinine ratio on a spot specimen is useful (normal <0.2 mg/mg, nephrotic-range >2 mg/mg) [74]. The use of a first-morning urine sample is recommended to remove the contribution of benign orthostatic proteinuria. The protein:creatinine ratio can be approximated to 24-h protein (g/m^2/day) by multiplying the ratio by a factor of 0.63 [75]. In addition to proteinuria, the urinalysis in idiopathic nephrotic syndrome may contain microscopic hematuria without cellular casts. In the ISKDC study, microscopic hematuria was reported in 49 % of children with FSGS and 23 % with MCNS [2]. Oval fat bodies and hyaline casts may also be found in the urinalysis.

Serum albumin concentration is decreased in nephrotic syndrome, and the diagnostic cutoff variably defined as 2.5 or 3 g/L. Paradoxically, some children with SRNS maintain a normal albumin level. Lipid abnormalities include increases in total cholesterol, LDL, VLDL triglycerides, and lipoprotein; HDL levels are unchanged. Hemoglobin and hematocrit are elevated due to hemoconcentration. Hyponatremia in children with nephrotic syndrome is dilutional due to excessive ADH secretion and water retention. Pseudohyponatremia secondary to nephrotic syndrome is no longer a concern because of the routine use of ion-selective electrodes to measure serum electrolytes in clinical chemistry laboratories. Low total calcium is related to hypoalbuminemia, but ionized calcium is usually normal. In patients with chronic nephrotic syndrome, there is persistent loss of vitamin D-binding protein in the urine and secondary hyperparathyroidism [76, 77]. These changes can lead to alterations in bone density that can be alleviated by oral supplements of calcium and vitamin D. Serum complement is normal in idiopathic nephrotic syndrome.

Renal Biopsy

The majority of children with nephrotic syndrome have MCNS which is generally responsive to steroids. Therefore, renal biopsy is not indicated in children 1–12 years old with typical features of idiopathic nephrotic syndrome [78, 79]. A trial of corticosteroids is recommended first. Renal biopsy is reserved for children with SRNS, renal failure, gross hematuria, low complement level, extrarenal disease, or hypertension. In the past, a kidney biopsy was considered routine in children less than 1 year of age. However, in view of the high likelihood of a genetic basis for disease in this age cohort [6, 7], it is advisable to defer a kidney biopsy until the results of genetic testing are available.

Complications

Complications of nephrotic syndrome are attributable to disease activity and as a result of treatment of the disease. Infection is a frequent complication with serious morbidity in children with nephrotic syndrome [80]. Spontaneous bacterial peritonitis and cellulitis as well as meningitis and pneumonia are well described. The rate of spontaneous bacterial peritonitis is 2–6 % [81]. Children with nephrotic syndrome are susceptible to bacterial and viral infections due to a combination of low IgG levels, low factor B (complement pathway), edematous tissue, immunosuppressive drugs, and impaired lymphocyte function. The most common bacterial pathogens include *Streptococcus pneumoniae*, *Escherichia coli*, and *Haemophilus influenzae*. The role of prophylaxis with penicillin has not been supported in the literature; however, vaccination with the 23-valent pneumococcal vaccine and influenza is recommended [73].

The incidence of thromboembolism in childhood nephrotic syndrome is 2–4.4 % [82, 83]. The risk of thromboembolism appears to be higher in SRNS than in SSNS. In a small series, six of nine patients with thromboembolism were SRNS [82]. The increased risk associated with

thromboembolism is multifactorial due to urinary losses of antithrombin 3, proteins C and S, and factors XI and XII; increased production of fibrinogen; hypovolemia; and immobilization. Prophylactic anticoagulation can be considered in children with prior history of clot, an underlying hypercoagulable condition, SRNS, and central venous catheter [84].

Acute kidney injury (AKI) occurs in some children with idiopathic nephrotic syndrome. AKI may be due to hypovolemia or renal swelling and compression of the tubulointerstitium. This complication is reversible when in remission. AKI may also be attributable to bilateral renal vein thrombosis, nephrotoxic medications, or increased interstitial pressure from edematous kidneys [85].

Treatment

Initial Therapy

Corticosteroids represent the time-honored initial therapy for presumed and biopsy-confirmed MCNS. The sensitivity of MCNS to steroid treatment prompts most pediatric nephrologists to empirically treat nephrotic children with glucocorticoids without a renal biopsy. Prednisone is the usual drug that is prescribed, and the standard dose in pediatric patients is 60 mg/m^2 or 2 mg/kg daily for 4–6 weeks followed by 40 mg/m^2 or 1.5 mg/kg every other day for 4–6 weeks. Compared to a shorter 8-week protocol—4 weeks daily and 4 weeks alternate day—the longer regimen delays the time until the first relapse [73]. Although there are conflicting data in the literature whether lengthening the course is beneficial, the consensus is that it is the preferred approach to the treatment of the initial episode of nephrotic syndrome and reduces overall exposure to steroids. Efficacy may vary depending upon the patient population, and the details of treatment should be guided by the experience at each center. In children, 70 % will achieve remission of the nephrotic syndrome after 10–14 days of treatment, and the vast majority will no longer have proteinuria after 4 weeks of therapy [86].

Relapse therapy involves similar doses but usually for a shorter period of time. Thus, corticosteroids are administered daily until the urine is negative or trace for 3 days, and then the medication is given every other day for 4 weeks. Various modifications in corticosteroid dosing such as extended tapering schedules, complete avoidance of every other day administration, and prolonged low-dose hydrocortisone to prevent adrenal insufficiency have been tried to prevent relapses and minimize side effects. Different formulations of steroids such a deflazacort have also been tried with mixed results; however, there is no high-quality evidence of the efficacy of these treatments.

Second-Line Therapy

Immunosuppression: Second-line therapy is utilized in patients with MCNS who relapse frequently (≥2 times in 6 months, ≥4 relapses in 12 months) or who are steroid dependent (relapse on corticosteroids or within 2 weeks of discontinuation) and manifest steroid side effects as a consequence of repeated exposure to the drug. The first class of drugs that were used under these circumstances was alkylating agents such as cyclophosphamide and chlorambucil. They generally achieve a prolonged remission of at least 1 year in 70 % of frequently relapsing patients [87]. Most patients who require an alkylating agent will be treated for at least 12 weeks based on the findings of the German collaborative group [88]. They should be carefully monitored for side effects including leukopenia, infection, hemorrhagic cystitis, gonadal toxicity, and malignancy. Alkylating agents can be given intravenously to patients in whom adherence may be a problem [89]. However, more than 25 % of patients with MCNS who were treated with cyclophosphamide were not in sustained remission after puberty and required prolonged immunosuppressive treatment [90–92]. Thus, because of the serious toxicity associated with the alkylating agents, reluctance to prescribe a second course, and the guarded long-term effect, there has been greater reliance on alternative medications for frequently relapsing or steroid-dependent patients with MCNS.

Antimetabolites such as azathioprine and mycophenolate mofetil have been evaluated in a number of open-label studies. Although they are not as effective as alkylating agents in inducing a permanent remission, they can reduce the overall relapse rate by approximately 50 % [93–95]. They are useful because they have a more favorable side effect profile, are not nephrotoxic, can be administered for an extended period of time, and require less intensive monitoring.

A third option is the calcineurin inhibitors such as cyclosporine and tacrolimus. These agents can induce a prolonged remission in nearly 80–90 % of patients, while the patient is taking the drug [96]. However, the optimal duration of therapy with calcineurin inhibitors is uncertain because relapses frequently occur shortly after stopping the drug. If treatment is prolonged, it is advisable to consider performing serial kidney biopsies to ensure that there is no structural damage that can occur in the absence of an increase in the serum creatinine concentration [97]. Calcineurin inhibitors can cause undesirable cosmetic changes such as hair growth and gingival hyperplasia. Tacrolimus is less problematic than cyclosporine in this regard and is favored in adolescents. Other important side effects include hepatotoxicity, hypertension, and nephrotoxicity. Therefore, patients taking calcineurin inhibitors require periodic blood tests and may benefit from serial kidney biopsies to monitor for evidence of calcineurin nephrotoxicity.

Finally, the newest agent that has been utilized to treat children with frequently relapsing or steroid-dependent MCNS who also have clinical evidence of steroid-induced side effects is rituximab. Administration of this monoclonal antibody to B-cells may induce remission in up to 80 % of cases [98–100]. This has been confirmed in study of 54 children (mean age 11 years) in which rituximab plus low-dose steroids and tacrolimus was as effective as treatment with standard doses of the latter two drugs. The likelihood of response is much lower in patients with SRNS, and based on a recent clinical trial involving 31 patients, it is not recommended unless future evidence suggests a more favorable outcome or specific subsample with a greater likelihood to respond [101]. Rituximab therapy is costly, and the long-term risks are unknown. Therefore, it is advisable that randomized clinical trials be performed and ongoing surveillance be maintained to gain perspective on the proper place of this biological agent in the therapeutic management of children with more difficult-to-treat NS. Finally, drugs such as levamisole are generally not available in the USA for use in patients with MCNS. This underscores the need to develop newer agents that can be used to control proteinuria in patients with MCNS especially in children with steroid toxicity and adults with relapsing disease.

The decision to recommend one of the medications in an effort to alleviate the adverse consequences of steroids must be weighed on an individual basis and take into account the patient's age, gender, and likely compliance with treatment. Consideration should be given to the severity of the side effect, the likelihood of reversal of the complication, and the odds that the MCNS will spontaneously resolve.

Long-Term Outcome of Childhood Onset Nephrotic Syndrome

Estimates ranging from 10 to 40 % of adult survivors of childhood onset nephrotic syndrome continue to relapse. Although reports vary, those at greatest risk for adult relapses are children with a greater number of relapses per year during childhood [90].

The risk for long-term adverse effects of steroid-sensitive nephrotic syndrome and related therapy has been evaluated in cohorts ranging in size from 15 to 102. The frequency and distribution of adverse events is likely related to the therapeutic regimen used in the treatment era and geographic region of the enrolled cohort, the inclusion criteria of the study of all steroid-sensitive patients or frequently relapsing only, and the sampling bias that accompanies the selected and available population of all children diagnosed in specified treatment years versus adults who were *referred* for ongoing care of childhood onset nephrotic syndrome versus adults who *remain in care* for childhood onset nephrotic syndrome versus adults who were in care and willing to attend a study-specific visit and related procedures. Despite the limitations that will influence the specificity of event frequency, there are clear long-term effects that have been observed. In adult survivors of steroid-sensitive nephrotic syndrome patients, ESKD is uncommon (1/43) (2 %) [90], diabetes mellitus in 2 % [90, 102], obesity/overweight in 10–20 % [90, 103, 104], cataracts 2–20 %; cancer (2/42) (4 %) [104]; and thromboembolic events (1/43) (2 %) [90].

Short stature was reported in approximately 20 % of patients in a cohort treated with 12–18 months of corticosteroids and a separate 15-member cohort of continually active adults in whom 12 were still receiving active immunomodulating therapy at the time of the study [103]. Osteoporosis of the lumbar spine or forearm regions has been reported in up to 30 % [90, 103] of adults with relapsing nephrotic syndrome. In other more generalized nephrotic syndrome cohorts, no children with nephrotic syndrome have met criteria for osteoporosis [105].

The risk for cardiovascular disease continues as an open question. Hypertension is reported in 7–45 % of adults with persistently relapsing nephrotic syndrome [90, 103]. The greatest frequency was observed in the 15-member cohort where the majority were still receiving cyclosporine A and/or prednisone. Two cohorts have reported myocardial infarctions/cardiovascular events without mortality, with individual frequencies of 2 and 8 % [90, 102]. Larger studies with more measurements and record capture in long-term monitoring will need to be conducted to fully understand the impact of childhood onset nephrotic syndrome, long-term steroid therapy, and dyslipidemia on cardiovascular risk.

Infertility is a concern for individuals exposed to cytotoxic therapies. In a single study that evaluated sperm count, motility, and structure in eight males with one or more courses of cyclophosphamide, 1/8 (12 %) had oligospermia, 4/8 (50 %) had decreased motility, and 6/8 (75 %) had teratozoospermia [103]. In a separate study comparing childlessness among adult survivors of childhood onset nephrotic syndrome, 8 % of adults exposed to cyclophosphamide and 33 % not exposed to cyclophosphamide had children as adults [104].

Children with steroid-resistant nephrotic syndrome are at risk for ESKD and the attendant additional complications related to kidney failure. The additional risk to these individuals due to long-standing immunomodulation, hypertension, and dyslipidemia during the nephrotic syndrome phase of therapy combined with ESKD remains to be evaluated.

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Focal and Segmental Glomerulosclerosis

4

Moumita Barua and Martin R. Pollak

Introduction

Focal and segmental glomerulosclerosis (FSGS) is a pathologic diagnosis, reflecting the end point of injury of a diverse group of clinical disorders and exists in primary and secondary forms (Table 4.1). FSGS can manifest heterogeneously with subnephrotic to nephrotic range proteinuria, with or without features of the nephrotic syndrome, depending on its etiology. As the name implies, FSGS is grossly defined by the pathologic finding of sclerosis in parts of the tufts involving some but not all glomeruli (Fig. 4.1). However, subtle features observed on light microscopy differentiate histologic variants of the disease, which some pathologists suggest can portend etiology and prognosis [1]. Though prevalence varies greatly across geography and race, FSGS has been reported to be increasing in frequency and is now a common cause of glomerular disease in adults worldwide, with a significantly higher incidence in individuals of recent African descent. FSGS primarily affects adults with less than 15 % of children with nephrotic syndrome reported to be due to FSGS [2]. Over the past two decades, our understanding of the disease has advanced significantly from the study of monogenic, albeit rare forms of FSGS. These studies have consistently pointed to dysfunction of the podocyte as central to the pathogenesis of disease. Nonetheless, FSGS remains a challenging disease to treat due to its frequent relapsing and unremitting course and high rate of progression to end-stage renal disease (ESRD). There is a paucity of rigorous clinical studies to define optimal treatment regimens and duration of therapy, which remain controversial. FSGS is the most common glomerular cause of ESRD in the USA, representing a significant burden to health care.

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Epidemiology

In adults, membranous nephropathy has traditionally been considered to be the most common histologic lesion underlying the nephrotic syndrome. Prevalences of glomerular disease, however, vary greatly by ethnicity. As a result, reported prevalences of glomerular diseases are difficult to apply to the general population, especially in certain countries with heterogeneous populations such as the USA. Furthermore, studies that report glomerular epidemiological data are inherently limited by changes in renal biopsy practices, changes in disease classification, and referral bias to urban tertiary centers. Nonetheless, there has been an emerging trend over the past three decades showing a disproportionate rise of FSGS in adults compared to other glomerulopathies in Europe, Australia, and the USA, with 15–25 % of nephrotic syndrome explained by this entity [3–5]. The most recent US population-based study using data from the United States Renal Data System (USRDS) and the National Center for Health Statistics showed that FSGS is the most common cause of ESRD due to a primary glomerular disease in both the black and white population during the period of 1980–2000 [6]. The proportion of ESRD due to FSGS increased 11-fold during the 21 year time period. The reason for this trend can be only partially explained by the rising rate of obesity and thus other factors, such as exposure to environmental triggers, need to be considered [7, 8]. Regardless of cause, this is a disturbing trend of clinical importance since FSGS is less amenable to treatment than membranous and carries a poorer renal prognosis.

A rise in the incidence of FSGS has also been observed in children, where minimal change disease is by far the predominant cause of glomerular disease [9]. It is a common clinical practice to empirically treat children with nephrotic syndrome with steroids, an effective treatment for minimal change disease. However, the rise of FSGS in children as well should keep the pediatric clinician mindful of this as a diagnostic possibility, especially in cases of steroid-resistant disease.

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Table 4.1 Etiologies of focal and segmental glomerulosclerosis

Primary (idiopathic) FSGS	**Secondary FSGS**
Classic (not otherwise specified) variant	Infection
Tip lesion variant	HIV (usually collapsing variant)
Collapsing glomerulopathy variant	Parvovirus B19
Perihilar variant	Medication and drug related
Familial	Pamidronate (usually collapsing variant)
Alpha-actinin-4 mutation	Interferon alpha
TRPC6 mutation	Lithium
INF2 mutation	Heroin
Podocin mutation	Intravenous drug abuse
Nephrin mutation	Reduced nephron mass (usually perihilar variant)
WT1 mutation	Unilateral renal agenesis
CD2AP mutation	Oligomeganephronia
ApoL1	Reflux interstitial nephritis
	Nephrectomy, surgical, traumatic ablation
	Solitary kidney, any cause
	Hyperfiltration (usually perihilar variant)
	Morbid obesity
	Sickle cell disease
	Cyanotic congenital heart disease
	Hypoxic pulmonary disease

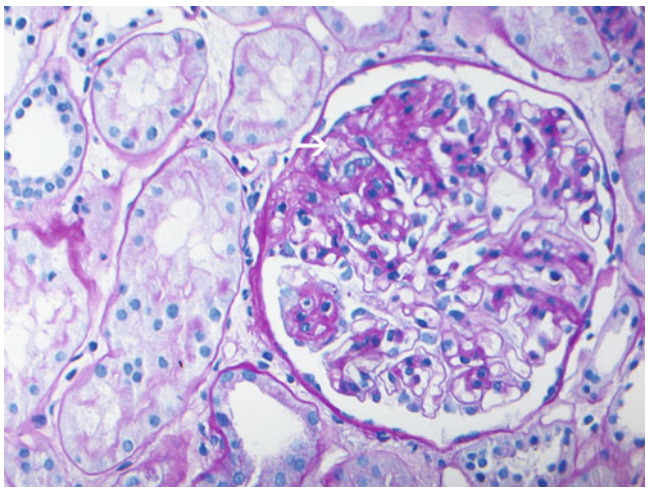

Fig. 4.1 PAS-stained section showing focal and segmental glomerulosclerosis with segmental consolidation of the glomerular tufts, endocapillary foam cells (*arrow*), and adhesion of the sclerosed segments to the Bowman's capsule (PAS ×40). Courtesy Sanjeev Sethi, MD

Certain studies have recently reported FSGS as the most common cause of primary glomerular disease including reports from Jamaica, Columbia, Brazil, and Jordan [10–13]. Collectively, these epidemiological data highlight the preponderance of FSGS for certain racial groups. Along with this data, observational studies in the USA demonstrate a clear predilection for individuals of African ancestry. The rise of FSGS has been reported to be more marked in African Americans and is 2–3 times more common compared to the European population [6, 14, 15]. Furthermore, nearly all patients with collapsing FSGS due to HIV are black [16]. These observations provide the rationale for population-based genetic studies of African Americans with FSGS, discussed below. Individuals of African ancestry also appear to have more malignant disease, with an earlier age of onset and an increased likelihood of progressing to ESRD. There is a disparity among genders as well, with males constituting the majority of individuals with FSGS [6].

Histologic Variants

FSGS can be a challenging diagnosis due to the nonspecific nature of the histologic findings. Segmental glomerular scarring in some, but not all, of the glomeruli is the signature lesion of FSGS observed on renal pathology, which can be idiopathic or related to a secondary cause (Fig. 4.1, Table 4.1). Furthermore, the pattern of focal and segmental glomerular scarring can also arise from a variety of inflammatory, proliferative, thrombotic, and chronic glomerulonephritides. The Columbia classification, described below, is a morphologic classification for FSGS and is based entirely on the assessment of glomerular light microscopic features, though it also requires immunofluorescence and electron microscopic examination to exclude these other causes of glomerular scarring [17].

Immunofluorescence shows neither immunoglobulin nor complement deposition but focal and segmental deposition of IgM, C3, and C1 can be observed within segmental glomerular sclerosis and hyalinosis. Weak mesangial

deposition of IgM and C3 can also be observed. Albumin and immunoglobulins IgA and IgG may be found in the podocytes and in the proximal tubule along with C3. Substantial glomerular deposits of IgG or IgA in mesangial, subendothelial, or subepithelial locations suggest an alternate diagnosis to FSGS.

On electron microscopy, there are changes in the podocyte including hypertrophy, microvillous transformation, detachment from the glomerular basement membrane (GBM), and accumulation of hyaline leading to occlusion of the glomerular capillary lumina. Sparse electron densities may be seen in the mesangium. The presence of sizeable or regular electron-dense deposits in the subepithelial location of nonsclerotic capillaries is also indicative of an alternate diagnosis. The glomerular basement membrane is of normal thickness in the nonsclerotic regions and diffuse thinning should raise the possibility of collagen IV disorders. Most notable, however, is the ultrastructural finding of the so-called foot process fusion, also known as effacement, which is observed on light microscopy as well.

Another diagnostic consideration is the quality of the biopsy sample and specifically the number of glomeruli sampled is particularly important given the focality of lesions. If a lesion that affects only 5 % of glomeruli is to be detected or excluded with 95 % confidence, then over 20 glomeruli are needed in the biopsy [18]. In cases where only a few glomeruli are sampled, minimal change disease may be erroneously diagnosed instead of FSGS.

A standardized classification scheme for FSGS was devised in 2000 at Columbia University, New York, at an international consensus meeting of renal pathologists.

There are multiple examples of consensus classifications as seen in the ISN classification of lupus nephritis, the Chapel Hill classification of systemic vasculitides, and the Banff classification of renal transplant pathology [19–23]. These schemas are formulated with the hopes of reflecting information on stage of disease, prognosis, and response to treatment. However, the significance of the standardized histologic variants in FSGS remains controversial. With that in mind, the Columbia schema defines five main light microscopic patterns of FSGS termed FSGS not otherwise specified (NOS), peripheral variant, cellular variant, tip variant, and collapsing variant (Table 4.2) [17, 18].

FSGS Not Otherwise Specified

FSGS (NOS) is the most common histologic variant. This form is also sometimes referred to as classic FSGS or FSGS of the usual type. The diagnosis of FSGS (NOS) relies on the exclusion of the other variants. On light microscopy, FSGS (NOS) is characterized on by the presence of focal and discrete segmental consolidation of glomerular tufts by sclerosis that obliterates some glomerular capillary lumens. The sclerotic lesions, characterized by increased matrix, affect variable parts of the glomerulus, including both the perihilar and peripheral areas. These changes occur first in the juxtamedullary glomeruli and thus superficial biopsies containing only cortex may miss these lesions. Hyaline deposits may be apparent within the sclerotic lesions, which also may form adhesions to Bowman's capsule. Podocyte effacement is observed above the sclerotic lesions along with separation from the glomerular basement membrane and intervening accumulation of new matrix leading to "podocyte capping." The degree of podocyte effacement generally correlates with the severity of proteinuria. Typically, podocyte hypertrophy or hyperplasia is mild in this variant. Mesangial hypercellularity, glomerulomegaly, and arteriolar hyalinosis may occur. Tubular atrophy and interstitial fibrosis are patchy and usually correlate in severity and to the distribution of the sclerosis, though sometimes have been observed to be disproportionately severe, presumably due to conditions of prolonged and heavy proteinuria which mediate tubular injury itself. In some cases, FSGS (NOS) may represent increasing chronicity and be an advanced stage of the other morphologic variants.

FSGS Perihilar Variant

The tip, cellular, and collapsing variants need to be first excluded. To meet criteria for this variant, greater than 50 % of segmentally sclerosed glomeruli must have sclerosis and hyalinosis adjacent to the vascular pole, otherwise known as the perihilar region. Other glomeruli may have randomly distributed lesions identical to those in FSGS (NOS). Glomerulomegaly and adhesions are common findings. Arteriolar hyalinosis is frequently observed and sometimes in contiguity with hyalinosis in the perihilar sclerotic segment. Podocytes may be hypertrophied and hyperplastic, but this is not as pronounced as with the tip, cellular, and collapsing variants. Mesangial hypercellularity is usually minimal or absent.

Though the perihilar variant can be found in primary FSGS, it is an especially common finding in FSGS due to secondary causes, namely, those related to low nephron number and hyperfiltering states. Such causes include obesity, cyanotic congenital heart disease, reflux nephropathy, renal agenesis, dysplasia, oligomeganephronia, or any advanced renal disease with a reduced number of functioning nephrons.

FSGS Cellular Variant

The diagnosis of cellular FSGS requires exclusion of the tip and collapsing morphologic variants. It is defined by the presence of at least one glomerulus with endocapillary hypercellularity involving at least 25 % of the tuft and causing

Table 4.2 Pathologic criteria for FSGS variants

Histologic variant	Positive criteria	Negative criteria
Collapsing	At least one glomerulus with: (1) Glomerular capillary tuft collapse (2) Overlying podocyte hypertrophy and hyperplasia Features of other variants may be present	
Tip	At least one glomerulus with: (1) Segmental lesion involving the outer 25 % of the glomerular tuft, otherwise known as the tip domain. Origin of proximal tubule must be identifiable (2) Adhesion or confluence of lesion in the tip domain (3) Foam cells or endocapillary cells in tip lesion or sclerosis	(1) Exclude collapsing variant (2) Exclude perihilar variant
Cellular	At least one glomerulus with: (1) Endocapillary hypercellularity, typically due to foam cells in any segment of glomerular capillary but involving at least 25 % of the glomeruli (2) Other glomeruli may have segmental sclerosis	(1) Exclude collapsing variant (2) Exclude tip variant
Perihilar	(1) More than 50 % of glomeruli with segmental lesions must have perihilar sclerosis (2) At least one glomerulus must have perihilar hyalinosis	(1) Exclude collapsing variant (2) Exclude tip variant
Not other specified	(1) Segmental glomerular capillary tuft lesion (2) May have segmental capillary wall collapse, without podocyte hyperplasia; peripheral or perihilar segmental sclerosis (3) Any number of glomeruli are involved	(1) Exclude all other variants

occlusion of the capillary lumina. These cells include endothelial cells, foam cells, and infiltrating leukocytes, such as monocytes, macrophages, and sometimes lymphocytes and neutrophils. Any segment can be affected. Extracapillary hypercellularity is also present, manifesting with podocyte hyperplasia and hypertrophy, which can enlarge and crowd to the degree that they resemble crescents. Adhesions may be present but glomerulomegaly and mesangial hypercellularity are uncommon.

The prognostic significance of this histologic variant is controversial, namely, because the published information regarding clinical course is again limited. Some published literature supports the notion that the cellular variant is characterized by severe proteinuria and represents an early phase in the evolution to segmental sclerosis [24–27]. This is based on the observation that there is a shorter time course from onset of proteinuria to renal biopsy. However, it is also possible these cases are actually collapsing FSGS, given the overlapping characteristic of hypercellularity but misclassified due to limited sampling.

FSGS Tip Variant

To make the diagnosis of the tip variant, the collapsing variant must be excluded. The name describes the location of the segmental lesion in at least one glomerulus—involving the quarter of the tuft adjacent to the origin of the proximal tubule—the glomerular tip. Sometimes, the affected segment may even appear to be herniated into the tubular lumen. Visceral epithelial cells next to the consolidated segment are hypertrophied and swollen, often becoming contiguous and attached to the nearby parietal and tubular epithelial cells at the origin of the proximal tubule. Adhesions between the tuft and Bowman's capsule occur. These lesions can expand toward the glomerular hilum, though they originate in the periphery.

The tip lesion was first reported in the literature by Howie and Brewer and described to be a steroid sensitive entity, thus resembling minimal change rather than FSGS [28]. Furthermore, autopsies of children in the presteroid era, who died of lipoid nephrosis, a term used at that time to encompass minimal change disease and FSGS, were found to have tip lesions but have no other histologic evidence of FSGS [29]. Based on this data, it is thought that prolonged proteinuria may cause tubular damage, resulting in the tip lesion. However, the data is scant and it is therefore difficult to draw definitive conclusions. A report of patients who had undergone serial biopsies shows an evolution of tip variant to classic FSGS in a subset of those individuals. Patients with tip variant FSGS have also been described to be steroid/calcineurin inhibitor unresponsive and to have recurrence of classic FSGS post transplant [30]. It is possible that the tip variant reflects two conditions, one an early form of classic FSGS and the other closely related to minimal change disease.

Collapsing FSGS

The presence of a collapsing lesion in even just one glomerular tuft is enough to make a diagnosis of collapsing FSGS, preempting all the other diagnostic subcategories. The collapsing lesion can be segmental or global and usually overlies shrunken, collapsed capillaries and a wrinkled glomerular basement membrane. There is a lack of appreciable increase in intracapillary or mesangial matrix, reflecting the acute nature of the injury. Overlying podocytes are markedly hypertrophied and hyperplastic, with enlarged vesicular nuclei, frequent nucleoli, occasional binucleated forms, and rare mitotic figures. Tubular atrophy and interstitial fibrosis can be disproportionately severe to the degree of collapse observed.

Collapsing FSGS is frequently associated with HIV but can occur idiopathically as well. The majority of individuals with HIV-associated collapsing FSGS are black. Endothelial tubular reticular inclusions observed on electron microscopy are not seen in primary disease and suggest concomitant HIV infection. There are other secondary causes of collapsing FSGS including drugs and other infections, which need to be excluded before arriving at a diagnosis of primary FSGS.

Since its original description in the literature in 1986, both primary and secondary collapsing FSGS have been described to have unique clinical and demographic characteristics compared to the other morphologic variants [31]. As with all FSGS, the collapsing variant is seen more in adults and in males. This morphologic variant has a particular preponderance for African Americans but affects the Caucasian population as well. Individuals with the collapsing variant have a more malignant course. Typically, there is a shorter time from onset of proteinuria to biopsy due to the fact that affected individuals present with higher grade proteinuria and more severe features of nephrotic syndrome and are more likely to have renal insufficiency [7, 16]. This morphologic variant also tends to have the most malignant clinical course and is commonly steroid resistant with progression to ESRD.

The Glomerular Filtration Barrier

The kidney filters approximately 180 L/day of plasma containing over 7,200 g of albumin, 99.9 % of which is retained. Plasma is filtered passively, with only the formed elements of the blood and the bulk part of the plasma proteins being retained [32]. Filtration occurs at the glomerular capillary wall, which separates the blood compartment from the urinary space. It consists of three layers; from the innermost layer to the outermost layer, they are the fenestrated endothelium, the glomerular basement membrane, and the podocyte (Fig. 4.2). This filtration barrier behaves as a size-selective sieve restricting the passage of macromolecules on the basis of their size, shape, and charge. Both size and charge influence filtration of macromolecules. Negatively charged molecules are more restricted in their passage due to the negative charge of the glomerular filter and filtration is also inversely proportional to size [33]. Disruption at any layer of the glomerular capillary wall can lead to passage of macromolecules and proteinuria. While it is logical to conceptually divide the glomerular filter into three parts based on structure, it has been difficult to differentiate the physiology of the filter along similar lines. However, as will be discussed below, advances in the biology of proteinuric renal disease suggest that the podocyte and its slit diaphragm are central to barrier function and disruption here can lead to FSGS.

Endothelium

Unlike the endothelial lining of vessels in other parts of the body, the glomerular endothelium is fenestrated with an apparent lack of bridging diaphragms. Therefore, in the past, the endothelium has been viewed as not significantly contributing to protein barrier function. This model is controversial, however, as it theoretically would result in massive convective movement of albumin and other macromolecules into the glomerular basement membrane, causing clogging of the filter [34]. Interestingly, the presence of filamentous plugs filling the capillary fenestrae has been recently described, and it is possible that postmortem artifactual tissue changes explain these apparent discrepancies [35].

In addition, there is now an emerging body of literature to support the concept of cross talk between podocytes, endothelia, and mesangial cells in maintaining glomerular capillary wall function. The production of vascular endothelial growth factor (VEGF) by podocytes is necessary for the integrity of the glomerular endothelium [36]. The upregulation and secretion of the podocyte protein angiopoietin-like 4 (Angptl4) into the glomerular capillary wall cause marked proteinuria in experimental models of nephrotic syndrome [37]. Clinically, VEGF inhibitors used as anti-angiogenic drugs in the treatment of malignancies have been observed to lead to glomerular disease manifesting with proteinuria and thrombotic microangiopathy [36].

The Glomerular Basement Membrane

Of the three layers, the GBM is unique in that it is a specialized extracellular matrix lying between two cellular layers. It is composed of a fibrous network consisting of type IV collagen (collagen α[alpha]3, α[alpha]4, and α[alpha]5 chains), laminin, and nidogen/entactin, together with negatively charged heparin sulfate proteoglycans such as agrin and perlecan.

It is the negative charge of the glomerular basement membrane that is traditionally felt to contribute to glomerular permselectivity, though the importance of this in the filtration barrier is debatable [38]. The GBM, however, does account for most of the restriction of fluid flux [39, 40]. Mutations in different components of the GBM lead to varied phenotypes. Human laminin beta2 deficiency gives rise to massive proteinuria but mutations in type IV collagen lead to Alport's or thin basement membrane disease, in which proteinuria is not a prominent symptom [41].

The Podocyte

Familial FSGS accounts for only a minority of disease, but nevertheless studies of inherited forms of disease have revealed insights into the molecular mechanisms underlying the development of FSGS. Since the discovery of nephrin as the culprit gene for congenital nephrotic syndrome of the Finnish (CNF) type by Karl Tryggvason and colleagues, subsequent studies have consistently pointed to podocyte dysfunction as central to the development of proteinuric kidney disease, including FSGS (Fig. 4.2) [42–49]. More specifically, the cell-cell junction between adjacent podocytes, the slit diaphragm, is made up of a multiprotein complex which dynamically controls foot process architecture via actin cytoskeleton signaling.

The podocyte is a terminally differentiated polarized epithelial cell that stems from precursor mesenchymal cells. During nephrogenesis, these cells undergo modification from a classic epithelial cell phenotype to a highly specialized octopus-shaped cell essential in glomerular filtration. The podocyte consists of a cell body and the major or primary processes, which branch into finger-shaped extensions called pedicles or foot processes. The podocytes cover the external surface of the capillaries, interacting with the GBM and interdigitate through their foot processes in a complex manner forming a network of narrow gaps. The gap spans a space of 30–40 nm and is bridged by a mesh-like network that stretches between these processes called the slit diaphragm. Originally described by Rodewald and Karnovsky in mice, the slit diaphragm forms the glomerular ultrafiltration barrier that allows for the passage of water and solutes while inhibiting loss of larger plasma molecules such as albumin and immunoglobulins [50]. The slit diaphragms (SD) appear early on in nephrogenesis at the capillary stage of development. A modified adherens junction, the SD is a zipper-like structure composed of a complex interplay of a number of proteins including ZO-1, FAT, and nephrin that are all transmembrane proteins shown to localize to the slit diaphragm. ZO-1 localizes to the cytoplasmic face of the SD and interacts with cell junction components as well as proteins in the cytoskeleton [51, 52]. FAT, a member of the cadherin superfamily, localizes at the slit diaphragm [53]. Within the kidney, the integral membrane protein nephrin is expressed exclusively in podocytes [54, 55]. Nephrin-deficient mice and humans develop severe nephrosis [55]. Additional slit diaphragm-associated proteins include podocin and CD2AP which both interact with nephrin and P-cadherin, which is involved in junction-cytoskeleton attachment [56].

The architecture of the podocyte and its unique shape are further characterized by a cytoskeleton composed of microtubules, intermediate filaments, and microfilaments. The cell body cytoskeleton is composed mostly of microtubules and intermediate filaments, with prominent vimentin and desmin. Cytoplasmic extensions or foot process extensions arise from the podocyte cell body. These extensions contain both a microfilament-based contractile apparatus and an actin cytoskeletal network. A group of intracellular cytoskeleton-associated proteins (talin, paxillin, and vinculin) link actin filaments to the cell membrane-associated integrins at the glomerular basement membrane [57].

The role of the podocyte and the slit diaphragm as functional barriers to protein filtration is further defined by its polarity. The exposed surface of the podocyte is covered with a cell coat that contains the sialic acid protein podocalyxin that is thought to give rise to its anionic charge [58]. A decrease in the content of sialic acid has been observed in rats with aminoglycoside nephrosis and in humans with proteinuria or glomerulonephritis [59]. In addition, neutralization of the anionic charge of the sialoprotein coat results in loss of epithelial foot processes that resembles the loss of foot processes seen in human with nephrotic syndrome [60].

FSGS as a Podocyte Disorder

Nearly all the products of genes identified as mutated in nonsyndromic hereditary forms of steroid-resistant nephrotic syndrome (SRNS) and FSGS localize to the podocyte and the slit diaphragm, thereby rationalizing our podocentric view of proteinuric kidney disease (Fig. 4.2c). Furthermore, relative or absolute podocyte number depletion in animal models has been shown to lead to initiation or progression of the lesions observed in FSGS [61–64].

Point mutations in the different genes lead to clinical manifestation of disease at different ages (Table 4.3). Genes that are responsible for recessive forms of nonsyndromic SRNS, which may or may not manifest histologically as FSGS, include nephrin (NPHS1), podocin (NPHS2), laminin-β[beta]2 (LAMB2), and phospholipase C-ε[epsilon]1 (PLCE1). These disorders occur during the first 3 months of life (congenital disease), during infancy (within 1 year), or during childhood. The earlier the onset

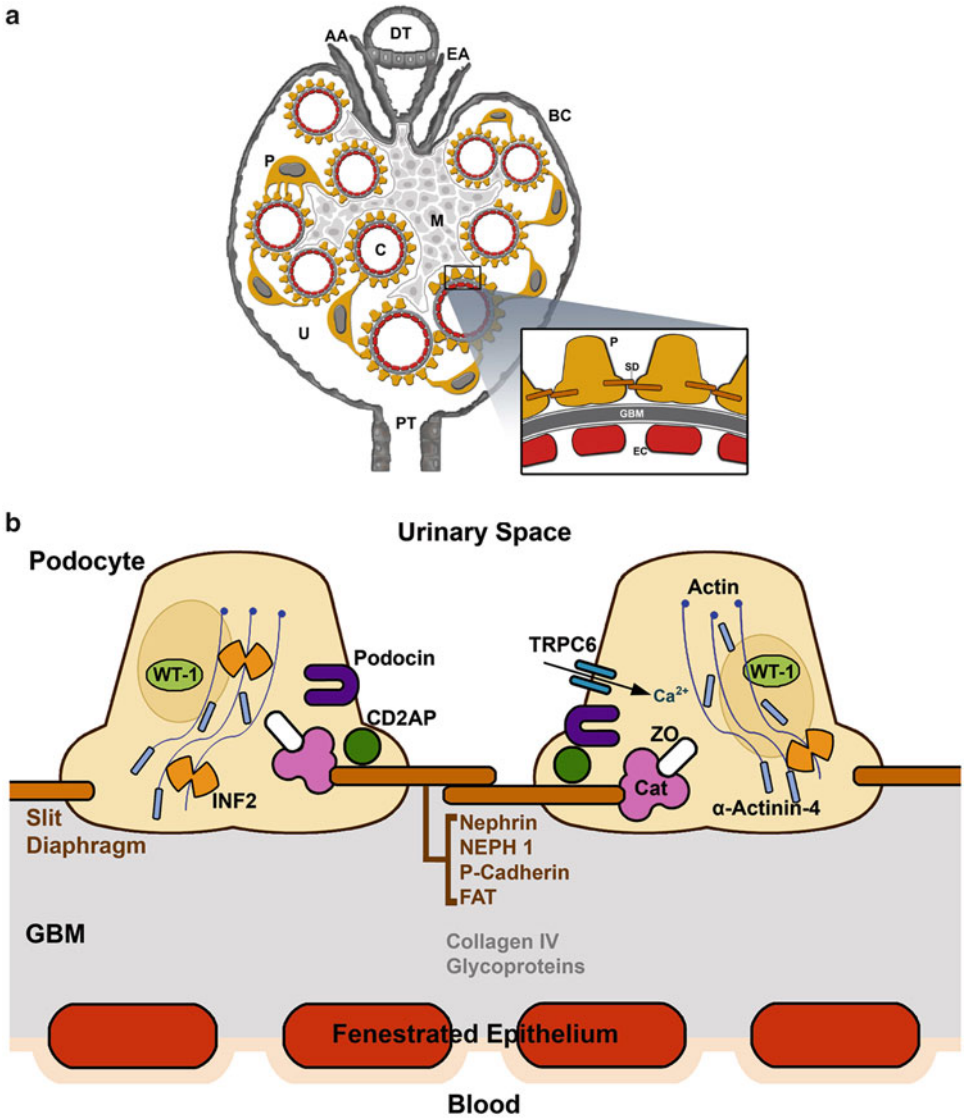

Fig. 4.2 (**a**) Glomerular filtration barrier structure. (A) Glomerular structure: (AA) afferent arteriole, (EA) efferent arteriole, (DT) distal tubule, (C) capillary loop, (P) podocyte, (FP) podocyte foot processes, (M) mesangium, (U) urinary space, (BC) Bowman's capsule, and (PT) proximal tubule. Details of the glomerular filtration barrier structure: (FP) podocyte foot process, (GBM) glomerular basement membrane, (EF) endothelial cell, and (SD) slit diaphragm. Inset indicates the glomerular filtration barrier formed by fenestrated endothelial cells, the GBM and podocytes. (**b**) The podocyte has a unique actin cytoskeleton made up of F-actin and non-muscle myosin such as MYH9 and actin-binding proteins including ACTN4 and INF2. WT1 is a nuclear transcription factor. The slit diaphragm is formed partially by nephrin, podocin, and CD2AP. These molecules at the slit diaphragm associate with TRPC6 and PLCE1

of SRNS, the more likely that it is of monogenic origin, as 85 % of all congenital SRNS and 66 % of all infantile SRNS is caused by mutations in one of four genes: nephrin, podocin, LAMB2, or WT1 [65]. In the minority of cases that are nongenetic, secondary causes like infections with syphilis and toxoplasmosis or toxin exposure to mercury ought to be ruled out. While those forms of SRNS that present at birth typically do not show evidence of FSGS, it seems appropriate to group these forms of inherited podocyte injury together given their related etiologies.

Point mutations in genes which cause autosomal-dominant nonsyndromic forms of adult-onset FSGS include α[alpha]-actinin-4 (ACTN4), TRPC6, and INF2. Other dominant genes are WT1 and CD2AP which have variable ages for onset of disease, from birth to adulthood. Most recently, two variants in the APOL1 gene have been shown to explain the increased rate of FSGS in individuals of recent African ancestry.

Table 4.3 Nonsyndromic hereditary causes of FSGS and nephrotic syndrome

Gene	Locus	Inheritance	Protein	Phenotype
Slit diaphragm				
NPHS1	19q13.1	AR	Nephrin	Congenital nephrotic syndrome of the Finnish type
NPHS2	1q25.2	AR	Podocin	Congenital-, childhood-, or late-onset SRNS. Juvenile- or adult-onset disease in affected individuals bearing the R229Q mutation along with a pathogenic mutation
PLCE1	10q23.33	AR	Phospholipase C epsilon 1	Early-onset SRNS with DMS and FSGS
CD2AP	6p12	AR(?)	CD2 associated protein	Unclear phenotype in humans. Mouse model develops severe proteinuria
TRPC6	11q22.1	AD	TRPC6	Adult-onset FSGS
Actin cytoskeleton				
ACTN4	19q13	AD	Alpha-actinin-4	Adult-onset FSGS
INF2	14q32.33	AD	INF2	Adult-onset FSGS
Nuclear				
WT1	11p13	AD	Wilms' tumor 1	Isolated NS or as part of Frasier or Denys-Drash syndrome
Glomerular basement				
LAMB2	3p21	AR	Laminin beta-2	Isolated NS or as part of Pierson syndrome
Other				
ApoL1	22q13.1	Recessive risk inheritance	Apolipoprotein 1	Risk haplotypes associated with increased risk of FSGS and ESRD in African Americans

SRNS steroid-resistant nephrotic syndrome, *DMS* diffuse mesangial sclerosis, *?* indicates unclear inheritance pattern

Nephrin (NPHS1)

Mutations in nephrin cause congenital nephrotic syndrome of the Finnish type (CNF), so named because it is most common in Finland but it does exist in other populations. CNF is inherited as an autosomal recessive trait, with both sexes being affected equally and leads to SRNS. Nephrin, encoded by NPHS1, is a transmembrane adhesion protein of the immunoglobulin superfamily, expressed almost exclusively in the podocyte. It forms homodimers and heterodimers with NEPH1 at the slit diaphragm and is involved in podocyte cell signaling to the actin cytoskeleton. To date, close to 200 nephrin mutations have been described in both the Finnish and non-Finnish population. These mutations include small deletions, insertions, nonsense, missense, splice site, and promoter variations and they are distributed throughout the gene [66]. However, 90 % of affected Finnish individuals harbor either the Fin-major (nt121delCT) or Fin-minor (R1109X) mutation due to a founder effect [67–70]. NPHS1 disease generally shows very little phenotypic variation, giving rise to congenital SRNS, but interestingly some NPHS1 mutations have been associated with a milder clinical course characterized by childhood onset of disease [66, 71, 72]. Missense mutations that affect nephrin trafficking to the plasma membrane and functionally act as a null allele result in a more severe phenotype, whereas those mutations in which normal trafficking are preserved results in the milder form of disease.

Most infants with NPHS1 disease are born prematurely with a low birth weight for gestational age. The placenta is markedly enlarged causing flexion deformities of large joints. Nephrotic syndrome is severe with profound urinary protein losses leading to marked edema, susceptibility to bacterial infections, thromboembolic complications, poor nutritional status, and retarded stature as well as hypothyroidism from urinary losses of thyroxine-binding protein. The kidneys are enlarged on ultrasound with loss of the corticomedullary border. No single histologic lesion is pathognomonic for NPHS1 disease. End-stage renal disease typically occurs in childhood, though some affected individuals may have a protracted course.

The treatment is challenging. NPHS1 disease is resistant to immunosuppressive therapy [73]. Treatment is initially supportive and includes albumin infusion, gamma globulin replacement, high protein but low salt diet, vitamin and thyroxine substitution, and close monitoring for infectious and

thrombotic complications. Sometimes the complications of nephrotic syndrome are unmanageable and bilateral nephrectomy may be performed to prevent ongoing protein losses to control symptoms. Renal transplantation is curative [73].

Podocin (NPHS2)

Mutations in the NPHS2 gene, encoding for the protein podocin, are a common cause of childhood-onset SRNS. However, screening of cases with congenital NS has shown that a significant proportion is due to mutations in the NPHS2 gene as well [74]. Mutations in podocin that result in nonfunctional and often truncated proteins lead to congenital and infantile disease. Homozygous missense mutations manifest in childhood, whereas individuals who carry one pathogenic NPHS2 mutation along with a common benign non-synonymous polymorphism in the population, the pR229Q variant, typically develop juvenile or adult-onset NS [75, 76]. Podocin is required for proper targeting of nephrin into the slit diaphragm and is expressed exclusively in the podocyte [77].

The clinical findings in patients with NPHS2 disease are more variable than in NPHS1 patients [78]. The renal histology is often but not always FSGS [65]. End-stage renal disease can occur at a wide range of ages, from childhood to adulthood.

Wilms' Tumor (WT1)

Wilms' tumor suppressor gene (WT1) encodes for the zinc finger transcription factor WT1, which functions both as a tumor suppressor and as a critical regulator of kidney and gonadal development [79]. It is expressed in podocytes and controls cellular functions, such as nephrin expression [80]. WT1 mutations have been associated with three renal-related clinical syndromes, often presenting in the first decade of life [81]: (1) Frasier syndrome, which is characterized by SRNS in childhood with histologic finding of FSGS and often slow progression to ESRD in the second or third decade of life, male pseudohermaphroditism, and a high incidence of gonadoblastomas; (2) Denys-Drash syndrome, which is characterized by infantile SRNS with histologic findings of mesangial sclerosis with rapid progression to ESRD usually by age 4, ambiguous genitalia, and WT; and (3) isolated SRNS. One important aspect of management is the issue of malignancy surveillance and consideration to prophylactic oophorectomies and nephrectomies. The management decisions are particularly challenging in individuals with isolated SRNS. Though quite limited, there is some data to suggest that affected individuals with isolated SRNS and a splice site mutation do not develop malignancy, whereas those with missense or nonsense mutations do [81].

Other Genetic Forms of Congenital Nephrotic Syndrome

Congenital nephrotic syndrome is clinically and genetically heterogeneous and the majority of cases, but not all, can be attributed to mutations in nephrin, podocin, and WT1. Positional cloning in consanguineous families with autosomal recessive inheritance led to the discovery of phospholipase epsilon (PLCE1) as a causative gene for an infantile and childhood form of SRNS called NPHS3 [48]. PLCE1 is expressed in the podocyte. Renal histology varies from diffuse mesangial sclerosis in infants to FSGS in children. Notably and distinct from the other inherited forms of NS and FSGS which are typically resistant to treatment, there have been cases of affected individuals responding to immunosuppressive therapy [81]. Laminin-β[beta]2 is a constituent of the GBM and mutations in its gene were first described to cause congenital NS as part of Pierson syndrome, which also includes distinct ocular anomalies with microcoria. However, there have been reports of isolated NS as well [41, 82].

Alpha-Actinin-4

In 2000, the first gene to cause adult-onset FSGS was identified in a family-based study [46]. Point mutations in the gene α[alpha]-actinin-4 (ACTN4) lead to a rare autosomal-dominant form of FSGS with variable penetrance. Alpha actinins are a family of ubiquitously expressed proteins but ACTN4 is the only one significantly expressed in the human podocyte. ACTN4 is a cytoskeletal protein which binds F-actin and mutant forms bind more tightly when compared to wild type leading to cytoplasmic aggregates [83]. The downstream effects by which disease is caused in unknown, though there is some evidence to suggest that mutant α[alpha]-actinin-4 alters podocyte cytoskeletal function [84]. Recent investigations report that α[alpha]-actinin-4 mutations associated with human FSGS may suppress transcriptional activity in nuclear hormone receptors in podocytes, which ultimately alters normal cytoskeletal actin filament organization.

Individuals who are heterozygous for ACTN4 mutations develop mild onset of proteinuria in their teenage years or onward and have slowly progressive kidney dysfunction which often leads to end-stage renal disease. Interestingly, mice homozygous for equivalent point mutations develop proteinuria, whereas heterozygous mice develop a much milder phenotype with subtle histologic renal abnormalities [85].

There are several defining ultrastructural features for ACTN4-related disease. In human and mouse kidney sections, cytoplasmic electron densities in the podocytes are seen. Furthermore, immunofluorescence with ACTN4

antibody, which is not routinely performed, reveals granular staining as opposed to linear staining observed in non-ACTN4-related disease.

Transient Receptor Potential Channel 6

Winn et al. demonstrated that mutations in the transient receptor potential channel 6 (TRPC6) can cause an autosomal-dominant adult-onset form of FSGS [47]. Multiple mutations in TRPC6 have now been identified [47, 86, 87]. TRPC6, a nonselective cation channel, belongs to the transient receptor potential (TRP) family of proteins and is expressed in the podocyte along with other TRP channels. Many of the mutations in this gene lead to higher voltage current across the channel due to increased cytoplasmic calcium flux and activate the NFAT-calcineurin pathway [88]. Interestingly, there is strong induction of TRPC6 in membranous nephropathy as well, suggesting that TRPC6 or its downstream effectors may represent therapeutic targets for a broader range of proteinuric kidney disease [89]. As a podocyte slit diaphragm ion channel, TRPC6 resides at an accessible site where drugs can reach before or after passing through the slit diaphragm, representing an ideal target for small molecules or biological modifiers, once available. Like ACTN4-related disease, affected individuals present with adolescent- or adult-onset proteinuria and have slowly progressive disease that often leads to ESRD.

Inverted Formin 2

The most recent gene to be identified as causing a form of autosomal-dominant FSGS is inverted formin 2 (INF2) [45]. Mutations in this gene have been demonstrated to be a major cause of autosomal-dominant FSGS, accounting for up to 17 % of familial cases [44]. To date, all FSGS-associated INF2 mutations alter highly conserved residues within a regulatory region of the protein. INF2 is highly expressed in the podocyte and belongs to the formin family, a group of heterogeneous actin nucleating proteins that regulate a variety of cytoskeleton-dependent cellular processes. Hence, it is postulated that INF2 mutations leads to a disturbance in actin dynamics [90–95]. Individuals with INF2 mutations present with disease in early adolescence or adulthood, typically with moderate proteinuria. Disease is progressive and often leads to ESRD. Pathology is typically FSGS.

CD 2-Associated Protein

CD 2-Associated Protein (CD2AP) is an adapter protein that was initially discovered because of its role in T cell immunity. CD2AP interacts with CD, a T cell and natural killer cell membrane protein that facilitates adhesion of T cells to antigen-presenting cells. However, CD2AP is widely expressed and interestingly CD2AP knockout mice develop massive proteinuria and die at 6–7 weeks of renal failure, with pathologic examination showing findings characteristic of FSGS [49]. Two heterozygous mutations were subsequently described in two patients with primary FSGS [96].

Other Genetic Conditions

Other genetic disorders are also associated with FSGS as well as other renal and/or extrarenal lesions. These include adult-onset cystinosis, nail-patella syndrome, Charcot-Marie-Tooth disease, and glucose-6-phosphatase deficiency.

Apolipoprotein 1

A widely quoted statistic holds that African Americans have about fourfold more kidney disease than European Americans. While old reports have hinted at this disparity for some time, it was neither widely appreciated nor reliably quantified until relatively recently [97]. In 2008, two studies took advantage of this renal disease prevalence disparity to demonstrate that a locus or region on chromosome 22 conferred most or all of the increased risk for nondiabetic kidney disease in African Americans [98, 99]. MALD (mapping by admixture linkage disequilibrium) uses differences in allele frequencies between populations with differing susceptibility to disease to determine chromosomal regions that are likely to harbor disease-causing genes. A large excess of western African ancestry among African Americans with either FSGS or hypertension-associated ESRD at this single locus on chromosome 22 confirmed a genetic basis for the observed ancestry-related disparity in renal disease prevalence.

Subsequent studies used new sequencing data to uncover a stronger association with two particular coding sequence variants in a neighboring gene, apolipoprotein 1 (APOL1) [100, 101]. This association appears to be conferred by two particular coding sequence variants in a neighboring gene, apolipoprotein 1 (APOL1). These two variants, a two amino acid substitution labeled G1 and a two amino acid deletion labeled G2, alter the amino acid sequence of the encoded APOL1, suggesting functionality. The association was found for African Americans with FSGS and hypertension-associated ESRD (H-ESRD). In these studies, individuals with one risk allele (G1 or G2) have no or only minimally increased kidney disease risk. Individuals with two risk alleles (one on each chromosome) have 7–10-fold increased risk of FSGS and H-ESRD [100]. Thus, APOL1-mediated kidney disease risk follows essentially an autosomal recessive

inheritance pattern. APOL1 circulates in the blood as part of the high-density lipoprotein complex (HDL). The mechanistic explanation for disease is not apparent.

APOL1 has the potential to serve as an important prognostic marker and guide clinical decision making. Clinical studies of the outcomes of renal transplant donors and recipients with high-risk APOL1 alleles are currently in progress and may define the utility of testing for the risk alleles in the donor and recipient population. Furthermore, the question of whether treatment aimed at slowing kidney disease progression will have different efficacy in individuals with or without APOL1 risk alleles deserves further clinical investigation.

HIV nephropathy (HIVAN), which is characterized by collapsing FSGS, is another clinical scenario where testing for APOL1 risk alleles may be clinically relevant. The vast majority of individuals with HIVAN are of recent African ancestry [102]. The development of HIVAN is an indication for the institution of antiretroviral therapy. Therefore, studies evaluating the risk of HIVAN in HIV-positive individuals carrying the APOL1 risk alleles will define whether treatment should be initiated even prior to the development of symptoms in APOL1 HIV-positive patients.

Interestingly, the G1 and G2 alleles are common, with an allele frequency (the percentage of chromosomes, rather than people) for a risk allele (either G1 or G2) of approximately 33 %. Wild-type APOL1 confers protection against the African sleeping sickness parasite, Trypanosoma brucei, by facilitating its lysis but not to certain subspecies that have evolved resistance. In vitro assays have demonstrated that the G1 and G2 alleles are lytic against these resistant subspecies. It is thus speculated that the G1 and G2 alleles rose recently in evolution because of the protective advantage it has conferred against the resistant Trypanosome subspecies, despite their deleterious consequences on the kidney.

While these two variants in APOL1 appear to confer most of the increased risk of kidney disease in blacks, some studies suggest an independent effect of the neighboring gene MYH9 [103].

Clinical Features

FSGS is a pathologically heterogeneous entity representing the end point of diverse pathogenetic mechanisms. Similarly, FSGS presents with heterogeneous clinical manifestations and affected individuals can have subnephrotic proteinuria, insidious or overt onset of nephrotic syndrome. Clinical features of the nephrotic syndrome include 3.5 or more grams of protein excretion per day, hypoalbuminemia, peripheral edema, hypercholesterolemia, and, less commonly, recurrent infections and thrombosis. Individuals with FSGS commonly have hypertension and may also have microscopic hematuria. The level of kidney dysfunction is variable and histologic subtype may predict clinical course and prognosis.

Individuals presenting with overt nephrotic syndrome or high-grade proteinuria are most likely to have primary FSGS. In contrast, secondary forms of FSGS have a more insidious onset with non-nephrotic proteinuria, along with the absence of the other distinguishing features of the nephrotic syndrome. Secondary FSGS is further distinguished by its lack of response to immunosuppressive therapy. The clinical history, as well as pathologic examination, is used to rule out secondary forms of FSGS. The perihilar variant is usually associated with adaptive structural responses to hyperfiltering states due to any cause. Electron microscope findings can also differ, with more diffuse foot process effacement in primary forms versus focal effacement associated with the sclerotic lesions in secondary forms.

Different studies report a broad range of estimates for progression to renal failure, remission rates of the nephrotic syndrome, and response to therapy of patients with FSGS. Untreated FSGS is progressive, with a spontaneous remission rate that is unknown but occurs rarely, and as a result ESRD is common. Remission is the most important variable for prognosis. Individuals who achieve a complete and partial remission have the best outcomes, with 5-year kidney survival rates approaching 100 % and 60 %, respectively [104–108]. Other predictors of ESRD for all FSGS patients are initial serum creatinine, percent global sclerosis, and chronic tubulo-interstitial injury score.

The prognostic significance of the histologic subtypes has been debated [109, 110]. The literature can be conflicting and is limited by lack of prospective studies, small sample sizes along with relatively short follow-up periods. The classification schema implies that there is a mechanistic difference underlying these subtypes, but some argue that these may reflect different stages of the disease process. It does appear that the collapsing, cellular, and tip variants represent more active disease, whereas the perihilar and NOS variant is more chronic in nature. The collapsing, cellular, and tip variants present more acutely, with higher degrees of proteinuria have shorter times to renal biopsy and have less tubular atrophy and interstitial fibrosis on pathologic examination. Of all the morphologic subtypes, the collapsing variant has the most malignant course and predominantly affects people of African origin. The collapsing form presents with marked proteinuria and higher serum creatinine, is less likely to be responsive to immunosuppressive therapy, and has a high rate of progression to ESRD.

The cellular variant shares features of hypercellularity with the collapsing variant leading to the question as to whether it is indeed a separate entity. The cellular variant, like the collapsing form, also has preponderance for people of African ancestry, though not as marked. The glomerular cellular lesion remission rate is intermediate to that of the collapsing variant and the tip lesion subtype, the latter having the most favorable prognosis. The glomerular tip lesion has a high remission rate and is highly responsive to steroids

and other immunosuppressive agents, leading some to hypothesize that the tip lesion is closely related to minimal change disease.

Given the rarity of monogenic adult forms of FSGS, a defined clinical course is difficult to describe. In contrast, monogenic congenital and infantile forms of nephrotic syndrome are more common, have defined clinical features as described earlier, are typically steroid resistant, and nearly always lead to ESRD [111].

Recurrence After Transplantation

FSGS can present in the posttransplant settings in one of two ways: (1) an early recurrence characterized by massive proteinuria within hours to days after implantation of the new kidney and (2) a late recurrence that develops insidiously several months or years after transplantation. The former clinical presentation is suggestive of the presence of a circulating factor causing FSGS, and several candidates have been identified thus far. FSGS recurs in about 30 % of grafts after transplantation [112–117]. The risk factors for recurrence of FSGS include childhood onset, age <15 years, rapid progression (within 3 years) from diagnosis to ESRD, diffuse mesangial hypercellularity in the native kidney, and recurrence of FSGS in a previous allograft [117]. Individuals harboring genetic mutations rarely develop posttransplantation recurrence with the exception of those with NPHS2-related disease, which has been cited to occur in up to 30 % of cases [118, 119]. Treatment of recurrent FSGS is anecdotal and can involve IVIG, plasmapheresis with cyclophosphamide or cyclosporine, and corticosteroids. A recently published small retrospective case series suggests that rituximab may help prevent recurrent FSGS in the allograft but additional studies are needed.

Treatment

There is a paucity of rigorous data to support current treatment practices of FSGS. The literature is dominated by small, retrospective observational studies, and there are only few small randomized control trials.

Initial evaluation of FSGS involves classification of the disease into primary or secondary forms, which has therapeutic implications (Table 4.1). Genetic testing is not a routine part of the evaluation given its rare occurrence. Secondary forms of FSGS are treated conservatively through a combination of lifestyle modification, management of hypertension and hyperlipidemia, and treatment of proteinuria with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (ACEi, ARB). Primary FSGS associated with clinical features of the nephrotic syndrome is treated in the same manner but in addition includes corticosteroid and immunosuppressive therapy. An important exception to this is primary FSGS with subnephrotic proteinuria, which is felt to have a more indolent course and is usually treated conservatively with close monitoring. There are conflicting reports about the effectiveness of immune-based therapy for genetic FSGS, but the likelihood of a positive response may be low [120].

The mainstay of treatment for primary FSGS is glucocorticosteroids. Non-randomized retrospective studies report a broad range of complete and partial remissions rates from 25 % to 80 % but relapse is common following discontinuation of therapy [104, 106, 121–126] The definitions of proteinuria used in adult nephrotic syndrome are listed in Table 4.4. Resistance to corticosteroids and immunosuppressive therapy is one of the, if not, strongest predictor of ESRD [127]. Prognosis is poor in patients who do not achieve remission, with 5-year kidney survival averaging 65 % (60–90 %) and 10-year kidney survival of 30 % (25–56 %) [126–130].

The optimal dose and duration of glucocorticosteroid therapy are unknown. Current practice regimens include prednisone at a daily single dose of 1 mg/kg, up to a maximum of 80 mg, or alternate-day dose of 2 mg/kg, up to a maximum of 120 mg, is effective. This high dose is administered for a minimum of 4 weeks up to 16 weeks as tolerated or until complete remission has been achieved. Prednisone is slowly tapered over 6 months after achieving complete remission. If a contraindication to high-dose corticosteroids exists, then calcineurin inhibitors can be used for first-line therapy. Since long-term steroid therapy may lead to serious toxicity, patient counseling and close monitoring for adverse

Table 4.4 Definitions of proteinuria used in the literature for adult FSGS

Complete remission	Reduction of proteinuria to <2.0 g/day or <20 mg/mmol (<2.0 g/g) creatinine and serum albumin >35 g/L
Partial remission	Reduction of proteinuria to 0.2–2.0 g/day or 20–200 mg/mmol (0.2–2.0 g/g) creatinine and stable GFR (change in creatinine <25 %) or Reduction of proteinuria to 0.2–4.5 g/day and decreased >50 % from baseline and stable GFR
Relapse	Proteinuria >3.5 g/day or >350 mg/mmol creatinine (>3.5 g/g) after complete remission has been obtained
Steroid dependent	Two relapses during or within 2 weeks of completing steroid therapy
Steroid resistant	Persistence of proteinuria despite prednisone 1 mg/kg/day or 2 mg/kg every other day for >4 months

effects are essential. Patient response is assessed by the presence or absence of edema, 24-h urine protein excretions, serum creatinine, creatinine clearance, serum albumin, and lipid levels.

Individuals who relapse are challenging to treat and there is very little data to guide practice. Current practice follows the same treatment as for relapsing minimal change disease: oral cyclophosphamide at 2–2.5 mg/kg/day for 8 weeks. For individuals who relapse on or have a contraindication to cyclophosphamide, calcineurin inhibitors (cyclosporine 3–5 mg/kg/day or tacrolimus 0.05–0.1 mg/kg/day in divided doses) are indicated. Again, patients need to be counseled regarding and monitored for the side effects of both of these therapeutic agents. The most concerning adverse effects of cyclophosphamide include hemorrhagic cystitis, malignancy, bone marrow suppression, and gonadal toxicity. Exposure to cyclophosphamide should be minimized, especially since the risk of malignancy increases with duration and cumulative dose [131]. One widely quoted study suggests that cumulative oral cyclophosphamide doses less than 36 g are not associated with increased risk of malignancy [132]. Cyclosporine is potentially nephrotoxic and should be used cautiously or avoided in individuals with significant vascular or interstitial disease on biopsy or glomerular filtration rate (GFR) less than 40 mL/min per 1.72 m^2. Cyclophosphamide may be an attractive alternative in these instances. The lowest therapeutic dose of cyclosporine to attain remission should always be used. Periodic monitoring of cyclosporine levels may also be useful in avoiding nephrotoxicity, targeting levels between 100 and 175 ng/mL.

Individuals with steroid-resistant (SR) FSGS are also challenging to treat, though the only therapeutic regimens to have been evaluated in randomized trials are ones for the treatment of this group of patients [133–135]. Cyclosporine with low-dose prednisone increases rate of complete and partial remissions, but there is a high relapse rate following discontinuation of therapy. Notwithstanding, there is still better long-term preservation of filtration function in cyclosporine-treated patients. In accordance with this data, SR FSGS is generally treated with cyclosporine at 3–5 mg/kg/day in divided doses for at least 4–6 months, along with low-dose prednisone at 0.15 mg/kg/day. Given the high relapse rate, it is advisable to treat people who respond for longer periods of time, at least 12 months, followed by a slow taper.

There is limited experience reporting the use of tacrolimus in the treatment of SR FSGS and thus it is not routinely used. Randomized trials using mycophenolate and chlorambucil have not demonstrated any treatment benefit for SR FSGS [135, 136]. Rituximab as a potential therapy for SR FSGS has been reported in case reports, but randomized trials are needed to evaluate its efficacy.

Conclusion

FSGS is a pathologic diagnosis characterized by a signature pathologic lesion, which can be primary or due to secondary causes. The incidence of FSGS has been increasing for unclear reasons and now it is the commonest glomerulonephritis underlying ESRD in the USA. Genetic studies have advanced our understanding of disease pathogenesis, pointing to injury in the podocyte as central to the disease. Despite this, our therapeutic options for primary FSGS remain limited and there is a lack of rigorous studies supporting current treatment practices. Ongoing studies are needed to improve our understanding of FSGS, both at the molecular level to identify therapeutic targets and in clinical trials to define optimal treatment regimens.

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Membranous Nephropathy

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Introduction

Membranous Nephropathy (MN) is a common immune-mediated glomerular disease characterized by the presence of immune deposits on the epithelial side of the glomerular capillary wall. It remains the leading cause of nephrotic syndrome in Caucasian adults [1]. Until recently, the majority of cases of MN were termed "idiopathic" because the etiology was unknown. Thanks to modern technology, major advances have occurred in our understanding of the autoimmune processes involved in the development of human MN. We now know that in approximately 70 % of cases, the disease is associated with antibodies against the M-type phospholipase A2 receptor (PLA2R) present on podocytes, thus establishing "primary" MN as an autoimmune glomerular disease [2]. Genetic studies are elucidating predisposing factors for development of the disease [3]. Although in most patients the disease progresses relatively slowly, approximately 40 % of patients eventually develop ESRD [4]. Different immunosuppressive therapies proved to be successful in reducing proteinuria and maintaining stable renal function, but a number of patients do not respond to these treatments and others may develop side effects or show relapse of proteinuria [5, 6]. In this chapter we will review clinical aspects, advances in our understanding of the pathogenesis of the disease, as well as therapeutic indications for patients with MN.

Etiology and Pathogenesis

Until recently, most of our understanding of the pathogenic mechanisms came from experimental models in rats, i.e., the Heymann nephritis model [7, 8]. In this model, megalin is the podocyte antigen involved but megalin is neither expressed in human podocytes nor detected in the subepithelial deposits in patients with idiopathic/primary MN. Thus, for years the MN target in human podocytes remained elusive. More recently, a number of additional podocyte antigens have been identified as potential targets for autoantibodies in patients with MN [9, 10]. Similarly, genetic studies are also starting to elucidate predisposing factors for development of the disease.

Anti-Neutral Endopeptidase Antibodies

Debiec and colleagues were the first to describe the case of a patient with neonatal MN due to the transplacental transfer of circulating anti-neutral endopeptidase antibodies to the fetus demonstrating that circulating antibodies against a podocyte protein could cause MN in humans [11]. Neutral endopeptidase (NEP) is a membrane-bound enzyme that is able to digest biologically active peptides and is expressed on the surface of human podocytes, syncytiotrophoblastic cells, lymphoid progenitors, and other many epithelials cells and polymorphonuclear leukocytes. Mothers with truncating mutations of the metallomembrane endopeptidase (MME) gene fail to express NEP on cell membranes. NEP-deficient mothers, who were immunized during pregnancy, were able to transplacentally transfer nephritogenic antibodies against NEP to their children causing MN in the newborn [12]. The fact that rabbits injected with the maternal IgG from these mothers also developed MN was additional proof that the disease was related to circulating anti-NEP antibodies and demonstration of a human counterpart to Heymann nephritis [13].

PLA2R Autoantibodies

Antibodies to the M-type phospholipase A2 receptor (PLA2R) are present in 70–82 % of the patients with primary MN [2]. The PLA2R is a transmembrane receptor belonging

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to the mannose receptor family and a receptor for the secreted phospholipase A_2, a lipolytic enzyme that cleaves the fatty acid bond of membrane glycerophospholipids [14]. A common functional feature of this family of receptors is their ability to undergo endocytosis and thus involved in the internalization of extracellular ligands. Sera from patients with primary MN contained IgG4 antibodies that specifically recognized PLA2R, but these antibodies are not present in the serum of healthy controls or in patients with other glomerular and autoimmune diseases [2, 15]. However, it is still unclear if these antibodies are specific for primary MN because cases of secondary MN with positive anti-PLA2R have been reported [16]. Levels of anti-PLA2R have been found to correlate strongly with the disease activity: disappearance of the antibody is associated with remission of proteinuria while reappearance of the antibody heralds a relapse of nephrotic syndrome [2, 15, 17]. How the presence of these antibodies leads to the development of proteinuria is unknown.

Potential discrepancies between circulating anti-PLA2R antibodies and detectable PLA2R in glomerular deposits in some patients have been reported. Debiec and Ronco evaluated the presence of PLA2R autoantibody in the serum and PLA2R in glomerular deposits in 42 consecutive patients with primary MN [18]. In 21 patients, anti-PLA2R antibodies were present in circulation and had PLA2R in glomerular deposits. However, three patients with high levels of circulating anti-PLA2R antibodies did not have detectable PLA2R in glomerular deposits. These cases suggest that antibodies were not nephritogenic or that epitopes were poorly accessible at the time of kidney biopsy. On the other hand, 18 patients had no detectable circulating anti-PLA2R antibodies, although 10 of them had PLA2R in glomerular deposits. Debiec and Ronco suggest that these apparently discordant findings might be due to rapid clearance from the circulation of antibodies deposited in the glomeruli, with consequent ultrastructural changes [18]. This may explain the persistent proteinuria in spite of an apparent immunological quiescence [17].

Antibodies to Superoxide Dismutase 2 (SOD2), Aldose Reductase (AR), and α[Alpha]-Enolase

Using a combined proteomic approach, Prunotto and colleagues identified specific IgG4 antibodies against the cytosolic proteins SOD2, AR, and α[alpha]-enolase in both serum and glomeruli of patients with primary MN but not from biopsies of patients with other glomerular diseases or normal kidney [19]. It is unclear what the role of these antibodies is in the pathogenesis of primary MN. As opposed to NEP and PLA2R, these are cytosolic proteins and are not present or minimally expressed on normal podocyte membranes but are "neo-expressed" in glomeruli of patients with MN. As in the case of anti-PLA2R antibodies, the predominance of IgG4 in the glomerular immune deposits supports the concept of an isotype-specific mechanism. Mechanisms of translocation of these intracellular molecules have been proposed as way of explaining the development of these autoantibodies. Preliminary in vitro data showed an increase of SOD2 expression on podocyte plasma membrane after treatment with hydrogen peroxide, suggesting that oxidative stress may drive glomerular expression of SOD2, whereas induction of AR seems less specific [19]. It is also possible that antibody spreading occurs, whereby the development of a particular autoantibody (e.g., anti-NEP, anti-PLA2R, etc.) induces the expression of other antigens that, in turn, form targets for the development of additional autoantibodies [20].

Most recently, glomerular α[alpha]-enolase has been identified as an additional target for autoantibodies in patients with MN [21]. Alpha-enolase is one of the most abundant cytosolic protein and is highly expressed in tubular kidney cells but it is absent in normal glomeruli. IgG4 antibodies against α[alpha]-enolase were present in circulation, were eluted from microdissected glomeruli, and colocalized with C5b-9 (membrane attack complex) in subepithelial deposits from kidney biopsies of patients with MN. Antibodies against α[alpha]-enolase have been reported in patients with primary and secondary MN [22] as well as in patients with a number of other autoimmune diseases including lupus erythematosus, ANCA-associated vasculitis, and inflammatory bowel diseases [23–27]. Whether these antibodies are simply a reflection of a nonspecific/molecular epitope spreading immune response in MN awaits further research.

Antibodies to Bovine Serum Albumin

Debiec and colleagues evaluated a cohort of 9 children and 41 adults with primary MN and found high levels of circulating anti-bovine serum albumin antibodies, of both IgG1 and IgG4 subclasses, in 4 children and 7 adults with MN [28]. These patients also exhibited high levels of circulating bovine serum albumin but without an increase of immune complex levels in circulation. Bovine serum albumin (BSA) immunopurified from these four children's serum differed from BSA purified from adult patients by migrating in the basic range of pH, whereas BSA from adults migrated in neutral regions and similar as native BSA. In children who were negative for anti-PLA2R antibodies but had both high levels of circulating cationic BSA and BSA-specific antibodies, BSA colocalized with IgG in subepithelial immune deposits. IgG1 and IgG4 eluted from kidney biopsy specimens showed reactivity that was specific for BSA.

The mechanism of BSA-induced MN in these patients is unclear. Human exposure to BSA is common (e.g., cow's milk) and antibodies to BSA are common in the general population [29]. A variety of modern food processing conditions could induce protein modifications affecting the proteolysis of BSA as well as facilitating the absorption and passage into the bloodstream. The relative elevated pH in infants (pH 3 or 4 vs. pH 2 in adults) and immaturity of the gastrointestinal tract could make children more susceptible to absorb undigested or only partially digested BSA, especially in the setting of childhood gastroenteritis. In this context, positively charged circulating cationic BSA could become attached to the negatively charged glomerular endothelial glycocalyx and heparan sulfate proteoglycans in the GBM acting as a target for the deposition of anti-BSA IgG and in situ formation of immune complexes. In animal models, cationic form of BSA can induce MN ("planted" antigen model) [30–32]. The studies by Ronco and Debiec suggest that the "planted" antigen model can also be applied to human disease [28].

In patients with BSA-mediated MN, the antibodies predominantly targeted the BSA peptide 147–161 (the two linear epitopes are not present in human albumin and have no cross reactivity to podocyte proteins), whereas controls with high anti-BSA antibodies but no MN had a broader spectrum of peptides reactivity. Debiec et al. suggest that the psychochemical properties of the BSA (e.g., charge; BSA modification during food processing/digestion) together with the amount of circulating BSA as well as a predominant T-helper type 2 (Th2) immune response resulting in production of IgG4 are the conditions necessary for the development of MN [28].

The four children with MN had both high levels of anti-BSA antibodies as well as BSA in circulation, and similar findings were seen in four of the seven adults with MN. BSA could specifically be detected in glomerular immune deposits only in patients who had both circulating cationic BSA and anti-BSA antibodies suggesting that both are required for the development of the disease [28]. Levels of anti-BSA IgG1 and IgG4 antibodies and circulating cationic BSA correlated with disease activity: high in patients with nephrotic range proteinuria and low in patients in remission.

BSA colocalized with IgG immune deposits only in four children with circulating cationic BSA, but in none of the 18 adults, patients with MN for whom biopsy specimens were available, implying that only cationic BSA can induce MN. On the other hand, positive PLA2R staining was detected in 14 of the 20 adult biopsy specimens again pointing to a different pathogenic process in adults with MN. Antibodies against other regions of BSA have been reported in patients with rheumatoid arthritis and multiple sclerosis but these patients do not have an associated MN [33, 34]. Whether or not dietary proteins could play a role in other cases of MN is currently unknown, but in children, the diagnosis of MN should raise the possibility of BSA-induced MN.

Genetic Susceptibility: The HLA-DQA1 and PLA2R1 Risk Alleles

Interaction of genetic susceptibility and environmental factors could play a role in the development of glomerular diseases such as IgA nephropathy and primary MN [35–38]. Using genome-wide association studies (GWAS), a group of international investigators recently linked single-nucleotide polymorphisms (SNPs) in the genes encoding M-type phospholipase A_2 receptor 1 (PLA2R) and HLA complex class II HLA-DQ alpha chain 1 (HLA-DQA1) in Caucasian patients with MN [3]. Although the risk for primary MN was higher with the HLA-DQ1 allele than with the PLA2R1 allele, it adds support to the findings of positive anti-PLA2R antibodies in the majority of patients with MN [2]. For person who is homozygous for both risk alleles, the odds ratio for developing MN is close to 80, with additive increase in the odds ratio, depending on the combination of genotypes [3]. Although these findings do not prove causality, they suggest that genetic background plays a significant role in the predisposition of the primary MN, as previously noted in cases of familial primary MN [39, 40]. The HLA region of the reported association spans a 6-Mb interval, extending far beyond the linkage-disequilibrium block of HLA-DQ1 raising the possibility that several independently associated variants account for this signal [41]. Further work is required to further examine the link between these alleles and MN as well as identify specific gene-environment interactions that "trigger" MN.

Clinical Manifestations

The disease affects patients of all ages and races but is more common in men than women and most often diagnosed in middle age with the peak incidence during the fourth and fifth decades of life [42]. At presentation 60–70 % of patients will have the nephrotic syndrome with remaining 30–40 % of cases presenting with proteinuria <3.5 g/24 h found at the time of a routine examination in an otherwise asymptomatic patient [42]. The presence of microscopic hematuria is common (30–40 %) but macroscopic hematuria and red cell casts are rare and suggest a different diagnosis. At presentation, the great majority of patients are normotensive, with hypertension present in 10–20 % of the cases, and only a small fraction of patients (<20 %) have significant renal insufficiency [42].

Secondary MN forms may account for up to one-third of cases and are associated with autoimmune diseases (e.g., SLE), infections (e.g., hepatitis B and C), medications (e.g., NSAIDs), and neoplasias (e.g., carcinomas) particularly in the elderly [43–45]. Idiopathic/primary and secondary forms have similar clinical presentations. Thus, the designation of

idiopathic should be made only after secondary causes have been ruled out. The disease is rare in children and, when it does occur, is commonly associated with an immunologically mediated disorder, e.g., SLE or secondary to BSA as previously discussed.

Pathology

Kidney biopsy in MN shows glomeruli with thickened capillary walls, although in early stages glomeruli may look normal. On trichrome stains, small fuchsinophilic deposits are often present on the subepithelial aspect of the glomerular capillary walls. On silver and periodic acid-Schiff stains, basement membrane spikes and pinholes can often be noted along the capillary walls. Spikes and pinholes may be absent in cases of early MN. Typically, proliferative features including mesangial proliferation and endocapillary proliferation are absent, even though numerous immune deposits are present along the capillary walls. This is due to the fact that the deposits are subepithelial (and not subendothelial) and are protected from the inflammatory cells by the barrier of endothelial cells and glomerular basement membrane. Immunofluorescence microscopy typically shows diffuse and global bright granular staining for IgG, C3, kappa, and lambda light chains. However, cases of segmental IgG and C1q deposits in a portion of the glomerulus have been reported in Japanese children [46]. It is unclear whether this segmental form of MN should be considered a separate entity. In the typical forms of primary MN, the deposits do not stain for IgM, IgA, or C1q in cases of primary MN. The presence of IgM, IgA, and C1q points to an autoimmune etiology. Electron microscopy shows numerous subepithelial deposits. The deposits are typically separated from each other by basement membrane material that results in basement membrane spikes on silver and periodic acid-Schiff stain. Subendothelial deposits and mesangial deposits are not present in primary MN. The mesangium is usually unremarkable in primary MN. Based on the location of the subepithelial deposits and glomerular basement membrane changes, MN is classified into four stages: stage I: sparse small deposits without thickening of the GBM, stage 2: more extensive subepithelial deposits with formation of basement membrane spikes between the deposits and thickening of the GBM, stage 3: combination of stage 2 along with deposits completely surrounded by basement membrane (intramembranous deposits), and stage 4: incorporation of deposits in the glomerular basement membranes and irregular thickening of the GBM. Most cases of MN show extensive effacement of foot processes, even in early stages of MN. Representative light microscopy, immunofluorescence microscopy, and electron microscopy are shown in Figs. 5.1, 5.2, and 5.3.

From the pathology standpoint, it is important to determine whether the membranous glomerulopathy may be due to a secondary cause such as an autoimmune disease, neoplasia, infection, or drugs. Although it is often difficult to determine whether the MN is primary or secondary, there are some features that are helpful in identifying a secondary cause. Features in favor of an secondary cause, in particular an autoimmune disease, include (1) proliferative features—mesangial or endocapillary, (2) full-house pattern of Ig staining including staining for C1q on immunofluorescence microscopy, (3) electron dense deposits in the subendothelial location of the capillary wall and mesangium or along the tubular basement membrane and vessel walls, and (4) endothelial tubuloreticular inclusions on electron microscopy [47]. Electron microscopy showing only few superficial scattered subepithelial deposits may suggest a drug-associated secondary MN. Furthermore, the location of the subepithelial deposits, i.e., subepithelial (homogenous) with subgroups superficial and deep versus subepithelial and intramembranous (heterogenous) deposits should be mentioned since the homogenous group with superficial deposits in one study has been shown to have a better prognosis than the homogenous group with deep deposits and the heterogenous group [48].

Finally, there is also diagnostic value in staining for IgG subclasses in MN that helps differentiate between primary MN and secondary MN due to lupus. IgG1, IgG2, and IgG3 tend to be highly expressed in lupus MN, while IgG1 and IgG4 tend to be highly expressed in primary MN [49].

Natural History

The natural course of MN is variable. Some patients achieve a spontaneous remission of proteinuria and usually maintain a normal renal function over the time; others have a persistent proteinuria without progression, and the remaining patients progress, usually slowly, to ESRD or die from complication related to the nephrotic syndrome. The proportions of these different outcomes are difficult to assess because most of the available data come from studies which are short term and which include not only patients with idiopathic and secondary MN but also treated and untreated patients, or nephrotic and non-nephrotic patients. As a consequence, it is not surprising that the reported outcomes are variable and that some authors consider MN as a relatively benign disease and feel that immunosuppressive agents should be avoided [50], while others stress the potentially poor outcome of MN and suggest an early specific treatment at least in nephrotic patients [51].

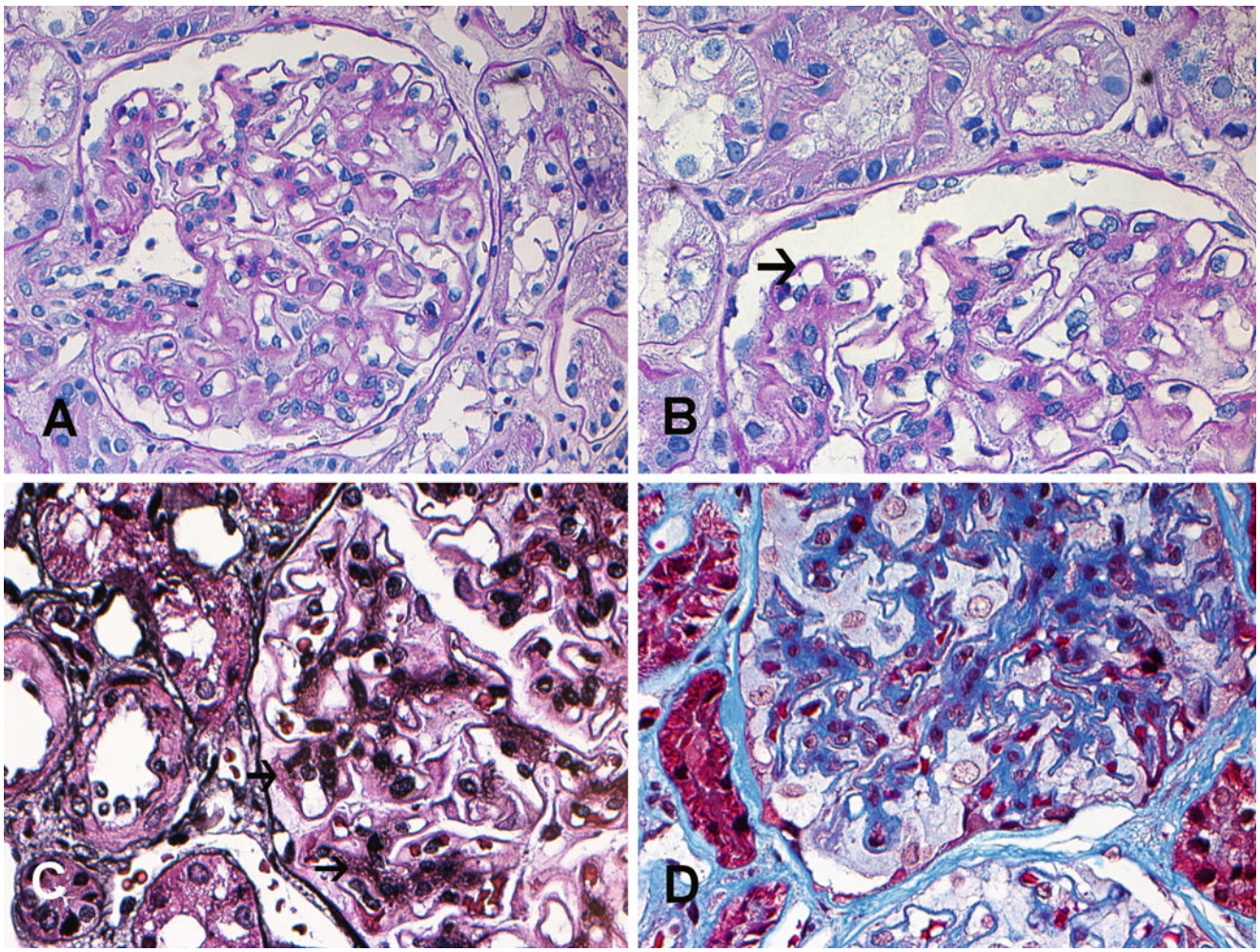

Fig. 5.1 Representative light microscopy showing thickened glomerular basement membranes with spikes and pinholes (*arrows*) on higher power. (**a**) PAS stain, 40×; (**b**) PAS 60×; (**c**) Silver stain 60×; (**d**) Trichrome stain 60×

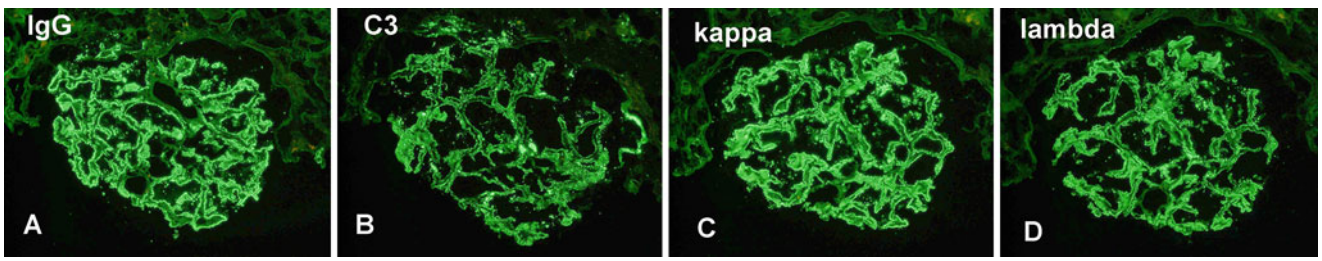

Fig. 5.2 Immunofluorescence microscopy showing (**a**) granular IgG, (**b**) C3, (**c**) kappa light chains, and (**d**) lambda light chains along the capillary walls

For example, Schieppati et al. reported a 72 % renal survival at 8 years for 100 untreated patients with MN [50]. However, in this study, 37 % of patients were non-nephrotic (56 % of patients had proteinuria <5 g/24 h), the median follow-up was only 39 months, and deaths were excluded from the analysis. Despite these exceptions and the "benign" presentation characteristics, 25 % of the patients reached end-stage renal disease (ESRD) by the end of 8 years [50].

Instead, the natural course of nephrotic patients is less favorable. Some 20 % of them may obtain a complete remission

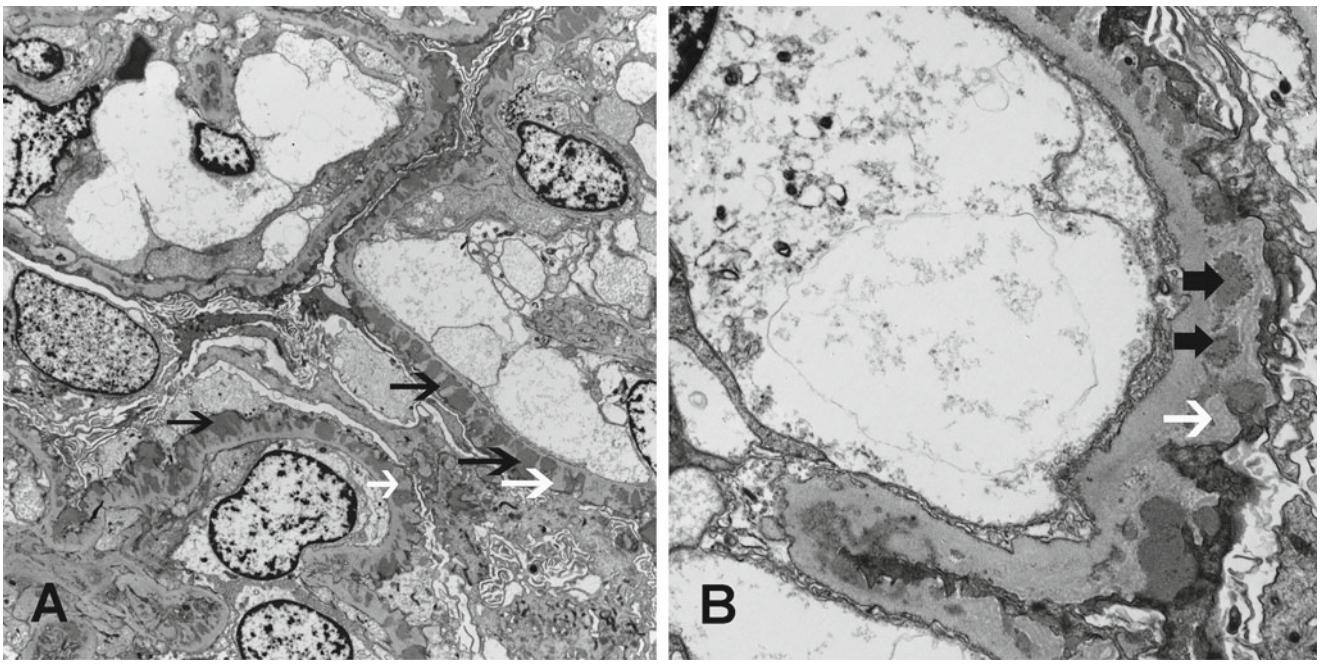

Fig. 5.3 Electron microscopy showing subepithelial electron dense deposits (*black arrows*) and basement membrane spikes (*white arrows*) separating the deposits. (**a**) Membranous nephropathy stage II, with spikes separating the deposits (×4730), and (**b**) Membranous nephropathy, stage III, with intramembranous deposits (*thick black arrows*) (×15100)

of proteinuria usually within 3–5 years [50–54]. However, a review of the papers reporting the long-term outcome of untreated nephrotic patients with MN found that 40–50 % of patients either died or progressed to end-stage renal failure 10–14 years after the clinical onset [55]. This can be illustrated by a recent paper reporting spontaneous remission developing in 32 % of the patients [56]. These authors suggested that patients that were treated conservatively and who did not go into spontaneous remission had final creatinine = 2.4 ± 2.2 mg/dl and eGFR 53 ± 35 ml/min. However, taking into consideration that the mean age at presentation was 51 years and the mean follow-up was 69 months (making these patients ~56 years at follow-up), the recalculated eGFR should be ~30 ml/min if males and ~22 ml/min if females, indicating that a significant loss of kidney function occurred. The paper further underestimates chronic kidney disease because their endpoint was restricted to the absence of chronic dialysis or need for renal transplantation. However, if one uses a different parameter, i.e., loss of kidney function on the basis of GFR, their disease trajectory would indicate a significantly higher incidence of ESRD over time.

Thus, it should not come as a surprise that until recently both in the USA and Europe, MN remained the second or third leading cause of ESRD among the primary glomerulonephritis types [57]. Even patients who do not progress but remain nephrotic are at an increased risk for life-threatening thromboembolic and cardiovascular events. However, if the natural course of the disease is probably worse than generally estimated, current recommendations for immunosuppression have improved outcomes in patients at risk of progression [58].

The progression of the disease is usually slow. However, in few cases, a rapid change in either the degree of proteinuria or in the rate of loss of renal function may occur. This event should raise the possibility of a superimposed condition, e.g., acute renal vein thrombosis, acute interstitial nephritis, or superimposed crescentic glomerulonephritis (ANCA-associated vasculitis or anti-GBM disease).

Predicting Factors:

Evaluating the prognosis is critical in making the decision regarding when and what to use in terms of treatment, e.g., conservative versus immunosuppressive treatment in patients with MN [59–61]. An accurate predictor of outcome of patients with idiopathic MN would allow the separation of those patients who are likely to have a long-term renal survival from those who are likely to progress. This would allow us to target immunosuppressive treatment to patients at high risk of renal disease progression. However, finding useful markers that predict this last group has been difficult. Pathology itself is not helpful in establishing prognosis or predicting response to immunosuppressive therapy, although the combination of severe interstitial fibrosis, vascular sclerosis, and focal glomerular sclerosis is usually associated with a

poor renal outcome [62]. Urinary excretion ratios of α[alpha]1-microglobulin, β[beta]2-microglobulin, IgM, and IgG have all been found helpful in assessing the severity of the overall renal injury and to predict outcome in MN [63–68]. Unfortunately quantification of urinary α[alpha]1-microglobulin, β[beta]2-microglobulin, IgM, and IgG is not widely available and thus limits their clinical use. Thus far, the best model for the identification of patients at risk was developed with data derived from the Toronto Glomerulonephritis Registry [69, 70]. This model takes into consideration the initial creatinine clearance (CrCl), the slope of the CrCl, and the lowest level of proteinuria during a 6-month observation period. This risk score assessment has good performance characteristics and has been validated in two geographically diverse MN populations, one from Italy and the other from Finland [70]. Based on this model, patients who present with a normal CrCl, proteinuria ≤4 g/24 h, and stable renal function over a 6-month observation period have an excellent long-term prognosis and are classified as at low risk of progression. Patients with normal renal function and whose CrCl remains unchanged during 6 months of observation, but continue to have proteinuria >4 g but <8 g/24 h, have a 55 % probability of developing chronic renal insufficiency and are classified as medium risk of progression, and patients with persistent proteinuria >8 g/24 h, independent of the degree of renal dysfunction, have a 66–80 % probability of progression to chronic renal failure within 10 years and are classified in the high risk of progression category [59]. Patients with MN who were never nephrotic or who achieved a complete remission of proteinuria have an excellent long-term renal survival. Even a partial remission has been recognized as a predictor of long-term positive outcome in patients with MN [71, 72]. Troyanov et al. reported data on 350 patients with MN and nephrotic syndrome and found that the 10-year renal survival was 100 % in the complete remission, 90 % in the partial remission, and 45 % in the no remission group [71]. Patients in complete or partial remission have similar rate of decline: −1.5 ml/min/year in the complete remission group and −2 ml/min/year in the partial remission group. In contrast, the no remission group lost GFR at a rate of −10 ml/min/year.

Therapy

Symptomatic Treatment

Initial therapy should be supportive and involves restricting dietary protein intake, controlling blood pressure, hyperlipidemia, and edema. In the Modification of Diet in Renal Disease (MDRD) study, patients with proteinuria >1 g/day had a significantly better outcome if their blood pressure was reduced to 125/75 mmHg [73]. Thus, in patients with proteinuric renal disease, the current target for blood pressure control is ≤125/75 mmHg. Reducing protein intake to about 0.6–0.8 g/kg ideal body weight per day also decreases nephrotic range proteinuria [74]. ACEi and/or ARBs are effective antihypertensive agents that can reduce proteinuria and slow progression of renal disease in both diabetic and nondiabetic chronic nephropathy patients, and for these reasons they are the preferred agents to treat hypertension in proteinuric renal diseases. However, evidence that such therapy is beneficial in MN is weak and largely inferential, and the following issues need to be considered: (1) the degree of renal protection is related to the degree of proteinuria reduction and if proteinuria is not lowered, the benefit is substantially attenuated [75, 76]. In the RENAAL trial the renal protective effect of angiotensin II blockade in patients with diabetic nephropathy was nearly fully explained by its antiproteinuric effect [77]. (2) In patients with MN, the antiproteinuric effect is modest (<30 % decrease) and is more significant in patients with lower levels of proteinuria [78–80]. (3) Thus, in contrast to diabetic renal diseases, ACEi may not offer the same degree of renal protection to patients with MN [81]. In fact, studies by du Buf-Vereijken et al. [82] and in a review by Troyanov et al. [71], the use of ACEi or ARBs by multivariate analysis did not show an independent value in determining the prognosis of patients with MN. Similarly, Praga et al. showed additional evidence that in patients with nephrotic syndrome (the majority with MN), ACEi were ineffective in reducing proteinuria and that this response in MN patients was associated with a poor renal function outcome [83, 84]. It is also difficult to handle hypercholesterolemia in patients with nephrotic syndrome. Dietary restriction is of little benefit and use of statins can usually obtain a reduction of 25–45 % from baseline values of serum cholesterol.

At any rate, although it is difficult to obtain a good reduction of proteinuria and normalization of hypercholesterolemia even with huge doses of ACE inhibitors, angiotensin receptor blockers, and statins, the current practice is to maximize the doses of these drugs in nephrotic patients. Loop diuretics are usually effective in solving the edema. In difficult cases a thiazide may be added to increase the diuretic activity. Anticoagulation is recommended in patients with severe hypoalbuminemia (<2 g/dl) and in those who already experienced thrombotic events and in bedridden or obese patients [85].

As discussed, the prognosis of patients with non-nephrotic proteinuria is usually excellent, and the conservative symptomatic approach described above should suffice. Obviously these patients should be monitored to ensure that the disease is not worsening since a significant number of patients who present with sub-nephrotic range proteinuria will progress to full nephrotic syndrome within 1–2 years from presentation.

Specific Treatments of Patients with Normal Renal Function

Corticosteroids

Four prospective randomized trials of corticosteroids therapy for MN have been published between 1970 and 1990. In two of these studies, prednisone was administered at low doses for 6 months, and in the other two studies, steroid was given at high doses for 8 weeks.

Black et al. randomly assigned 19 patients with MN and nephrotic syndrome either to symptomatic therapy or to 20–30 mg per day of prednisone for 6 months. After 2 years, 20 % of controls and 40 % of treated patients had daily proteinuria lower than 1 g, but the difference was not significant and side effects of treatment were frequent. The conclusion of the study was that the risks of therapy outweighed the possible benefits [86]. A more solid 6-month therapeutic protocol was evaluated in a Canadian study, in which 158 patients with or without nephrotic syndrome were assigned to receive symptomatic therapy or 45 mg/m^2 of prednisone on alternate days. After a mean follow-up of 4 years, there were no differences in remission rates or renal function between the two groups [87]. A problem with this study was that untreated controls had such a benign course that it would have been difficult to find any significant difference even if treatment was effective. In a controlled US Collaborative Study, patients assigned to treatment with prednisone 125 mg every other day for 2 months obtained a higher, but transient, number of remissions (22 vs. 11) and a slower average rate of decline in GFR (−2 % per year vs. −10 % per year) when compared with untreated controls [88]. Two major points of criticism of this study were the short follow-up period and the poorer than expected outcome in the placebo group. A few years later a British Medical Research Trial evaluated the same therapeutic protocol on 107 patients with nephrotic syndrome followed for 3 years [89]. This study was unable to demonstrate any difference between the two groups at any time in either the mean proteinuria or mean plasma creatinine.

Taking together, these controlled trials did not show a benefit of corticosteroids in patients with MN. The lack of response has been confirmed by a meta-analysis published in 1995, which did not find any difference either in the probability of remission (odds ratio 0.97) or in the 5-year renal survival (80 % for both groups) between treated and untreated patients [90]. In addition, a recently published Cochrane meta-analysis showed that, in MN, corticosteroids compared with symptomatic therapy had no beneficial effect on total mortality or ESRD [91]. However, it is important to realize that in these studies, prednisone was given in limited dosage or during a limited period. Thus, one cannot completely exclude that corticosteroids may be of some benefit when using different schedules. Actually, corticosteroids may regulate the actin cytoskeleton of podocytes and their foot processes so improving the permselectivity of the glomerular barrier [92].

Alkylating Agents

A few randomized trials evaluated the role of alkylating agents. Donadio et al. reported on 22 patients randomly assigned to receive oral cyclophosphamide, 1.5–2.5 mg/kg/day for 12 months, or no specific therapy [93]. At the end of treatment, there was a trend to a greater decline of proteinuria in treated patients than in controls (mean reduction 4.7 g per day vs. 2.6 g per day, respectively), but the difference was not significant, and cyclophosphamide was considered ineffective. In a trial including several forms of primary glomerulonephritis, Lagrue et al. randomly allocated 41 nephrotic patients with MN either to chlorambucil (0.2 mg/kg/day for 6 months, then 0.1 mg/kg/day for a further 6 months) or to azathioprine (3 mg/kg/day for 6 months, then 2 mg/kg/day for a further 6 months) or to placebo [94]. At the end of treatment period, chlorambucil-treated patients showed a significant reduction of proteinuria, while no changes were observed in patients given azathioprine or placebo. One year after the treatment period was completed, 81 % of patients assigned to chlorambucil were in complete or partial remission, compared with 9 % of patients treated with azathioprine and 21 % of patients assigned to placebo. However, the prolonged use of chlorambucil was loaded by severe complications, including neoplasia. Two Australian trials investigated the role of cyclophosphamide associated with anticoagulant and antiplatelet agents. Tiller et al. randomly allocated 54 patients to symptomatic therapy or to cyclophosphamide plus warfarin and dipyridamole for 36 months [95]. Patients who completed the treatment obtained a significantly lower proteinuria and higher serum albumin when compared with untreated controls. However, many patients had to stop therapy because of complications. Murphy et al. randomized 40 patients either to symptomatic therapy or to cyclophosphamide for 6 months plus dipyridamole and warfarin for 2 years [96]. Treatment was well tolerated. Compared with controls, treated patients showed a greater reduction of proteinuria and a higher incidence of complete remission compared with controls (9/13 vs. 4/13, respectively).

These controlled trials would show that cytotoxic agents may reduce proteinuria and can improve the chances of remission. The follow-ups of the available studies were too short, however, to evaluate the impact of these agents on renal function and their potential long-term morbidity. Good results have also been reported in noncontrolled studies by using 6–12-month courses of cyclophosphamide and corticosteroids [97].

Alternating Corticosteroids and Alkylating Agents

An Italian multicenter controlled trial evaluated whether giving alkylating agents and steroids in an alternate fashion could achieve therapeutic results while sparing the side effects of these agents. The treatment consisted of three consecutive cycles of 2-month therapy. Each cycle began with an intravenous administration of methylprednisolone, 1 g intravenously daily for 3 consecutive days, followed by oral prednisolone 0.5 mg/kg daily for the rest of the month. Then steroid was stopped and replaced by chlorambucil 0.2 mg/kg/day for 1 month. The 2-month cycle was repeated three times until a total of 6 months of therapy had been given (Table 5.1). Eighty-one adult patients with MN and nephrotic syndrome were randomized to receive either the combined therapy or the symptomatic treatment. The two groups were homogeneous at randomization. The early results of this study were published in 1984 [98], but patients continued to be followed up to 10 years [51]. The probability of having a remission of the nephrotic syndrome, either complete or partial, was significantly higher in treated patients than in untreated (83 % vs. 38 %, respectively). At 10 years, 40 % of treated patients compared with only 5 % of untreated controls were still in complete remission; the slope of the reciprocal of plasma creatinine with time reduced from 1.0 to 0.51 in controls and from 1.0 to 0.84 in treated patients; the probability of surviving without developing ESRD was 92 % in patients given the combined therapy versus 60 % in controls. One patient in the treated group died of lung cancer a few months after randomization and two patients in the control group died, respectively, of cardiac infarct and of hepatorenal failure. Four patients had to stop treatment because of peptic ulcer (two cases), gastric intolerance, and pneumonia. In the long-term, one treated patient became obese and another developed diabetes.

Table 5.1 Therapeutic protocol used in the three Italian multicenter controlled studies

Methylprednisolone + chlorambucil versus symptomatic therapy	
Months 1, 3, 5	Methylprednisolone 1 g IV for 3 days, followed by oral prednisone 0.5 mg/kg/day for 27 days
Months 2, 4, 6	Chlorambucil 0.2 mg/kg/day for 30 days
Methylprednisolone + chlorambucil versus methylprednisolone alone	
Months 1, 3, 5	Methylprednisolone 1 g IV for 3 days, followed by oral prednisone 0.5 mg/kg/day for 27 days
Months 2, 4, 6	Chlorambucil 0.2 mg/kg/day for 30 days
Months 1, 3, 5	Methylprednisolone 1 g IV for 3 days, followed by oral prednisone 0.5 mg/kg/48 h for 27 days
Months 2, 4, 6	Oral prednisone 0.5 mg/kg/48 h for 30 days
Methylprednisolone + chlorambucil versus methylprednisolone + cyclophosphamide	
Months 1, 3, 5	Methylprednisolone 1 g IV for 3 days, followed by oral prednisone 0.5 mg/kg/day for 27 days
Months 2, 4, 6	Chlorambucil 0.2 mg/kg/day for 30 days
Months 1, 3, 5	Methylprednisolone 1 g IV for 3 days, followed by oral prednisone 0.5 mg/kg/day for 27 days
Months 2, 4, 6	Cyclophosphamide 2.5 mg/kg/day for 30 days

IV intravenous

Another Italian multicenter controlled study compared the combined cyclical combined therapy with methylprednisolone alone given for 6 months at the same cumulative dosage [99] (Table 5.1). At the end of a mean follow-up of 54 months, 64 % of patients given combined therapy versus 38 % of patients given steroids alone were without nephrotic syndrome. There was also a trend toward a better slope of the reciprocal of plasma creatinine in patients given the combined therapy, but the difference was not significant. One patient per group died. Four patients in the group assigned to the combined therapy had severe side effects that reversed completely after treatment was stopped (two cases of pneumonia, one of liver dysfunction, and one of gastric intolerance to chlorambucil). One patient assigned to steroids alone stopped therapy because of pulmonary embolism. A third Italian controlled study compared the effects of the regimen based on steroids and chlorambucil with those of a regimen using the same schedule of steroids but giving cyclophosphamide (2.5 mg/kg/day) instead of chlorambucil [100] (Table 5.1). Of 43 patients assigned to methylprednisolone and cyclophosphamide, 40 (93 %) entered complete or partial remission of the nephrotic syndrome versus 36 of 44 (82 %) for those assigned to methylprednisolone plus chlorambucil. In both groups the reciprocal of plasma creatinine remained unchanged in patients followed up for 2 and 3 years when compared with baseline. Six patients in the chlorambucil group developed severe side effects (two leukopenia, two pneumonia, one anemia and thrombocytopenia, and one nausea). Two patients stopped cyclophosphamide, one because of nausea and one because of a transient ischemic attack. All side effects reversed after treatment was stopped.

Pooling the results of these 3 Italian studies, 174 patients with MN and nephrotic syndrome received a 6-month therapy with corticosteroids alternated with chlorambucil (131 patients) or cyclophosphamide (43 patients). Of them, 72 (41.3 %) obtained complete remission and 72 (41.3 %) had a partial remission as a first event. After a mean follow-up of 54 months, 74 % of patients were in complete (34 %) or partial (41 %) remission. Four patients (2.4 %) progressed to ESRD and two died. Sixteen patients (9 %) suffered from severe side effects that completely reversed after stopping therapy. The results of these trials have been confirmed by an Indian randomized, controlled trial on 93 patients [101]. Of the 47 patients treated with a 6-month course of alternating prednisolone and cyclophosphamide, 34 achieved remission

(15 complete and 19 partial remission), compared with 16 (5 complete, 11 partial remission) of 46 in the control group treated with symptomatic therapy. The 10-year dialysis-free survival was 89 % and 65 %, and the likelihood of survival without death, ESRD, and doubling of serum creatinine were 79 % in the treated versus 44 % in the control group. The incidence of infections was similar in the two groups [101]. The long-term studies by Jha and Ponticelli clearly showed that the combined regimen may protect the long-term renal function, with treatment being well tolerated in the majority of patients [51, 101].

Since the most frequent side effects of alkylating agents are leukopenia and infection, we suggest that blood cell count should be checked at least every 7–10 days. The dose of chlorambucil or cyclophosphamide must be halved when leukocytes fall below 5,000 and should be stopped if they fall below 3,000/mm^3. Moreover, both chlorambucil and cyclophosphamide also can cause azoospermia; thus young males should be encouraged to deposit their semen in a sperm bank before starting therapy. A main concern with the use of cytotoxic drugs is the potential risk of neoplasia in the long term. Leukemia and, more rarely, lymphoma [102, 103] are the most frequent types of cancer that may develop in patients given chlorambucil, whereas bladder cancer and lymphoreticular tumors [104, 105] are the most frequent malignancies in patients treated with cyclophosphamide. These severe complications appear to be related to the cumulative doses and to the duration of treatment [106–108]. No case of hematological neoplasia has been reported in patients treated with a cumulative dose of chlorambucil of less than 1 g or for a cumulative period shorter than 6 months, and bladder complications are exceptional in patients given cyclophosphamide at doses of 2–3 mg/kg per day for not more than 12 weeks. In the Italian trials none of the patients enrolled developed leukemia, lymphoma, or bladder cancer. Three patients were recognized to have a cancer only after randomization. In order to evaluate whether these cancers represented a chance event or a complication of treatment, the cumulative risk of cancer in patients given methylprednisolone and chlorambucil for 6 months was compared with that of a general white population [100]. Considering together the patients who received methylprednisolone and chlorambucil in the three Italian trials, it was possible to collect information of 662 patient/years. In this population the cumulative risk of developing cancer was 4.53/1,000 patients per year (95 % CI, 0.935–13.241), which is concordant with the average annual incidence of primary cancer of 4.33/1,000 for men and 3.40/1,000 for women among white general population [109].

In a recent study, Hownan et al., randomized 108 patient with progressive MN (defined as a 20 % decline in eGFR before study entry), to treatment with either alternating chlorambucil and corticosteroids for 6 months, cyclosporine monotherapy for 12 months, or supportive care only [110]. The risk of reaching the primary endpoint (a further 20 % decline in eGFR) was significantly lower in the chlorambucil group versus the cyclosporine or supportive care group. Although this study provides support for the use of cyclic corticosteroids/chlorambucil treatment in patients with renal function deterioration, the high progression rate (58 %) in chlorambucil-treated patients, as compared to the low progression rates of 5–8 % in the early randomized controlled trials, may suggest that starting treatment once a significant degree of renal injury had occur may be less effective. It could also be argued that the surrogate renal endpoint used, i.e., a mere 20 % change of eGFR, is inadequate. Too many variables, i.e., lowering of blood pressure, use of diuretics, and change in kidney creatinine handling during nephrotic syndrome, may contribute to slight changes in serum creatinine values. Similarly, cyclosporine starting dose of 5 mg/kg may be too high especially in patient already showing signs of renal compromise, and prompt sudden drop of eGFR could thus be considered as treatment failure. There is also the question of how many patients with unusually rapid loss of renal function entered the study since inclusion criteria stated "a 20 % or greater decline in GFR that was based on at least three measurements over a period of 3 months or longer within the 2 years before the study." Thus, it is conceivable that a patient that lost eGFR during a 3–6-month period could be included, but it would be atypical for a patient with MN and would require ruling out a superimposed event, e.g., acute renal vein thrombosis, interstitial nephritis, etc. Unfortunately, these concerns cannot be answered by the data provided in the manuscript.

Calcineurin Inhibitors

A number of uncontrolled studies showed that cyclosporine may obtain remission of the nephrotic syndrome in about 60 % of patients with MN [111–119] (Table 5.2). However, in a number of patients, relapse of nephrotic syndrome occurred after cyclosporine was withdrawn. Cattran et al. reported the results of a randomized trial of cyclosporine in MN [120]. Fifty one patients with steroid-resistant MN and

Table 5.2 Results of studies using cyclosporine in mn

Author	No. of patients	Complete or partial remission
de Santo [111]	5	4
Meyrier [115, 119]	73	32
Guasch [114]	14	10
Rostoker [116]	15	11
Goumenos [113]	16	14
Fritsche [112]	41	14
Alexopoulos [118]	51	44
Kalliakmani [117]	32	28
Total	247	157 (63 %)

nephrotic syndrome were randomized to either 3.5 mg/kg/day of cyclosporine plus low-dose prednisone or placebo plus prednisone for 26 weeks. At the end of the treatment period, 75 % of the treated patients versus 22 % of the placebo patients had a partial or complete remission. After 6 months, relapses of nephrotic syndrome occurred in 43 % of treated and in 40 % of untreated patients. At the end of the study (78 weeks), despite the high relapse rate, the incidence of remissions was still significantly higher in the cyclosporine group than in controls (39 % vs. 13 %). Renal function remained unchanged in both groups during the treatment period but later deteriorated in two patients for each group.

Although short-term, the available studies indicate that cyclosporine may be effective in inducing remission of the nephrotic syndrome. It is possible that a prolonged administration may increase the chances of response to cyclosporine. A German multicenter study reported, showing that in patients with MN, the mean time to response to cyclosporine was in the range of 8 months [112]. It is important to emphasize that although reduction of proteinuria usually occurs within a few weeks, the majority of complete remission occurred after more than 6 months of treatment. On the other hand, if after 3–4 months of cyclosporine therapy at adequate doses, proteinuria is not significantly reduced, it is unlikely that the therapy will be effective. The optimal dose and duration of treatment remain a controversial issue. It should be noted, however, that good results have been obtained with doses of 2–3 mg/kg/day and that the addition of small doses of prednisone may improve the probability of response [118]. Unfortunately, most responders develop relapses of the nephrotic syndrome after the end of cyclosporine administration. However, a number of patients may be maintained in remission if cyclosporine is tapered off very gradually [121]. It remains still unclear whether the use of cyclosporine in MN may have long-term benefits. Repeat biopsy examinations performed in patients responsive to cyclosporine revealed the persistence of immunoglobulin and complement deposits, suggesting that the disease process was not halted by treatment [80].

The main problem with cyclosporine is the risk of nephrotoxicity, which may render the patient more susceptible to the risk of progressive renal failure. This risk is dose-dependent and is particularly increased in patients with elevated plasma creatinine levels and tubulointerstitial lesions at renal biopsy [122]. Moreover, the higher the increase in plasma creatinine levels during cyclosporine treatment, the higher the risk of irreversible nephrotoxicity [122]. Thus, patients with a creatinine clearance lower than 60 ml/min and/or those with severe interstitial fibrosis and tubular atrophy should not be treated with cyclosporine, and caution is recommended in patients with subnormal levels of creatinine clearance and/or moderate tubulointerstitial lesions. In addition, whenever plasma creatinine rises by more than 30 % over the baseline, cyclosporine should be reduced or stopped for at least 1 month in case of further creatinine increase; cyclosporine may be reintroduced only if plasma creatinine returns to normal or to values no higher than 10 % over baseline. In MN the starting dosage of cyclosporine microemulsion should not exceed 4 mg/kg/day. If nephrotic syndrome goes into remission, the doses may be reduced slowly (0.5 mg/kg/day every month) to a maintenance dosage of 2.5–3.5 mg/kg/day. After 1–2 years a cautious trial of stopping cyclosporine may be attempted, by tapering the doses very gradually. If the indications and contraindications are respected, the nephrotoxic risk of cyclosporine treatment appears to be low [121]. Prolonged low-dose cyclosporine (~1.5 mg/kg/day) could be considered for long-term maintenance of patients with preserved renal function who achieve a complete remission or partial remission but that relapse once cyclosporine is discontinued or the blood trough levels are maintained below 100 ng/ml. With such a strategy, there is a little risk of nephrotoxicity [118, 123].

Tacrolimus has also been used. In a pilot study, 21 patients received low doses of oral steroids and tacrolimus with the addition of mycophenolate mofetil in nine patients with proteinuria >1 g/day after 3 months. At the end of the treatment, eight patients (38.0 %) had complete remission and seven (33.3 %) partial remission, but 11 (73 %) responders relapsed [124]. In a randomized trial, 25 patients received low-dose tacrolimus (0.05 mg/kg/d) over 12 months with a 6-month taper, whereas 23 patients were assigned to the control group [125]. The probability of remission in the treatment group was 94 % after 18 months versus only 35 % in the control group. However, nephrotic syndrome reappeared in almost half of the patients who were in remission by the 18th month after tacrolimus withdrawal.

In summary, calcineurin inhibitors represent a promising and perhaps useful therapeutic option for patients with MN but their administration should be prolonged since relapses are frequent after treatment cessation.

Adrenocorticotrophic Hormone

Another approach to the treatment of MN consists in the prolonged administration of synthetic adrenocorticotrophic hormone (ACTH). Berg et al. treated nine patients with an 8-week course of i.m. synthetic ACTH given three times a weeks and gave ACTH twice a week for 1 year to five patients with resistant MN and severe nephrotic syndrome [126]. All patients obtained a median reduction of 80 % in the urinary protein excretion. However, after withdrawal of ACTH, proteinuria relapsed in the patients treated for 8 weeks, whereas it did not relapse in any of the five patients treated for 1 year, during 18 months of observation following the end of treatment.

On the basis of these promising results, a few small-sized studies have been performed. In a pilot study seven patients

with MN, nephrotic syndrome, and normal renal function received synthetic ACTH 1 mg twice weekly for 1 year [127]. Two patients had to stop treatment due to allergy and malaise, respectively. All the five patients who completed the ACTH course obtained a complete remission of nephrotic syndrome, which persisted up to 26 months after the end of the ACTH course. An exploratory multicenter randomized trial compared the efficacy and safety of a 6-month treatment with methylprednisolone alternated with a cytotoxic drug, versus 1-year synthetic ACTH treatment at a dose of 1 mg twice a week in 32 patients with biopsy-proven MN and nephrotic syndrome [128]. Complete or partial remission as a first event was attained by 93 % of the 16 patients assigned to the combined therapy and by 87 % of the 16 patients assigned to ACTH. At the last observation, 75 % of patients given the cyclical therapy were in remission (four complete and eight partial) compared with 87 % of patients treated with ACTH (eight complete and six partial). Median proteinuria significantly decreased from the basal value in both groups without any difference between the two groups. In the group receiving the cyclical therapy, two patients did not complete treatment because of severe leukopenia. One patient in the group assigned to ACTH had worsening renal function and had to undergo regular dialysis 18 months after randomization. A patient did not tolerate ACTH because of dizziness, and another patient stopped therapy because of lack of response. Two patients from each group developed glucose intolerance, which reversed after completion of treatment.

In a trial of Arnadottir et al. reported in form of abstract, 30 patients with idiopathic MN and nephrotic syndrome were given ACE inhibitors and statins and were randomized to add synthetic ACTH subcutaneously twice a week for 9 months or to continue with symptomatic therapy [129]. At a follow-up of 21 months, 11 of 15 patients given ACTH were in complete remission and 3 in PR, while only 1 control reached complete remission and another one entered PR. Serum creatinine decreased significantly in the ACTH group and increased significantly in the group treated with ACE inhibitors and statins alone.

All these studies were conducted using a synthetic version of ACTH (Synacthen®; not available in the USA), but a retrospective case series of patients with nephrotic syndrome using a natural, highly purified ACTH gel formulation (H.P. Acthar Gel®), currently approved in the USA for remission of proteinuria in the nephrotic syndrome, reported similar encouraging results [130]. Bomback and colleagues retrospectively reviewed data on 21 patients identified as receiving treatment with Acthar for resistant idiopathic nephrotic syndrome [130]. Of these, 11 patients were identified as having MN that did not respond to previous treatments. The most common treatment regimen used was Acthar Gel® 80 units (U) subcutaneous twice weekly for 6 months. Dosing intervals varied from 2 to 3 times weekly. Most patients were treated for a minimum of 6 months, with the longest treatment period being 14 months. Nine of the eleven patients with MN achieved a complete or partial remission. No severe infections were reported in the entire cohort. A multicenter, randomized, double-blind, 4-arm, placebo-controlled, comparing Acthar® Gel with placebo in treatment-resistant patients with MN and nephrotic syndrome is currently underway (ClinicalTrials.gov Identifier: NCT01386554).

The exact mechanism by which ACTH mediates its effects in proteinuria is not completely understood, but is likely independent of its induction of cortisol production, as its production remains low and there is concomitant evidence that steroids alone do not affect the outcome of the disease [90]. Ponticelli et al. hypothesized that by modifying apolipoprotein metabolism, ACTH might restore glomerular expression of apolipoprotein J (also called clusterin) which is inappropriate in patients with MN [128, 131]. Experimental studies showed that clusterin competes with the terminal components of complement, C5b-9, for the same receptor in podocytes, namely, megalin, which has been identified as the target of the C5b-9 injury in experimental models of MN [132]. Therefore, the presence of clusterin in glomeruli would represent a limitation to the lesions caused by complement, whereas deficient expression could enhance these lesions [128]. More recently, it has been suggested that ACTH may mediate its effects via the α[alpha]-melanocyte-stimulating hormone (α[alpha]-MSH). ACTH is derived from the pro-opiomelanocortin (POMC) precursor. POMC is proteolytically cleaved by endopeptidases to yield various polypeptide fragments with varying physiological activity such as ACTH and α[alpha]-MSH. To date, five forms of melano-corticotropic receptor (MCR) have been cloned, each with different tissue distributions, affinities, and physiological roles. MCR1 is located on various cells, including B cells, T cells, antigen-presenting cells, and human podocytes. In a recent study, treatment with ACTH, α[alpha]-MSH, or MS05, a specific MCR1 agonist, showed similar but significant reduction in proteinuria in rats with passive Heymann nephritis [133]. These results suggest that ACTH mediates its effects via α[alpha]-MSH interaction on MCR1 on podocytes and may explain why patients who are resistant to previous immunosuppressive therapies respond to ACTH. Acthar®, which is obtained from the processing of porcine pituitary, contains a highly purified form of ACTH with smaller amounts of other POMC peptides and ACTH fragments/analogues. Acthar is known to have activity at all five MCRs and all MCRs are known to be activated by more than one melanocortin ligand [134]. The steroidogenesis associated with Acthar and ACTH is mediated by activation of the melanocortin 2 receptor expressed in the adrenal cortex and other tissues. The functional roles of all the peptides in Acthar, however, have not been fully elucidated.

ACTH may also work by modulating autoantibody production. In a pilot study, 14 patients with MN treated with

Acthar® Gel for 6 months were evaluated for its effects on anti-PLA2R levels [135]. Twelve patients (86 %) were anti-PLA2R positive at baseline. All 12 patients experienced a reduction in anti-PLA2R (17–100 %) by 6 months with disappearance of anti-PLA2R in five cases. There were five partial remissions at 6 months: three in patients who cleared their anti-PLA2R and two in those lacking baseline anti-PLA2R. Another patient later achieved a 96 % reduction in anti-PLA2R without further treatment while two others, after receiving rituximab for perceived failure of Acthar® Gel, fully cleared anti-PLA2R. With long-term (12–18 months) follow-up of those with undetectable anti-PLA2R at baseline or after Acthar® Gel ± rituximab, there were four complete and two partial remissions, one nonresponder, and a final patient who achieved partial remission but relapsed coincident with a return of anti-PLA2R. Two other patients also showed an increase in anti-PLA2R after discontinuing treatment with Acthar® Gel. Thus, measurement of anti-PLA2R provides useful information relating immunological and clinical disease activity in MN patients treated with new agents such as Acthar® Gel. This study suggests that Acthar® Gel may work in part by suppressing autoantibody production although the duration and degree of this response need further study [14–16].

Intravenous Immunoglobulins

The mechanism of therapeutic effects of intravenous immunoglobulin (IVIg) in MN is not well established. It has been speculated that IVIg afford its immunomodulatory effects through several possible Fc- and F(ab)-mediated mechanisms [136, 137]. There is also evidence that IVIg interferes with complement-mediated immune damage by binding to C3b and C4b. Such an effect would reduce complement-mediated glomerular injury. This mechanism may be involved in MN as suggested by a study in passive Heymann nephritis in which treatment with systemic immunoglobulin obtained a decrease in proteinuria, associated with a decreased glomerular deposition of C3c and C5b-9, without changes in the amount, size, or distribution of the subepithelial immune complexes [138].

Kida et al. first reported the results of two regimens of IVIg therapy in patients with MN [139]. Four patients received a single bolus of 0.5 g/kg repeated for 6 days, and 19 patients were given a single dose of 1 g/kg. Only one of the four patients treated with the first regimen obtained a remission, while 14 out of the 19 patients given 1 g/kg developed remission within 1 year. Palla et al. treated nine patients with MN (five with normal and four with moderate renal insufficiency) with pulse doses of IgG (0.4 g/kg body weight) for 3 consecutive days, repeated three times at 21-day intervals for 10 months [140]. Complete or partial remission of proteinuria was obtained in all of the five patients with normal renal function and in three of four patients with mild renal insufficiency. Interestingly, a second renal biopsy in five responders showed the disappearance of glomerular deposits of IgG and C3 at immunofluorescence, a regression from stage II to stage I in two patients and a complete recovery of the glomerular lesions in three patients. No information about the long-term outcome of these patients was provided. Another retrospective study compared the outcome of 30 patients treated with IVIg, plus corticosteroids and/or alkylating agents in five patients, with the outcome of 56 control patients who received either no treatment (30 %) or treatment with corticosteroids alone or in combination with alkylating agents [141]. The IVIg regimen consisted of 1–3 courses of 5–10 g/day for 6 consecutive days. At 6 months, patients treated with IVIg obtained a statistically significant higher rate of complete remission (57 % vs. 10 %). However, the difference disappeared later. At 12, 24, or 60 months of follow-up, the rate of remission was similar between the two groups. Moreover, the benefits of IVIg were limited to a subgroup of patients with a homogenous (synchronous) pattern of immune deposits.

Although usually well tolerated, treatment with IVIg has potential complications such as acute renal failure, vascular thrombosis, contamination with infective agents, and respiratory distress. Moreover, the cost of intravenous immunoglobulin is elevated.

Mycophenolate Mofetil

The effectiveness of mycophenolate mofetil (MMF) in some animal models has encouraged its evaluation in the treatment of human glomerular disease, including MN. Penny et al. showed that MMF can prevent the induction of active Heymann nephritis in rats treated at the beginning of the disease course, while it did not have a beneficial effect when given later in the course of the disease [142]. In another study, rats given MMF before the induction of active Heymann nephritis developed less proteinuria than untreated rats and showed an attenuated antibody response persisting after cessation of treatment [143].

Some authors have evaluated in small uncontrolled studies the efficacy of MMF in human MN. Obviously, in contrast with the experimental model, human clinical studies examined the response to MMF among patients with established MN. In a study by Miller et al., 16 patients with resistant MN were treated, for a mean of 8 months, with MMF (0.5–2.0 g/day), plus prednisolone in 5 of them [144]. At 6 months, six patients experienced a halving of proteinuria, but only two patients developed a partial remission, and none obtained a complete remission of the nephrotic syndrome. There were not significant changes in the serum creatinine during the study. Eighteen percent of treated patients developed relevant side effects. In another retrospective study, 17 patients

received MMF for 6–25 months, at a dosage ranging between 0.5 and 1.5 g twice a day [145]. At the end of treatment, mean proteinuria significantly decreased in all patients. Among the 15 nephrotic patients, 2 achieved a complete remission and 8 a partial remission. In addition, 14 of 15 patients with either corticosteroid or cyclosporine dependency were able to successfully withdraw either agent. However, MMF dependency developed in four patients and three patients had to stop therapy because of severe side effects. However, the efficacy of MMF treatment was difficult to evaluate, since only three patients received MMF monotherapy, whereas most patients were also given variable doses of steroids, cyclosporine, or cytotoxic agents. In another small study, eight nephrotic patients with stage III–IV MN were treated with 2 g/day of MMF for 9 months [146]. After 3 and 9 months of treatment with MMF, proteinuria significantly decreased from the basal value of 4.4 g/day to 2.0, and 1.9 g/day, respectively. Renal function did not change significantly.

Branten et al. compared treatment with IV methylprednisolone 1 g for 3 consecutive days at the beginning of months 1, 3, and 5 followed by alternate-day prednisone, 0.5 mg/k/48 h, for 6 months plus MMF 2 g/day for 12 months (32 patients) or cyclophosphamide 1.5 mg/kg/day for 12 months (32 historical controls) [147]. There were no significant differences in remission of proteinuria at 12 months or in adverse events between the two groups. However, 75 % of the patients treated with MMF relapsed within 2 years after the end of treatment. Similarly, Senthil Nayagam et al. reported that a 6-month course of combined corticosteroids with MMF is as effective as alternating monthly cycles of steroids and cyclophosphamide for 6 months for primary treatment of MN in the short term [148]. On the other hand, Dussol et al. randomized 36 patients with MN and nephrotic syndrome to receive conservative therapy plus MMF (2 g/d) ($n=19$) or conservative therapy alone ($n=17$) for 12 months and found that the probability of complete or partial remission did not differ between the two groups [149].

In summary, looking at these studies, the impression is that in contrast with the results obtained in the experimental model, treatment with MMF in clinical MN had moderate efficacy, but better results might be obtained by combining MMF with steroids.

Rituximab

In MN, experimental data suggest that B cells are involved in the pathogenesis of the disease [150]. To date, the best proven therapy for patients with MN consists of the combined use of corticosteroids and cyclophosphamide. The mechanism of action of cyclophosphamide includes suppression of various stages of the B cell cycle including B cell activation, proliferation, and differentiation and inhibition of immunoglobulin secretion, supporting the hypothesis that B cell abnormalities are involved in the pathogenesis of MN [151, 152]. Given the key role of IgG antibodies in IMN [2], it is reasonable to postulate that suppression of antibody production that targets glomerular antigens by depleting B cells may improve or even resolve the glomerular pathology. This is the theory underlying the application of B cell targeting with rituximab in MN. Rituximab is a chimeric monoclonal antibody which contains a human Fc IgG1 region and a murine variable region specific for the CD20 B cell antigen. This agent acts through a ligation with the membrane receptor CD 20 of B cells and inhibits their activation, proliferation, differentiation, and immunoglobulin secretion. In contrast to cyclophosphamide that has striking but nonselective effects on B cell function, rituximab offers a more targeted approach to B cell depletion.

Remuzzi et al. studied eight consecutive patients with MN treated with full-dose ACE inhibition, who had persistent nephrotic syndrome for at least 6 months [153]. All patients received 4 weekly infusions of 375 mg/m^2 of rituximab. Treatment obtained a significant decrease of urinary protein excretion from 8.6 to 3.8 g/24 h. Two patients achieved a decrease of proteinuria to 1 g/24 h or less and 3 had a decrease to 3.5 g/24 h or less. None of the remaining patients had any worsening of proteinuria. CD20+ lymphocytes decreased to undetectable levels by month 1 after rituximab infusion and remained below normal ranges up to the end of study. Of note, after the completion of the study, one patient had progressive recovery of circulating CD20 lymphocytes to normal ranges that was paralleled by progressively increasing proteinuria to pretreatment values, opening the question if rise in CD20 lymphocytes is inevitably associated by an increase in proteinuria in all patients. In a subsequent paper, Ruggenenti et al. reported that rituximab proved to be ineffective in patients with severe tubulointerstitial lesions at renal biopsy [154].

Two subsequent studies involving a total of 35 patients (about half of them refractory to prior therapy) treated with rituximab 1G IV 2 weeks apart or 375 mg/m^2 for 4 weeks showed 50 % complete or partial remission of proteinuria at 1 year and 80 % at 2 years [155, 156]. The response in proteinuria was gradual and sustained and there was no difference in the effectiveness at 1 year between the two different dosing regimens. Total B-cell counts started to recover at 3 months, which is faster than in patients with ANCA-associated vasculitis, rheumatoid arthritis, or non-Hodgkin's lymphoma suggesting that heavy proteinuria resulted in decreased levels in rituximab, though there was no correlation between rituximab levels, degree of proteinuria, or response to drug. A B-cell-titrated protocol using a single dose of rituximab 1 g has shown to be similarly effective as the 4-dose protocol but at a lower cost [157]. A beneficial effect was also reported in a matched-cohort study compared 2-year outcomes of 11 consecutive patients with primary

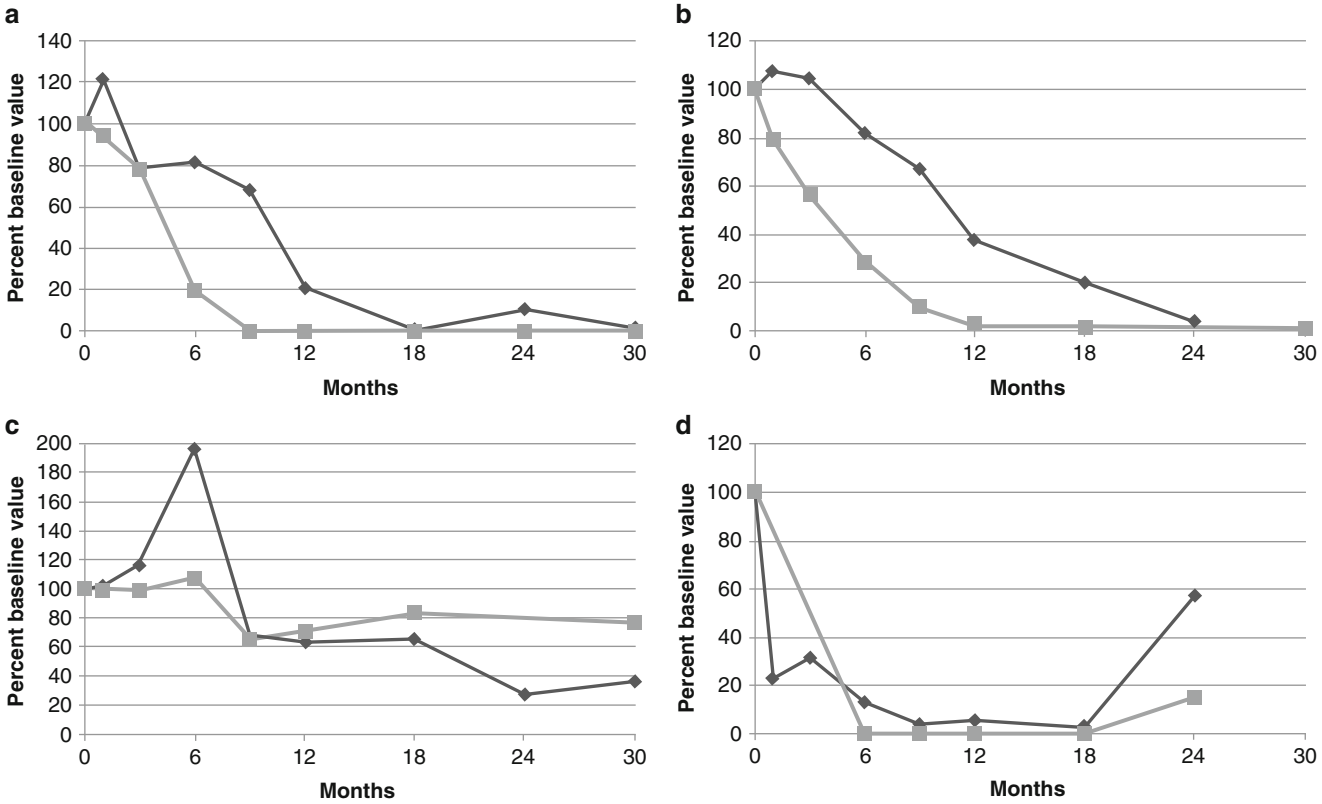

Fig. 5.4 Representative plots of anti-PLA2R (*gray squares*) and proteinuria (*black diamonds*) versus time following initial rituximab (RTX) treatment. Values are plotted as percent of baseline value. Panels (**a**) and (**b**) depict the typical reduction and disappearance of anti-PLA2R followed by resolution of proteinuria exhibited by the majority of patients. Panel (**c**) is representative of patients in whom anti-PLA2R did not substantially decline following treatment and the associated with persistence of proteinuria. Panel (**d**) depicts the single patient whose anti-PLA2R level returned with relapse of his disease after having initially disappeared. From Beck et al. [17] with permission

MN who received rituximab as second-line therapy for persisting nephrotic syndrome or relapsing disease [158]. These encouraging results have been further supported by recent results in the largest study to date which included 100 consecutive patients with nephrotic syndrome treated with rituximab [159]. During a median follow-up of 29 after administration of rituximab, 65 patients achieved complete or partial remission of proteinuria. Remission rates were similar between patients with or without previous immunosuppressive therapy. No serious adverse events were attributed to rituximab [159]. Rituximab may also allow successful withdrawal in calcineurin-inhibitor-dependent patients [160]. These results suggest that rituximab is effective in inducing remission of proteinuria in a large number of patients with MN, either as initial treatment or for patients refractory to previous therapeutic attempts. The short-term side-effect profile and compliance issues of this selective therapy seems much more favorable than the currently used immunosuppressive regimens, although some concerns about the long-term effects of rare and fatal complications such as progressive multifocal leukoencephalitis potentially related to B cell depletion therapy have been reported.

Monitoring anti-PLA2R levels may be also helpful to monitor response to therapy in patients treated with rituximab. Hoxha et al. treated five patients with MN with rituximab; in two of them disappearance of anti-PLA2R from circulation anticipated a complete or partial remission of proteinuria [161]. Patients who failed to clear anti-PLA2R from circulation did not achieve remission of proteinuria. Similar results have been reported in a study involving 35 patients with primary MN treated with 2–4 doses of rituximab [17]. Pretreatment samples contained antibodies against anti-PLA2R in 25 of 35 (71 %) patients. Autoantibodies declined or disappeared in 17 of 25 (68 %) patients within 12 months after rituximab. Those who demonstrated this immunologic response fared better clinically with 59 % and 88 % of the patient attaining complete or partial remission of proteinuria at the end of 12 and 24 months, respectively. This compared with 0 % and 33 % among those patients with persistent anti-PLA2R levels. Changes in antibody levels preceded changes in proteinuria. One subject who relapsed during follow-up had a concomitant return of anti-PLA2R [17] (Fig. 5.4). The relationship between reduction in anti-PLA2R levels and response to rituximab points toward a direct effect on the

pathophysiology of the disease process and emphasizes the future of more specific targeting therapy in MN.

A multicenter randomized control study comparing the use of rituximab versus cyclosporine in the treatment of MN is currently on the way. If available data is confirmed, rituximab could become the new standard of care for patients with MN (ClinicalTrials.gov Identifier: NCT01180036).

Eculizumab

Eculizumab is a new, humanized anti-C5 monoclonal antibody designed to prevent the cleavage of C5 into its pro-inflammatory by-products. In a recent randomized controlled trial (currently reported in abstract form only), 200 patients with MN were treated every 2 weeks with two different intravenously dose regimens and compared to a placebo group, over a total of 16 weeks [162]. Neither of the active drug regimens of eculizumab showed any significant effect on proteinuria or renal function when compared to placebo. It was later determined that adequate inhibition of C5 was seen in only a small percentage of patients suggesting that the doses given were inadequate. More encouraging results were seen in a continuation of the original study in which eculizumab was used for up to 1 year, with a significant reduction in proteinuria in some patients (including two patients who went into complete remission). Whether complement inhibition with higher doses of eculizumab will prove to be more effective, as well as safe, in the treatment of MN remains a question for the future.

Treatment of Patients with Deteriorating Renal Function

A number of noncontrolled studies focused on specific treatment of patients with progressive renal failure [163–177] (Table 5.3).

Corticosteroids alone did not show a clear benefit in progressive MN. In a small study by Short et al., treatment with high-dose intravenous methylprednisolone pulses followed by oral prednisone achieved only a transient improvement in serum creatinine. At the last follow-up about 50 % of treated patients either died or progressed to ESRD [163].

Five studies evaluated the response of deteriorating MN to methylprednisolone and/or prednisone alternated with chlorambucil. The results of these studies showed that 61 % of treated patients responded with an improvement or stabilization of renal function [164, 167–170]. Four other studies reported an improvement or stabilization of serum creatinine in 85 % of patients given oral cyclophosphamide associated with methylprednisolone and/or prednisone [171–174].

Three small controlled studies on treatment of deteriorating MN are also available. Falk et al. found no difference in renal survival and in mean plasma creatinine between 13 patients assigned to receive alternate-day prednisone for 8 weeks, and 13 patients treated with alternate-day prednisone plus 3 pulses of intravenous methylprednisolone plus monthly intravenous pulses of cyclophosphamide for 6 months [175]. Reichert et al. compared a 6-month treatment with methylprednisolone and chlorambucil alternated every other month with a 6-month treatment with intravenous pulses of cyclophosphamide. Mean plasma creatinine significantly decreased in patients given chlorambucil whereas significantly increased in those given intravenous cyclophosphamide [176]. Cattran et al. randomly allocated 17 patients with nephrotic syndrome and renal dysfunction, either to placebo or to 3.5 mg/kg/day of cyclosporine for 12 months [177]. Creatinine clearance reduced by 2.0 ml/min per month in the placebo group and by 0.73 ml/min per month in the cyclosporine group, the difference being significant. Twenty months after, cyclosporine withdrawal creatinine clearance remained stable in 6 of the 8 patients. Daily proteinuria also improved significantly in the treated patients (−4.5 g/day vs. +0.7 g/day, respectively).

In summary uncontrolled and controlled studies suggest that immunosuppressive therapy may be effective even when started in patients with established renal insufficiency, although the response to treatment may depend on the therapeutic regimen used, as well as on the severity of the underlying histologic lesions. The better results were obtained with corticosteroids alternated with cytotoxic agents. However, it must be pointed out that most papers did not report information about the baseline renal biopsy. When data were available, patients had only mild tubulointerstitial lesions suggesting that the damage of the kidney was less severe than suggested by serum creatinine [164]. All the studies reported a high incidence of side effects, suggesting that patients with renal insufficiency are more susceptible to iatrogenic morbidity. Thus, as already pointed out more than 25 years ago [178, 179], a reduction of the dosage of steroids and cytotoxic agents is necessary in patients with declining renal function, and a careful assessment of the benefit and the risks of a specific treatment should be done, considering that even in favorable cases, renal function does not return to normal values and most patients eventually progress to ESRD in the long term.

Treatment of Older Patients

MN represents the most frequent cause of idiopathic nephrotic syndrome in the elderly [180–186]. The clinical presentation is similar in older and in younger patients, with the majority of cases presenting with the nephrotic syndrome. There are conflicting opinions about the prognosis of MN in aging patients. Davison et al. reported that 8 of 9 patients aged over 60 had a slow or a steady deterioration

Table 5.3 Results of the therapeutic trials in patients with deteriorating MN

Study	No. pts	Agent	Duration	Results	Side effects
Steroids alone					
Short et al. [163]	15	MP + prednisone	6 mo	Better serum creatinine in six patients	Pneumonia one patient Cataract two patients
Steroids and chlorambucil					
Mathieson et al. [164]	8	MP + chlorambucil	6 mo	Mean creatinine clearance from 52 to 81 ml/min Mean proteinuria from 15 to 2 g/24 h	Numerous and severe
Wetzels and Koene [165]	8	Prednisone + chlorambucil	6 mo	Better serum creatinine in 2 patients	Numerous (50 %) and severe
Araque et al. [166]	4	MP + chlorambucil	6 mo	Mean serum creatinine from 2.2 to 1.3 mg/dL Mean proteinuria from 14 to 2.8 g/24 h	Pneumonia one patient Oral herpes one patient
Warwick et al. [167]	21	MP and/or prednisone + chlorambucil	6 mo	Better serum creatinine in seven patients	Numerous (50 %) and severe
Brunkhorst et al. [168]	17	MP + chlorambucil	6 mo	Mean serum creatinine from 162 to 127 µmol/L Mean proteinuria from 17 to 5.5 g/24 h	Mild and rare
Stirling et al. [169]		MP and/or prednisone + chlorambucil	6 mo	Remission in 26 % of patients End stage renal disease in 26 % of patients	Numerous and severe
Torres et al. [170]	19	Prednisone + chlorambucil	6 mo	Mean serum creatinine from 2.3 to 2.0 mg/dL Mean proteinuria from 11.2 to 5.2 g/24 h	Numerous and severe
Steroids and/or cyclophosphamide					
Bruns et al. [171]	11	Cyclophosphamide or Prednisone + cyclophosphamide	1 year	Mean plasma creatinine from 2.2 to 1.5 mg/dL Mean proteinuria from 12 to 4.8 g/24 h	Cushingoid features in all Pneumonia two patients Leukopenia two patients
Jindal et al. [172]	36	Cyclophosphamide Prednisone + cyclophosphamide	23 mo	Mean serum creatinine from 2.6 to 2.9 mg/dL Mean proteinuria from 11.1 to 1.9 g/24 h	Numerous
Branten et al. [173]	39	MP and/or prednisone + cyclophosphamide	1 year	Mean serum creatinine from 226 to 143 µmol/L Mean proteinuria from 10.3 to 2.2 g/24 h	Numerous
du Buf-Vereijken and Wetzels [174]	21	MP and prednisone + cyclophosphamide or chlorambucil	1 year	Complete remission 5 patients, partial remission 13 Patients, Nephrotic syndrome 1 patient, end stage renal disease 1 patient, death 1 patient	
Controlled studies					
Falk et al. [175]	13	Prednisone MP + IV cyclophosphamide	3 mo 6 mo	No difference at 2 years in renal survival	mild
Reichert et al. [176]	9	MP + chlorambucil Cyclophosphamide	6 mo	Mean serum creatinine from 260 to 189 µmol/L Mean serum creatinine from 218 to 297 µmol/L	Numerous
Cattran et al. [177]	9	Cyclosporine plus low dose prednisone	26 weeks	Remission (first event) 75 % Remission (last follow up) 39 %	Mild

MP methylprednisolone, *IV* intravenous, *mo* months

of renal function [187], while Donadio found that age was not associated with a poor renal outcome [188]. However, independently of the renal outcome, elderly patients are more prone to the thrombotic and cardiovascular complications of the nephrotic syndrome when compared with younger adults [189].

The results of specific treatment in older patients are difficult to assess since there are no controlled trials in the elderly. Information is available on 51 patients with MN older than 60, treated with corticosteroids [182, 190–192]. After follow-ups of various lengths, 22 patients obtained a remission of the nephrotic syndrome. However, it is not clear whether the remission represented a first event or was still persistent at the last observation, nor information about the progression to renal failure is available. Passerini et al. treated 41 patients with MN older than 65 years either with corticosteroids alone for 3–12 months (group A 14 patients) or with a 6-month course of methylprednisolone alternated with chlorambucil every other month (group B 15 patients). Other 12 patients (group C) never received any specific treatment. After a follow-up ranging between 1 and 16 years, there were significantly more remission in group B than in groups A and C (73 % vs. 21 % and 25 %, respectively). Three patients in group C and one in group B died. During the follow-up, five patients in group A, six in group C, and two in group B developed renal function deterioration. Severe side effects developed in 40 % of patients treated with the combined therapy and in 30 % of those receiving steroids alone [193]. Rollino et al. treated 18 patients older than 65 years with alternated steroids/chlorambucil treatment for 6 months [194]. Complete remission was obtained in 28 % and partial remission in 39 % of the patients. Minor complications were observed in 77 % of patients.

In summary, the clinical response to treatment seems to be similar in young adults and in old patients with MN. Thus, in older patients with severe nephrotic syndrome and/or incipient renal failure, a therapeutic trial seems to be justified. A regimen with steroids and cytotoxic agents can increase the chances of remission and protect renal function. However, such a regimen may be poorly tolerated by older patients. Thus, to minimize iatrogenic morbidity, the doses of methylprednisolone pulses and cytotoxic agents should be halved. Such a strategy obtained good results in patients with MN and severe nephrotic syndrome [168]. Moreover, as cases of cardiac arrhythmia after high-dose methylprednisolone pulses have been reported in [195, 196], some cautions should be used in older patients: (1) IV methylprednisolone should be infused over at least 1 h; (2) hypokalemia should be corrected before steroid pulse therapy; (3) patients with preexisting cardiac lesions should have continuous electrocardiographic monitoring during methylprednisolone administration; (4) to prevent thrombotic complications, a treatment with low-dose subcutaneous heparin for a week should be given starting from the first day of high-dose methylprednisolone administration; (5) glycemia should be controlled a few days after steroid pulse administration since some patients may develop diabetes, this complication being more frequent in the elderly.

For those patients in whom corticosteroids are contraindicated, a 6-month trial with chlorambucil or cyclophosphamide may be offered. A rescue treatment may be also offered to older patients with renal insufficiency, but patients should be strictly monitored to prevent or promptly treat possible complications, rendered more frequent by the concomitance of elderly age and renal failure.

Recurrent MN Post-Transplant

MN can recur after kidney transplantation, causing proteinuria, allograft dysfunction, and graft failure. More than 300 cases of recurrent MN have been reported in renal transplant patients, most cases occurring in adults [197]. Surveillance graft biopsies in patients with MN showed that initial clinical manifestations of recurrent MN were mild or absent, whereas light microscopic changes were subtle or absent at the time of diagnosis [198]. All patients had granular glomerular basement membrane deposits of IgG but little or absent C3 by immunofluorescence. Subepithelial deposits were observed in all cases by electron microscopy. In that series, 42 % of patients showed a recurrence in the allograft, and the recurrence most often occurred during the first year following transplantation. Thus, the initial clinical and histologic manifestations of recurrence are subtle but the disease may be progressive. Actually, a review of 35 renal transplants performed in patients with idiopathic MN showed that the long-term graft survival was lower in comparison with well-matched controls, although the difference was at borderline significance. Recurrence occurred in one-third of the patients and caused graft loss in half of them [199].

The role of anti-PLA2R antibodies has also been evaluated in recurrent and de novo MN after kidney transplantation [200]. Anti-PLA2R antibodies were present in five of ten patients with recurrent MN but in none of the nine patients with de novo MN. Some patients with ESRD due to MN and circulating anti-PLA2R antibodies at the time of kidney transplant did not develop recurrence. At Mayo Clinic we have a similar experience in our own transplant population in which positive anti-PLA2R antibodies at the time of transplant do not predict recurrence of MN. Because anti-PLA2R antibodies are not always associated with recurrence of the disease, the presence of these antibodies should not preclude kidney transplantation in patients with ESRD secondary MN and positive anti-PLA2R antibodies.

Rituximab has also been tried in recurrent MN [201]. Eight patients with recurrent MN diagnosed by protocol

biopsy were treated with RTX after their proteinuria increased from a median of 211 mg/day at diagnosis to 4,489 mg/day. Twelve months post-rituximab, 75 % of patients had either partial or complete remission. After 24 months, 6/7 (86 %) cases were without nephrotic proteinuria and one patient relapsed. Posttreatment biopsies showed resorption of electron dense immune deposits in 6/7 cases and were negative for C3 (4/7) and IgG (3/7). Treatment of recurrent MN with rituximab is promising and should be evaluated in a prospective randomized controlled trial.

Conclusions

Antibodies against PLA2R account for approximately 70 % of the cases of MN. In addition, to anti-PLA2R antibodies, a number of other antibodies, including antibodies against common food products may be involved in some cases of MN. Genetic susceptibility is also involved in the development of MN as demonstrated by the frequent linkage of these patients to HLA-DQ gene loci and to specific PLA2R SNPs.

Complete remission of nephrotic syndrome is associated with prolonged renal survival and a slower rate of renal disease progression; also partial remission may improve the prognosis of patients with MN. There are no standard or universal first-line specific therapeutic options for idiopathic MN. Supportive or conservative care should be given in all cases with significant proteinuria and should include the use of ACEi and ARB therapy as well as a lipid-lowering agent. In patients who are at low risk of progression, this approach should suffice, given their excellent prognosis. However, these patients need to be followed long term to ensure that there is no disease progression. On the other hand, patients at medium or high risk of progression are candidates for immunosuppressive therapy, since there is overwhelming clinical evidence that higher sustained levels of proteinuria predict an increased risk of extrarenal complications, more rapid decline in renal function, more pronounced tubulointerstitial injury, and eventual kidney failure.

Controversy exists on whether inpatients with nephrotic syndrome treatment should be started at diagnosis or only after a patient shows deterioration of renal function. The advantage of the first approach is that waiting until renal insufficiency develops may reduce the chances of a response to treatment and may increase iatrogenic morbidity when treatment is started. Moreover, if treatment is begun early, the risk of the complications of the nephrotic syndrome can be reduced. The advantage of the second approach is to restrict a potentially toxic therapy to those patients who clearly need it. Unfortunately it is difficult to identify at presentation which patient will have a progressive disease and which patient will not. One can predict that patients with elevated serum creatinine and/or those with severe tubulointerstitial lesions are at high risk of developing end-stage renal failure, but this also means that it is probably too late to modify the progressive course of MN. It is also worth emphasizing that patients with persistent nephrotic syndrome proteinuria have significant abnormalities in their lipid profile and although these abnormalities can be improved by the use of an HMG-CoA-reductase inhibitor, it will not be completely corrected unless nephrotic syndrome goes into remission. Thus, allowing a heavy proteinuric state to remain long term is likely to place the patient at an increased risk for cardiovascular complications.

A compromise between these two diverging approaches could be an early treatment for patients with persistent, severe, symptomatic nephrotic syndrome (usually patients with proteinuria exceeding 8 g per day or with plasma albumin lower than 2 g/dl), while starting treatment later in relatively asymptomatic patients who maintain proteinuria >4 g per day over 6 months [58, 59].

The efficacy of a treatment based on a 6-month course alternating methylprednisolone and an alkylating agent (chlorambucil or cyclophosphamide) every other month has the highest level of evidence among the various regimens suggested. This treatment not only favors remission of the nephrotic syndrome but also protects the renal function in the long term in a large proportion of patients and is well tolerated by more than 90 % of patients. The use of synthetic ACTH is another therapeutic option. ACTH has proven to induce remission of the nephrotic syndrome in the majority of the few treated patients, and it was usually well tolerated. However, in elderly patients, diabetics and osteoporosis may occur. Cyclosporine may induce remission in 60–70 % of patients, but in most cases proteinuria increases when the drug is stopped. Although it is likely that patients who enter a complete remission may maintain stable renal function over the time, still there is no evidence that either cyclosporine or ACTH may have a favorable impact on the possible development of renal failure in the long term. Preliminary evidence suggests that rituximab is another agent that may be effective and safe in the treatment of MN. However, rituximab is expensive, not covered by insurance, and before it can be recommended for routine use, it needs to be evaluated by rigorously randomized controlled trials. Additional information will also be needed regarding long-term safety and efficacy, before it can be recommended as first-line therapy for patients with MN.

In patients with established renal insufficiency, good results have been reported with cytotoxic drugs, often associated with corticosteroids, but side effects were frequent and severe. Thus, in order to reduce iatrogenic morbidity, the daily dose should not exceed 1.5 mg/kg per day for cyclophosphamide and 0.1 mg/kg per day for chlorambucil, and the doses of IV methylprednisolone pulses should not exceed 0.5 g each. It must be also remembered that the therapeutic

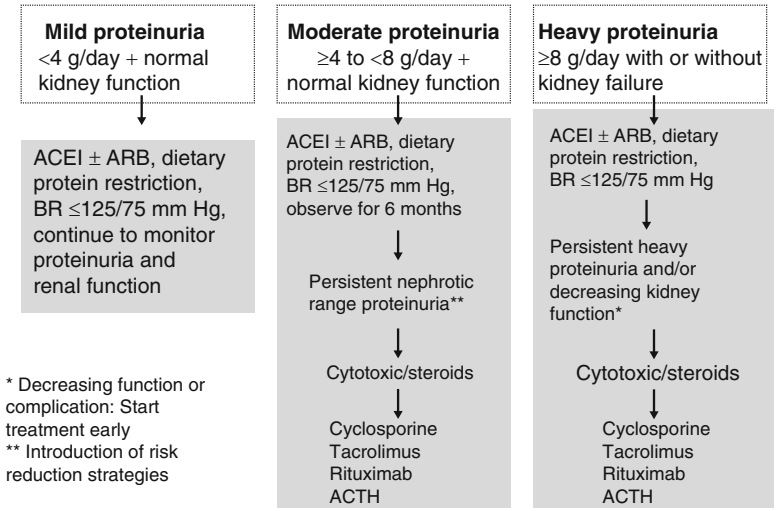

Fig. 5.5 Treatment algorithm for membranous nephropathy. *ACEI* angiotensin-converting enzyme inhibitor, *ARB* angiotensin receptor blocker, *BP* blood pressure. Modified from: Fervenza FC, Cattran DC. Membranous Nephropathy. In Molony DA, Craig JC, eds. Evidence-Based Nephrology. Oxford: Wiley-Blackwell; 2009

results largely depend on the severity of the underlying disease. In patients with a serum creatinine higher than 3 mg/dl, shrunken and hyper echogenic kidneys at ultrasonography, severe glomerular sclerosis, and tubulointerstitial lesions at renal biopsy, any form of treatment is probably useless.

Older patients with severe nephrotic syndrome can also benefit of specific therapy, since the response to treatment is comparable to that of younger adults. However, older patients are particularly susceptible to the side effects of treatment. Thus, if treatment with methylprednisolone plus chlorambucil or cyclophosphamide is chosen, the doses of the drugs should be reduced, and the alkylating agent should be definitely stopped if severe leukopenia occurs. Alternatively, ACTH given twice a week for 1 year may be tried but a prolonged administration may favor adverse events in the elderly subjects. In patients with contraindications to glucocorticoids, a 6-month course with cyclophosphamide (1.5 mg/kg per day) or chlorambucil (0.1 mg/kg per day) might also be tried. Caution is required with cyclosporine because the older kidney is particularly vulnerable to the nephrotoxic effects of this agent [48].

The recognition that proteinuria may persist despite the absence of immunological disease activity but as a consequence of an altered/remodeled glomerular basement membrane due to long-standing disease is of great clinical relevance. In this situation, further immunosuppression would be unnecessary and potentially harmful, and management should be conservative. Alternatively, the persistent proteinuria in the presence of circulating anti-PLA2R could be due to ongoing immunological disease and continued, increased, or altered immunosuppressive therapy should be considered [17]. Taken all together, these observations suggest that detection of circulating anti-PLA2R antibodies and PLA2R in biopsy samples and quantification of circulating anti-PLA2R levels may provide a tool for monitoring disease activity and treatment efficacy in patients with MN. When commercially available, quantification of antibodies (e.g., anti-PLA2R antibodies) may help monitoring disease activity and response to therapy more efficiently than proteinuria alone. Soon, we should be able to reach the goal of "personalized" therapy in patients with MN.

A treatment algorithm that combines the predictive factors and best evidence for immunosuppressive therapy is presented in Fig. 5.5. The recommendation is based on evidence from trials conducted in patients in these respective categories but the physicians must take into account the individual patient and their wishes in order to make the best decision regarding which therapy should be initiated. These treatments are not mutually exclusive and may follow one after the other (with a drug holiday) if the first one chosen does not succeed in reducing the proteinuria to the desired range and/or adverse side effects makes completion of a course of therapy untenable. Patients who do not respond well or relapse after a first course of immunosuppression therapy may benefit from a second course of immunosuppression. Patients with severe renal insufficiency (serum creatinine ≥3 mg/dl) are less likely to benefit from immunosuppression therapy, and the risk of treatment is significantly higher and these patients should be consider for conservative therapy only and plans made for transplantation in the future.

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Membranoproliferative Glomerulonephritis

6

Sanjeev Sethi and Fernando C. Fervenza

Membranoproliferative glomerulonephritis (MPGN) is a pattern of glomerular injury resulting from predominantly subendothelial and mesangial deposition of immune complexes and/or complement factors and their products. It is histologically defined on light microscopy by mesangial hypercellularity, endocapillary proliferation, and capillary wall remodeling with double-contour formation, all of these changes resulting in a lobular accentuation of the glomerular tufts.

Pathology of MPGN

Glomerular injury resulting in MPGN results from deposition of immunoglobulin (Ig) and/or complement factors in the mesangium and subendothelial region of the capillary wall. This triggers a phase of acute injury followed by an inflammatory (cellular or proliferative) phase with influx of inflammatory cells. The inflammatory cells include neutrophils that are followed by mononuclear cells. The cellular phase then evolves into a reparative phase (membrano-phase) in which new basement membranes are formed along capillary walls and in the mesangium resulting in double contours and mesangial expansion, respectively. The reparative phase also results in the entrapment of immunoglobulin and complement within the new basement membranes.

Ig and complement factors are easily identified on immunofluorescence microscopy (IF), which shows immunoglobulin and/or complement factors depending on the underlying etiology of MPGN. Electron microscopy (EM) typically shows mesangial and subendothelial deposits and, depending on the etiology, intramembranous and subepithelial deposits. During the proliferative phase, endothelial injury is evidenced by swelling and loss of fenestrations. Double contours are not well formed at this stage. However, during the reparative phase, new basement membrane formation results in the entrapment of the capillary wall deposits and cellular elements derived from inflammatory cells and mesangial and endothelial cells within the new basement membrane material resulting in thickening and double-contour formation along the capillary walls. Similar to the capillary wall changes, there is mesangial expansion with formation of matrix material and deposition of mesangial deposits and cellular elements derived from both mesangial and inflammatory cells.

Current Classification of MPGN

Based on electron microscopy findings, MPGN is traditionally classified into MPGN type I, MPGN type II, and MPGN type III, and secondary MPGN [1, 2]. Secondary MPGN is due to hepatitis C and other infections, rheumatologic diseases, and malignancies, particularly lymphoproliferative disorders [3]. Based on this classification, MPGN type I is the most common form and includes cases that show an MPGN pattern of injury, with strong C3 staining with or without Ig on IF, and subendothelial deposits with evidence of double-contour formation on EM. MPGN type III is similar to MPGN type I except that subepithelial deposits are noted as well as subendothelial deposits on EM, and the IF shows similar strong C3 staining with or without Ig. Based on the EM findings, MPGN type III is further classified into two principle variants: the Strife and Anders variant and the Burkholder variant, describing different patterns of electron-dense deposits and disorganization of the glomerular basement membrane. The Burkholder variant shows both subendothelial deposits and subepithelial deposits, sometimes with spike formation (as seen in membranous nephropathy, stage II) [4]. In the Anders and Strife variant, the deposits have an electron-lucent appearance, with multilayering and complex disruption of the lamina densa, due to

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deposition of several generations of subepithelial and subendothelial deposits [5, 6]. MPGN type II, also known as dense deposit disease, is somewhat distinct in that in addition to the MPGN pattern on LM and C3 staining on IF, wavy sausage-shaped subendothelial deposits replacing the GBM are present along the capillary walls on EM [1, 2]. With the exception of dense deposit disease, EM does not help in identifying the underlying etiology or pathogenesis of MPGN.

Pitfalls in Current MPGN Classification

Both MPGN types I and III are often considered immune complex-mediated glomerulonephritis because of the presence of "immune-type" electron-dense deposits on EM [1]. However, as noted above, many cases currently classified as MPGN type I and III show only C3 deposits with no Ig deposits on IF. This suggests that in cases of MPGN in which only C3 is present on IF, the deposits observed on EM consist of complement and complement products (and not Ig deposits). It has been known for a long time that the alternative pathway of complement is abnormal in many patients with MPGN type I and III, as manifested by markedly depressed serum level of C3 (even more so than patients with dense deposit disease), while serum levels of components of the classical complement pathway (C4 and C1q) are normal or decreased mildly [1, 5]. This suggests that many cases of MPGN type I and III may be related to abnormalities of the alternative pathway of complement. Indeed as discussed in the next section, many cases of MPGN are associated with abnormalities of the alternative pathway of complement. The current classification is even further complicated by the fact that some authors have suggested removal of DDD from MPGN altogether [7]. As currently classified, MPGN type I and III are likely to include cases of both: (a) Ig deposition that are likely Ig mediated and (b) complement deposition that are likely complement mediated due to dysregulation of the alternative pathway of complement. *Thus, the current classification fails by not distinguishing between the two major pathophysiologic factors leading to the development of an MPGN, i.e., abnormalities resulting in Ig deposition and abnormalities resulting in complement deposition.* The correct understanding of the differences between these two entities is of paramount importance because the underlying etiology is different and thus both management and prognosis may also be different [8].

Another shortcoming from the current classification is that it implies that MPGN type I and III are primary or idiopathic. This approach may deter the clinician from the extensive work up that every case of MPGN requires, since the clinician may recognize MPGN type I or type III as an idiopathic disease entity.

MPGN by the Last Three Decades to the Current Decade

MPGN and Autoimmune Diseases (1970s–1980s)

Autoimmune diseases such as systemic lupus erythematosus and occasionally Sjögren's syndrome, mixed connective tissue disorders, and rheumatoid arthritis are well-recognized causes of MPGN [9, 10].

Hepatitis C and Other Infections (1990s)

Chronic viral infections such as hepatitis C and hepatitis B, with or without circulating cryoglobulins, are an important and common cause of MPGN. Hepatitis C was recognized as a common cause of immune complex-mediated MPGN in the 1990s [3, 11, 12] and is now the main cause of a viral infection causing MPGN [3, 13–15]. In addition to viral infections, chronic bacterial (e.g. endocarditis, shunt nephritis, abscesses), fungal, and parasitic infections [16–18] are also an important cause of MPGN worldwide. Bacteria associated with an MPGN pattern of injury include Staphylococcus, Mycobacterium tuberculosis, Streptococci, Propionibacterium acnes, Mycoplasma pneumoniae, Brucella, Coxiella burnetii, Nocardia, and Meningococcus [19–32]. Figure 6.1 shows an example of MPGN resulting from shunt nephritis due to Propionibacterium acnes.

Monoclonal Gammopathies (2000s)

Recently, it was shown that monoclonal gammopathies, with or without cryoglobulins, are another important cause of MPGN [33, 34]. Sethi et al. showed that 41 % of MPGN patients without an autoimmune process had serum and/or urine electrophoresis studies positive for a monoclonal gammopathy. Based on the bone marrow biopsy findings, the majority of these patients were classified as having a monoclonal gammopathy of undetermined significance. Other less common causes of MPGN due to deposition of monoclonal Ig included multiple myeloma, low-grade B cell lymphoma, and chronic lymphocytic leukemia. This was a retrospective study, and many of these patients were initially labeled as MPGN type I based on the MPGN pattern on LM, Ig deposits that were often monoclonal on IF, and subendothelial deposits with double-contour formation on EM. Many of these cases were not ascribed to an underlying monoclonal gammopathy even after the renal biopsy showed immunoglobulin restriction on IF [33]. Based on this study, it was suggested that in patients with MPGN

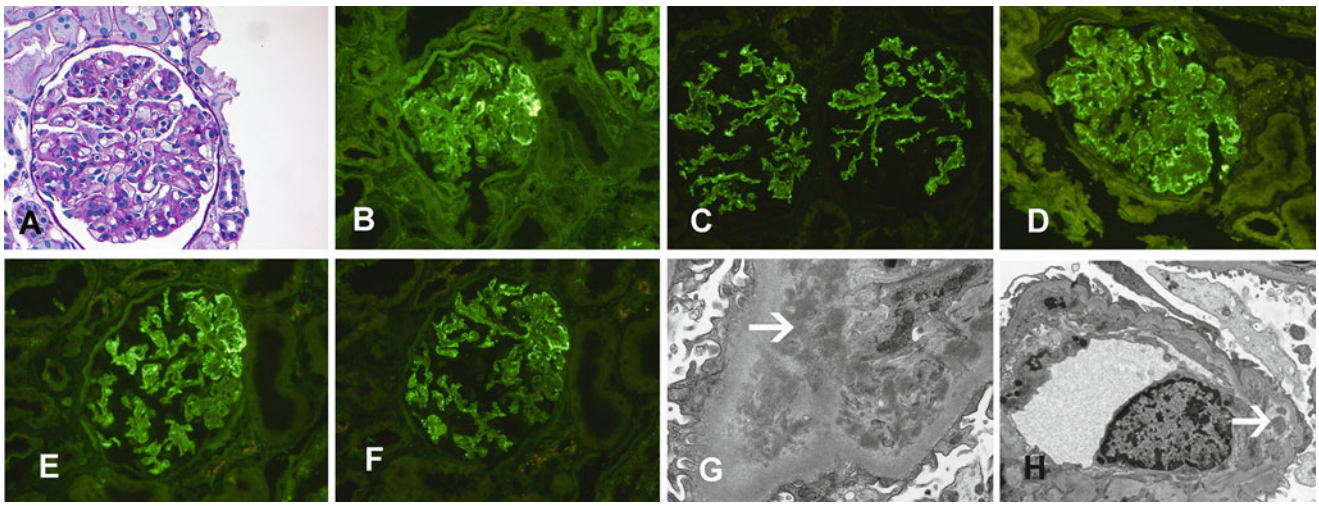

Fig. 6.1 MPGN due to chronic infection (shunt nephritis). (**a**) Light microscopy showing an MPGN pattern of injury (PAS ×40). Immunofluorescence microscopy showing granular capillary wall staining for (**b**) IgG, (**c**) IgM, (**d**) C3, (**e**) kappa light chains, and (**f**) lambda light chains. Electron microscopy showing (**g**) capillary wall deposits and (**h**) subendothelial expansion with cellular elements and electron-dense deposits. *Arrows* point at deposits

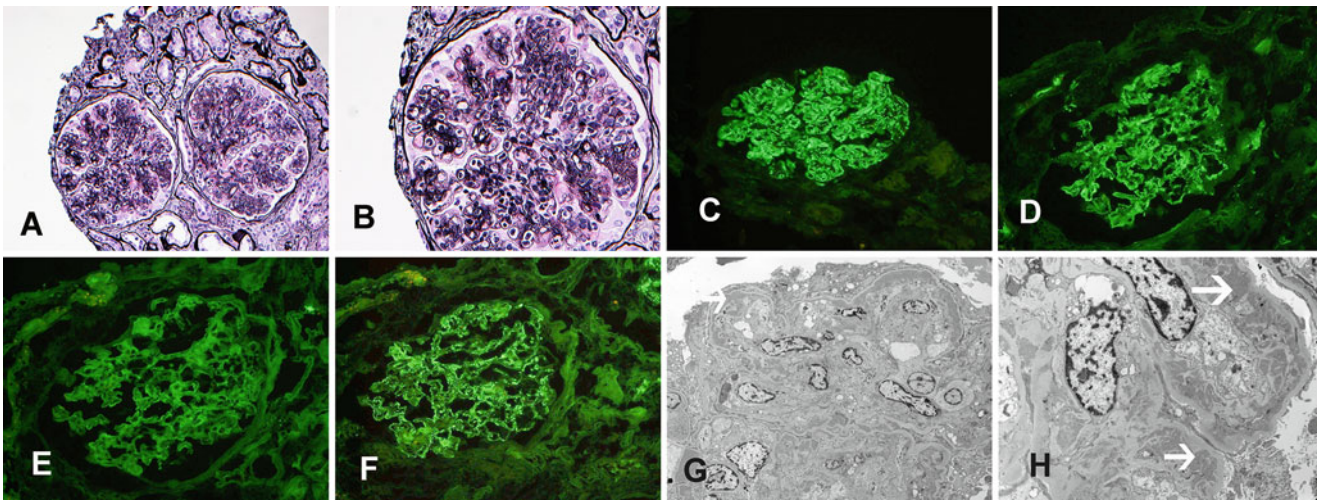

Fig. 6.2 MPGN due to monoclonal immunoglobulin deposition. MPGN due deposition of monoclonal immunoglobulins. (**a**, **b**) Light microscopy showing an MPGN pattern of injury (silver **a** ×20, **b** ×40). Immunofluorescence microscopy showing granular capillary wall staining for (**c**) IgG, (**d**) C3, and (**f**) kappa light chains. Note negative (**e**) lambda light chains. Electron microscopy showing (**g**, **h**) capillary wall deposits and subendothelial expansion with cellular elements and new basement formation resulting in double contours. *Arrows* point at deposits

and immunoglobulin restriction on IF, the monoclonal gammopathy should not be called of "undetermined significance" or MGUS, but rather these patients should be diagnosed as having a "monoclonal gammopathy related or monoclonal gammopathy associated MPGN" [33]. Other recent studies have also shown that monoclonal deposition of Ig, in particular IgG3, results in a proliferative glomerulonephritis [35]. It should be pointed out that in our experience deposition of monoclonal Ig other than IgG3 such as monoclonal IgA or IgM also result in MPGN [36]. Indeed, deposits composed of light chains alone in absence of any heavy-chain component can result in MPGN. Figure 6.2 shows an example of MPGN resulting from deposition of monoclonal immunoglobulins.

Dysregulation of the Alternative Pathway of Complement (2000s)

Activation of the alternative pathway (AP) of complement occurs in a sequential manner that can be divided into four main steps: initiation of complement activation, C3 convertase

Table 6.1 Abnormalities of the AP of complement

Complement factor	Abnormality	Pathology	References
C3	C3 mutation	DDD	[42]
Factor H	Factor H mutation	DDD and C3 glomerulonephritis	[38, 40, 43–45]
Factor I	Factor I mutation	C3 glomerulonephritis	[38, 46]
Factor H	Antibody	DDD and C3 glomerulonephritis	[40, 47]
Factor H-related protein 5	CFHR5 mutation	C3 glomerulonephritis	[39, 48]
Factor B	Antibody	DDD	[49]
C3NeF	Antibody	DDD and C3 glomerulonephritis	[50, 51]
Membrane cofactor protein/CD46	Mutation	C3 glomerulonephritis	[38]
Factor H alleles (p.Y402H, p.V62I)	Allele variant	DDD and C3 glomerulonephritis	[51–53]
C3 alleles (p.R102G, p.P314L)	Allele variants	DDD and C3 glomerulonephritis	[46, 47]

activation and amplification, C5 convertase activation, and terminal pathway activity with assembly of the terminal complement complex or membrane attack complex (MAC). Once activated, the AP generates effector compounds that are delivered to all surfaces indiscriminately, mandating control over progression of the cascade and the action of these molecules. Multiple complement regulators and inhibitors operate at every level to prevent self-mediated damage. For example, proteins that regulate C3 convertase (C3bBb) assembly, activity, and half-life include factor H, factor I, factor B, decay-accelerating factor, factor H-related proteins, membrane cofactor protein (CD46), and complement receptor 1. Mutations in, or antibodies against, these proteins therefore can alter AP control and lead to the dysregulation of the AP.

Whatever the mechanism, dysregulation of the AP results in activated complement products that are delivered indiscriminately to all surfaces, including the glomerular capillary walls with subendothelial and mesangial deposition of the complement products and debris. For example, dysregulation of AP occurs when antibodies develop to proteins that regulate C3 convertase (C3bBb) assembly and activity such as factor H, factor I, factor B, decay-accelerating factor, factor H-related proteins such as factor H-related protein 5, membrane cofactor protein, and complement receptor 1 [37–41]. Similarly, mutations in these complement-regulating proteins, including C3 itself, can alter AP control leading to deposition of complement products along the glomerular capillary walls. Antibodies to C3 convertase (C3 nephritic factor or C3Nef) also prolong its half-life resulting in activation of AP of complement. Genetic background also is another risk factor for development of disease. Best studied are the Tyr402His allele variants of factor H. His402 (H402) is overrepresented in MPGN patients with alternative pathway abnormalities as compared to Tyr402, and functional studies have shown that the former provides poorer factor H-mediated regulation of the C3 convertase on cell surfaces (Table 6.1) [38–40, 42–54].

Once complement products and debris are deposited in mesangium and subendothelial region of the capillary walls, it leads to glomerular inflammation, manifested as a proliferative GN followed by reparative changes and development of MPGN.

The prototypical example of a glomerular disease associated with dysregulation of the AP is dense deposit disease (DDD) [50, 55, 56]. It is characterized by an MPGN pattern on LM, C3 deposition in the mesangium and along capillary walls on IF, and osmiophilic sausage-shaped wavy dense deposits along the GBM and in the mesangium on EM. The absence of significant Ig by immunofluorescence and the location and character of the dense EM deposits distinguish DDD from Ig-mediated MPGN. Laser microdissection of glomeruli from DDD patients followed by mass spectrometry of the glomerular proteins shows large amounts of complement factors of the AP and terminal pathway.

On the other hand, many cases labeled as MPGN type I or type III that show mesangial and capillary wall C3 staining (and no significant Ig deposits) with subendothelial (type I) and subepithelial deposits (type III) on EM also result from dysfunction of AP due to mutations or antibodies to the complement-regulating proteins [40]. These proliferative lesions are termed C3 glomerulonephritis or C3-GN [37, 57]. Figure 6.3 shows an example of MPGN resulting from complement deposition, in the absence of Ig deposits.

Data from laser microdissection and mass spectrometry analysis of glomeruli obtained from a number of patients with MPGN type I and type III with C3 deposits, i.e., C3 glomerulonephritis, is consistent with unrestricted activation of the AP; the proteomic profile in these cases is similar to that found in patients with DDD [40, 54]. That DDD and C3-GN are part of a continuum is further supported by cases of MPGN with strong C3 deposition on IF that show features that are intermediate between DDD and C3-GN, with some capillary loops showing sausage-shaped intramembranous deposits, while other loops show subendothelial and subepithelial deposits on EM.

The limited data on C3-GN suggests that it carries a better prognosis than DDD [38, 40]. It has been hypothesized that increase in C3 convertase activity is associated with the DDD phenotype while increase in C5 convertase activity is

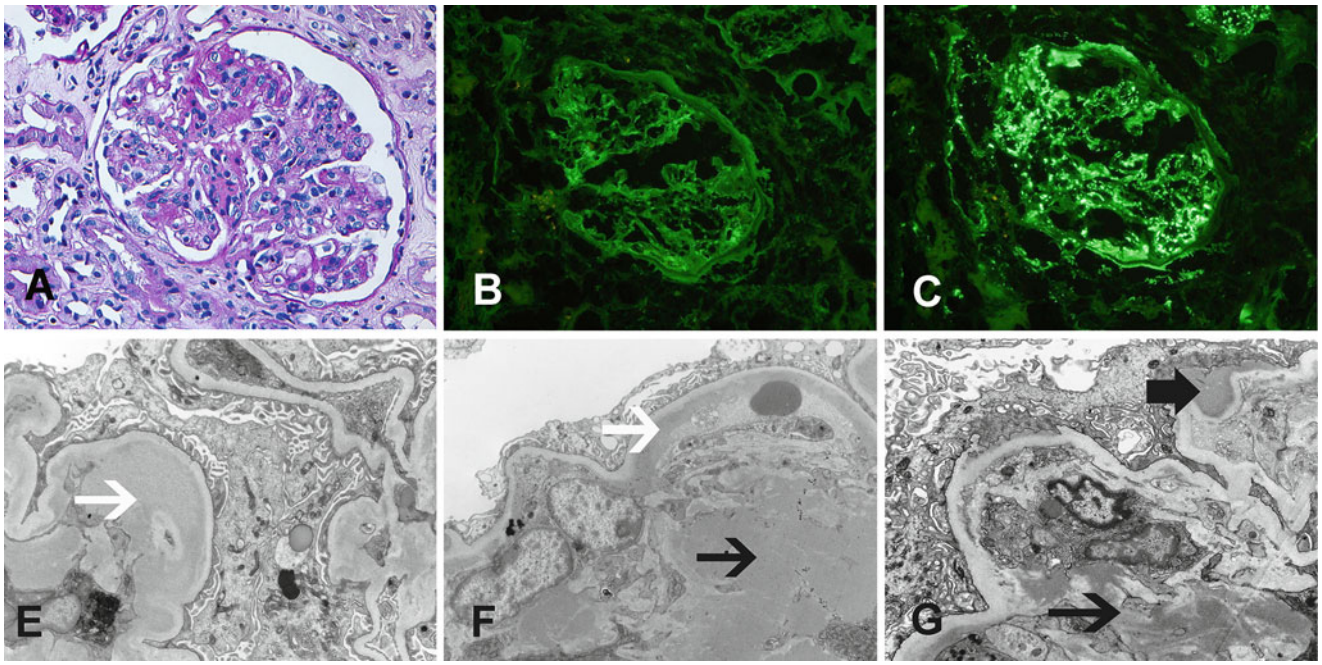

Fig. 6.3 MPGN due to C3 glomerulonephritis. MPGN due deposition of monoclonal immunoglobulins. (**a**) Light microscopy showing an MPGN pattern of injury (PAS ×40). Immunofluorescence microscopy showing (**b**) no staining for IgG, (**c**) bright mesangial and capillary wall staining for C3. Electron microscopy showing (**d**, **e**, **f**) numerous subendothelial, and mesangial deposits and occasional subepithelial deposits. *Black arrows* point at subendothelial deposits, *white arrows* point at mesangial deposits, and *thick black arrow* points at subepithelial deposit

associated with the C3-GN phenotype [58]. In addition, it is also likely that certain allele variations of complement-regulating proteins may be associated with DDD while others may be associated with C3-GN.

The differential diagnosis of MPGN due to AP dysfunction includes postinfectious glomerulonephritis (due to the presence of subepithelial humps) and autoimmune disease (due to subepithelial, subendothelial, and mesangial deposits). In fact, most of the cases of MPGN with C3 deposits that we receive in our practice have previously been labeled as either idiopathic MPGN type I/III or postinfectious glomerulonephritis (often healing or resolving). However, there is often no history of infection in these cases of MPGN that have been labeled as postinfectious GN. Subsequent evaluation of these cases in fact has shown that these patients have an MPGN due to dysfunction of the AP of complement. In these cases, the lack of significant Ig deposition on IF should alert the pathologist that they are dealing with complement-mediated MPGN instead of a postinfectious GN. Another helpful histological difference is that MPGN due to AP dysfunction shows large and abundant mesangial, subendothelial, and occasionally subepithelial deposits that have a homogenous amorphous lobular quality and typically few intramembranous deposits can be found (similar to DDD but not as dense), whereas capillary wall deposits in a postinfectious GN are often sparse, sharp, and well demarcated.

Finally, even though MPGN with C3 deposits due to AP dysfunction is being now recognized more frequently and is often separately classified as C3-GN, many cases of MPGN from AP dysfunction are still likely to be classified as MPGN type I and type III [37–40, 57, 59]. This is because based on the current classification, there is considerable overlap and some cases can belong to either group. Thus, it is important to understand that based on the current classification, MPGN with C3 deposits belonging to either MPGN type I/III or C3GN can all result from dysfunction of the AP of complement. Thus, this has necessitated the recent proposal of a new classification (next section).

Thrombotic Microangiopathy

Lastly, an MPGN pattern of injury can also result from damage to the endothelial cells in cases associated with thrombotic microangiopathies (TMA) [60]. In the acute phase, mesangiolysis, endothelial swelling, and fibrin thrombi are present in the glomerular capillaries. As the process evolves into a reparative and chronic phase, mesangial expansion and glomerular capillary wall remodeling with double-contour formation takes place, resulting in the classical double-contour pattern associated with late stages of MPGN. Thus, the healing phase of TTP/HUS, cases of aHUS associated with complement abnormalities [61],

antiphospholipid antibody syndrome, drug-induced TMA, nephropathy associated with bone marrow transplantation, radiation nephritis, malignant hypertension, and connective tissue diseases can all present with an MPGN pattern of injury. In these cases, however, Ig and complement are typically absent on IF, and EM does not show electron-dense deposits along the capillary walls.

Proposal for a New Classification of MPGN

There is a recent proposal to classify MPGN based on the IF findings rather than the EM findings. Thus, MPGN has been classified as immune complex mediated and complement mediated [62]. Immune complex-mediated MPGN shows Ig and/or complement factors on IF studies. Complement-mediated MPGN shows complement factors and the lack of any significant Ig on IF studies. Immune complex-mediated MPGN includes MPGN resulting from chronic infections, autoimmune diseases, and dysproteinemias. On the other hand, complement-mediated MPGN results from dysregulation of the AP of complement. It should be noted that extensive complement (C3 and C4) deposition is also present in immune complex-mediated MPGN. However, the complement deposition in these cases results from activation of the classical and terminal pathway of complement by the immune complexes.

We recommend the terms MPGN types I–III should be abandoned since it implies a primary/idiopathic MPGN, while it is likely that an underlying etiology can be found in the majority of cases of MPGN.

Clinical Presentation

The clinical presentation of MPGN is variable. During the injury and proliferative phase, the presentation is often as nephritic syndrome. As the disease progresses and reparative changes set in, the presentation evolves to a nephrotic syndrome. In fact, many cases present with a nephritic-nephrotic syndrome. Thus, the phase of the disease process often dictates the clinical presentation. In addition, the different pathophysiological mechanisms that result in MPGN, i.e., immune complex versus complement deposition also likely contribute to the heterogeneity in the clinical presentation. This is further complicated by a variable histology, since immune complex and/or complement deposition results not only in an MPGN but can present as milder form with mesangial proliferative lesion or severe lesion with crescents [63, 64].

Thus, microscopic hematuria, mild proteinuria, or full-blown nephritic or nephrotic syndrome may occur depending on the type of the glomerular lesion as well as timing of the biopsy in relation to the clinical presentation. The same is true regarding the presence of hypertension and/or degree of kidney impairment.

Evaluation of MPGN

In view of the evidence discussed above, we propose that cases in which renal biopsy shows an MPGN pattern of injury, the work-up should be directed towards two major pathophysiologic factors: immune complex mediated and complement mediated. If Ig is present on IF, the evaluation should include evaluation for infections, autoimmune diseases, and monoclonal gammopathies, including cryoglobulins. On the other hand, if IF shows predominantly C3 and is negative or shows no significant staining for Ig, then the MPGN should be worked up as a complement-mediated MPGN, which can be further subdivided into DDD or C3-GN based on the results of the electron microscopy examination. Complement-mediated MPGN warrants an in-depth study of the AP, regardless of whether the renal biopsy shows DDD or C3-GN. We predict that Ig-mediated MPGN is more likely to be present in adults whereas complement-mediated MPGN is more likely to be present in children and young adults. It is likely that a genetic mutation in the complement regulatory proteins of the AP accounts for the many cases of a complement-mediated MPGN noted in children and young adults, while cases occurring in adults are more likely to be secondary to the development of autoantibodies to one of the complement-regulating proteins. Furthermore, in few cases of complement-mediated MPGN, an infection, autoimmune disease, or a monoclonal gammopathy may result in the development of antibodies or monoclonal proteins that are directed against epitopes on complement-regulating proteins, thus leading to dysfunction of the AP of complement. However, in such cases, the MPGN will be due to AP dysfunction not due to Ig deposition, and the renal biopsy will show C3 without any significant Ig deposition [51]. On the other hand, few cases of immune complex-mediated MPGN may also have dysfunction of the AP of complement. Finally, it is possible that even after extensive evaluation of the two groups, we will be unable to determine the underlying etiology in a small number of immune complex-mediated MPGN or complement-mediated MPGN [65].

We believe that new tests/techniques will continue to reveal new causes for a lesion that until recently was considered to be idiopathic in a significant number of patients. Table 6.2 shows the proposed evaluation of the AP of complement.

Evaluation of MPGN according to the underlying pathophysiologic process will allow us to better characterize and treat patients with an MPGN based on etiology, instead of grouping these cases together into MPGN types I–III.

Table 6.2 Proposed evaluation of MPGN secondary to AP dysfunction

Screening tests	C3 and C4 levels AH50 and CH50 sMAC levels AP functional and hemolytic assay
Mutations/genetic analyses, including allele variants	C3 Factor H Factor I Factor B MCP/CD46 CFHR1–5
Antibodies	C3NeF Factor H Factor B Factor I
Concentration of complement factors	Factors H, I, B

AH50 alternative pathway functional assay, CH50 total complement functional assay, sMAC soluble membrane attack complex, *CFHR* complement factor H-related proteins, *MCP* membrane cofactor protein

Treatment of MPGN

Treatment recommendations are difficult to make in MPGN due to different underlying pathogenic mechanisms. It is also important to realize that earlier reports of treatment of MPGN should be viewed with caution since they did not take into account that the various pathogenic mechanisms of MPGN were unknown. Pragmatic considerations would suggest that patients with MPGN due to chronic infections should undergo treatment of the infection, while those with MPGN due to an autoimmune disease should undergo treatment of the autoimmune disease. Similarly, patients with MPGN due to a monoclonal gammopathy should undergo treatment aimed to attain remission of the hematologic dyscrasia. A recent study of patients with MPGN associated with monoclonal Ig deposits and no overt hematologic malignancy showed a good response to rituximab [66]. Patients with normal kidney function, no active urinary sediment, and non-nephrotic range proteinuria can be treated conservatively with angiotensin II blockade in order to control blood pressure and reduce proteinuria, since the long-term outcome is relatively benign in this setting [67, 68]. A better understanding of the etiology and pathogenesis of complement-mediated MPGN would logically set the stage for the possible use of newer drugs including anticomplement drugs. For example, patients with MPGN due to autoantibodies to complement-regulating proteins may benefit from immunosuppressive therapy (e.g., glucocorticoids, rituximab), while those cases due to a genetic mutation in complement-regulating proteins may benefit from treatment with drugs that inhibit formation of terminal complement complex (MAC), e.g., eculizumab [69, 70].

The role of such anticomplement agents in MPGN is not delineated but offers exciting possibilities for the future. It is also conceivable that patients with elevated serum levels of MAC may be more likely to respond to treatment with eculizumab than those with normal serum MAC levels. Patients with MPGN secondary to factor H deficiency might benefit from plasma infusion [71] or factor H infusions. Patients who present with advanced renal insufficiency and severe tubulointerstitial fibrosis of renal biopsy are unlikely to benefit from immunosuppressive therapy.

Kidney Transplantation

With regard to kidney transplantation, immune complex-mediated MPGN such as infection and autoimmune diseases are less likely to recur, whereas immune complex-mediated MPGN such as those secondary to a monoclonal gammopathy are more likely to recur unless the monoclonal gammopathy is adequately treated prior to transplantation [72]. With regard to complement-mediated MPGN, it is possible that those associated with genetic mutations in complement-regulating proteins are more likely to recur than those associated with antibodies to the complement-regulating proteins [73].

Conclusion

We conclude that MPGN results from two major pathophysiologic factors: immune complex mediated and complement mediated. Immune complex-mediated MPGN should lead to evaluation for infections, autoimmune and rheumatologic diseases, and monoclonal gammopathies. Complement-mediated MPGN is further subdivided into DDD or C3-GN depending on the electron microscopy findings and should lead to further evaluation of the AP of complement. A clear understanding of the pathophysiologic process will allow us to better characterize and treat patients with an MPGN.

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Cryoglobulinemias

7

Dario Roccatello and Antonello Pani

Introduction

Cryoglobulinemia refers to the presence in the serum of immunoglobulins that reversibly precipitate and form a gel at temperatures below 37 °C and redissolve upon re-warming. They are usually classified into three subgroups according to Ig composition: type I cryoglobulinemia is composed of only one isotype or subclass of immunoglobulin. Types II and III are referred to as mixed cryoglobulinemias because they consist of both IgG and IgM components. Both types II and III cryoglobulins are immune complexes composed of polyclonal IgGs, the autoantigens, and mono- or polyclonal IgMs, respectively. The IgMs are the corresponding autoantibodies with rheumatoid factor (RF) activity. With the use of more sensitive methodologies, such as immunoblotting or two-dimensional polyacrylamide gel electrophoresis, or advanced techniques of immunofixation, type II mixed cryoglobulins frequently shows a micro heterogeneous composition; in particular, oligoclonal IgM or a mixture of polyclonal and monoclonal IgM can be detected. This particular serological subset, termed types II–III mixed cryoglobulinemia (MC), may represent an intermediate evolution from types III to II MC [1]. Type III mixed cryoglobulin is commonly detected in a great number of infectious or autoimmune disorders. In primary Sjögren's syndrome, type III mixed cryoglobulinemia is associated with extraglandular involvement, an enhanced risk of B-cell lymphoma, and poor survival. Type III cryoglobulins are detected in nearly 10 % of patients with systemic lupus erythematosus and rheumatoid arthritis, but cryocrit values are generally lower compared with those in patients with Sjögren's syndrome, and the clinical manifestations of cryoglobulinemic vasculitis are much less common [2–5]. Type II and type II–III, and less commonly type III mixed cryoglobulins, could result in a distinct disorder which can be classified among the systemic vasculitides affecting small and sometimes medium-sized vessels. They are related to a hepatitis C virus (HCV) infection in the vast majority of cases [6] and, not infrequently in the type II variety, also to low-grade proliferative B-cell lymphomas [6, 7].

This chapter will review the etiology, clinical manifestations, diagnosis, and treatment of these disorders with particular emphasis to their renal involvement.

Type I Cryoglobulinemia

Type I cryoglobulinemia is usually composed of a monoclonal Ig, mainly IgG (IgG1 or IgG3) and occasionally IgM or IgA, and it accounts for 10–15 % of all cryoglobulin cases [8]. It is almost invariably associated with hematological disorders, such as Waldenström's macroglobulinemia, multiple myeloma, or chronic lymphocytic leukemia. It is usually asymptomatic per se. Sometimes it is associated with rheological disturbances due to a hyperviscosity syndrome. It is related to clinical and biological features of vasculitis including necrotizing skin ulcers, gangrenous toes, purpuric rash, and petechial hemorrhages in extremely rare cases [8].

Types II and III: Mixed Cryoglobulinemia Syndromes

The term mixed cryoglobulinemia syndrome refers to primary or idiopathic cryoglobulinemia as well as cryoglobulinemia associated with connective tissue disorders, lymphoproliferative disorders, chronic infections, noninfectious hepatobiliary diseases, or immunologically mediated glomerular diseases and can be caused by either type II or type III cryoglobulins.

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In 10–20 % of cases, no underlying disease can be found, and cryoglobulinemia is referred to as "idiopathic."

Type II cryoglobulins usually consist of a monoclonal IgM–k with rheumatoid activity against polyclonal IgG, while type III cryoglobulins, which also share rheumatoid activity, are characterized by a combination of polyclonal immunoglobulins [9]. In the majority of cases, a cross-idiotype WA monoclonal rheumatoid factor, firstly isolated from serum of a patient with Waldenström's macroglobulinemia, can be found. The WA cross-idiotype (XId) is the major cross-idiotype among human monoclonal rheumatoid factors (mRF). It is a conformational antigenic determinant involving both H and L chains and appears to be located in the antigen-binding site. It is generally accepted that there is restricted expression of germline genes with little or no somatic mutation in the WA mRFs [10]. The monoclonal RFs that bear the WA cross-idiotype are involved in most cases of cryoglobulinemic vasculitis in patients with HCV infection [11]. In rheumatologic surveys, patients with type III mixed cryoglobulins outnumbered those with type II mixed cryoglobulins [12]. On the contrary, surveys based on renal involvement indicate a large prevalence of type II mixed cryoglobulins [13–15].

The clinical syndrome of mixed cryoglobulinemia (MC) was first described by Meltzer et al. in 1966 [9]. Many subsequent reports have further defined this syndrome, indicating that its incidence varies in different geographical areas, with the majority of cases having been reported in the Mediterranean area, namely, in Italy, France, Spain, and Israel [16, 17].

The prevalence of the syndrome ranges from 1 to 7/100,000 with a female-to-male ratio from 1.5:1 [15] to 3:1 [12]. However, the actual prevalence of MC might be underestimated due to its clinical polymorphism. A single manifestation is often the only apparent feature leading the patient to different specialties. Diagnosis could be either delayed or overlooked.

An important step forward the knowledge of the disease came from the discovery of the presence of HCV infection in the majority of cases that were previously defined as "essential" [18–23].

The primary role of HCV infection in the development of mixed cryoglobulinemia is supported by the presence of an HCV concentration from 20 to 1,000 times higher in the cryoprecipitate than in the serum supernatant [24].

It has been estimated that the HCV entered the human host several 100 years ago. Genotype 1 has a worldwide distribution, whereas genotypes 2 and 3 originated more recently and are geographically more restricted, with genotype 2 being relatively common in Western Africa and genotype 4 in the Middle East, and in Northern and Central Africa. HCV infection causes prolonged and persistent diseases in virus carriers, often leading to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. About 150 million people worldwide are infected with HCV [25] with a seroprevalence rate of about 1 % in Western Europe but 2–3 % in some Mediterranean areas. Seroprevalence in the USA is on the rise and is approaching the current levels that are found in **Southern** Europe. The extent of this social problem is amplified by the recently discovered occult hepatitis C infection that is characterized by the presence of HCV-RNA in the liver, and in 70 % of these cases, also in the peripheral blood mononuclear cells in the absence of both HCV-RNA and anti-HCV antibodies in the serum [26].

About 40 % of patients with chronic hepatitis C have circulating cryoglobulins, which are asymptomatic in the vast majority of cases. These patients should not be confounded with those showing clinical features of mixed cryoglobulinemia. A cryoglobulinemic syndrome develops only in 1–2 % of cases, and nephropathy occurs in 0.1–0.2 % of HCV-infected persons. The predisposing factors for the expression of cryoglobulinemic vasculitis in patients with chronic hepatitis C are unclear. Some links with older age and, mainly, with longer duration of hepatitis have been observed (glomerulonephritis appearing several years, and sometimes decades, after HCV infection) [27]. Genetic background is probably important in determining which subjects are susceptible to developing cryoglobulinemic manifestations.

The clinical symptoms of mixed cryoglobulinemia range from mild palpable purpura, arthralgias, and fatigue to severe vasculitis with skin necrosis, as well as peripheral neuropathy and, less frequently, involvement of the central nervous system, gastrointestinal tract, lungs, and myocardium. The kidney is rarely spared in the long term, and glomerulonephritis represents a key factor affecting prognosis. Cryoglobulinemic nephritis is a unique nephropathy which is undoubtedly pathogenetically related to HCV infection [17]. Case reports and even small series of presumed HCV-related, non-cryoglobulinemic nephropathies, specifically those with membranoproliferative patterns, are often misleading and lacking adequate procedures of cryoglobulin detection or electron microscopy studies of renal histology, so that the role of cryoglobulins in promoting tissue damage in these cases cannot be ruled out with certainty.

The relationship between HCV infection and cryoglobulinemic-associated nephritis was specifically addressed in a recent multicenter study which described the clinical characteristics, the etiology related to viral specificities, and the outcome of the biggest cohort of cryoglobulinemic glomerulonephritis patients ever examined worldwide [15]. In this report, about 88 % of subjects had been infected with HCV and 83 % were RNA positive, with genotype 1b being more frequent than genotype 2. Only 3 % of patients

had been exposed to hepatitis B virus (HBV) (usually occurring as a coinfection). Some genetic markers were found to be relevant. DRB1*11 was associated with nephritic involvement, while DRB1*15 was found to be protective. The prevalence of type II cryoglobulins was significantly higher in nephritic patients than in non-nephritic HCV-positive controls (74 % vs. 27 %), showing that clonal restriction is important for the development of renal involvement. Unlike rheumatologic and dermatologic surveys [12, 28], no clinical or laboratory differences could be detected between HCV-positive and HCV-negative (truly "essential") cryoglobulinemic cases with nephritis, except for hepatic involvement.

Over the last few years, several attempts have been made to identify an etiologic agent in the small sample of non-HCV-infected cryoglobulinemic patients. At first, GB virus C, another member of the Flaviviridae family, was indicated as the possible agent in these cases [29], but these initial observations were not confirmed [30]. Some evidence concerning the role of the TT virus, i.e., a DNA hepatotropic virus, is limited to coinfectious conditions [31]. As regards to this particular subset of patients, the so-called French multicenter CryoVas survey collected data from 242 cases of noninfectious type II (84 %) and type III mixed cryoglobulinemic vasculitis. Clinical manifestations were similar to HCV-related disorders showing purpura (75 %), peripheral neuropathy (52 %), arthralgia or arthritis (44 %), glomerulonephritis (35 %), cutaneous ulcers (16 %), and cutaneous necrosis (14 %). A connective tissue disease was diagnosed in 30 % and B-cell non-Hodgkin lymphoma in 22 %. The remaining 48 % were classified as "essential" cryoglobulinemic vasculitis [32].

Pathogenesis of HCV-Related Cryoglobulinemia

It is presently believed that HCV infects B lymphocytes, while infecting hepatocytes due to the common expression of the CD81 receptors [33]. More specifically, active HCV replication has been demonstrated in CD19-positive B cells [34]. Moreover, it has recently been shown that HCV-RNA and HCV core and NS3 proteins can only be detected in CD19-positive, but not CD19-negative peripheral blood mononuclear cells [35]. Of note, HCV replicates in other peripheral blood mononuclear cell types, including peripheral dendritic cells, monocytes, and macrophages [36, 37]. Lymphocytes that are chronically stimulated by HCV are assigned to widespread autoantibody production related to HCV-induced lowering of the cell activation threshold. This favors the development of a number of immune manifestations associated with HCV infection, which are variably assembled in clinical pictures that have been collectively called "HCV syndrome" [15]. HCV syndrome includes manifestations apparently distal to the characteristic picture of MC, such as autoimmune thyroiditis (frequently observed in MC patients), sicca syndrome, thrombocytopenia, hemolytic anemia, autoimmune diabetes, and pulmonary fibrosis.

Furthermore, B cells, which are protected from apoptosis by an HCV-dependent gene translocation, develop oligoclonal monotypic lymphoproliferation [38]. Distinct lymphoid infiltrates with cells expressing oligo- or monoclonal rheumatoid factor in the portal tracts, spleen, and bone marrow (on occasion evolving towards an overt B-cell non-Hodgkin lymphoma) can be detected in these patients. Thus, mixed cryoglobulinemia would appear to be a cross between classic autoimmune disorders and malignancies (i.e., B-cell lymphoma). Indeed the persistent stimulation of B cells by viral antigens and the enhanced expression of lymphomagenesis-related genes (particularly the activation-induced cytidine deaminase which is critical for somatic hypermutation [39]) could lead to polyclonal and later monoclonal expansion of B cells and ultimately induce a lymphoproliferative disorder that may eventually evolve into B-cell non-Hodgkin lymphoma. More specifically, a strong association has been found with some B-cell non-Hodgkin lymphoma subtypes, including diffuse large B-cell lymphoma, marginal zone lymphoma, and lymphoplasmacytic lymphoma [40].

The pathogenetic implication of HCV in the formation, transport, and removal from circulation of cryoprecipitable immune complexes (ICs) in MC has been studied extensively in recent years.

Under the trigger effects of chronic HCV infection, oligo- or monoclonal IgM that shares rheumatoid activity is produced by a permanent clone of B cells and favors the appearance of ICs, formed by circulating HCV, anti-HCV polyclonal IgG, and the monoclonal IgM itself. Due to the clonally restricted IgM, these cryoprecipitable ICs also escape the erythrocyte transport system [24] and directly impact hepatic and splenic macrophages, which are unable to process them due to abnormalities in the biogenesis of lysosomal enzymes [41]. The same abnormality (possibly due to the HCV infection of phagocytic cells) is likely to occur in circulating monocytes which, when examined by electron microscopy, are usually found to be engulfed with cryoglobulins [17], but that are unable to digest phagocytosed immune material [41].

The exact role of monocytes/macrophages, i.e., whether the phagocyte influx to the glomerulus that characterizes cryoglobulinemic nephritis is deleterious or beneficial (favoring cryoglobulin removal and disease stabilization with injury repair), has been a controversial issue for several years. A previous hypothesis based on in vitro studies [41], stating that monocyte/macrophage entrapment of cryoglobulins reflects ineffective cryoglobulin clearance and could be associated with perpetuating glomerular damage, has recently been supported by an elegant study on a murine

model of cryoglobulinemic membranoproliferative glomerulonephritis [42]. It has been shown that macrophage ablation confers protection from mesangial expansion and collagen accumulation and that macrophages do not affect cryoglobulin removal since macrophage ablation has no effect on serum levels of cryoglobulins. Therefore, we can assume that macrophage influx to the glomeruli may be due to events related to amplification of injury following immune complex deposition rather than to an attempt to clear cryoglobulins [42]. Moreover, these cells showed a detrimental role in the progression of kidney injury in this experimental model [42].

Defective maturation of lysosomal enzymes, specifically pro-cathepsin D [41], and /or danger-associated molecular patterns (DAMPs) released from injured resident cells [42] could attenuate macrophage innate function to clear immune complexes via Fc gamma receptors. The extracellular activation of released pro-cathepsin D [41] and/or the release of pro-inflammatory cytokines from DAMPs-activated macrophages [42] could drive mesangial expansion and activation.

In addition to the classical pathway of immune complex deposition, the clonally restricted IgM was shown to share strong affinity for the glomerular matrix components, especially fibronectin, and was thought to deposit in the glomerulus together with the IgG anti-HCV that was previously bound in circulation or subsequently fixed through an "in situ" binding mechanism [43].

In conclusion, the pathogenetic scenario of the disease is dominated by the chronic stimulation by HCV infection sustaining the synthesis of IgM rheumatoid factor and consequently of cryoprecipitable ICs, an abnormal kinetics with tissue deposition of the HCV-containing ICs conjugated with an ineffective cryoglobulin clearance by monocyte/macrophages implicated in perpetuating glomerular damage, and a subclinical, smoldering lymphoproliferative disorder.

Clinical Features

Kidney Involvement. A consistent proportion of patients present with isolated proteinuria (<3 g/24 h) usually with microscopic hematuria (about 35 % of cases). Nephrotic syndrome (20–25 %) and acute nephritic syndrome (10–20 %) are other possible clinical features at presentation. Some patients present with a mixed nephrotic and nephritic syndrome. Ten percent of patients *present* with macroscopic hematuria, 10–20 % show chronic renal insufficiency, 10 % have acute renal failure, and 5 % present with oligoanuria [15].

The majority of patients with renal involvement (and, more specifically, about 80 % of patients with diffuse membranoproliferative glomerulonephritis) have a monoclonal IgM–k with polyclonal IgG cryoglobulin [15].

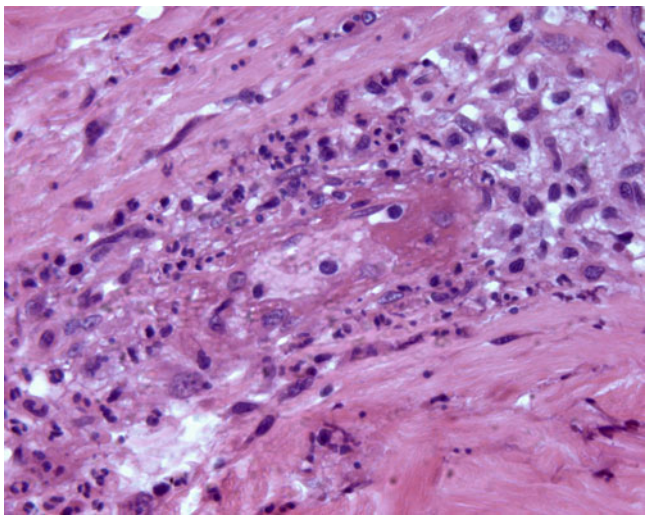

Fig. 7.1 Skin histology. Cryoglobulinemic vasculitis with fibrinoid necrosis of the capillary walls with pyknotic nuclei and nuclear debris of granulocytes and mononuclear cell infiltrates

Skin Involvement. Purpura, a very common manifestation with a prevalence at presentation of 70 %, frequently leads the patients to the first clinical observation. Lesions range from 2 to 10 mm in diameter, are initially bright red, then darken and gradually fade over a week. The most common localization is the lower extremities, but the abdomen is also frequently affected. Purpura is orthostatic, generally intermittent, ranging from isolated petechiae to severe vasculitic lesions, and is often complicated by torpid ulcers of the leg and the ankle. They are favored by a number of cofactors, including chronic venous insufficiency, prolonged standing, muggy weather, and hemorheological disturbances due to high cryoglobulin concentration. The leukocytoclastic vasculitis is the histopathological hallmark of mixed cryoglobulinemia. The term refers to the breakdown of white blood cells in lesional tissue, and, specifically, to the characteristic nuclear debris, called "nuclear dust," observed at the optic microscopy examination (Fig. 7.1). This lesion is not specific for mixed cryoglobulinemia. However, immunofluorescence studies reveal vascular deposition of IgM and complement components in the affected skin, and electron microscopy examination confirms the presence of structured electron-dense deposits.

Rheumatologic Manifestations. Arthralgias, usually bilateral and symmetric, are present in 40–90 % of patients. The clinical pattern may vary largely among patient series referred to different tertiary care facilities [12, 14, 15]. Arthritis is relatively rare, characterized by two main patterns: oligoarthritis [12] usually mild and nonerosive involving medium-sized and large joints and symmetrical polyarthritis mimicking rheumatoid arthritis. Xerostomia and xerophthalmia are present in 30 % of cases, but very few cases fulfill the classification criteria for Sjögren's syndrome.

Gastrointestinal Involvement. Chronic hepatitis is the rule in HCV-infected patients, evolving in cirrhosis in a quarter of patients. The clinical course of chronic hepatitis is generally mild to moderate. Hepatocarcinoma is a less frequent complication than the subjects with HCV-related hepatitis without MC [12]. Episodes of diffuse abdominal pain can be observed in any time of the disease, especially during the flare-up episodes, and range from 7.5 to 11 %. Abdominal pain is an expression of mesenteric vasculitis as confirmed in occasional laparotomy [15].

Neurological Disorders This is a major cause of clinical disability making the quality of patient's life severely compromised. Peripheral neuropathy is clinically present in 10–30 % of cases at the onset of the disease and increases in the follow-up. If peripheral neuropathy is searched by mean of electrophysiology techniques, it can be detected in the vast majority of the patients. The common symptoms are paresthesias with painful or burning sensations of the lower limb with nocturnal exacerbation. Peripheral neuropathy is sensory or sensory motor and mainly asymmetric, more often presenting as a mononeuritis multiplex. Axonal damage is thought to be related to cryoglobulin occlusion or perivascular inflammation of vasa nervorum. Anti-neuronal antibodies could play an additional role [44]. Seizures and dysarthria have been reported on occasion [15]. Exceptionally, cerebral hemorrhage, as an expression of brain vasculitis, can occur [13, 15].

Cardiovascular Manifestations. Myocardial infarction, angina, and myocardial insufficiency are generally absent at the onset of the disease but became progressively more frequent in the follow-up as a consequence of a high prevalence of hypertension. Coronary vasculitis, congestive heart failure, and episodes of pericarditis can occur in the course of the disease [15].

A hyperviscosity syndrome related to high levels of cryoglobulins, which represents a major symptom of a monoclonal cryoglobulinemic syndrome related to a hematologic disorder, is rare in mixed cryoglobulinemia [12].

Hypertension The prevalence of hypertension at the time of renal biopsy is about 75 %. During the follow-up, the arterial pressure is hardly controlled. More than 50 % of the patients involved in a multicenter trial who were followed up for at least 10 years showed pathologic values [15].

Bone Marrow Abnormalities This is the most frequent neoplastic complication and is related to the lymphoid infiltrates, in the form of "early lymphomas," found in liver, spleen, and bone marrow. These infiltrates tend to remain unchanged even for decades. The term "monotypic lymphoproliferative disorder of undetermined significance" has been coined. In about 10 % of cases, an overt lymphoma can develop.

Lung Involvement It is usually asymptomatic, but diffuse interstitial pulmonary fibrosis can be observed on occasion. Alveolar hemorrhage is a rare event.

Laboratory Markers

Biochemical analyses usually reveal type II cryoglobulins (IgM–k, polyclonal IgG), IgM RF ranging from slight to extreme increase, and low values of C3, C4, and C1q. C4 levels are usually very low, sometimes undetectable, and represent a valid surrogate marker of cryoglobulinemia. Anemia is the rule, due to chronic inflammation and, when present, renal failure. C-reactive protein and erythrocyte sedimentation rate are usually high [15]. Inexplicable fluctuations of erythrocyte sedimentation rate and/or immunoglobulin levels sometimes reflect untimely processing of the blood specimen. A sudden disappearance of cryoglobulin and rheumatoid factor or a spontaneous increase of C4 can announce the development of an overt lymphoma.

A critical issue is cryoglobulin detection. A negative cryoglobulin detection does not *a priori* rule out the diagnosis, since cryoglobulins precipitate when the test sample cools below 37 °C during transit to bedside to laboratory, leading to false-negative results.

A recommended method for detection of cryoglobulins is as follows. Venous blood is collected without anticoagulation in a pre-warmed syringe, transferred in warmed tubes, and stored at 37 °C until it clots. After centrifugation at 37 °C, the serum is collected and stored at 4 °C for 1 week. After separation and washing, the cryoprecipitate is quantified and expressed as percentage of precipitate/serum volume. The components of cryoprecipitate are determined by immunoelectrophoresis or immunofixation. Processing of anticoagulated blood can produce false-positive results due to cryofibrinogen or heparin-precipitable proteins. If the disease is highly suspected and the cryoglobulins cannot be detected by this method, a simple technique of precipitation in hypoionic medium to detect the so-called hypocryoglobulins can be used [45]. Could you give a reference for this? Please refer to n° [15]. Serum collected after centrifugation is mixed volume to volume with be-distilled water before storage [15].

The levels of serum cryoglobulins do not correlate with the severity of the disease. Low levels of cryocrit, even in trace amounts, such as those found using the method of hypocryoglobulin detection, can be associated with severe cryoglobulinemic syndrome. Conversely, high cryocrit values can be related to a mild clinical course.

With sensitive methodologies, such as immunoblotting or two-dimensional polyacrylamide gel electrophoresis, oligoclonal IgM with polyclonal IgG can be detected. This particular pattern represents an intermediate, possibly evolutive state from types III to II mixed cryoglobulin and is called "types II–III cryoglobulin."

Renal Histology

Light Microscopy

Despite the variety of clinical presentations, three main glomerular patterns can be recognized [15].

Diffuse Membranoproliferative GN (About 80 % of Cases). This is the typical pattern of cryoglobulinemic glomerulonephritis (Figs. 7.2 and 7.3) characterized by duplication of glomerular basement membrane, interposition by mesangial cells, and especially mononuclear leukocytes/macrophages, subendothelial (but also mesangial) deposition of immune reactants, mesangial expansion and proliferation with intracapillary leukocyte accumulation, and endoluminal hyaline thrombi (corresponding to cryoglobulin precipitates). More than 50 % of glomeruli are involved. Immune deposits are eosinophilic and PAS positive, red/orange by trichrome stain. A centrolobular sclerosis can be detected in half of cases. Extracapillary proliferation is relatively uncommon and usually affects a minority of glomeruli. Exceptionally, crescents involve more than 50 % of glomeruli. Necrosis of the glomerular tuft can be found on occasion.

Focal Membranoproliferative Glomerulonephritis (About 10 % of Cases). The pattern of cryoglobulinemic glomerulonephritis is typical but involves less than 50 % of glomeruli. Cellular proliferation and exudation and thickening of the capillary wall are mild, with irregular distribution within the same glomerulus and among glomeruli. Endoluminal thrombi are less frequently found than in the diffuse form.

Mesangial Proliferative Glomerulonephritis (10 % of Cases). It is characterized by diffuse mesangial expansion and proliferation without exudation and endocapillary proliferation. Rarely isolated proteic endoluminal thrombi can be detected.

Interstitial and Vascular Lesions Interstitial leukocyte infiltration, usually focal, is present more frequently in the membranoproliferative forms and is correlated with the intensity of the proliferative glomerular lesions. Interstitial fibrosis, mainly focal, is almost invariably present in the membranoproliferative forms. Arteriosclerotic lesions are present in one third of cases with no differences among groups. Arteritis is relatively rare.

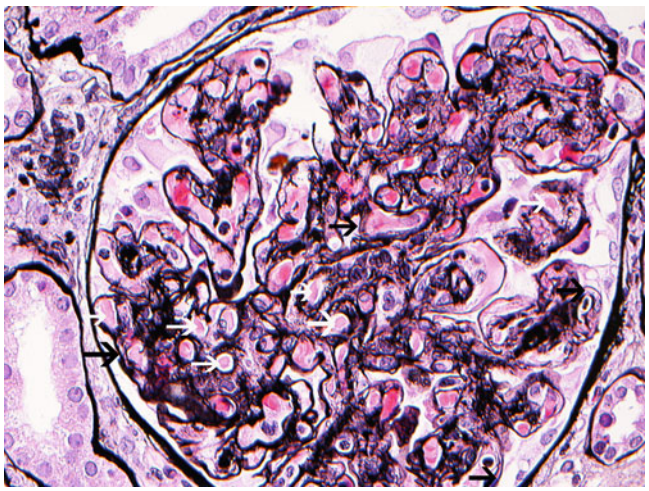

Fig. 7.2 Light microscopy. Silver stain (×40) showing a membranoproliferative pattern of injury with double contour formation (*black arrows*). Many loops contain pale eosinophilic material consistent with cryoglobulins (*white arrows*)

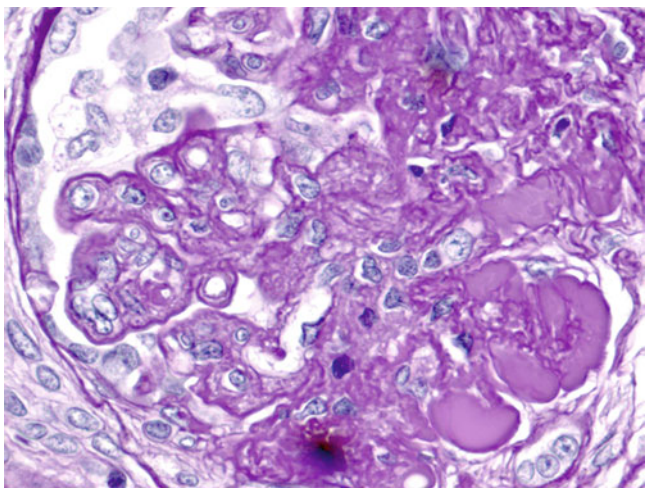

Fig. 7.3 Light microscopy ×1,000. PAS + ve endoluminal pseudothrombi and reduplication with cellular interposition of the glomerular basement membranes

Immunofluorescence Microscopy

Diffuse, pseudolinear peripheral capillary wall and mesangial staining for IgM, IgG, and C3 are usually found, with a relatively stronger staining for IgM and k (as compared to lambda) light chain, which reflects the typical clonal restriction of type II cryoglobulins (Fig. 7.4). Prominent IgM and IgG staining is detected in thrombi. Fibrinogen is found in vessel walls when a vasculitis is present.

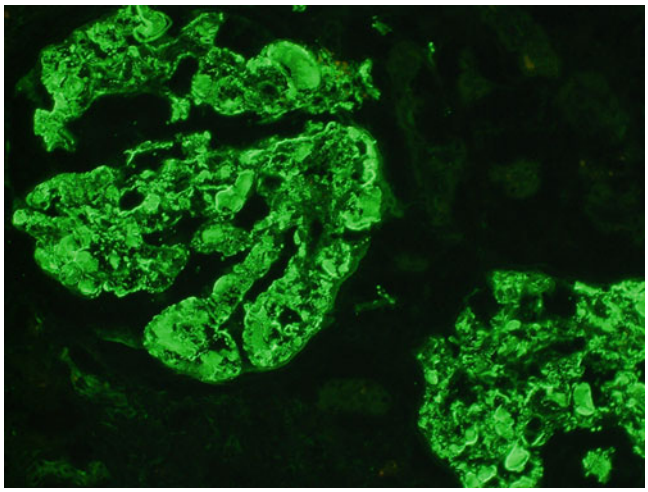

Fig. 7.4 Immunofluorescence studies showing cryoglobulins in many capillary loops that are positive for IgM

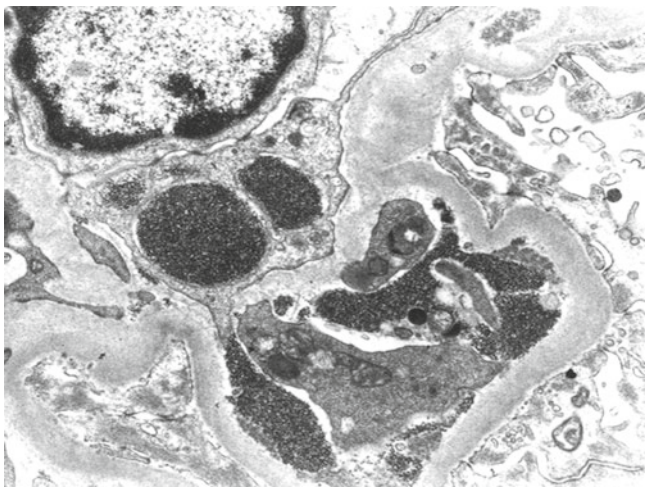

Fig. 7.5 Electron microscopy. Monocyte interposition into the glomerular basement membrane. Monocytes are engulfed with cryoglobulins

Electron Microscopy

Electron-dense deposits are detected in subendothelial and mesangial areas. Segmental subepithelial deposits are occasionally seen. Deposits can be also detected within phagolysosomes of intracapillary macrophages. Interposition of glomerular basement membrane by monocytes is seen (Fig. 7.5). As discussed above, the role of these cells in cryoglobulin removal is a controversial issue. Cryoglobulin deposits often display an organized substructure: short, curved, thick-walled tubular structures with a diameter of about 30 nm which appear annular on cross sections (Fig. 7.6).

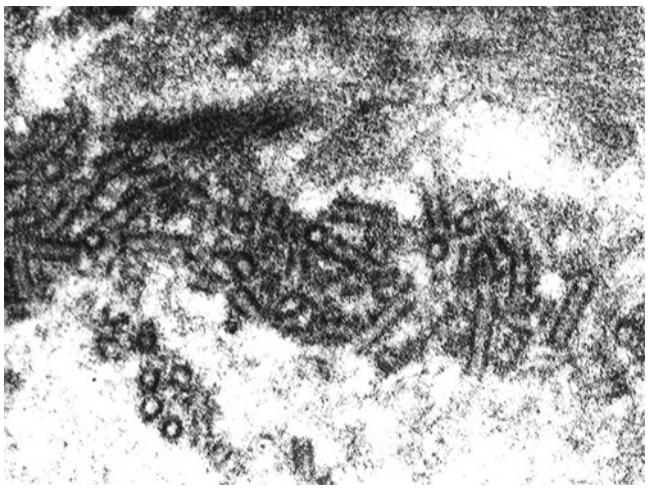

Fig. 7.6 Electron microscopy. Magnified pictures of the characteristic tubular and anular structures (about 30 nm of diameter) of mixed cryoglobulins

Atypical Features

A few cases of membranoproliferative glomerulonephritis present, in addition to the common immunofluorescence pattern, deposits of IgA with prevalent mesangial but also parietal localization [15]. In a small percentage of patients, a pattern of membranous nephropathy can be detected. It is characterized by the same morphology of the primary form, with prevalent subepithelial deposits of IgM, IgG, and C3 (with crystalloid structured deposits at the electron microscopy examination). Segmental features of membranoproliferative glomerulonephritis together with membranous nephropathy can be detected in rare cases.

Clinical-Pathologic Correlations

The patients with diffuse membranoproliferative glomerulonephritis show a stronger C4 hypocomplementemia and higher levels of proteinuria than the patients with other patterns [4]. Hematuria and hypertension are equally distributed among the various histologic groups. Serositis, hepatosplenomegaly, leukopenia, peripheral neuropathy, and cardiac involvement are more frequently observed in association with the diffuse membranoproliferative pattern [15].

Prognostic Factors

Significant prognostic variables include age, male gender, creatinine and proteinuria at the time of renal biopsy, number of syndromic relapses, and poor blood pressure control [15]. Survival at 10 years is about 80 % nowadays [15], much better than a decade ago [13]. Kaplan–Meier survival curves are worsened by basal creatinine value >1.5 mg/dL (133 μ[mu]mol/L). Cardiovascular disease is the cause of death in over 60 % of cases.

Non-Cryoglobulinemic HCV-Associated Glomerulonephritis

Due to the diverse histologic patterns and the specific therapeutic implications (see below), biopsy should be done, if not specifically contraindicated, in every patient with mixed cryoglobulinemia who presents with urinary abnormalities or otherwise unexplained renal insufficiency.

A number of alternative renal manifestations have been reported in hepatitis C-infected patients, including membranous nephropathy [46], focal segmental sclerosis [47], IgA nephropathy and other proliferative glomerulonephritis [48], non-cryoglobulinemic membranoproliferative glomerulonephritis [49], fibrillary and immunotactoid glomerulopathies, and anti-cardiolipin-associated thrombotic microangiopathy [50].

Treatment

Antiviral Therapy

Discovery of an association between mixed cryoglobulinemia and HCV and the possible pathogenetic implications prompted researchers to develop new approaches to disease control by eradicating the infection. Interestingly, due to its antiproliferative and immunomodulatory effects, interferon-α[alpha] (IFN) was used, and it achieved favorable results in the treatment of mixed cryoglobulinemia even before HCV had been identified [51].

Antiviral treatment of mixed cryoglobulinemia followed the evolution of treatment of chronic hepatitis C. However, studies on antiviral therapy of mixed cryoglobulinemia are more difficult to compare because of a greater heterogeneity in treatment regimens, patient selection, response evaluation, and short follow-up [52–60]. The presence of circulating cryoglobulins should not influence the response to IFN in patients with chronic HCV infection [53–55], albeit cryoglobulinemia probably favors HCV entrapment inside mega complexes which are phagocytosed, but not degraded by infected phagocytes [41] that are potential virus reservoirs. Actually, in a very large cohort of nephritic patients, less than 10 % of subjects reached sustained viral response (SVR), i.e., undetectable HCV-RNA 24 weeks after the end of treatment [56], using standard interferon [15].

With regard to the effects of IFN on syndromic symptoms, a number of retrospective studies have reported some clinical improvement in about 60 % of patients with mixed cryoglobulinemia [57–62]. In responsive patients, reduction of HCV-RNA heralded the decline of cryocrit, IgM, and RF activity. Notably, in Dammacco's study [59], the concomitant administration of prednisone resulted in earlier responses of cryoglobulinemic symptoms than with interferon-α[alpha] alone.

The attempts to identify clinical or serological pre-therapy variables predictive of interferon-α[alpha] response are controversial. In Mazzaro's study [62], the lack of response was associated with genotype 1b, liver cirrhosis, and high cryoglobulin levels. The HCV-RNA levels might also affect response. Casato et al. [61] found a correlation between detection of anti-HCV core antibodies and good response. Purpuric lesions tend to respond rapidly while neuropathy and nephropathy are the slowest to respond.

In these studies, the benefit of IFN monotherapy was transient, and relapse was very frequent after therapy discontinuation [53, 57–72] and often combined with the reappearance of viral RNA, occasional worsening of skin ulcers (possibly due to the antiangiogenic effects of alpha interferon), precipitation of renal failure and nephrotic syndrome, psychiatric manifestations, peripheral neuropathy, and autoimmune hepatitis.

Trials with IFN plus ribavirin (RBV) showed better performance in both viral eradication and cure of symptoms [73–78]. Ribavirin, an oral guanosine nucleoside analog that induces a significant decrease in serum aminotransferase, is unable to achieve a long-term virological and biochemical response when given alone. It has rarely been used as monotherapy in patients with HCV-associated glomerulopathy, and it has achieved limited response in both immunocompetent and transplanted patients.

At present, ribavirin is almost only used in combination with interferon-α[alpha] [73–75, 77], but only few studies have been reported in the literature. Calleja et al. [74] treated 13 patients with interferon-α[alpha] and ribavirin for 12 months. All patients had previously received interferon-α[alpha] in monotherapy. Five of the eight nonresponders to interferon alone showed an initial response, but this was sustained in only three cases after 12 months. Loss of response was accompanied by the reappearance of HCV-RNA, worsening of clinical manifestations and an increase in the levels of cryoglobulins and hepatic enzymes.

All nine patients who were refractory to interferon-α[alpha] treated by Zuckerman et al. [75] with interferon-α[alpha] and ribavirin for 6 months achieved a substantial improvement in mixed cryoglobulinemia-related symptoms although polyneuropathy was resistant to treatment.

Sustained response has also been reported by Donada et al. [73]. In Mazzaro's report [77], 18 % of patients who were "relapsers" to the first interferon-alpha treatment had complete recovery from viral infection and from all signs and symptoms of the disease after 1 year of combined treatment. A marked clinical improvement occurred in the

majority of cases within 2 or 3 weeks of treatment. Regrettably, 70 % of patients relapsed within a few weeks of the end of treatment.

Finally, a single study showed an improvement in renal histology in a few patients with biopsy-proven GN who underwent antiviral combination therapy with alpha interferon and ribavirin [78].

Care should be taken when administering ribavirin in patients with severe renal impairment as this drug is not dialyzable. Mild dose-related hemolytic anemia is commonly observed in patients treated with the combination therapy.

Antiviral treatment with IFN-α[alpha] also proved to be effective in determining some regression of monoclonal B-cell expansion. Several series reported that antiviral treatment with interferon-α[alpha] alone or with ribavirin is effective in HCV-associated indolent and marginal zone lymphoma accompanied with cryoglobulinemia [79–82].

Further progress with regard to response to therapy has been obtained using pegylated interferon (PEG-IFN) which is endowed with improved pharmacokinetic characteristics. PEG-IFN alone or combined with ribavirin has proven to be more effective than interferon-α[alpha] alone, or plus ribavirin, in patients with HCV infection, especially those infected with genotype 1b. Currently, PEG-IFN in combination with ribavirin is the standard treatment for chronic hepatitis C, with 41–48 % SVR in genotype 1 and approximately 80 % SVR in genotypes 2 and 3.

This combined therapy also opened new opportunities for the treatment of mixed cryoglobulinemia and is presently regarded as the first-line antiviral treatment for these patients.

Indeed, patients with HCV-related mixed cryoglobulinemia seemed to benefit from this new combination therapy much more than from standard therapy, even though, as in Mazzaro's study [83], 44 % of patients relapsed a few weeks after the end of therapy. However, Cacoub et al. [84] obtained remarkable results in clinical, virological, and serological parameters in 9 patients with cryoglobulinemic vasculitis.

Nowadays, the therapeutic strategy for HCV eradication in chronic hepatitis C with the combination of PEG-interferon and ribavirin is based on genotype. Genotypes 2 and 3 can be eradicated in 75–80 % of patients (obtaining sustained virological response) by a 6-month combined regimen. Conversely, genotype 1b requires a 48-week course of therapy with weekly injections of either PEG-IFN-α[alpha]2a or α[alpha]-2b and 1,200 mg of oral ribavirin daily. In this case, the rate of sustained virological response is only 41–48 %, and in the absence of a decrease in HCV-RNA concentration of 2 logs at week 12, the chances of responding is further reduced (up to 20 %). This figure is even lower in African Americans and individuals with advanced fibrosis or HIV coinfection. Therefore, a considerable percentage of patients with HCV-associated mixed cryoglobulinemia are expected not to achieve virological response and to have persistent cryoglobulins and symptoms [85, 86]. Moreover, as emphasized above, due to interferon immunomodulatory properties, antiviral treatment can induce immune-mediated adverse events, such as peripheral sensory-motor neuropathy, thyroiditis, rheumatoid-like polyarthritis, and other vasculitic manifestations [57, 87–92] that may precipitate or exacerbate preexisting, subclinical disorders. Unfortunately, there are no available parameters for predicting these complications. Thus, antiviral therapy should be very carefully administered to patients with mixed cryoglobulinemia-related peripheral neuropathy, active skin ulcers, or severe nephritis. In addition, several patients may present clinical conditions, including advanced age, decompensated cirrhosis, major uncontrolled depressive illness, significant coronary heart disease, and untreated thyroid disease [56], which make antiviral treatment not recommended.

In conclusion, acute nephritic syndrome, extensive cutaneous ulcers, widespread vasculitis, and hyperviscosity syndrome actually do require more prompt and aggressive treatment, even due to the uncertain response to antiviral therapy. In these settings, antiviral therapy may only have a role in sequential or combined treatment schedules [52, 93–95].

In clinical practice, antiviral treatment of mixed cryoglobulinemia should be tailored to the individual patient according to the progression and severity of clinical manifestations [93, 94].

Randomized controlled trials of adequate size and follow-up are needed to clarify whether higher virological or clinical response rates can be obtained by extending treatment duration (up to 48 weeks for HCV genotypes 2 or 3 and 72 weeks for HCV genotypes 1 or 4).

Lastly, the role of Telaprevir, the promising peptidomimetic inhibitor of NS3–4A protease, that is able to increase the rate of SVR in patients with genotype 1 from 41–48 % to 61–68 % when given in combination with standard INF/Ribavirin regimen [96], has not yet been explored in cryoglobulinemic patients.

Standard Immunosuppression

Despite the unquestionable evidence of a viral etiology, immunosuppression is still regarded as the first-line intervention if renal involvement is severe. Immunosuppression may also be taken into consideration in patients failing to respond to interferon treatment or in acute immunological flares. In these cases antiviral treatment is usually either insufficient to control renal disease or even detrimental. In many centers, high-dose corticosteroids, plasmapheresis, and, in more severe cases, cytotoxic therapy are commonly administered, but no trials have prospectively evaluated these treatments. These therapeutic approaches may cause

an increase in the levels of viremia, thus exacerbating chronic hepatitis C disease. Nevertheless, renal disease is often a compelling indication for these treatments. Purpura is the most frequent indication for corticosteroids, which remain the most amenable to therapy. Another indication is peripheral neuropathy, even though its general refractoriness to any treatment is widely recognized. Patients who need such therapies usually require multiple courses. Symptoms often recur after periods of quiescence or flare after periods of stability.

By examining the data of a robust sample of patients with cryoglobulinemic nephritis [15] and comparing them to a similar cohort described 10 years earlier [14], a better survival rate (80 % vs 50 % at 10 years) was shown with cardiovascular events as predominant cause of death instead of infections and hepatic failure observed in the previous study. There could be several reasons for this changing feature. The better survival profiles which have been obtained over the last decade could be the consequence of aggressive but time-limited intervention on the acute renal involvement, together with the long-term antiviral therapy that was used to control moderate manifestations of renal and extrarenal involvement.

More specifically, with regard to glucocorticoids, long-term administration of low-medium doses (0.1–0.5 mg/kg/day) of steroids alone was largely used in clinical practice for vasculitis symptoms or pain, and data from small case series support their effectiveness in controlling the flares of disease [59, 97–99]. However, the result of controlled studies have been conflicting [59, 99–101], and no reports evaluating the effectiveness of long-term glucocorticoid administration are available, while side effects of long-lasting steroid therapy are known to be serious and irreversible. Glucocorticoids, also in pulse doses, are frequently used in association with other drugs [59, 97–103]. As far as cytotoxic drugs are concerned, while there is a lack of significant experience with the use of cyclosporine, azathioprine, methotrexate, and mycophenolate mofetil, and what there is remains anecdotal, cyclophosphamide was frequently used in mixed cryoglobulinemic patients [104–112], especially in association with apheresis. The rationale of cyclophosphamide administration in this setting is based on the assumed need to obtain temporary immunosuppression after acute apheretic treatment, but neither randomized controlled data nor large cohort studies support this postulate. Cyclophosphamide has been taken into consideration mainly for the treatment of membranoproliferative glomerulonephritis or severe polyneuropathy. The majority of data was collected before HCV was identified as a major determinant for mixed cryoglobulinemia, and the risks of using cyclophosphamide in patients with HCV infection are poorly defined. The use of cyclophosphamide in an i.v. bolus is currently believed to be safer than oral administration, **reducing** the cumulative dose [113]. Careful monitoring of HCV infection and liver function after cyclophosphamide administration is recommended.

With regard to plasma exchange, several different apheretic procedures have been applied in the treatment of various clinical conditions associated with mixed cryoglobulinemia. No controlled trials or large cohort studies are available on this issue. Data can only be drawn from case reports [97, 104–120], which were often published before the association between HCV and mixed cryoglobulinemia was discovered, and furthermore, the apheresis methods significantly differ in the various studies.

Several observations support the role of apheresis both in improving acute renal disease [115–117] and in treating neuritis [102, 115] and ulcers [97, 120]. With regard to hyperviscosity syndrome, despite the lack of data from controlled randomized studies, which are obviously difficult to obtain in this rare condition, apheresis procedures remain the treatment of choice. A combination of immunosuppressant drugs in association with apheresis has been supported by some clinical reports [107–112], but is no longer considered mandatory.

Among the available apheretic approaches, double filtration (which selectively removes molecules greater than 600 kDa) is the procedure of choice [120]. No agreement exists about intensity, duration, and frequency.

In clinical practice, Cyclophosphamide (1.5–2 mg/kg/day given orally for 3 months, or 0.5–1 g administered intravenously every 2–4 weeks), in association with oral steroids (0.5–1 mg/kg/day for 1 month with subsequent tapering by 2.5–5 mg/week) often preceded by 3 pulses of 10–15 mg/kg methylprednisolone, represents the most widespread combination therapy in the severe manifestations of mixed cryoglobulinemia or in patients with uncontrolled symptoms.

The less toxic mycophenolate mofetil given for 6 months can be an alternative option.

It has recently been claimed that Cyclosporin is able both to interfere with the polymerase binding affinity to viral RNA [121] and to achieve a significant decrease in viral load after a few months of administration in patients with HCV-associated non-cryoglobulinemic arthritis.

Plasma exchange, especially double-filtration plasmapheresis, continues to be successfully used in escalation protocols for rapidly progressive glomerulonephritis, skin ulcers, sensory-motor neuropathy, widespread vasculitis, and hyperviscosity syndrome. A recommended scheme consists of a procedure every other day for 2 weeks followed by two procedures a week for 2 weeks and one procedure a week for a month [113].

A standard course of combined antiviral therapy is indicated following immunosuppression in order to attenuate viral replication.

Novel Therapeutic Strategies

A critical aspect in the development of novel therapeutic strategies is the validation of treatment options that are alternative to steroids given alone or in combination with immunosuppressive drugs in the acute manifestations of cryoglobulinemia. Such alternatives could further prolong patient survival (which has already been improved in the last decade [15]) by reducing cardiovascular risks.

Among the biologic drugs currently used in immunopathology that have raised hopes for new therapeutic approaches for patients with systemic signs of severe vasculitis, Rituximab (RTX) and Infliximab are the only two that have been employed in the treatment of mixed cryoglobulinemia. Encouraging results have been obtained with Rituximab in open studies and single case reports [122–129]. Initial results with Infliximab [130–132] did not support the extensive use of this drug in mixed cryoglobulinemia.

Rituximab is a humanized mouse monoclonal antibody directed at CD20, a B-cell-specific membrane protein. Presently, the published cases of mixed cryoglobulinemia treated with Rituximab account for more than 300 patients, deriving from 20 case reports, 13 clinical studies with at least five patients [122–124, 126, 129, 133–140], three trials in which Rituximab was associated with PEG-IFN plus Ribavirin [95, 139, 141], one retrospective study on the effects of the combination of Rituximab with antiblastic therapy [142], and, finally, three studies [135, 143, 144], two randomized [143, 144], that will be considered separately. General data have been collected in [140].

The lymphoma protocol, consisting of 4 weekly infusions of 375 mg/m^2, was usually used. Two more doses at 1-month intervals were administered in two studies [124, 129]. No additional therapy was given in 19 nephritis patients [124, 126, 129], suggesting that some severe cases could be treated ab initio without steroids.

Rituximab was shown to improve or cure various clinical manifestations of mixed cryoglobulinemia, including fatigue and arthritis (in almost all patients), skin manifestations (purpura, skin ulcers), and glomerulonephritis in 75–90 % of cases, peripheral neuropathy in 70 %, abdominal vasculitis in 80 %, and hyperviscosity syndrome. Rituximab was also reported to be effective in some life-threatening conditions.

Glomerulonephritis and skin ulcers usually improve within 3 months after the beginning of therapy. Complete healing takes longer. One study showed that both sensitive and motor neuropathy improved 1–5 months after RTX, and the electromyography picture was stable or had improved [136]. Moreover, RTX treatment reportedly decreases serum cryoglobulins and rheumatoid factor and increases C4 levels, with the disappearance of bone marrow B-cell clonal expansion [95, 124, 129, 145, 146].

Short-term infusion reactions following RTX administration do not appear to be more frequent in mixed cryoglobulinemia than in other immunological-mediated disorders including rheumatoid arthritis. The risk of serum sickness [147] is negligible. Severe adverse effects (1 %) and disease worsening (2 %) are relatively rare [140]. Dropout due to adverse events is less than 5 %. One French group reported that patients with high cryocrit may experience severe flares of vasculitis within 2 days of RTX infusion, especially if the rheumatoid arthritis scheme (two infusions of 1 g/day 2 weeks apart) is used [148]. Plasma exchange before RTX administration has been suggested in patients with high cryocrit levels [148]. However, in clinical practice, slow administration of the lymphoma dose (375 mg/m^2) over 12–24 h or administration of half a dose per day in two consecutive days given together with a premedication with steroids, antihistamine drugs, and paracetamol reduces the risk of such reactions [124, 129, 140]. Similar precautions should be taken for patients with a history of heart failure or arrhythmia. Aspirin administration has been proposed for cardiovascular risk [122, 124]. RTX treatment does not significantly affect HCV viral load nor parameters of liver impairment. Presently, there are no data supporting a substantial risk of liver toxicity directly caused by RTX or due to HCV reactivation, even in the long term [129]. Moreover, RTX was successfully prescribed to mixed cryoglobulinemia patients with liver cirrhosis. Patients showed improvement of cryoglobulinemia-related symptoms as well as liver function despite a transient increase in serum HCV-RNA [137, 149]. By contrast, RTX is definitely contraindicated in HBsAg-positive patients because of the possible severe reactivation of HBV infection. In HBsAg-negative/anti-HBc-positive patients (potential carriers [150]), RTX should be used in association with antiviral therapy even if HBV DNA is negative.

Severe infections (lethal disseminated cryptococcosis and severe bacterial pneumonia) were reported after RTX administration in severely immunocompromised renal transplant recipients with type III cryoglobulin-related graft dysfunction [128, 133]. However, except for transplanted patients, no life-threatening infections have been reported in typical HCV-related mixed cryoglobulinemia after RTX administration.

Special care should be taken for the prevention and management of infections, especially in patients who have previously been treated with immunosuppressants or steroids, or those with low serum levels of immunoglobulins. On the contrary de novo hypogammaglobulinemia has rarely been described after RTX in mixed cryoglobulinemia or in other autoimmune diseases [151]. Panniculitis [122], neutropenia [95, 122, 126, 152], and retinal vascular occlusion [122] have seldom been reported as side effects of RTX treatment in mixed cryoglobulinemia.

Duration of response is difficult to define due to the lack of long-term follow-up data in most reported studies. Short-term relapse within 3–4 months was reported in a minority of patients, while long-term response lasting more than 1 year is the most frequent outcome. A proposed strategy [124] to reduce or delay relapse is to give two more infusions of Rituximab 1 and 2 months after the standard 4-week course. Relapses that usually occur after 6–15 months may be delayed until 12–36 months using the "4 plus 2" protocol [124, 129].

Re-treatment with RTX at disease relapse proved to be effective again in most cases [122, 125, 126, 138, 153, 154].

Maintenance therapy with Rituximab may be taken into consideration in subjects with severe nephritis or untreatable gastrointestinal manifestations [126, 128, 138].

Results of a phase II, single-arm, multicenter study of low-dose Rituximab, i.e., 250 mg/m^2 given twice at 1-week intervals, was recently published [135]. Twenty-seven patients were enrolled, but clinical response was evaluable in 19. The 13 patients who did not reach the end of follow-up at 12 months (8 because of relapse and 5 due to lack of response) were given additional therapy. Only six patients completed the 12-month follow-up. The complete response rate of nephropathy (and also neuropathy) was 39 %. This scheme that was previously designed for patients thought to be too compromised for receiving a standard dose [125] is not recommended for either polyneuropathy or especially for cryoglobulinemic nephritis.

A large multicenter, randomized, long-term controlled trial was recently carried out on mixed cryoglobulinemic patients who failed or were not eligible for antiviral therapy. The trial compared Rituximab (with or without low-dose steroids) to conventional immunosuppressive treatment (corticosteroids, cyclophosphamide, azathioprine, or plasma exchange). The results of the trial support the superiority of RTX [143]. A single-center open-label, randomized controlled trial of Rituximab compared to the best available immunosuppressive treatment (maintenance or increase in therapy) confirmed the greater effectiveness of Rituximab in patients in whom antiviral therapy had failed to induce remission [144].

Selective depletion of IgM-producing B cells represents the basis for RTX treatment in mixed cryoglobulinemia, a disorder in which the autoimmune process can become independent from the virus triggering and may take on a predominant role in the pathogenesis of the disease. Rituximab acts downstream of the disease trigger more selectively than the conventional immunosuppressive treatments [124, 129]. In this context mixed cryoglobulinemia-associated nephritis represents a unique condition of immune-mediated disorder in which Rituximab specifically targets the nephrotoxic Ig-producing cells. Moreover, since peripheral CD20-positive B cells serve as a reservoir for persistent HCV infection [35], administering an anti-B-cell antibody such as Rituximab might be beneficial to patients with chronic hepatitis C in order to eliminate the lymphoid reservoirs. When given together with antiviral agents, this would assure a synergistic effect on HCV clearance from the blood. Finally, RTX has been reported to restore B-cell homeostasis and reverse Th1/Th2 imbalance by decreasing clonal VH1–69 memory B cells and by expanding regulatory T cells [155].

In conclusion, Rituximab given at the standard dose of 375 mg/m^2 four times at 1-week intervals appears to be a safe and effective therapeutic option in symptomatic patients with HCV-associated mixed cryoglobulinemia with glomerulonephritis and/or severe vasculitis affecting the skin and peripheral nervous system. These are potentially invalidating manifestations and their typical clinical course is characterized by frequent flares, requiring repeated interventions. These patients are at high risk of cumulative toxicity from current treatments and also of increasing damage due to active disease. Limited experiences with other novel agents (including Imatinib and Tocilizumab) have been recently reported [156, 157].

Combined Antiviral and Anti-CD20 Therapy

It has been suggested that the combination of antiviral and RTX therapy exerts a synergistic effect [94, 95, 146, 152]. These studies are retrospective and the examined cohorts are heterogeneous. They include both patients with severe vasculitis who were nonresponders to antiviral therapy, and IFN-naïve subjects who received Rituximab plus PEG-IFN 2 alpha and ribavirin.

A prospective, nonrandomized cohort study showed that as compared to patients receiving PEG-IFN + RBV [95], patients treated with combined antiviral drug-Rituximab therapy required less time to reach clinical remission and had a higher rate of cryoglobulin clearance, and that 50 % of patients achieved sustained virological response. These results have been confirmed by another group [139].

The optimal order of sequential treatment remains undefined and should be related to the condition of the individual patient. Administering combined therapy ab initio is poorly tolerated. Rituximab should be given first and antiviral therapy should be added later (once the efficacy and safety of Rituximab have been assessed), especially in naïve patients with severe clinical manifestations.

A Therapeutic Algorithm

Based on general experience, milder forms of cryoglobulinemia can be managed without immunosuppression.

Combined antiviral therapy with PEG-interferon and ribavirin appears logical, targets the viral trigger, and can be favorably used to control mild to moderate manifestations

Table 7.1 Therapeutic options according to the severity of disease: a proposed algorithm of treatment of mixed cryoglobulinemia and related glomerulonephritis

Disease severity	Cardinal features	First-line treatment
Moderate	Purpura, arthralgia, mild polyneuropathy, and mild mesangial GN	Peg-IFN alpha plus ribavirin[a]
Severe	Membranoproliferative GN, severe multiplex mononeuritis, and skin ulcers	Rituximab[b]
Life threatening	Rapidly progressive GN; CNS, digestive and /or lung involvement, skin necrosis	Steroids, immunosuppressors, PE (+/– PEG-IFN plus ribavirin), and rituximab[b]

GN glomerulonephritis, *CNS* central nervous system, *PE* plasma exchange

[a]PEG-IFN alpha 2a (180 μg) or alpha2b (1.5 μg/Kg) plus ribavirin (1,00–1,200 mg) given temptatively up to 48 weeks for HCV genotype 2 or 3 and 72 weeks for HCV genotype 1 or 4

[b]Rituximab administered preferentially according to lymphoma protocol (4 infusions of 375 mg/sm one week apart). Two more infusions 1 and 2 months later have been proposed to prolong response duration [114, 126]

including mesangial glomerulonephritis with moderate signs of inflammation. Standard therapy for chronic hepatitis C, however, does not achieve sustained effects. Depending on the genotype, some clinicians claim that a 1-year schedule (for genotypes 2 and 3) or a 1½-year schedule (for genotypes 1 and 4) are needed in cryoglobulinemic patients.

In acute immunological flares, antiviral treatment cannot be used alone and in some cases it can even be deleterious. Steroids and immunosuppressive drugs, mainly cyclophosphamide, and plasma exchange are needed. Besides the concerns surrounding the use of immunosuppressants in a virus-triggered multiorgan disease, especially in cases of refractory disease or with disease characterized by a relapsing course requiring prolonged immunosuppression, several patients complain of one or more of the several side effects of conventional immunosuppressive drugs.

Rituximab has earned a definite role in these cases, but its appropriate position in the treatment strategy of mixed cryoglobulinemia remains controversial. The main indications for its use include severe worsening of renal function, mononeuritis multiplex, extensive skin ulcers, and distal necrosis (Table 7.1) [114, 126]. Moreover, it could even be indicated in life-threatening conditions or fulminant presentations, i.e., peripheral necrosis of the extremities, in which plasma exchange and immunosuppressive drugs were considered the only therapeutic tools until a few years ago.

The duration of effects appears to be finite, with mean response duration of 6–12 months. The "4 plus 2" protocol seems to favor a longer lasting response.

While antiviral drugs administered with Rituximab may possibly be synergic, they multiply the adverse effects, thereby making treatment poorly tolerated. Until recently it was believed that staggered administration after a critical phase of vasculitis was mandatory in order to avoid the risk of exacerbating HCV infection, but in most cases, it is probably unnecessary.

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IgA Nephropathy and Schöenlein-Henoch Purpura Nephritis

8

Antonello Pani and Dario Roccatello

Introduction

IgA nephropathy (Berger's disease) is a form of glomerulonephritis characterized by predominantly immunoglobulin A (IgA) mesangial deposits [1–3]. The disease may present with multiorgan involvement (Henoch-Schöenlein purpura nephritis) or in conjunction with other conditions [1, 2, 4, 5].

The main clinical features of Henoch-Schönlein purpura nephritis (HSPN) include purpura, arthritis, abdominal pain, gastrointestinal bleeding, and nephritis, but virtually every organ system can be involved, albeit less commonly [6]. These clinical features are the consequence of systemic, small vessel leukocytoclastic vasculitis caused by deposition of IgA1 in vessel walls.

Some associations with IgAN are well established. These include dermatological (dermatitis herpetiformis and psoriasis), connective tissue diseases (particularly ankylosing spondylitis, rheumatoid arthritis, mixed connective tissue disease, postinfectious arthritis), carcinomas (bronchogenic, laryngeal, mucin secreting), lymphoma (Hodgkin's lymphoma and T-cell lymphomas, including mycosis fungoides), hematologic (cyclic neutropenia, mixed cryoglobulinemia, immune thrombocytopenia, polycythemia), disseminated tuberculosis, bronchiolitis obliterans, inflammatory bowel disease, cirrhosis, celiac disease, and HIV infection (Table 8.1).

Renal manifestations may occur before or during the clinical course of the associated diseases, and a wide variety of glomerular lesions may be present. A heavy proteinuria in IgAN is usually indicative of advanced disease, while a true nephrotic syndrome can be due to the combination of IgAN with a "minimal change disease like" podocytopathy or a membranous nephropathy.

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Epidemiology

For over 40 years, IgAN has been recognized as a major cause of progressive glomerular disease. In 1987 IgAN was defined as the "commonest glomerulonephritis in the world" [7]. However, its prevalence varies considerably among countries, and it is influenced by sex and ethnicity. It is found more often in men and distinctly less in blacks [8]. Renal biopsy and Registry data of all primary glomerular diseases show that the prevalence of IgAN ranges from 20 % up to 50 % in Asia (45 % in China; nearly 50 % in Japan) and 20–40 % in Europe (37 % in Italy, 29 % in Romania, 19 % in Spain). Percentages as low as 2–4 % have been reported in some parts of the USA. However, a prevalence of over 35 % has been observed in American Indians in the Southwest and 14 % in young American Caucasians. In Australia, the general estimated prevalence is 12 %, but in Victoria, due to a relatively liberal policy of renal biopsy, the prevalence is higher, and the incidence is 87 cases per million per year [9].

Local indications for renal biopsy and health screening practices had influenced the epidemiologic data. Early studies carried out in 1975 reported rates of less than 5 % [10] in Great Britain, while 12 years later, Propper et al. reported that in Scotland, 37 % of patients affected by isolated hematuria had IgAN [11]. Based on autopsy studies on the general population, asymptomatic deposition of IgA is estimated at an average of about 10 % [12].

The majority of studies show a male predominance of at least 2:1 [13]. Unlike other glomerular diseases, IgAN and HSPN are uncommon in both African-Americans and in Africans [14]. Some authors have attributed this difference to a different structural property of IgA2 according to different allotypes, i.e., $A_2m(1)$ and $A_2m(2)$. The $A_2m(2)$ allotype that predominates in blacks is more resistant to cleavage and could provide a protective role against IgAN. Nevertheless, the IgAN clinical course in blacks does not differ significantly from what is observed in whites [15–18].

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Table 8.1 Reported diseases or conditions associated with IgAN

Gastrointestinal diseases	Celiac disease Crohn's disease Ulcerative colitis
Hepatic diseases	Alcoholic liver disease Hepatic B viral infections Chronic schistosomiasis Cryptogenic cirrhosis Primary biliary cirrhosis Portosystemic shunts
Autoimmune diseases	Granulomatosis with polyangiitis Uveitis Episcleritis/scleritis Goodpasture's disease Chronic thyroiditis Myasthenia gravis Vogt–Koyanagi–Harada syndrome
Rheumatological diseases	Ankylosing spondylitis Reactive arthritis (Reiter syndrome) Rheumatoid arthritis Behcet's syndrome Sjögren's syndrome Systemic lupus erythematosus Takayasu's arteritis
Malignancy	Renal cell carcinoma Bronchial carcinoma
Infections	HIV Hepatitis A Hepatitis B Staphylococcus Brucellosis Cytomegalovirus
Dermatological diseases	Dermatitis herpetiformis Psoriasis
Kidney disease	Nephrotic syndrome with minimal change disease Membranous nephropathy
Hematologic diseases	IgA monoclonal gammopathy Mycosis fungoides Sézary syndrome
Pulmonary diseases	Sarcoidosis Pulmonary hemosiderosis
Genetics	X-linked thrombocytopenia Wiskott–Aldrich syndrome Cystic fibrosis

Genetic Susceptibility

After the initial diagnosis of IgAN in identical twins in 1978 [19], scattered family studies and a number of studies on sporadic cases strongly suggested a genetic predisposition for at least some patients with IgAN and familial primary IgAN. The true frequency of familial IgAN remains uncertain because no serologic markers are currently available. Out of 269 cases from 48 families of IgAN patients, urinary abnormalities have been detected in 23 % [20]. In a second study, the same group examined a cohort of 110 patients with biopsy-proven IgAN and checked for both urinalysis and estimated renal survival. The 20-year renal survival rate from the apparent onset of the disease was significantly shorter in patients with familial (41 %) than sporadic IgAN (94 %). Furthermore, 15-year renal survival from the renal biopsy was reported to be significantly worse in familial disease: end-stage renal disease (ESRD) was present in 64 % of patients with familial and 8 % with sporadic IgAN [21]. Characterization of IgAN1 gene linked to IgAN in some Italian and American multiplex families has remained elusive [22].

A number of immunologic abnormalities suggesting a common genetic substrate affecting B-lymphocyte function in patients and their relatives have been reported: increased serum IgA, multimeric IgA and IgA rheumatoid factor, increased IgA1 production by peripheral blood mononuclear cells, detectable amounts of IgA–IgG complexes, and increased interleukin-2 and interleukin-4.

As regard to immunogenetics, IgAN was initially reported to be associated with HLA Bw35, but this association has not been confirmed. A benign course of IgAN has been associated with HLA DR4 in Japanese patients [23]. Because of the complex genetic architecture of IgAN, efforts to map disease susceptibility genes have not achieved definite results, and no causative mutations have been identified to date. Linkage analysis studies in families with IgAN have identified multiple loci and risk alleles, indicating that IgAN is likely a polygenic disease. These include a major disease locus (IgAN1) on chromosome 6q22–23 in Caucasian families [24] and a number of potentially significant gene polymorphisms, including uteroglobin [25], TGF beta [26], and atherosclerosis-associated genes [27]. A strong association with the HLA locus on chromosome 6p has been recently identified in a genome-wide analysis of familial and sporadic cases of IgAN in white Europeans [28].

Henoch-Schöenlein purpura (HSP) is the most common form of vasculitis in children (with an incidence of 10–20 cases per 100,000 children/year, <10-year-old in 90 % of cases). HLA B35 has been associated with an increased risk of nephritis. HLA DRB1*01, DRB1*11, and DRB1*14 also seem to increase susceptibility to glomerulonephritis.

Recent studies have focused on polymorphisms of genes encoding proinflammatory adhesion molecules associated with endothelial cell activation, TNF-α[alpha] , IL-1β[beta], IL-8, TGF-β[beta], and VEGF [29]. These studies are often limited by the relatively small cohorts.

HSP is a feature of the genetically determined familial Mediterranean fever (FMF) which is often observed in Israel and Turkey [30]. FMF is characterized by neutrophil activation and migration to the serosal surfaces. Neutrophil infiltration of the small vessels is a key histological feature of HSPN.

Observation that blood and urinary leukotriene B_4 (LTB_4) levels are higher while lipoxin A_4 (LXA_4) levels are lower in patients with HSPN compared to patients without is consistent with this association [31]. Indeed, recruitment of neutrophils and chemotaxis are activated by LTB_4 and inhibited by LXA_4.

Pathogenesis

Abnormalities in IgA1 Glycosylation

A galactose deficiency or diminished sialic acid content in the hinge region O-linked glycans of IgA1 has been reported in patients with IgAN and HSPN [32]. These results have been recently confirmed in experiments using mass spectrometry proteomic analysis that allows for accurate molecular weight estimation of the various O-glycans [33].

IgA_1 has a unique structure at the hinge region and possesses a variable number of O-linked oligosaccharides composed of *N*-acetylgalactosamine and galactose, which may be sialylated. Studies on immortalized circulating B cells from patients with IgAN have suggested a decreased β[beta]-1, 3 galactosyltransferase enzyme activity that could result in an impaired galactosylation of the core GalNaC residues [34] and an aberrantly exposed GalNac moiety. The origin of the defects in the enzymatic glycosylation of GalNac is unknown. Both genetic abnormalities in enzyme structure or function and acquired perturbations due to microenvironmental influences on IgA1 maturation might contribute. Incidentally, intercurrent infections could determinate the release of microbial neuraminidase, and the epitopes produced by the conformational changes may mimic those of environmental bacterial or viral glycoproteins.

An alternative emerging explanation for the apparent excess of undergalactosylated IgA1 in the circulation of IgAN patients could be that, differently from the response to systemic antigen challenge, the immune response to mucosal antigens is characterized by mucosally derived, relatively galactose-deficient IgA1 [35].

Consequences of the Underglycosylation

Decreased sialic acid and/or diminished content of galactose makes IgA1 molecule susceptible to auto-aggregation, thus forming molecular species whose properties resemble "immune complexes" in the circulation [36]. Galactose-deficient IgA1 is also the target of an "autoimmune" response carried out both by IgA and IgG autoantibodies, thus forming galactose-deficient-IgA1/IgA1 and galactose-deficient IgA1/IgG immunocomplexes [36]. An alternative concept has been proposed in that the increased plasma levels of galactose-deficient IgA1 would not result of an aberrant B-cell production of IgA1 but due to an "aberrant" distribution in which mucosal IgA1-committed plasma cells are misdirected to systemic sites and secrete "mucosal-type" IgA1 into the circulation [35]. The "right" mucosal-type, underglycosylated, IgA1 molecules could circulate in the "wrong" place concomitantly to the "right" anti-GalNac IgG antibodies at the "wrong" time, i.e., when galactose-deficient IgA1 is in circulation. Galactose-deficient IgA1 has a tendency to bind to a variety of glycoproteins, including constituents of normal glomeruli, leading to "planted" antigens, which promote in situ immune complex formation. Moreover, desialylation enables IgA to activate complement through a C4-independent alternative complement pathway promoting deposition and assembly of the membrane attack complex.

Impaired Removal of IgA1 Containing Immune Material from Circulation

Removal from circulation of I-labeled aggregated IgA or I-labeled IgA1–IgG aggregates is delayed in the IgAN patients [37, 38]. The hepatic Ashwell-Morell receptors, originally named asialoglycoprotein receptors (ASGPRs), represent a major pathway of IgA catabolism. ASGPRs bind to desialylated galactose or *N*-acetylgalactosamine residues in the hinge region of IgA1 governing the removal from circulation of IgA1 and IgA1-containing complex. Studies on the kinetics of intravenously injected neuraminidase-treated radiolabeled orosomucoid have shown normal liver expression of ASGPRs [39]. Undergalactosylated IgA1 could make the normally expressed ASGPR unable to remove IgA1 aggregates or IgA1-containing complexes from circulation. Aberrantly glycosylated IgA1 has been also thought to form immune complexes too large to enter the space of Disse and reach the ASGPRs [40]. However, ASGPRs are known to be the main route mediating the removal of long-term refrigerated platelets [41] casting doubt that immune complexes size prevents access to ASGPRs.

Other removal pathways include macrophage Fc alfa receptors (Fcα[alpha]R). Fcα[alpha]R expression on phagocytes is decreased in patients with IgAN [38], possibly due to continuous receptor occupation resulting in receptor down-regulation or shedding [42].

Potential Role of Intercurrent Infection as Cofactors Triggering the Disease

The likelihood of a genetic predisposition of aberrant IgA is supported by the presence of higher levels of serum galactose-deficient IgA1 in patients with IgAN and in their relatives than in healthy controls. However, relatives with high serum

levels of underglycosylated IgA1 are mostly asymptomatic, and serum levels of galactose-deficient IgA1 are not elevated in a significant proportion of IgAN patients. Glomerular mesangial IgA deposits in transplanted kidney from donors with subclinical IgAN can disappear in patients without IgAN. Moreover, mesangial IgA deposition is incidentally encountered in asymptomatic individuals: examination of kidney donors and nonselected autopsy series reveal mesangial IgA deposition without clinical evidence of renal disease in 4–16 % of subjects. Thus, it is likely that additional hits are needed for the development of clinical disease.

Environmental factors could contribute. Some patients with IgAN have heightened mucosal sensitivity to certain food antigens [43].

Factors that promote disease expression include high affinity of IgA1-containing immunocomplexes to the mesangium and decreased clearance of IgA-containing immune reactants. Polymeric IgA1 in patients with IgAN binds to mesangial cells with higher affinity than monomeric IgA1 and induces mesangial cellular proliferation. Galactose-deficient IgA1 has a high negative charge and tends to bind to fibronectin, type IV collagen, and laminin.

Infection may trigger autoimmune responses favoring the appearance in circulation of galactose-deficient IgA1/IgA1 and galactose-deficient IgA1/IgG immunocomplexes [36]. A mechanism of molecular mimicry has also been postulated [32]. The conformation of GalNac, which is exposed in galactose-deficient IgA1, is similar to same bacterial and viral epitopes. This reaction could be broadened through intramolecular and intermolecular epitope "spreading" where the primary response against the dominant initiating epitope extends to other epitopes within the same molecule or among different molecules.

Intercurrent infection could also exacerbate disease through activation of Toll-like receptors (TLRs-4). TLRs have been shown to be involved in the switch from IgM to IgA production in B cells. Expression of TRLs-4, whose activation is associated with bacterial lipopolysaccharides, is enhanced in IgAN [44].

Taken all together, the data supports the idea that the abnormalities of the hinge region O-linked glycans make the abnormal IgA1 molecules inherently "sticky" and promote the formation of IgA1 aggregates or immune complexes which escape the physiologic removal systems. Furthermore, these abnormalities increase mesangial deposition of IgA1-containing immune reactants, which is favored by interactions with transferring receptor, fibronectin, or Fcα[alpha] receptor on mesangial cells. Subsequent events include complement activation via the alternative and mannose-binding lectin pathways and release of cytokines that lead to glomerular hypercellularity, matrix production, podocyte injury, and scarring. A numbers of mediator systems are engaged in these processes, including PDGF and TGF-β[beta]. PDGF is intimately involved in the production of mesangial cell proliferation in IgAN. TGF-β[beta] promotes transcription of extracellular matrix glycoproteins and promotes transdifferentiation of tubular epithelial cells to pro-fibrotic myofibroblasts.

Natural History

The clinical course of IgAN is variable. Studies from around the world have shown that about 10 % (3–25 %) of all patients with IgAN have complete resolution of hematuria and proteinuria (although regression of renal lesions and disappearance of mesangial deposits are rare). The most common clinical course is an indolently slow progression to renal insufficiency which occurs in 40–45 % of patients at 20 years of follow-up, half of whom reaching ESRD (i.e., 20–25 % of the total). The actuarial renal survival at 10 years of 4,153 patients recruited from 23 studies in Europe, Australia, Asia, and North America ranged from 77 % to 94 %, 87 % to 93 %, 74 % to 91 %, and 57 % to 67 %, respectively.

This variability is due to diverse diagnostic approaches since a strategy of early renal biopsy in patients with mild urine abnormalities and normal renal function will increase the proportion of IgAN patients with good prognosis. Performing renal biopsy at a late stage will select patients with advanced disease and poor prognosis.

Stable renal function is generally detected in subjects with mild forms of mesangial proliferation, while deterioration of renal function occurs in patients with advanced histological lesions. IgAN prognosis is also more favorable when observation begins at the time of the first symptoms rather than at the renal biopsy [45].

A multicenter study on IgAN collecting data from three continents showed that the overall 10-year renal survival rate was 77 % with a wide range from 93.3 % in Helsinki to 61.4 % in Toronto. Median slope creatinine clearance ranged between −1.24 ml/min/year in Helsinki and −3.99 ml/min/year in Toronto. Taking into account age, creatinine clearance, proteinuria, and mean arterial pressure, this study suggests that variability is mainly related to "lead-time bias" and to the inclusion of milder cases in centers with apparently good outcomes [46]. Further studies are needed to determine whether geographic differences may also be related to genetic factors, environmental factors, or differences in medical management.

Older age at diagnosis has an adverse influence on outcome, while episodes of macroscopic hematuria do not confer a worse prognosis.

A small proportion of patients present with macroscopic hematuria, acute nephritic syndrome, and rapid progression to ESRD over 2–3 years. The main histological features of these cases are crescents (often focal and segmental).

Arterial hypertension, severe proteinuria, impairment of renal function at presentation, as well as histological evidence of glomerular sclerosis and interstitial fibrosis have been reported as strong predictors of unfavorable outcome [45]. Hyperuricemia, increased body mass index, and hyperlipidemia are independent factors of progression [47].

Prognostic formulas that use simple clinical and laboratory data have also been proposed [48, 49].

Berthoux et al. calculated the absolute renal risk (ARR) of dialysis. ARR was obtained by counting a number of risk factors present at diagnosis: hypertension, proteinuria >1 g/day, and severe pathological lesions (overall optical score >8) [50]. The cumulative incidence of death or dialysis at 10 and 20 years in adequately treated patients was 2 % and 4 % for ARR = 0, 2 % and 9 % for ARR = 1, 7 % and 18 % for ARR = 2, and 29 % and 64 % for ARR = 3.

Achieving hypertension control and reducing proteinuria lowered the risk of death or dialysis [50].

ARR scoring at diagnosis proved to be useful in estimating at an early stage the final risk of dialysis/death of IgAN patients.

A more complex score using proportional hazard risk models has been proposed to determine the predictors of ESRD in IgAN [51]. In this study, systolic hypertension, proteinuria, hypoproteinemia, azotemia, and high histological grade at initial renal biopsy were independently associated with the risk of ESRD. Mild hematuria was found to predispose patients to ESRD more than severe hematuria. Using eight clinical and pathological variables, a scoring system was developed to estimate 7-year ESRD risk by ROC curve analysis. Patients were subdivided into five groups according to estimated risk (ER), minimum risk (ER = 0–0.9 %), low risk (ER = 1.0–4.9 %), moderate risk (ER = 5.0–19.9 %), high risk (ER = 20.0–49.9 %), and very high risk (ER = 50.0–100 %). The incidence of ESRD over 7 years was 0.2, 2.4, 12.2, 40.2, and 80.8 %, respectively [51].

HSPN is usually a self-limited condition that lasts an average of 4 weeks. A considerable minority of patients relapse. However, relapses usually subside after 6 months. Nephritis occurs in 40 % of patients (within 1 month from onset in 85 % of cases and in nearly all within 6 months). Persistent purpura, severe abdominal symptoms, and an older age are significant risk factors for later nephropathy. Nephritis is a unique feature with potentially chronic consequences that influences the long-term prognosis. It could be estimated that 2 % of all HSPN patients from unselected series will develop chronic kidney disease, but the risk of progression increases up to 20 % at 20 years in tertiary centers. In general, patients with microscopic hematuria and low-grade proteinuria have an excellent prognosis. By contrast, nephritic or nephrotic syndrome has a poor prognosis, with 20–40 % of patients developing long-term renal impairment. A poor correlation between histological features and ultimate outcome has been reported [29].

Clinical Presentation and Diagnosis

The broad clinical spectrum of IgAN includes isolated urinary abnormalities (IUA), acute nephritic syndrome, nephrotic syndrome, acute renal failure (ARF), chronic renal failure (CRF), and rapidly progressive renal failure (RPRF).

Approximately 40–50 % of patients with IgAN present with one or more recurrent episodes of macroscopic hematuria, beginning 1–3 days after an upper respiratory tract infection (synfaringitic hematuria). The episode usually ends after a few hours or a few days, although microscopic hematuria may persist indefinitely. During the episodes the patient usually presents low-grade fever, loin pain (stretching of the renal capsule) mimicking urinary tract infection, or urolithiasis [8, 52–54]. Less commonly, patients may present with acute renal failure. Patients in whom acute renal failure is associated with macroscopic hematuria (ARF-MH) show a 75 % rate of complete recovery [55]. The most striking histological abnormalities in ARF-MH are signs of acute tubular necrosis with a high proportion of tubules that are filled with red cells casts.

The risk factors for incomplete recovery of renal function after the disappearance of MH include MH lasting longer than 10 days, patient's age >50 years, decreased baseline estimate glomerular filtration rate (eGFR), absence of previous episodes of MH, and severity of tubular necrosis [55]. When IgAN presents with a rapidly progressive glomerulonephritis, the histological pattern is usually a crescentic GN. Most of these patients progress to ESRD within 2–3 years [3, 56, 57].

Thirty percent of IgAN patients show an indolent course characterized by microscopic hematuria and mild proteinuria [58] incidentally detected on routine examination [59, 60]. Gross hematuria occurs in 20–25 % patient.

Patients with persistent microscopic hematuria without proteinuria, normal renal function, and no episodes of macroscopic hematuria will have a diagnosis of thin basement membrane nephropathy (TBMN) or variants of Alport's syndrome in 43 % of cases and IgAN in 20 % [59]. Conversely, patients with microscopic hematuria associated with low grade of proteinuria will have a diagnosis of IgAN in 46 %, TBMN in 7 %, and other nephropathies in 26 % of cases.

Routine renal biopsy is indicated for asymptomatic microscopic hematuria with low-grade proteinuria [59], and some countries have started routine screening for urinary abnormalities among the general population and promoting a policy of early renal biopsy [12].

In children, IgAN is the second cause of nephritic syndrome after postinfectious glomerulonephritis [61].

Nephrotic syndrome associated with mild mesangial proliferation and diffuse fusion of the foot processes at electron microscopy may result from two simultaneous diseases, i.e., minimal change disease plus a mild form of IgAN [62]. This

hypothesis is supported by a rapid remission of proteinuria induced by corticosteroids [63].

By contrast, Kim et al. [64] examined clinical features and long-term outcome of more than 1,000 biopsy-proven IgAN patients. In almost a 100 patients, nephrotic syndrome occurred in any subclass of IgAN, and corticosteroids administration did not result in complete reduction of heavy proteinuria. These findings suggest that MCD is not entirely responsible for the development of NS in patients with IgAN. Complete remission (CR), partial remission (PR), and no response (NR) occurred in 48 %, 32 %, and 20 % of patients, respectively. The prognosis of NS in IgAN was not favorable unless PR or CR was achieved. The risk of doubling the baseline serum creatinine was significantly higher in the NS group than in the non-NS group ($p < 0.001$) [64].

Overlapping IgAN and membranous disease have rarely been described [65].

In a few cases, IgAN was described in patients with granulomatosis and polyangiitis [66].

In an adult male with gross hematuria, especially if he is a smoker or complains of loin pain and low-grade fever, a urological cause of macroscopic hematuria should be ruled out: kidney, bladder or prostate cancer, stones, or urinary tract infections including tuberculosis. In these patients, a CT urogram and cystoscopy should be considered. If the urological study is negative and the patient shows persistent prevalently dysmorphic microscopic hematuria and mild proteinuria, renal biopsy is recommended. Elevated serum IgA levels as well as elevated serum IgA/C3 concentration ratios (>4.0–4.5) can be seen in about 50 % of patients. Serum IgA/C3 concentration ratio >3, in the presence of overt proteinuria and elevated blood pressure, is said to distinguish IgAN from other causes of hematuria in over 75 % of cases [67].

Circulating IgA rheumatoid factor and IgA immunocomplexes, IgA–fibronectin complexes, IgA deposits in the dermal capillaries at skin biopsy, elevation of plasma polymeric IgA1 levels, measurement of poorly galactosylated IgA1 o-glycoforms in the serum, and urinary proteomics have all been proposed as diagnostic biomarkers. None of them have proven to be useful in clinical practice [68–73]. Serum complement levels (C3, C4, and C1q) are normal and C-reactive protein (CRP) levels are not increased [74]. In some patients, positive ANCA test (IgA subclass) has been reported using purified antigens [75].

Pathological Features

Light Microscopy

IgAN can show different patterns of glomerular injury, although the mesangial area is predominantly involved [76].

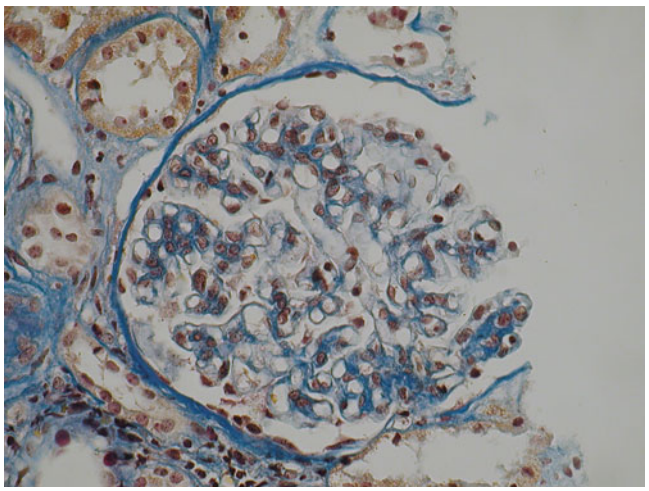

Fig. 8.1 Mild mesangial hypercellularity (AFOG, ×400 magnification) (Class. Oxford: M1, S0, E0, T0)

The clinical syndrome is often suggestive of a specific histological lesion [1]. Hematuria, mild proteinuria, and normal renal function are often related to mild mesangial proliferation. Moderate to heavy proteinuria and/or acute renal impairment is associated with varying degrees of either endocapillary or extracapillary proliferation, while chronic renal impairment is often observed when glomerular sclerosis is present.

The diverse prevalence of minimal glomerular changes in worldwide studies possibly depends on the biopsy policy [77].

Glomeruli. All forms of glomerular injury have been described in IgAN. In most cases, the disease appears as a mesangial proliferative GN with focal (less than 50 % of glomeruli involved) (Fig. 8.1) or diffuse (more than 50 %) mesangial hypercellularity (defined as more than three mesangial cells per mesangial area), which is almost always accompanied by reactive mesangial matrix expansion. If patency of the capillary lumens is compromised by the expanded matrix, it is defined as segmental or diffuse sclerosis.

Another pathological pattern is focal or diffuse endocapillary proliferative GN (Figs. 8.2 and 8.3). This differs from mesangial proliferative GN by the presence of nonresident cell infiltrates (lymphocytes, monocytes, or granulocytes, according to the phase of the inflammatory process) that may result in narrowing of the capillary lumen. Occasionally, subendothelial deposits can be seen and are characterized by nonresident and mesangial cell infiltration of the capillary wall. Glomerular hyperlobulation and "double contour" membranoproliferative appearance can be rarely observed.

In some biopsies, huge deposits of IgA/IgG can be identified by LM stained by fuchsin and PAS. Segmental necrosis of the loop and fibrin deposition, which evolves into adhesions or crescents, can be also observed (Fig. 8.4).

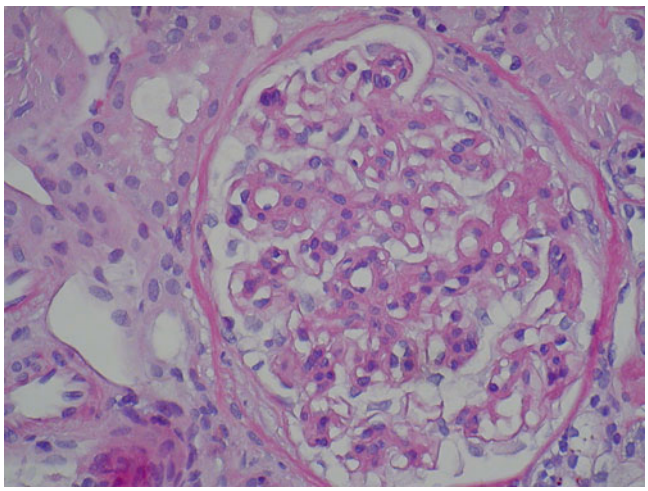

Fig. 8.2 A glomerulus with diffuse mesangial hypercellularity and mesangial matrix with a focal membranoproliferative pattern. Segmental adhesion to the Bowman capsule (H&E, ×400) (Class. Oxford: M1, S1, E1, T1)

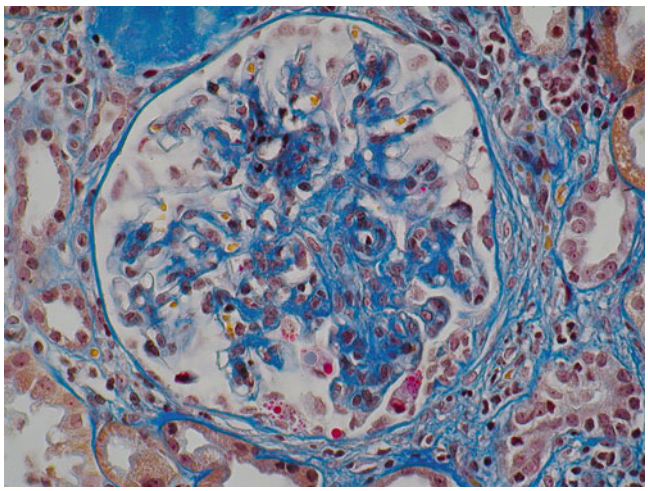

Fig. 8.3 Mesangial expansion and focal endocapillary proliferation (AFOG, ×400) (Class. Oxford: M1, S0/1, E1)

Extracapillary GN can be found in 25 % of IgAN cases. An ANCA-associated vasculitis [78–80] should be ruled out in these cases.

Severe inflammation, especially if untreated, results in focal or diffuse glomerulosclerosis.

The heterogeneity of IgAN makes it possible to find nonspecific lesions with negative IgA fluorescence in early biopsies and clear-cut features in subsequent biopsy specimens (i.e., lead-time bias).

Tubuli and Interstitium. Tubuli and interstitium are involved mainly in chronic stages. Tubulointerstitial injury is on occasion characterized by eosinophilic cell infiltration as well as

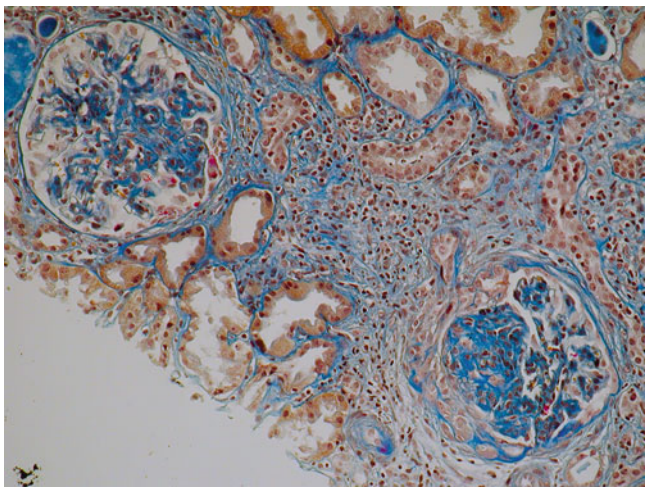

Fig. 8.4 Mesangial hypercellularity with expanded matrix and segmental fibrosis. In the lower right glomerulus, a segmental cellular crescent with adhesion and fragmentation of Bowman capsule. There is also an interstitial fibrosis with inflammatory infiltration associated with tubular atrophy (AFOG, ×400) (Class. Oxford: M1, S1, E1, T1)

mononuclear and plasma cell infiltration, all of which may lead to chronic interstitial fibrosis (Fig. 8.4). In cases of macro- and less frequently microscopic hematuria, stacked casts of dysmorphic red blood cells can be seen within the tubules. Occasionally, massive hematuria and acute renal tubular damage can lead to an increase in serum creatinine. Persistent micro-/macro-hematuria is suggested by the presence of hemosiderin in the interstitium, and especially in the medulla.

Vessels. Vessels are usually normal in the early stages of disease, but vascular sclerosis may be present in more advanced cases.

Immunofluorescence

On immunofluorescence IgAN is characterized by diffuse IgA mesangial immune deposits (Fig. 8.5) which are dominant or codominant compared to concurrent IgG and IgM deposits [81]. Deposits can be also segmentally distributed along the glomerular basement membrane. IgG deposits are found in up to 85 % of the cases. IgM is detectable in sclerotic areas, mainly because of entrapment phenomena. C3 complement fraction is a common finding (90 %). Occasionally, C4d (30 %) and C1q (10 %) can overlap with IgG or IgM deposits, suggesting activation of the lectin complement pathway [82–84]. There is usually a prevalence of lambda chain [85].

Fibrinogen and fibrin are found in extracapillary forms.

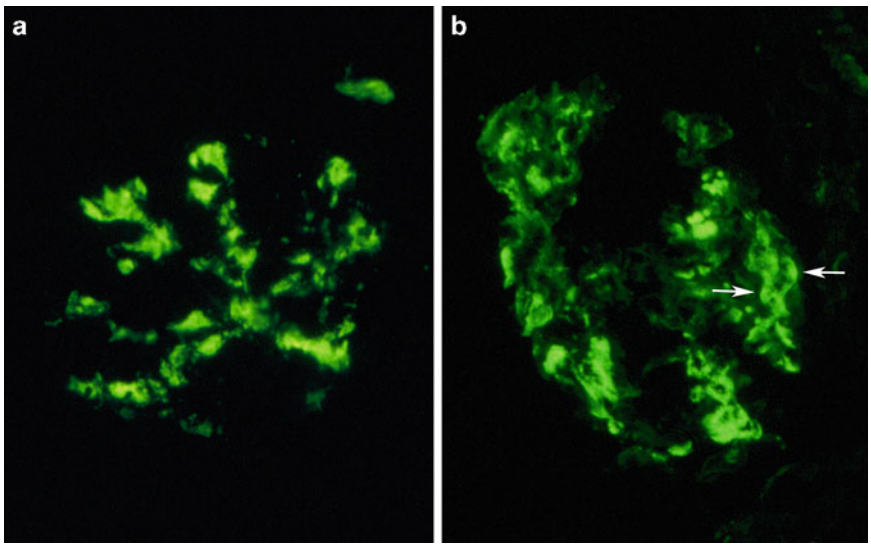

Fig. 8.5 Deposition of IgA immunoglobulin in the mesangium (**a**) and within mesangium and along capillary membrane (**b**). Paramesangial prominent deposition (*arrows*) can form images resembling arc, named "arc de cercle" by some authors (original magnification ×400)

Electron Microscopy

Ultrastructural analysis can be important to define the nature and the site of the deposits. The deposits can be located in mesangium and para-mesangium (100 %) (Fig. 8.6) in subendothelial (11 %) or subepithelial areas (6 %). Rarely, intramembranous (2 %) and hump-like deposits can also be seen. An association between IgAN and TBGM has been described, with TBGM present in 5 % of patients with IgAN [86].

Prognostic Impact of Histological Classification

Glomerulosclerosis, tubular atrophy, and tubular fibrosis as well as mesangial and endocapillary proliferation and extensive crescents are histological predictors of bad prognosis. A number of classifications have been proposed to identify prognostic factors in IgAN [3, 87–89].

Recently, the Renal Pathology Society together with the International Group of IgAN Network supported an international consensus on the predictive role of the main histological lesions, the Oxford classification [90, 91]. Two hundred and sixty-five biopsies from patients with IgAN with proteinuria greater than 0.5 g/24 h and eGFR more than 30 ml/min/1.73 m^2 at the time of diagnosis who had been followed up for at least 1 year were included. Twenty histological lesions were identified, and six of particular relevance were selected on the basis of frequency and reproducibility: mesangial proliferation and endocapillary proliferation, crescents, segmental sclerosis and synechiae, tubular atrophy and interstitial fibrosis, and arterial score. Four of these variables, (1) the **M**esangial hypercellularity score, (2) **S**egmental glomerulosclerosis, (3) **E**ndocapillary hypercellularity, and (4) **T**ubular atrophy/interstitial fibrosis, were able to predicting renal outcome independent independently of clinical assessment (Table 8.2). Validation of the Oxford classification has been carried out in children as well as in adults [92–94]. Verification is necessary because premature implementation of biased conclusions can harm patients and mislead future research. In some studies mesangial hypercellularity and segmental glomerulosclerosis were weaker predictors than others [93, 94]. In a retrospective analysis of 187 adults and children with IgAN, the four individual variable of MEST offered the same predictive value in both cohorts except mesangial hypercellularity, which was a weaker predictor than in the original Oxford classification paper [94]. Bellur et al. compared the immunofluorescence pattern with optical findings categorized according to the Oxford classification score system and demonstrated the supportive role of IgG and the deposition of IgA in the capillary wall in predicting the development of proliferative glomerulopathy [95]. The Oxford classification has also been validated for in children with IgAN [93, 96]. Recently, Alamartine et al. carried out a validation in 183 adult patients who had IgAN and were followed for an average of 77 months. In univariate time-independent analyses T, E, and S lesions of MEST score were strongly associated with doubling creatinine or ESRD. Conversely, M lesion was not associated with renal outcome. In the multivariate model, only estimated GFR at baseline was a risk factor, and pathological lesions had no independent influence [97]. Proteinuria and blood pressure also failed to predict survival independently from a combined event when factoring in the initial eGFR.

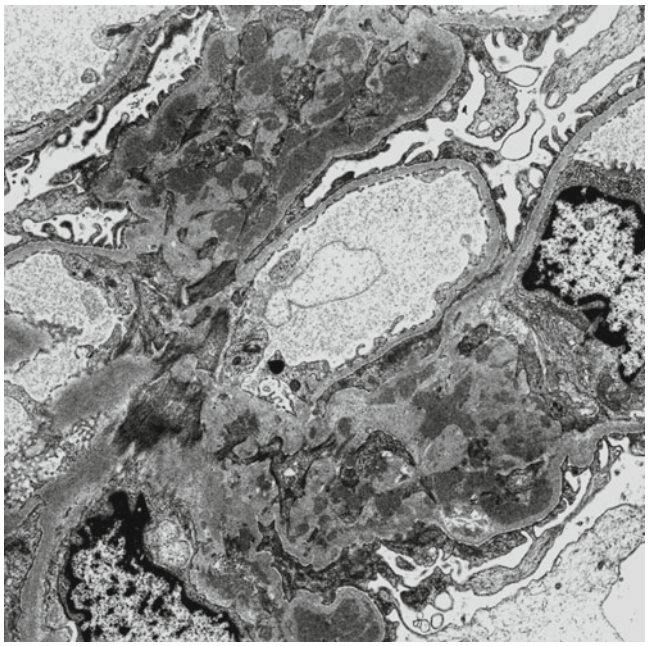

Fig. 8.6 At the electron microscopy glomerular IgA deposition appears as fine granular electron dense material localized within mesangium, with marked accumulation along paramesangial basal membrane (*arrows*) (original magnification ×3,000)

Table 8.2 Past and present IgAN classifications

• Lee histological classification (1982)
○ I: Focal mesangial expansion
○ II: Moderate focal proliferative
○ III: Mild diffuse proliferative
○ IV: Moderate diffuse proliferative; crescents in ≤45 % of glomeruli
○ V: Severe diffuse proliferative; crescents in >45 % of glomeruli
• Haas histological classification (1997)
○ I: Minimal or no mesangial hypercellularity
○ II: Focal and segmental glomerulosclerosis without active cellular proliferation
○ III: Focal proliferative glomerulonephritis
○ IV: Diffuse proliferative glomerulonephritis
○ V: ≥40 % globally sclerotic glomeruli or area of cortical tubular atrophy
• Four key pathological features of the Oxford classification (2009)—MEST
○ **M**esangial hypercellularity
(a) Mean < 4 mesangial cells/mesangial area (M0 score)
(b) Mean ≤ 4 mesangial cells/mesangial area (M1 score)
○ **E**ndocapillary hypercellularity
(a) Present (E0 score)
(b) Absent (E1 score)
○ **S**egmental glomerulosclerosis or adhesions
(a) Present (S1 score)
(b) Absent (S0 score)
○ **T**ubular atrophy/interstitial fibrosis
(a) 0–25 % (T0 score)
(b) 26–50 % (T1 score)
(c) >50 % (T2 score)

Validation studies include categories of patients that are similar but not identical to the original study so that duplicating every result is not expected [98]. Despite the rigor of the methodology adopted, the Oxford classification remains the result of a retrospective observation, and although special attention was paid to search for independent risk factors, confounding factors cannot be ruled out. Prospective studies are therefore needed to further evaluate these results. For instance, since cases who had been followed up for less than 1 year have been not examined, a number of patients with rapidly progressive IgAN or advanced CKD have been excluded. Conversely, excluding patients with proteinuria <0.5 g/24 h means excluding cases with more favorable prognosis. The involvement of patients from eight different countries made the cohort more varied and representative, though it was not possible to guarantee the homogeneity of the laboratory findings. Analyzing renal survival using two different statistical methods of multivariate analysis provided different results for segmental glomerulosclerosis and for mesangial hypercellularity. The histological study was carried out with PAS staining alone, which, by itself, is not ideal for all the lesions observed in the first stage of the study. Besides, no immunofluorescence or electron microscopy correlations have been investigated. These issues could be the aims of further refinement of the classification.

Treatment

As previously discussed, IgAN is characterized by a variable clinical profile (ranging from a benign course to rapidly progressive renal failure) and a wide spectrum of histological lesions.

Up to 23 % of patients, i.e., those who do not develop proteinuria >300 mg/24 h or hypertension during the follow-up, will have complete clinical remission [99]. Patients whose urinary protein excretion increases during the follow-up are more likely to develop renal impairment compared to patients without proteinuria [99].

Proteinuria is the most important predictor of the decline of GFR rate in studies using multivariate analysis [100]. Five patterns of progression have been identified on the degree of proteinuria: <0.3 g/day, 0.3–1 g/day, 1–2 g/day, 2–3 g/day, and >3 g/day. The kidney survival curve at 15 years was 96 %, 87 %, 65 %, 55 %, and 30 %, respectively. Nevertheless, patients presenting with baseline proteinuria >3 g/day who achieved partial remission, especially if proteinuria dropped to levels less than 1 g/day, had a course similar to patients presenting with <1 g/day at diagnosis [100]. A treatment goal of <0.5 g/day per 1.73 m^2 is recommended in children [101]. Values of proteinuria equal or less than 0.5 g/day [102] have been reported as a threshold of risk in adults as well.

Uncontrolled hypertension is associated with greater proteinuria and faster decline of GFR [89, 103], while reducing blood pressure has protective effects especially in mild renal insufficiency [103]. In order to achieve maximal renal and cardiovascular protection, blood pressure goals <130/80 mmHg in patients with less than 300 mg/24 h of proteinuria, and <125/75 in patients with proteinuria >1 g/24 h are recommended [104–106].

Low GFR at presentation was also listed among the clinical factors of poor prognosis [45], but this issue remains controversial [89, 107].

KDIGO guidelines recommend the assessment of all patients with biopsy-proven IgAN for secondary causes of disease, emphases on the role of pathological findings in assessing prognosis, and the evaluation of the risk factors of progression (i.e., proteinuria, blood pressure, and eGFR) at the time of diagnosis and during the follow-up [108–113].

Conservative Treatment

Conservative treatment is based on hypertension control with a low salt diet, the use of angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, and the use of statins. Tonsillectomy and fish oil have been also proposed for patients with IgAN.

Angiotensin-Converting Enzyme Inhibitors and Angiotensin II Receptor Blockers

Angiotensin-converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARBs) reduce the risk of progressive renal disease in a number of proteinuric diabetic and nondiabetic glomerulopathies [114]. One observational study [115] and three small prospective trials [102, 116, 117] have confirmed initial observations [118, 119] that ACEi protect long-term renal function in patients with severe IgAN and reduce proteinuria in normotensive IgAN patients. Four more recent studies provided evidence that ACEi and ARBs are more effective than other antihypertensive drugs in reducing proteinuria and slowing the progressive decline in GFR in IgAN. A 3-year prospective follow-up study showed that the decline in creatinine clearance was lower in ACEi-treated group as compared to control patients treated with amlodipine [115]. Positive effects of reducing proteinuria in slowing the progression of renal damage can be seen with proteinuria values greater than 500 mg/day. In these cases ACE inhibition should be given even in normotensive patients.

The advantage of administering ARB or ACEi in patients with IgAN has been also emphasized in recent metanalysis [120, 121]. However, there are no randomized trials reporting benefits from the combination therapy.

Fish Oil

The rationale for using n-3-polyunsaturated fatty acids (omega-3 fatty acids) is the potential anti-inflammatory effect and reduction of glomerulosclerosis. Donadio et al. studied 106 patients with a mean baseline creatinine clearance of 82 ml/min and protein excretion of 2.5–3.2 g/day who were randomly assigned to therapy with either 12 g of fish oil or a similar amount of olive oil (placebo) for 2 years [122]. Baseline clinical characteristics were similar between the two groups. At 4 years, 6 % of the patients in the fish oil-treated group vs. 33 % of placebo group showed a >50 % increase in serum creatinine. ESRD occurred in 10 % of the patients in the fish oil-treated groups vs. 40 % of placebo groups and 15 % vs. 37 % at 6 years of follow-up. In a trial of Southwest Pediatric Nephrology Study Group [123] 96 patients were randomly assigned to one of three treatment arms: a purified preparation of omega-3 fatty acids (4 g/day) for 2 years, alternate-day prednisone (60 mg/m^2 per dose for 3 months, 40 mg/m^2 per dose for 9 months, and 30 mg/m^2 per dose for 1 year), or placebo. The superiority of omega-3 fatty acids over placebo in slowing progression of renal disease was not confirmed at 3 years. A Cochrane meta-analysis of four trials showed no beneficial effect of fish oil on renal outcomes including serum creatinine, creatinine clearance, or change in proteinuria [124]. However, fish oil supplements have shown a number of cardiovascular beneficial effects: lowering of systolic blood pressure and triglyceride values, reduction of resting heart rate, improvement in several markers of endothelial damage, and reduction in the risk of sudden cardiac death in patients with established coronary heart disease. KDIGO suggests the use of fish oil in patients with IgAN and persistent proteinuria > 1 g/day [108].

Lipid Control

In a meta-analysis that included 13 prospective controlled trials in 404 patients, mostly diabetics, Fried et al. examined the effects of lipid-lowering agents on GFR and proteinuria or albuminuria in patients with renal disease [125]. The pooled outcome showed only a trend towards benefit by lipid-lowering therapy (mainly, but not exclusively, statins) on protein or albumin excretion. Several other studies have failed to confirm any effect. Long-term statin treatment failed to reduce proteinuria in 16 patients with NS and renal insufficiency [126]. No effects in children with steroid-resistant NS or in nephrotic patients with MN have been observed [127–129]. Taken all together, the anti-proteinuric effect of statins appears to be negligible. Statins are perhaps more effective when used in combination with ACEi, although the majority of the data has been obtained from patients without NS. On the other hand, proteinuria has been

reported as a complication of statin therapy [130–132] as a consequence of statin inhibition of protein uptake by tubular cells [133, 134] which could be dose related.

There is no large, randomized, controlled study that proves whether statins are deleterious or beneficial to patients with proteinuric kidney disease.

Tonsillectomy

Tonsillectomy, a widespread practice in France and Japan, might be beneficial in IgAN patients who present with recurrent tonsillitis and macro-hematuria. Several retrospective studies suggested it had a beneficial effect on renal survival in IgAN. In a retrospective study on 118 patients who received steroid pulse therapy and/or immunosuppressive therapy, Kaplan-Meier curve predicted a better renal survival in the subset of 48 patients who underwent tonsillectomy than in the 70 remaining patients who did not underwent tonsillectomy [135]. However, it took >10 years of follow-up for any significant difference to be noted. In a more recent, nonrandomized, prospective, controlled study, 55 patients received methylprednisolone pulses followed by oral prednisone (0.5 mg/kg/day) for 12–18 months alone or combined with tonsillectomy. At 24 months, remission of proteinuria and hematuria was higher in the tonsillectomy group than in control patients: 76.5 % vs. 41.2 % and 79.4 % vs. 17.6 %, respectively. Eighteen patients in the tonsillectomy group (but none of patients receiving corticosteroids alone) who repeated renal biopsy at 24 months follow-up had a decrease in mesangial proliferation. The Cox regression model showed that combined therapy was approximately six times more effective at making urinary protein disappear than steroid pulse monotherapy [136]. This study provides some evidence that tonsillectomy may be effective in inducing clinical remission in patients with normal renal function and proteinuria >500 mg/day. KDIGO suggests that tonsillectomy should not be performed for IgAN [108].

Immunosuppressive Therapy

Several therapeutic approaches have been attempted in IgAN. Corticosteroids have been used in the treatment of glomerulonephritis since the 1960s without a clear understanding of the pathogenic pathway to be modified. In the 1980s, studies on the effects of corticosteroids in IgAN led to controversial results because of remarkable differences in drug regimens as well as characteristics of the patients (e.g., dose schedule, patient population, histology, renal function, and proteinuria). Since then, three Italian trials on the use of corticosteroids in IgAN have been reported [137–139]. The first trial was a prospective, randomized, controlled multicenter study aimed to assess effects and safety of a regimen based on the use of corticosteroids for 6 months. Eligible patients had biopsy-proven IgA nephropathy, proteinuria of 1.0–3.5 g/day, and plasma creatinine of ≤1.5 mg/dl. Patients were randomly assigned either supportive therapy alone or steroid treatment (intravenous methylprednisolone 1 g/day for 3 consecutive days at the beginning of months 1, 3, and 5 plus oral prednisone 0.5 mg/kg on alternate days for 6 months). The primary end point was deterioration in renal function defined as a 50 % or 100 % increase in plasma creatinine concentration from baseline. Nine of 43 patients in the steroid group and 14 of 43 in the control group reached the primary end point (a 50 % increase in plasma creatinine) by year 5 of follow-up [137]. All 43 patients assigned to steroids completed the treatment without experiencing any important side effects. A subsequent study by the same group evaluated the long-term effectiveness of steroids in IgAN [138]. Ten-year renal survival was significantly better in the steroid than in the control group (97 % vs. 53 %). In 72 patients who did not reach the end point (doubling in baseline serum creatinine), median proteinuria significantly decreased (1.9 g/24 h at baseline, 1.1 g/24 h after 6 months, and 0.6 g/24 h after a median of 7 years). In 14 progressive patients, proteinuria increased from a median of 1.7 g/24 h at baseline to 2.0 g/24 h after 6 months and 3.3 g/24 h after a median of 5 years. These studies support the notion that patients with relatively well-preserved renal function and moderate proteinuria may benefit from 6-month steroid therapy.

Two RCT studies were recently published on the use of oral prednisone plus ACEi to improve kidney survival and decrease proteinuria, and both documented some renal protection [140, 141]. The Southwest Pediatric Nephrology Study Group trial, which included both adults and children, all of whom received ACEi, showed that administration of 60 mg/m^2 of prednisone every other day tapered to 30 mg/m^2 at 12 months reduced proteinuria but did not influence kidney function at 2 years [123]. Also low doses of prednisone (20 mg/day) gradually tapered to 5 mg/day within 2 years showed no benefit in preserving kidney function despite some reduction in proteinuria [142].

All studies showed decreases in proteinuria, but improvement in renal function seemed to be achieved only by high doses of corticosteroids over a medium-term course of therapy. A recent meta-analysis confirmed the beneficial effects of corticosteroids on renal function [143].

It should be remembered that approximately 30 % of patients show a poor response to corticosteroids. Moreover, the positive effects of corticosteroids on the natural course of IgAN seem to decrease over time, and in a number of patients, IgAN progressed despite steroid treatment.

KDIGO guideline suggests a 6-month course of corticosteroid therapy in IgAN patients with GFR > 50 ml/min per 1.73 m^2 and persistent proteinuria ≥1 g/day despite 3–6

months of optimized supportive care (including ACE-I or ARBs administration and blood pressure control) [108].

Cytotoxic Drugs

The rationale for using immunosuppressive treatment in IgAN is based on the pathogenesis of the disease involving immune complex deposition, complement activation, cell-mediated immune-mechanisms, and inflammatory mediators that could potentially be blocked by cytotoxic drugs.

Three immunosuppressive drugs have been used in IgAN, i.e., cyclophosphamide, azathioprine, and mycophenolic acid.

In a prospective trial on 38 patients with progressive IgAN, 19 were treated with cyclophosphamide 1.5 mg/kg/day for 3 months, and then switched to azathioprine 1.5 mg/kg/day for 2 or more years, in combination with prednisolone 40 mg/day slowly tapered to 10 mg/day within 2 years [109]. The control group consisted of the remaining 19 patients. All 38 patients had serum creatinine ranging between 1.5 and 2.8 mg/dl. Arterial blood pressure target (145/85 mmHg) was not reached by any of the patients, and the use of ACEi/ARBs was not clearly stated. No crescent was present in biopsies. At 60 months, cumulative renal survival was 72 % and 6 % in the treated and control groups, respectively, and the delta of the reduction of renal function decreased from -5.19 ± 0.66 to -1.07 ± 0.47 in the treated group, whereas remained unchanged in the control untreated group (-4.85 ± 0.67 and -5.12 ± 0.81). At a 48-month follow-up, proteinuria decreased from 3.39 g/24 h at entry to 0.8 ± 0.3 g/24 h in the treated group and remained unchanged (4 g/24 h) in the control group [109]. Critical points in this study were the relatively small patient samples, the failure to reach the arterial pressure target, the unusually poor course of the control group, and the azathioprine treatment period (2 years) which was thought to be long.

A larger, retrospective study followed up 74 patients for more than 10 years [144]. Forty-one patients were treated with prednisolone (initially 60 mg/day) and azathioprine (initially 2 mg/kg BW/day) in gradually reduced doses for 24± months, whereas 33 patients received no immunosuppressive drugs. The primary end points were doubling of baseline serum creatinine and/or ESRD. The overall clinical courses of both groups of patients showed a rather similar pattern. Doubling of serum baseline creatinine was observed in 9 of 41 treated (22 %) and in 10 of 33 untreated (30 %), whereas ESRD developed in 6 treated (15 %) and 6 untreated patients (18 %). However, treated patients with proteinuria >3 g/24 h had a significantly better outcome compared to untreated regarding doubling of serum creatinine (29 % vs. 78 %) and ESRD (17 % vs. 55 %). Proteinuria, mean blood pressure, baseline serum creatinine, and severity of interstitial myofibroblast expression were identified as independent risk factors related to a poor outcome by multivariate analysis. Side effects of treatment were not uncommon and observed in 10 (24 %) patients. The incidence of ESRD in patients with crescentic IgAN was 15 % in the treated vs. 50 % in the control group. Treatment had no effect on patients with chronic renal lesions and serum creatinine >2.5 mg/dl. Adverse events included a case of squamous cell carcinoma and a low-grade non-Hodgkin's lymphoma [144].

In another study, 26 out of 45 patients with proteinuria greater than 3 g/day and renal failure (as defined by a serum creatinine of at least 1.5 mg/dl) received prednisolone 30 mg/day tapered to 5 mg/month, plus cyclophosphamide 50 mg/day for 3 months and then 25 mg/day for an additional 3 months. After a mean observation period of approximately 3.5 years, serum creatinine was 3.4 mg/dl vs. 9.1 mg/dl, while proteinuria was 1.3 g/24 h vs. 2.7 g/24 h, in treated vs. untreated patients, respectively. Adverse effects in patients receiving immunosuppressive therapy included diabetes, thrombocytopenia, and gastric and colon cancer [145].

A recent trial aimed to establish whether addition of azathioprine to prednisone improves long-term renal survival included 207 patients who were enrolled among 28 Italian centers: 106 were allocated in the steroid regimen, while 101 patients received corticosteroids plus azathioprine 1.5 mg/kg/day for 6 months [139]. Both groups were given 1 g of i.v. methylprednisolone for 3 days at the beginning of months 1, 3, and 5 followed by 0.5 mg/kg of oral prednisone given every other day for 6 months. The primary end point was progression of renal disease as defined as a 50 % increase in serum creatinine from baseline. Secondary end points included profile of proteinuria over time, frequency of relapses and re-treatments, and side effects. Both groups had serum creatinine ≤2.0 and proteinuria >1.0 g/day. Patients were followed up for a median of 4.9 years (IQR 3.0–6.0). The intention-to-treat analysis showed that the primary end point was reached by 13 patients (12.9 %) receiving corticosteroids alone and 12 (11.3 %) given azathioprine plus corticosteroids. The 5-year cumulative renal survival of the two groups was similar (88 % vs. 89 %). Multivariate Cox regression analysis revealed that gender, systolic blood pressure, number of antihypertensive drugs, administration of ACEi, and proteinuria during follow-up predicted the risk of reaching the primary end point. Treatment significantly decreased proteinuria from 2.0 to 1.1 g/day during follow-up in both groups. Eight patients (7.5 %) in the steroid group and six (5.9 %) in the steroid plus azathioprine group started dialysis after a mean of 35.6 months of follow-up. A better renal function at 6 years was observed in patients on group I than group II (71 %). Treatment-related adverse events were more frequent among subjects receiving azathioprine (15.8 % vs. 6.6 % in the steroid alone group). This trial confirmed the effectiveness of corticosteroids in IgAN, but showed that the administration of low-dose azathioprine to corticosteroids

for 6 months provides no additional benefits and may increase the risk of adverse events [139].

Cytotoxic drugs were also used in adults and children who had normal renal function and proteinuria. Seventy-four normotensive children with asymptomatic hematuria, proteinuria (1 g/24 h), and normal renal function were studied [146]. At renal biopsy, more than 50 % of the patients had glomeruli with sclerosis, crescents, or adhesions. Forty patients were treated with prednisolone 1–2 mg/kg/day plus azathioprine 2 mg/kg/day, as well as anticoagulant therapy plus dipyridamole, while 34 patients were treated with anticoagulant therapy plus dipyridamole (control group) for 24 months. At the end of follow-up, only the group treated with cytotoxic drugs showed almost complete remission of proteinuria from 1.35 g/24 h to 0.22 g/24 h, as well as lower extent of hematuria and lower IgA serum levels. Biopsy was performed at the end of the follow-up and revealed that treated patients had a significantly lower number of crescents. However, similar results were seen in the control group treated with anticoagulant and dipyridamole [146]. Important limitation of the study was the very high incidence of severe adverse effects, i.e., 25 %. A similar controlled prospective study was performed on 48 adult patients with normal renal function and mean proteinuria of 2.0 g/24 h [147]. Twenty-seven of them received triple therapy with cyclophosphamide 1.5 mg/kg/day for 6 months plus warfarin and dipyridamole for 36 months. The results showed a greater reduction of proteinuria (from 2.4 to 1.0 g/24 h) in patients treated with cyclophosphamide. Limitations of the study include lack of information regarding adverse events and short follow-up [147].

Cytotoxic drugs are useful in patients with crescentic lesions and rapidly progressive renal failure. Roccatello et al. published a retrospective study on 20 patients with crescentic IgAN [148]. Twelve of them were treated with three pulses of 1 g of methylprednisolone followed by prednisone 0.8 mg/kg/day for 2 weeks and then 0.4 mg/kg/day for 4 weeks, followed by tapering at 5 mg/month until complete discontinuation in 8 months. Cyclophosphamide 1.5 mg/kg/day was also administered for 2 months. The cumulative renal survival curve at 5 years was 100 % in treated patients vs. less than 50 % in non-treated patients. The study suggested that in patients with crescentic IgAN, the combination of corticosteroids plus cyclophosphamide can be effective in arresting the inflammatory process and prevent progressive renal damage [148].

A similar experience was reported by Tumlin et al. in a prospective, open label trial on 24 patients with crescentic IgAN and renal failure (serum creatinine 1.7 mg/dl). Most of them had heavy proteinuria (>3 g/24 h) and hypertension. Twelve patients were treated with three pulses of methylprednisolone, 15 mg/kg/day, followed by oral prednisone (1 mg/kg/day for 2 months, 0.6 mg/kg/day for 2 more months, 0.3 mg/kg/day for 2 additional months, and then 10 mg/day) plus intravenous cyclophosphamide at 0.5 g/m^2/month for 6 months. Treatment resulted in an improvement in serum creatinine (from 2.69 to 1.85 mg/dl) at 36 months and a drop in proteinuria from 4.6 to 1.46 g/day, as well as a regression of endocapillary and extracapillary proliferation after 6 months, together with an improvement in activity score at control renal biopsy [113].

In a prospective, non-controlled study, Rasche et al. treated 21 patients with crescentic IgAN who had serum creatinine >2 mg/dl with 750 mg/m^2 i.v. pulses of cyclophosphamide (one pulse every 4 weeks for 6 months) plus low doses of oral corticosteroids. Combined immunosuppression slowed the loss of renal function from −16 %/year before therapy to −4 %/year after therapy [149].

As emphasized by Barratt and Feehelly [150] crescentic IgAN should be treated with cytotoxic therapy (1) when crescents are present in more than 10 % of glomeruli, (2) in the presence of active glomerular inflammation, (3) with rapid worsening of renal function, and (4) in the absence of advanced chronic lesions.

KDIGO guideline suggests not to treat IgAN patients with corticosteroid and cyclophosphamide or azathioprine unless is a crescentic IgAN with rapidly deteriorating kidney function. Immunosuppressive therapy is also not recommended in patients with GFR < 30 ml/min per 1.73 m^2 unless patients have a crescentic IgAN.

Corticosteroids and cyclophosphamide are suggested in patients with rapidly progressive crescentic IgAN (>50 % of glomeruli) analogously to the treatment of ANCA vasculitis.

Mycophenolic Mofetil

Tang et al. [151] reported the long-term results of their first experience on 40 Chinese patients randomized to receive either mycophenolic mofetil (MMF) for 6 months or to continue angiotensin II blockade. The actuarial renal survival curve in the immunosuppressive-treated group at 6 years was better than in controls: 90 % vs. 65 %. The anti-proteinuric effect was greater in treated group, but this effect tends to disappear 2 years after the end of treatment.

The only study that tested MMF in association with corticosteroids was published by Roccatello et al. [152]. They evaluated a subset of patients who had acute inflammatory histological changes with diffuse mesangial proliferation and florid crescents in at least 10 % of glomeruli together with heavy proteinuria (2.4 g/24 h) and renal failure (serum creatinine 1.6 mg/dl). They treated the patients with three pulses of methylprednisolone (15 mg/kg) followed by oral prednisone 0.8 mg/kg/day tapered until discontinuation in 4 months plus MMF 2 g/day for 6 months. Serum creatinine,

proteinuria, and microscopic hematuria significantly dropped at 6 months compared with baseline values and remained lower at the end of follow-up 51 months later. The authors concluded that MMF together with steroid administration may be useful for treating the subset of IgAN patients with florid inflammatory histological changes, heavy proteinuria, and renal failure.

Two RCTs evaluated the efficacy of MMF therapy in IgAN. Maes et al. studied 34 patients treated with ACE inhibition and MMF 2 g/day ($N=21$) or placebo ($N=13$). After 36 months there was no difference between the two groups in terms of percentage of patients with a decrease of at least 25 % of the inulin clearance or increase in serum creatinine more than 50 % over 3 years [153]. Frisch et al. study included 32 patients with advanced IgAN (baseline serum creatinine 2.4 mg/dl) and who had failed previous immunosuppressive therapy. Patients were randomized to receive 1 year of MMF or placebo. Total follow-up lasted 2 years. All patients received angiotensin II blockade. Five out of 17 patients who were given MMF (1,000 mg bid for 1 year) vs. 2 of 15 patients in the placebo group reached a 50 % increase in serum creatinine ($p=0.4$). Ten who received MMF vs. 7 who received placebo had a 0.5 mg/dl increase in serum creatinine ($p=0.7$). Only three MMF and two placebo patients had a 50 % reduction in 24-h proteinuria. MMF was shown not to have beneficial effects in these patients with relatively advanced stage of diseases [154].

A recent systematic review [155] as well as a recent meta-analysis [156] confirmed that currently available evidence does not support the routine use of MMF, and there is no benefit in treating advanced IgAN with MMF. It is important to highlight that MMF is associated with an increased fetal risk and should not be used in women who are or might become pregnant. KDIGO guideline does not recommend the use of MMF in IgAN.

Plasma Exchange

Plasmapheresis has been used in some cases of IgAN that presented with rapidly progressive glomerulonephritis and diffuse extracapillary proliferation. Roccatello et al. [157] described a cohort of six patients with IgA and 40–90 % of glomerular crescents who received a combined treatment with three pulses of methylprednisolone (15 mg/kg) followed by oral prednisone (1 mg/ kg/day for a month with subsequent tapering), oral cyclophosphamide (2.5 mg/kg/day for 2 months), and therapeutic apheresis (10 procedures with one plasma volume exchange). A substantial clinical improvement was observed, and two patients recovered from dialysis. However, a second biopsy revealed a persistence of florid crescents, and in the long term (24 months), the initial improvement reverted in three out of six patients.

Clinical Practice Recommendations for Primary IgA Nephropathy

Based on the available data and our own clinical experience, the following indications are suggested [106–158].

No specific therapy is recommend for isolated hematuria or recurrent macroscopic hematuria with normal renal function and proteinuria <0.5 g/24 h unless florid inflammatory features are present at the renal biopsy. Patients should be monitored regularly (every 6–12 months) to detect new onset of hypertension, worsening proteinuria, and renal impairment. If they develop hypertension, ACEi/ARBs therapy is recommended [158] at the dose which assures the achievement of pressure values less than 130/80 mmHg.

For patients with hematuria and persistent proteinuria ranging between 0.5 and 1 g/24 h ACEi or ARBs therapy should be titrated to the maximum tolerated dose in order to achieve proteinuria values <1 g/24 h.

If proteinuria persists at levels >1 g/24 h despite 3–6 months of supportive care, especially if renal function is relatively preserved, a 6-month course of corticosteroid therapy might be attempted. An alternative regimen that avoids pulses of MP consists of oral prednisone 2 mg/kg every other day for 2 months, tapering to 0.5 mg/kg (approximately 30–40 mg) every other day for an additional 4 months. Combination with azathioprine provides no additional benefits in these patients.

In patients with hematuria and proteinuria >1 g <3 g/24 h renal impairment (serum creatinine >1.5 mg/dl) and no significant signs of chronic damage at the kidney biopsy, a combined therapy with oral steroid and cyclophosphamide (1.5 mg/kg/day) or MMF (1,000 mg twice a day) is suggested.

Even in the absence of RCTs, patients with crescentic IgAN and rapidly progressive course should be treated with three pulses of methylprednisolone (15 mg/kg), followed by oral prednisone and either cyclophosphamide (1.5–2 mg/kg/day or 0.5 g/m^2 i.v. monthly) for at 2–3 months followed by azathioprine (1.5 mg/kg/day), or MMF (1,000 mg twice a day for at least 6 months tapered to 1,000 mg a day for another 6 months).

In diffusely extracapillary IgAN, as defined by the presence of crescents in more than 50 % of glomeruli, some additional benefit of plasma exchange is generally recognized.

Patients with IgAN and nephrotic syndrome should be treated either with high-dose corticosteroids or a combined therapy of corticosteroids and cyclophosphamide.

Patients with gross hematuria and features of ATN and tubular erythrocyte casts at the renal biopsy could be managed with supportive therapy alone. A repeat biopsy should be considered in those patients who failed to show spontaneous functional improvement in order to rule out a crescentic glomerulonephritis.

Treatment of HSPN

Corticosteroids have been widely thought to be helpful in treating both the abdominal and the joint pains. Instead, both retrospective and randomized controlled studies, which were focused on the value of corticosteroids in decreasing the risk of kidney involvement, obtained conflicting results [159–162]. Although nephritis is the most serious long-term complication of HSP, little data is also available to determine the best treatment. Corticosteroids alone do not provide consistent benefit in treating patients with severe HSP nephritis. Azathioprine, cyclophosphamide, cyclosporine, and mycophenolate have been used in combination to corticosteroids, and general indications are similar to primary IgAN [163–165]. In a randomized, double-blind, placebo-controlled trial, administration of prednisone 1 mg/kg/day for 2 weeks, tapered over 2 more weeks, resulted in resolution of renal involvement and reduced severity and duration of abdominal and joint pain [160]. This scheme was not effective in purpura. A retrospective study failed to demonstrate a superiority of the combination of corticosteroids plus cyclophosphamide compared to corticosteroids alone [166]. One randomized controlled trial in a small number of patients suggested that cyclosporine may be more beneficial than corticosteroids alone in patients with nephrotic range proteinuria [167]. In a large French series, methylprednisolone pulses followed by oral prednisone and cyclophosphamide resulted in clinical recovery in about 70 % of children with nephritic syndrome or diffuse (>50 %) glomerular crescents [168]. In the last decade, plasma exchange, given alone or in conjunction with immunosuppressive therapy in children with >60 % glomeruli with crescents, has claimed to obtain good short- and medium-term outcome [157, 169, 170]. The beneficial effects of mycophenolate mofetil [152] and rituximab [171] need confirmation in larger series of patients.

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Anti-glomerular Basement Membrane Disease

9

Alan D. Salama

Introduction

Anti-glomerular basement membrane (GBM) disease is also commonly termed Goodpasture's disease, an eponym coined by Stanton and Tange in 1958 after their report of patients with the condition [1] and their recognition that they were similar to the cases published 37 years earlier by Ernest Goodapsture [2]. Goodpasture was an American pathologist who described the clinical constellation of pulmonary hemorrhage and renal failure during the influenza pandemic of 1918–1919. While Goodpasture believed the pathology he observed was due to influenza infection (see potential disease triggers below), he clearly understood the link between pulmonary and renal systems which provided the subsequent basis for the molecular understanding of the condition that now bears his name. It is debatable as to whether the patient Goodpasture described had anti-GBM disease or small vessel vasculitis, a common differential diagnosis presenting as a pulmonary-renal syndrome. Some therefore differentiate Good-pasture's disease (anti-GBM disease) from Goodpasture's syndrome (pulmonary-renal syndrome); however, it may be clearer to only use one eponymous title, Goodpasture's disease as a particular form of pulmonary-renal syndrome, caused by a specific autoimmune response directed against the GBM.

Although uncommon, the rapid evolution of anti-GBM disease, leading to irreversible kidney failure and life-threatening lung hemorrhage, requires that it is a diagnosis that it made quickly so that timely treatment may be initiated. It is clear that the severity of the renal involvement at presentation correlates with long-term patient and kidney outcome. Anti-GBM disease has been extensively studied, despite its rarity, since it remains one of the few human autoimmune diseases in which the autoantigen has been identified.

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Epidemiology

Anti-GBM disease has an incidence of 0.5–1 case/million population/year in white populations, making it 10–20 times less common than pauci-immune systemic, antineutrophil cytoplasm antibody (ANCA)-associated vasculitis, which represents one of the most significant differential diagnoses. Anti-GBM disease is a rare pulmonary-renal syndrome but is responsible for up to 20 % of cases of acute renal failure presenting due to rapidly progressive glomerulonephritis [3]. There is a bimodal age distribution with peaks in the third and sixth decades and slight male predominance, with men presenting at a younger median age than women (35 vs. 45 years, respectively) [4]. Although early reports consisted of mostly white patients, it is clear that other ethnic groups are also susceptible [5–8]. There are both genetic and environmental factors that have been demonstrated to be associated with disease (see below), and there is one of the strongest human leukocyte antigen (HLA) associations, with over 90 % of patients carrying the HLA DR1501 or 0401 alleles. Although anti-GBM disease does not form part of a generalized autoimmune phenotype, there are associations with ANCA positivity, in approximately one third of patients, most commonly in a perinuclear fluorescent pattern (P-ANCA) with anti-myeloperoxidase reactivity [9, 10]. In addition, there are associations with other immune-mediated diseases such as membranous glomerulopathy [11] (though etiologically this may relate more to damage of GBM rather than autoimmune reactivity) and case reports of associations with uveitis [12], dermatomyositis [13], bullous pemphigoid [14], and systemic lupus erythematosus [15], among others.

Clinical Features

Patients may present with isolated renal failure, lung hemorrhage, or most commonly both (in over 60 % cases) [4, 16], especially in those who smoke, which is a significant

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Table 9.1 Clinical and pathologic features of anti-GBM disease compared with pauci-immune ANCA-associated vasculitis

Anti-GBM disease	ANCA-associated vasculitis
• May present with rapidly progressive renal failure and anuria • Systemic prodromal symptoms are uncommon • Dialysis dependency at presentation rarely associated with renal recovery • Pulmonary hemorrhages in 50–70 % cases • Anti-GBM Ab positive (some serum anti-GBM antibody negative cases reported) • ANCA positive in 30–47 % cases • Glomerular crescents of similar age • Linear immunoglobulin deposition	• May present with rapidly progressive renal failure • Systemic prodromal symptoms are common, except in cases of renal limited vasculitis • Dialysis dependency at presentation associated with renal recovery in 40–60 % cases • Pulmonary hemorrhage in 25 % • Anti-GBM negative, ANCA positive in 95 % cases • Glomerular lesions of various ages • Pauci-immune

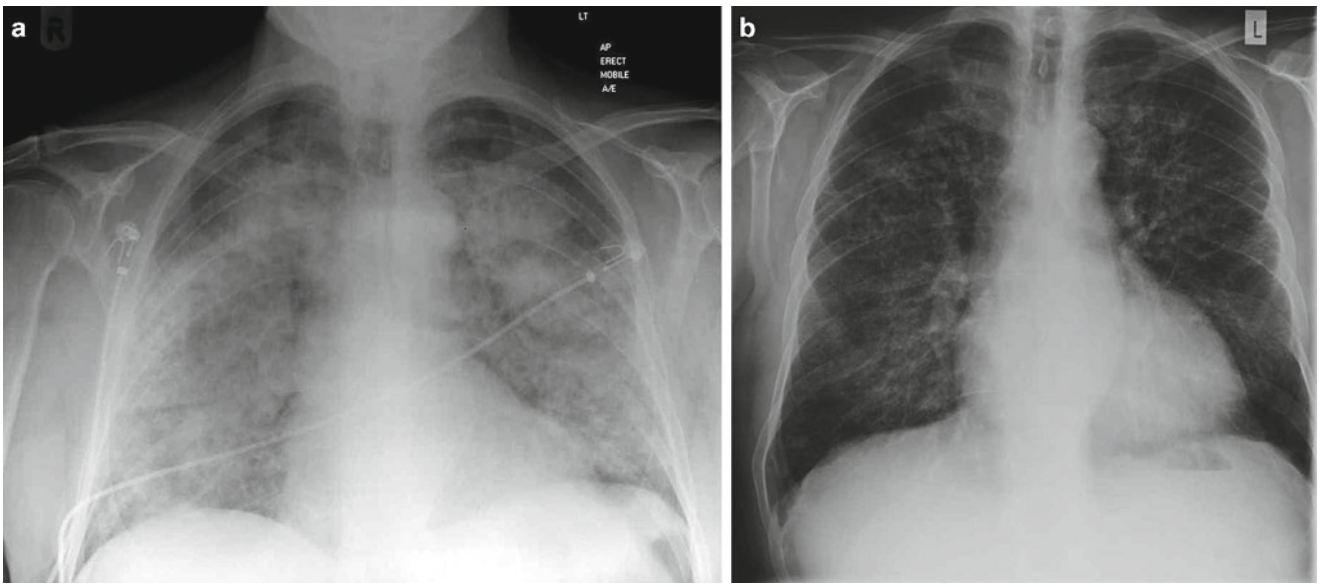

Fig. 9.1 (**a**) Chest radiograph of a patient who presented acutely with anti-GBM disease demonstrating pulmonary hemorrhage with relative sparing of costophrenic angles and (**b**) 3 days later following immunosuppression and plasmapheresis

risk factor for lung hemorrhage. Unlike systemic ANCA-associated vasculitis, a long prodrome with systemic symptoms is unusual (see Table 9.1) and disease relapses rarely occur. An important clinical feature that may alert clinicians to the likelihood of anti-GBM disease is complete anuria, found in almost 20 % cases [17], which is more common than systemic vasculitis, and should prompt rapid radiological assessment of the renal tract to exclude other causes such as thrombosis and obstruction. While symptoms related to renal failure tend to occur late and with advanced disease, some patients may report macroscopic hematuria or loin pain related to renal edema [16]. Although the target autoantigen is present in other organs such as the eye and ear, symptoms related to these systems are generally limited.

Lung hemorrhage may be subclinical and present as an iron deficiency anemia or as breathlessness with cough. In more advanced cases, hemoptysis is a presenting feature, and there may be a drop in hemoglobin which is not explained by any other signs or symptoms. Radiological examination demonstrates diffuse alveolar airspace shadowing, which cannot easily be distinguished from pulmonary edema, although it is said that apical and costophrenic spaces are less often affected (Fig. 9.1). Pulmonary function tests may be useful to confirm hemorrhage through the finding of elevated corrected gas transfer coefficient (KCO). This may also provide a non-radiological means of monitoring resolution of lung hemorrhage. In a recent series from China, patients older than 65 years were reportedly less likely to have hemoptysis and had marginally better presenting creatinine, although they had a greater mortality than their younger counterparts during follow-up [6]. These data confirm findings in earlier series that there was a higher incidence of pulmonary hemorrhage in younger male patients [4].

Those patients with double positivity (ANCA and Anti-GBM antibodies) have a renal prognosis similar to that in

isolated anti-GBM disease, but their presentation may encompass significant constitutional symptoms and their tendency to relapse is more in keeping with ANCA-associated vasculitis [10, 18–20]. Patients older than 65 were more often double positive for ANCA and anti-GBM antibodies in a large Chinese cohort [6].

Pathology

The characteristic pathologic feature of the disease is a crescentic glomerulonephritis, with the majority of glomeruli showing crescents of a similar age (unlike the pattern found in primary systemic vasculitis; see Table 9.1) (Figs. 9.2 and 9.3).

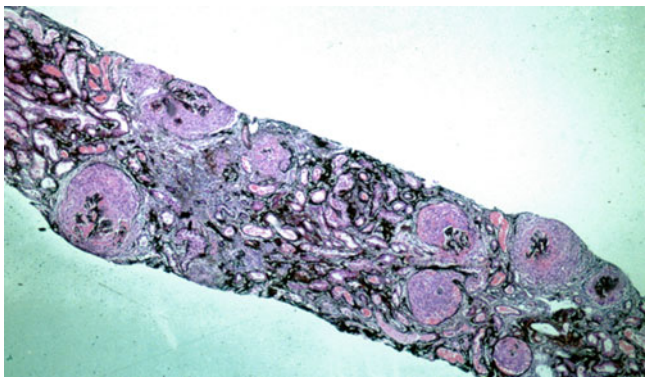

Fig. 9.2 Low power silver methenamine stain of a renal biopsy demonstrating diffuse crescentic glomerulonephritis with cellular crescents of similar age (×100)

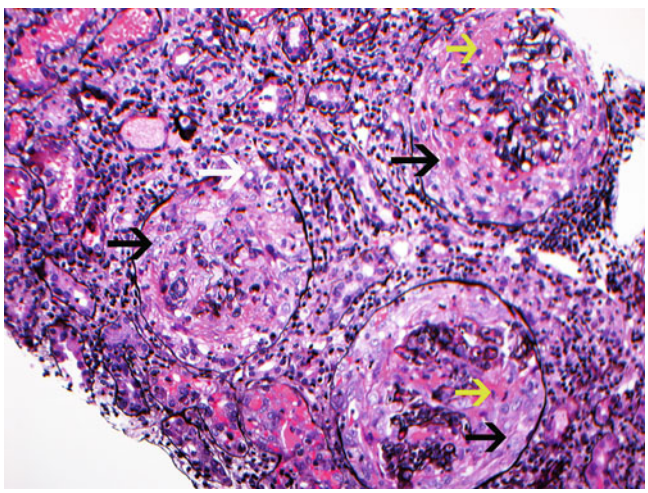

Fig. 9.3 Silver stain showing 3 glomeruli with large circumferential cellular crescents with necrosis. Note disruption of Bowman's capsule. There is prominent interstitial inflammation present. Red blood cells are present in tubules. (black arrow points at cellular crescents, yellow arrow points at fibrinoid necrosis, and white arrow points at disruption of Bowman's capsule. Jones silver methanamine stain 200x). Courtesy of Dr. Sanjeev Sethi, Mayo Clinic

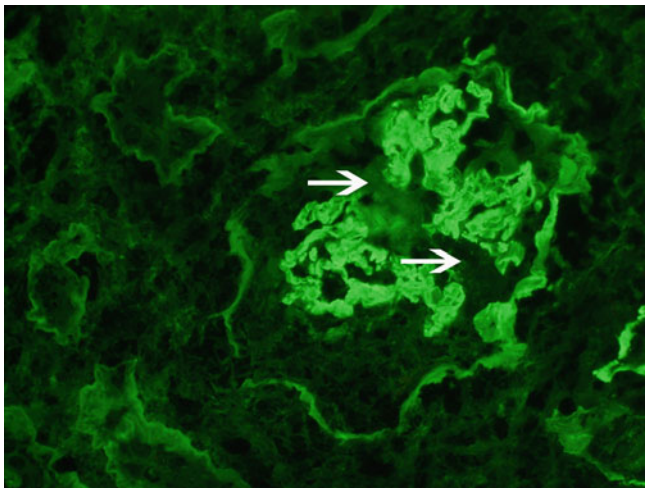

Fig. 9.4 Immunofluorescence microscopy showing linear staining for anti-IgG along the glomerular capillary walls. Note disruption of the linear staining necrosis/crescent (white arrow points at disruption, 400x). Courtesy Dr. Sanjeev Sethi, Mayo Clinic

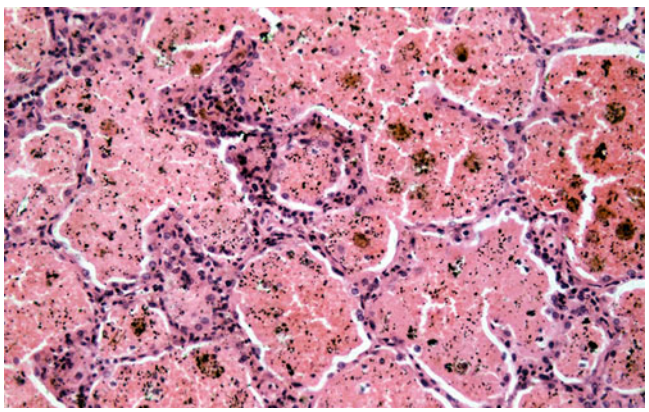

Fig. 9.5 Section of lung from a patient with Goodpasture's disease and alveolar hemorrhage demonstrating hemosiderin-laden macrophages and red blood cells filling the alveolar space

Fibrinoid necrosis may also be present. Furthermore, there may be a florid alveolitis, associated with pulmonary hemorrhage, found in over half the patients (Fig. 9.4). Immunohistochemistry reveals linear immunoglobulin deposition, generally IgG with C3, while IgA and IgM are deposited less commonly (Fig. 9.5). The diagnosis relies on the demonstration of linear immunoglobulin deposition along the GBM in the context of a crescentic glomerulonephritis. Serum anti-GBM antibodies, detected by ELISA, multiplex-based methodology, or Western blotting have high sensitivity and specificity for disease. Rare cases in which circulating anti-GBM antibodies are not detected by conventional means have been described [21]. A number of different ELISA assays and multiplex-based detection systems are available, with most assays demonstrating similar degrees of

sensitivity (>95 %) but variable specificity, ranging from 91 to 100 % [22, 23]. These autoantibodies are almost never found using conventional techniques in normal individuals and thus their detection represents a highly sensitive and specific assay for the disease [24]. However, patients with HIV or HCV infection, and polyclonal B cell activation, may be anti-GBM positive without any signs of disease, making the test interpretation more problematic in these scenarios [25]. Overall, since there may be false-positive and false-negative cases of anti-GBM antibody, over-reliance on serum analysis may be misleading and confirmation of the diagnosis necessitates renal biopsy.

Recently, following methods of purification using autoantigen affinity columns, low titers of predominantly IgG4 and IgG2 anti-GBM antibodies have been found in sera from normal Chinese and Swedish populations [9, 26]. The significance of these "natural" anti-GBM antibodies in healthy individuals is uncertain, although they suggest that healthy individuals have effective methods of regulation, preventing these low-titer antibodies from inducing disease.

Serum ANCA has to be tested for any patient with anti-GBM disease as they may warrant different management strategies with maintenance immunosuppression to prevent relapsing vasculitic symptoms.

Outcome

Both patient and renal prognosis are dependent on the severity of disease at presentation, with the presenting creatinine and extent of glomerular involvement clearly relating to the likelihood of renal recovery [4, 16, 27]. Again, unlike systemic ANCA-associated vasculitis, where 50–60 % of dialysis-dependent patients will recover independent renal function even temporarily [28], in anti-GBM disease dialysis dependency at presentation with 100 % glomerular involvement on biopsy is generally associated with irrecoverable renal failure, even with adequate therapy [4]. Overall, in patients with more severe renal dysfunction at presentation, in particular those requiring dialysis, renal recovery is less likely (Table 9.3 [4, 16, 17, 27, 29]). Other degrees of severity generally respond with some degree of renal recovery following immunosuppression and plasmapheresis (see treatment below) [4, 16, 27].

Risk Factors

Genetic Susceptibility

Human Leukocyte Antigen Genes

The association of anti-GBM disease with HLA DR15 (previously known as HLA-DR2) was first reported over 20 years ago and remains one of the strongest HLA associations for autoimmune disease. Since then a number of studies, including a large meta-analysis using more refined genotype-based techniques, have confirmed this association and gone on to reveal a hierarchy of susceptibility and resistance alleles that map to the HLA system [30, 31]. The strongest linkage of anti-GBM disease is with HLA-DRB1*1501, being found 3.5 times more in patients than in the control groups. By excluding the effect of DRB1*1501, subsequent analysis demonstrated an increased frequency of DRB1*03 and DRB1*04 and a decreased frequency of DRB1*01 and DRB1*07. Furthermore, an increased frequency was found of DQB1*06 (some alleles of which are in linkage disequilibrium with DRB1*1501) and DQB1*03 and negative associations with DQB1*05 alleles. Similar HLA associations have now been reported in Japanese populations [32].

The recent meta-analysis [31] demonstrated that both inherited DRB1 alleles exert an effect on disease susceptibility, but that if one of these alleles was DRB1*1501, the effect of the second allele was either neutral (DRB1*04 and DRB1*03) or diminished the susceptibility to disease (DRB1*01 and DRB1*07) caused by DRB1*1501. Moreover, by comparing the structure of the susceptibility (DRB1*1501) and protective (DRB1*0701) alleles, the authors found that a particular region of the peptide-binding groove of the HLA-DRB1 (pocket 4) was significantly different and may therefore play a role in disease susceptibility. Interestingly, protective DRB1*07 and DRB1*01 alleles bind the majority of peptides corresponding to α[alpha]3(IV) NC1 sequence with greater affinity than the DRB1*15 alleles [33]. This suggests that the mechanism of disease susceptibility with DRB1*15 is not due to more efficacious presentation of an α[alpha]3(IV)NC1-derived peptide but may be due to competition for the autoreactive peptide epitope by the protective (DRB1*07 and DRB1*01) alleles. However, the precise molecular basis of disease susceptibility conferred by HLA-DRB1*15 alleles remains unknown.

Non-HLA Genes

Experimental anti-GBM models demonstrate that Th1 predominant strains appear to be susceptible to disease induction whereas Th2 strains are resistant, while other non-major histocompatibility (MHC) genes also confer susceptibility since disease can be induced in some, but not other, strains carrying the same MHC alleles [34]. These differences do not appear to be related to levels of expression of α[alpha]3(IV)NC1 in the thymus, nor to differences in the COL4a3 gene, which encodes rat α[alpha]3(IV)NC1 [35].

A role in maintaining B cell tolerance to the autoantigen has recently been shown for the inhibitory FC gamma receptor IIB (FCλ[gamma]RIIB), since mice deficient in this receptor developed severe pulmonary and renal lesions following immunization with type IV collagen, unlike wild-type animals [36]. In addition, recent data from patients with

anti-GBM disease demonstrates that FCλ[gamma]RIIB polymorphisms(232T/I) are present at significantly elevated frequencies when compared with healthy controls [37], at least in Chinese populations. While the inhibitory receptor appears to attenuate disease, deficiency of all FC λ[gamma] receptors (FCRλ[gamma]) is associated with diminished disease, suggesting that stimulatory receptors are necessary for disease induction [38] and indeed copy number variation of the activatory FcgRIIIA receptor is found at higher frequency in patients compared to matched controls [39].

Environmental Factors

Although there is a strong disease association with HLA-DRB1*1501, this allele is present in almost a third of the white population and thus cannot solely explain the susceptibility to such a rare disease. It has therefore been proposed that a "second hit" occurs in susceptible individuals, which may be genetic or environmental.

In his original description of the clinical condition, Goodpasture believed he was studying the pathology of influenza infection, and although it has been shown that infectious episodes may precede clinical signs of autoimmunity [40], identifying a specific etiological infectious agent has not been successful.

Pulmonary hemorrhage in Goodpasture's disease has been associated with a history of cigarette smoking, and disease relapses have been temporally linked with episodes of smoking [41]. Furthermore, there are human and experimental data suggesting that disease may be related to inhalation of hydrocarbons, although in some cases the exposure may simply have provoked a manifestation of an already existing disease [42, 43]. It is possible that pulmonary injury (through cigarette smoke or inhaled hydrocarbons) exposes the cryptic epitope within the alveolar basement membrane, thus triggering an immune response towards the α[alpha]3(IV)NC1 with subsequent anti-GBM disease. This suggestion is indirectly supported by the finding that exposure of α[alpha]3(IV)NC1 hexamers to reactive oxygen species is followed by significant anti-GBM antibody binding, which is absent using intact hexamers [44].

Autoimmunity

Humoral Immunity

The first demonstration that anti-GBM autoantibodies may be pathogenic dates back to 1967 when, in a classic series of experiments, Lerner, Glassock, and Dixon transferred antibodies eluted from the kidneys of patients with Goodpasture's disease to squirrel monkeys, which subsequently developed proliferative glomerulonephritis [45]. Adoptive transfer experiments such as these have been repeated using a number of animal models demonstrating that serum from diseased animals to syngeneic recipients leads to development of disease [46]. The pathogenicity of the autoantibodies is supported by clinical data from patient studies. For example, rapid disease recurrence occurs if transplantation is performed in the presence of circulating anti-GBM antibody [47], and there are correlations between disease severity and antibody titer [48].

The majority of patients' anti-GBM antibodies bind to the non-collagenous-1 domain of the α[alpha]3 chain, and in some cases the α[alpha]5 chain, of type IV collagen (α[alpha]3(IV)NC1 and α[alpha]5(IV)NC1, respectively), molecules that are found only in specialized basement membranes in sites such as the kidney, lung, choroid plexus, retina, and cochlea [47, 49, 50]. The clinical features are therefore a result of the tissue distribution of the antigen with pulmonary and renal disease predominating [51]. Rare cases with neurological involvement have been reported, but it is not clear if these are due immune effects on cerebral targets (such as the choroid plexus) [52]. The α[alpha]3 chain forms a triple helix with the α[alpha]4 and α[alpha]5 chains, which combines with another triple helix, through the NC1 domains, to form a NC1 hexamer, reinforced through disulphide bonds. Some patients may also form additional antibodies directed towards other collagen chains (such as the α[alpha]1, α[alpha]4, or α[alpha]6 chains of type IV collagen) [49, 53, 54]. The majority of the autoantibodies are of IgG1 subtype [55], while data from Chinese patients suggest that greater degrees of renal impairment are associated with a greater proportion of IgG1 and IgG3 [56]. The specificity of circulating and tissue-bound antibodies is identical, shown following elution of antibodies from affected organs [54]. Two immunodominant antigenic epitopes have been identified, termed epitopes A(E_A) and B(E_B) [57–59] in α[alpha]3(IV)NC1, to which the antibody binds. These epitopes are in close proximity in the intact molecule and contain a high degree of homology, leading to predictions of high degrees of autoantibody cross-reactivity and stabilization occurring, which may explain the high-affinity interaction [60]. Although antibodies may bind these sites separately, some autoantibodies recognize an epitope formed by combination of both antigenic sites [61]. Importantly, anti-GBM antibodies do not bind the intact NC1 hexamer but do so only following its dissociation [61]. Moreover, it appears that in vivo the epitopes are sequestered under normal circumstances, by α[alpha]4 and 5 chains, and only exposed following some physicochemical disruption of the GBM—such as exposure to hydrocarbons, smoking, infection, lithotripsy [62], degradation by reactive oxygen species [44], or basement membrane disruption consequent to some other glomerulonephritis [11, 63]. These may disrupt the NC1 hexamer and in turn induce conformational changes in

the E_A and E_B epitopes, allowing priming of the immune response [54]. This is in contrast to anti-GBM disease that can arise in transplanted kidneys in patients with Alport's disease, in whom the α[alpha]5 molecule is recognized as foreign and in which the alloantibodies can bind the epitope within the intact hexameric structure [54].

Cellular Immunity

Autoantibodies may be sufficient to transfer disease to primates, but in other model systems, cellular-based immunity is necessary, since transfer of autoantibodies to T cell receptor knockout mice does not result in disease, and disease can be transferred using mononuclear cells in both murine and avian models [46, 64]. Furthermore, generation of anti-GBM antibodies that bind in the kidney occurs in both susceptible and resistant strains of mice following immunization [65]. Thus, although autoantibodies are centrally important to disease pathogenesis, autoreactive T cells appear to be both necessary and sufficient to induce disease in animal models.

Evidence demonstrating a role for cellular immunity in human disease pathogenesis include the striking HLA-DRB1*1501 and DRB1*04 association [30], the class-switched (IgG1 and IgG4) autoantibody isotypes in the majority of patients (implying T cell-mediated help), and the finding of both CD4+ and CD8+ T cells within affected glomeruli [66, 67]. Furthermore, in rodent models, disease can not only be transferred by mononuclear cells but is also inhibited by anti-T cell therapies, including CTLA4-Ig co-stimulatory blockade [68] anti-CD4 and anti-CD8 monoclonal antibodies, and cyclosporin A [69]. Disease is absent in CD4+ and CD8+ knockout animals, requires a Th1 pattern of cytokine expression [65], and is attenuated following oral administration of GBM, inducing a form of mucosal tolerance [70]. Moreover, it appears that disease can be dissociated from autoantibody generation, since a number of animals may develop anti-GBM autoantibodies but neither nephritis nor alveolitis, while disease in others may be accompanied by circulating but not deposited anti-GBM antibody [71].

Evidence of autoreactive T lymphocytes directed against the α[alpha]3(IV)NC1 antigen has been demonstrated in patients by proliferation of peripheral blood mononuclear cells taken at the time of disease presentation [72]. Of note, healthy control subjects also demonstrated some proliferative capacity to these antigens, despite an absence of anti-GBM autoantibodies. This has been confirmed recently using peptides derived from α[alpha]3(IV)NC1, to which most control subjects had some response [73]. These data are similar to those described in multiple sclerosis patients and healthy subjects tested for T cell reactivity to myelin basic protein. However, there is a significantly different frequency of α[alpha]3(IV)NC1-specific autoreactive T cells in acute patients and controls [74]. Furthermore, this frequency diminishes with time from disease onset and reaches control levels after a number of years, mirroring the decline of anti-GBM autoantibodies in untreated patients.

The rarity of α[alpha]3(IV)NC1-specific autoreactive T cells in healthy individuals is in part due to the deletion of these cells during thymic ontogeny. In keeping with data on other tissue-specific autoantigens, a number of groups have found that human thymus expresses the α[alpha]3(IV)NC1 autoantigen, making it likely that negative selection of α[alpha]3(IV)NC1-reactive T cells does occur [74, 75]. Whether there is a difference in levels of α[alpha]3(IV)NC1 expression in the thymus between patients and healthy individuals, as has been suggested for other autoantigens (e.g. in human diabetes), has not yet been established.

The epitopes within α[alpha]3(IV)NC1 to which the autoreactive T cells respond to have been harder to define. From animal models a single peptide (containing three critical amino acids) is sufficient to induce disease, and this resembles peptides derived from a number of infectious agents, implicating molecular mimicry as a potential pathogenic mechanism and thus linking infection with subsequent disease [76]. Interestingly, the peptides that bind the susceptible HLA molecules most avidly are not the ones to which the T cells respond [77, 78]. Indeed rapid destruction of the autoreactive peptides by proteases (such as Cathepsin D) during antigen processing may serve to limit the exposure of autoreactive T cells to its antigen and may explain why these low frequency cells escape thymic selection [73].

Although the factors responsible for initiating the proliferation of autoreactive T cells in patients but not in healthy people remain unknown, they may relate to the exposure of neo-epitopes following NC1 hexamer dissociation, altered antigen processing allowing the pathogenic epitopes to be presented, or altered T or B cell regulatory mechanisms. This is in the context of the individual's MHC class II alleles, as well as to other genetic and environmental factors.

Induction of Autoimmunity

The isolation of anti-GBM antibodies, albeit at low titer, and of circulating α[alpha]3(IV) NC1-specific T cells in healthy controls suggested that there may be a failure to appropriately delete these autoreactive cells during immunological development and that the cryptic nature of the autoantigen or the rapid degradation of the autoreactive epitopes prevents disease induction under normal circumstances. However, recent experiments using transgenic mice expressing α[alpha]3(IV) NC1-reactive immunoglobulin have demonstrated that autoreactive B cells are effectively deleted during maturation. These data suggest that generation of autoreactive B cells may result

as a failure of central tolerance through IgG editing or following somatic mutation of mature B cells [79]. These data assumed that some autoantigen is expressed or delivered to the bone marrow during ontogeny. However, even in the absence of autoantigen (achieved by cross breeding the transgenic α[alpha]3(IV) NC1-Ig reactive mice with α[alpha]3(IV)-deficient animals), it appears that autoreactive B cells are eliminated during development, implicating a more complex regulatory network and suggesting that another (bone marrow-expressed) antigen may promote deletion of the α[alpha]3(IV) NC1-autoreactive B cells [80]. The nature of this potential tolerizing antigen remains to be elucidated. Interestingly, in patients (in whom tolerance has failed), this antigen or a cross-reactive antigen may be responsible for episodes of immune activation and disease.

Using a prospective analysis of sera collected from patients who had samples stored in the defense department repository, some of whom went on to develop anti-GBM disease, Olson et al. reported that asymptomatic anti-GBM antibodies are found in patients up to 10 months before diagnosis [5], but at low levels, up to many years in advance. They found a sudden increase in titer immediately preceding clinical disease. Interestingly, they also described low-level ANCA that preceded the development of anti-GBM antibodies in a significant proportion of patients, up to 5 years in advance, and postulated a possible pathogenic role for ANCA inducing GBM damage and exposing a cryptic epitope.

The need to break multiple regulatory mechanisms and expose the cryptic epitope may explain the rarity of clinical disease and the reason for a paucity of disease relapses, unlike other autoimmune diseases. Recent reports of anti-GBM disease arising following depletional biologic therapy with alemtuzumab (Campath-1H) also suggest that autoreactive B cells may be found in some susceptible individuals, but only when regulatory circuits are damaged do increased B cell responses arise followed by clinical disease [81].

Therapeutic Advances

Untreated, the disease was rapidly fatal in cases with pulmonary hemorrhage or in those with advanced renal failure prior to the availability of dialysis. However, with the introduction of dialysis, mortality rates were significantly reduced, and these were further improved, as was renal recovery, with the introduction of immunosuppressive regimens. In those patients presenting dialysis dependent and with 100 % crescentic change on renal biopsy, many practitioners avoid immunosuppression as renal recovery is unlikely, and the risks of immunosuppression outweigh the benefits [82]. However, in the face of other degrees of renal involvement, there is still a chance of regaining independent renal function [4] and this tips the balance in favor of treatment, assuming the patient is assessed as being robust enough to withstand immunotherapy. The other situation in which treatment may be appropriate in someone with dialysis dependency is if there is a living donor willing to donate a kidney, in which case more rapid antibody removal will be achieved (generally within 2–8 weeks) [8], allowing earlier transplantation (see below). Without immunosuppression, anti-GBM antibodies may persist for variable periods of time ranging from a few months to years [82].

Therapy with cyclophosphamide, corticosteroids, and plasmapheresis has remained the gold standard for over 30 years. Standard regimens utilize oral daily cyclophosphamide for 3 months; oral corticosteroids, tapered over 6 months; and plasmapheresis (Table 9.2). Whether pulsed intravenous cyclophosphamide is equivalent to daily oral therapy has not been demonstrated in any study, but as with dialysis-dependent ANCA-associated vasculitis, it appears some practitioners have already changed to this modality with various cases describing its use [52, 83, 84]. It would clearly be of interest to compare outcomes of oral and pulsed

Table 9.2 Recommended treatment regimen for anti-GBM disease

Treatment	Dosing
Plasmapheresis	• Daily 3–4 L exchanges for 5 % human albumin solution. Addition of fresh frozen plasma for patients undergoing invasive procedure (e.g., biopsy) within 72 h • Should be continued until antibody level is undetectable • Withhold if platelet count <70, fibrinogen <1 g/L • Watch for hypocalcemia, hypokalemia, and coagulopathy
Prednisolone	Daily oral dosing at 1 mg/kg, with maximum at 60 mg. Reduce dose weekly to 20 mg by week 6, then more slowly. Aim to stop all steroids by 6 months
Cyclophosphamide	Daily oral dosing 2–3 mg/kg. Reduce dose in older patients (1 mg/kg in >70 years). Withhold if WCC $< 4 \times 10^9$ Pulsed intravenous cyclophosphamide may be as good but no evidence for this
Prophylactic therapies	• Oral antifungals (nystatin/amphotericin or fluconazole) • Gastric protection with H2 antagonists or proton pump inhibitors • Oral co-trimoxazole for prevention of *Pneumocystis jiroveci* pneumonia • Calcium/D3 bone protection

intravenous regimens, the latter of which clearly provides a benefit with regard to infectious risks and reduced cumulative doses of cyclophosphamide.

Plasmapheresis was first introduced by Lockwood and colleagues in the 1970s, when they described the outcome of seven patients treated with steroids, cyclophosphamide, and plasmapheresis [85] and demonstrated a rapid removal of anti-GBM antibodies, with improvement in renal function in those not presenting dialysis dependent. There has only been one limited study examining the additional benefit of plasmapheresis to conventional cytotoxic and steroid therapy. This enrolled a total of 17 patients, all treated with cyclophosphamide and steroids and eight treated with additional plasmapheresis, every 3 days. Antibody removal was more rapid and renal function had improved to a greater extent in those in the plasmapheresis arm. However, disease was less severe (as judged by presenting serum creatinine and percentage crescent involvement on biopsy) in the plasmapheresis group [27]. Using a standard protocol including plasmapheresis, renal recovery rates are generally good in those with some degree of renal function preservation (Table 9.3).

Small numbers of case reports have suggested efficacy with rituximab [86, 87] while other reports of successful outcome with mycophenolate mofetil [84, 88] and ciclosporin [89] in patients unresponsive to or intolerant of standard therapy have been published. However, no controlled trials have been carried out and thus these treatments could not be recommended as first-line therapy.

Post-transplant anti-GBM disease

Early reports of kidney transplantation in patients with anti-GBM disease were notable for recurrent disease which resulted in rapid graft loss [47]. These cases were likely transplanted in the presence of circulating anti-GBM antibodies. However, if transplantation is deferred until a time when there are no circulating anti-GBM antibodies detectable, it is extremely rare to develop recurrent disease, with none of 16 transplanted patients developing recurrence in a large UK series [10]. Occasional cases [90, 91] of late disease relapse in transplants have been reported; in one case relapse occurred when the patient stopped all immunosuppression, suggesting that autoimmunity was only prevented by the use of maintenance immunosuppression [90]. Whether allograft rejection following cessation of immunotherapy was the initial trigger for augmenting the autoimmune response cannot be ascertained. In another, anti-GBM antibody titer was consistently at low positive levels prior to transplantation but did become negative following transplantation. Subsequent herpes zoster infection predated the recurrent anti-GBM disease, again suggesting that an immune trigger may have provoked augmented autoimmunity [91]. In cases of low-titer anti-GBM antibodies, others have successfully performed pre-transplant plasmapheresis to remove antibody, achieving negativity prior to transplantation, with no signs of recurrence in the transplanted graft. Unsuccessful removal of antibody precluded transplantation [82]. Overall, in a review of six reported graft recurrences, only two were successfully treated with augmented immunosuppression and plasmapheresis and resulted in graft survival [91].

Our policy has been to wait for 6 months from the time of antibody negativity before considering transplantation, although there are no firm data to support this particular time period. By contrast in ANCA-associated vasculitis, transplantation earlier than 1 year from time of remission is associated with greater mortality [92], but relapses are not related to ANCA positivity at the time of transplantation.

A second consideration is the development of de novo anti-GBM disease in patients transplanted for Alport's syndrome. In classical X-linked Alport's syndrome, there is a mutation in the gene coding α[alpha]5 chain of type IV collagen, while in autosomal recessive or dominant forms, abnormalities in genes coding for either α[alpha]3 or α[alpha]4 chains of type IV collagen exist. In all these cases, expression of the α[alpha]3–4-5 heterotrimer molecule in the kidney is defective. However, in the transplanted organ, the normal α[alpha]5 (or α[alpha]3/α[alpha]4) chain is recognized as an alloantigen and initiates an anti-GBM immune response, which can lead to the development and deposition

Table 9.3 Reported renal recovery in patients with anti-GBM disease

		Percentage with independent renal function at 1 year according to presenting serum creatinine (μ[mu]mol/L)	
Study	Number	<600	>600
Levy et al. [4]	71	95[a]	8[b]
Daly et al. [16]	40	20	0
Bouget et al. [29]	14	50	0
Walker et al. [17]	22	82	18
Johnson et al. [27]	17	69	0

[a]In this study less than 500 μ[mu]mol/L
[b]Dialysis dependent with creatinine >500 μ[mu]mol/L

of anti-GBM antibodies. Linear antibody binding to the GBM in the renal allograft can be found in up to 20 % of Alport's patients; however, crescentic anti-GBM glomerulonephritis occurs in only a small fraction (5 %) [93]. The majority of alloantibodies in X-linked Alport's syndrome are directed against the α[alpha]5 chain of type IV collagen [94]. These will be detectable by immunohistochemistry in a kidney transplant biopsy but may not be detectable in serum if ELISA or multiplex assays utilize recombinant α[alpha]3 chain of type IV collagen as the target antigen [93]. Treatment of de novo anti-GBM disease in this setting is usually unsuccessful and graft loss frequent. In addition, if Alport's patients are re-transplanted following graft failure due to anti-GBM disease, more rapid recurrence ensues (as a result of the sensitization to the appropriate collagen alpha chain) and these patients are effectively rendered un-transplantable using conventional protocols [93].

Conclusions

Anti-GBM disease is an uncommon, rapidly progressive, pulmonary-renal syndrome which occurs in susceptible individuals, with a particular HLA-type, through T and B cell-dependent immune responses directed towards cryptic epitopes of the α[alpha]3- and α[alpha]5-(IV)NC1 autoantigens. It requires early recognition for effective therapy to be instituted with a reasonable chance of reversing renal dysfunction and arresting pulmonary hemorrhage. Therapeutic protocols have changed little over the last 30 years, but unlike ANCA-associated vasculitis, maintenance therapy is not required and only in exceptional circumstances, where cyclophosphamide avoidance is required, should alternative regimens be tried.

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Pathogenesis and Management of ANCA-Associated Vasculitis

10

Ulrich Specks

Introduction

Three systemic autoimmune small vessel vasculitis syndromes are collectively known as ANCA-associated vasculitides (AAV) because the majority of afflicted patients have circulating antineutrophil cytoplasmic antibodies (ANCA) [1]. The three syndromes are granulomatosis with polyangiitis (Wegener's; GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (Churg–Strauss; EGPA) [1]. They share many clinical disease manifestations caused by necrotizing small vessel vasculitis and capillaritis, including a variable propensity for renal involvement [1]. Focal segmental necrotizing glomerulonephritis is the most common renal manifestation of these disorders [1]. Immune complexes, autoantibodies, or other immune deposits are scarce or not detectable in affected tissues including the kidneys, and therefore this type of inflammation is called "pauci-immune" [1]. Any single organ can be affected first or in isolation in these syndromes. Accordingly, isolated pauci-immune glomerulonephritis should be considered a systemic disease, just as any other clinical presentation of AAV.

Two different types of ANCA are associated with these syndromes [2]. ANCA directed against the neutrophil serine protease proteinase 3 (PR3) cause a cytoplasmic (C-ANCA) staining pattern on ethanol-fixed neutrophils by indirect immunofluorescence microscopy (Fig. 10.1). PR3-ANCA are the predominant ANCA type in GPA, less common in MPA, and the rare exception in EGPA [2]. In contrast, ANCA directed against myeloperoxidase (MPO) generating a perinuclear (P-ANCA) fluorescence pattern are found in the majority of patients with MPA, but only in about 5–10 % of patients with GPA [2] (Fig. 10.1). In EGPA, ANCA are usually of the MPO-ANCA type and can be detected in 30–70 % of patients [3–5]. Patients with renal involvement are usually ANCA positive in all three syndromes [6–8].

Despite the many clinical similarities, there are substantial clinical differences between these three syndromes, and the different ANCA types portend different prognostic implications. Consequently, a nuanced clinical management approach to these patients is warranted. With emphasis on renal manifestations, this chapter aims to highlight the current understanding of the pathogenesis of AAV as well as clinical differences between the syndromes and different ANCA types as they impact treatment decisions and prognosis.

Epidemiology

Together, the AAV have a reported incidence of 10–20 cases per million per year [9]. The distribution of the specific syndromes varies geographically and with ethnicity [10]. GPA is more common in Northern than in Mediterranean Europe, where MPA is more common [10]. GPA is also exceedingly rare in African-Americans and in Japan, where it is almost exclusively encountered on the northern island of Hokkaido [11, 12]. A similar latitude-dependent incidence gradient has also been documented in New Zealand [13]. Furthermore, in New Zealand as well as in France, the incidence of AAV is higher in the populations of European ethnicity than among non-Europeans, Asians, or Pacific Islanders [13, 14].

Etiology and Pathogenesis

The etiology of the AAV syndromes remains unclear. Like for many other polygenic systemic autoimmune diseases, GPA, MPA, and EGPA are in all likelihood the result of complex interactions between genetic factors *predisposing* for the loss of self-tolerance and autoimmunity and *triggering* environmental exposures. These factors induce and maintain

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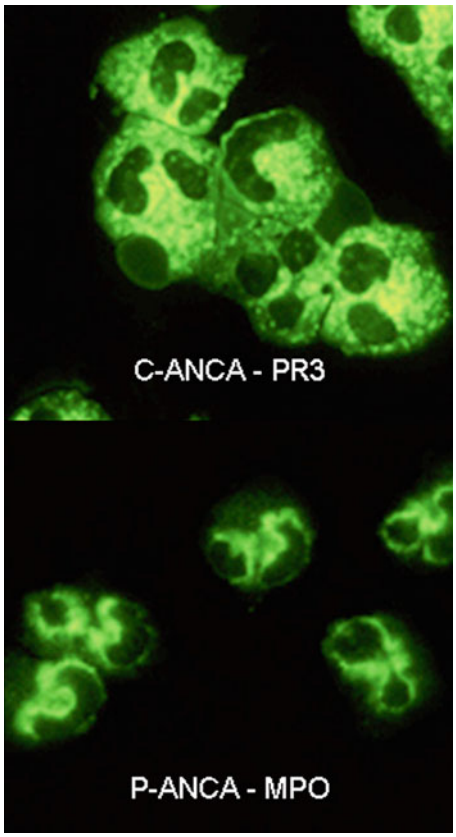

Fig. 10.1 On ethanol-fixed neutrophils, proteinase-3 (PR3) ANCA cause a characteristic cytoplasmic granular centrally accentuated immunofluorescence pattern, referred as c-ANCA (*top*), while myeloperoxidase (MPO)-ANCA causes a perinuclear immunofluorescence pattern, referred to as p-ANCA (*bottom*)

inappropriate lymphocyte activation and autoantibody production which lead to tissue injury and predispose to disease relapses.

Genetic Predisposition

A familial aggregation study found a relative risk of 1.56 for GPA among first-degree relatives of patients with GPA, which is similar to the risk seen in rheumatoid arthritis [15]. Familial clustering has also been reported with other AAV syndromes [16, 17]. Several candidate genes have been investigated in several different cohorts of patients with AAV, and single nucleotide polymorphisms (SNPs) have been found in several genes coding for proteins involved in the immune response [18].

Variants most strongly and most reproducibly associated with AAV are found in the major histocompatibility complex (MHC) and *PTPN22* genes [18]. Most genetic studies in AAV comprise only GPA cohorts, or the number of GPA patients included in the studied cohort is much larger than those with MPA and EGPA. Nevertheless, interesting genetic differences are emerging between the different syndromes. For instance, the *HLA-DPB1**0401 variant represents a strong and reproducible genetic risk factor for GPA, but not for MPA or EGPA [19–21]. Interestingly, African-Americans with PR3-ANCA-associated vasculitis had a 36-fold higher likelihood to have the *HLA-DRB1*15* genotype than community-based controls, and the *HLA-DRB*1501* allele, which is of Caucasian rather than African descent, conveyed a 73.3-fold higher risk for PR3-ANCA-associated vasculitis [22].

In contrast, an increase in the *PTPN22* 620W allele, which has been associated with other autoantibody-associated autoimmune diseases and has thus been implicated in the regulation of B lymphocyte activity, was found to be associated with both GPA and MPA [23–25].

Cytotoxic T lymphocyte antigen 4 (CTLA4), expressed mostly on CD4-positive T lymphocytes, exerts an inhibitory function on T lymphocytes by binding to CD80 and CD86 on antigen-presenting cells. CTLA4 competes with the co-stimulatory molecule CD28, which exerts a stimulatory effect on T lymphocytes, for binding to CD80 and CD86. If T lymphocytes are activated via the T cell receptor and CD28, CTLA4 expression is increased, probably as a regulatory mechanism. Via these mechanisms CTLA4 is thought to maintain peripheral self-tolerance [26]. Elevated levels of CTLA4 have been found in GPA, undoubtedly a reflection of T lymphocyte activation [27]. CTLA4 is coded for by the *CTLA4* gene, and *CTLA4* polymorphisms, which may negatively affect expression or function of CTLA4, have been associated with autoimmune diseases including GPA [24, 28, 29].

Another gene of interest is *PRTN3*, which codes for PR3, the most prominent target antigen for ANCA in GPA. Eight SNPs have been identified in the *PRTN3* promotor region and exons, but their function for PR3 expression is unclear. An increased frequency of one promotor polymorphism (A546G) was found among patients with GPA [30]. PR3 is not only stored in granules and released during neutrophil activation, but it is also expressed on the neutrophil membrane, where it may be engaged by PR3-ANCA (see below). The membrane expression of PR3 varies between individuals, remains constant over time, and appears genetically determined [31, 32]. One study found a link between membrane PR3 expression and HLA antigens [33]. Individuals with high membrane PR3 expression are significantly more frequent among patients with GPA than in the normal population, and patients with GPA and high membrane PR3 expression are at higher risk for relapses than those with low membrane PR3 expression [34, 35].

The major natural inhibitor for PR3 is alpha 1-antitrypsin, coded for by the *AAT* gene. Of the many polymorphisms of *AAT*, the Z and S alleles are responsible for the majority of alpha 1-antitrypsin deficiency cases. An increased frequency of heterozygosity for the Z and S alleles in association with GPA, but not MPA, has been reported by several groups in

small studies and recently confirmed in larger studies [36, 37]. The clinical significance of this association is not entirely clear, but heterozygosity for the Z allele has been associated with a worse prognosis of the disease [38]. This may be because Z-allele carriers, but not S-allele carriers, have increased levels of pro-inflammatory polymers circulating in their blood [37]. Deposits of these polymers have been documented in kidney biopsy specimens from patients with active GPA, and these polymers can prime neutrophils and augment the activation of neutrophils by PR3-ANCA in vitro [37].

Unconfirmed or conflicting results have been reported for the association with either GPA or MPA and polymorphisms of other genes including those coding for the high-affinity soluble interleukin 2 receptor (*IL-2RA*), interleukin 10 (*IL-10*), leukocyte immunoglobulin-like receptor A2 (*LILRA2*) and CD226 (*CD226*), as well as for the Fc receptors FcRIIa and FcRIIIb [18].

Another, yet unclear, factor to be considered in the interpretation of genetic SNP association studies is the issue of copy number variation [39]. Copy number variations as a result of gene duplication, triplication, or exon shuffling may occur at a higher frequency in the genome than SNPs and may be more important for evolution [39]. The study of effects of copy number variations on the disease phenotype of complex autoimmune diseases including the AAV syndromes is just beginning.

Environmental Triggers and Infection

Environmental triggers for the onset of AAV in susceptible individuals remain unknown for most patients. A significant association between MPA with MPO-ANCA and exposure to silica has been reported [40].

The onset of AAV (predominantly MPA) has also been reported following exposure to a variety of therapeutic agents including propylthiouracil, hydralazine, and penicillamine [41, 42]. These agents have polyclonal B lymphocyte stimulatory properties, which may induce the production of ANCA. However, there are several features distinguishing drug-induced AAV from typical AAV. First, the ANCA response is often targeting several different antigens at the same time, whereas in typical AAV ANCA target either PR3 or MPO, but not both and not other additional antigens such as human neutrophil elastase or lactoferrin. Second, drug-induced AAV usually subsides after discontinuation of the offending agent. Thus, the cases of drug-induced AAV support the hypothesis of the pathogenic role of ANCA in the development of vasculitis (see below), but they do not explain the loss of tolerance to ANCA target antigens characterizing GPA and MPA.

Infections have been implicated as triggers as well as persistent drivers of various autoimmune diseases including AAV. A variety of different and often interrelated mechanisms by which infections trigger and perpetuate the disease in predisposed patients have been proposed. Many infectious agents have been reported to induce an ANCA response. In most instances these ANCA are directed against target antigens other than MPO or PR3. Furthermore, these infection-associated ANCA usually disappear once the infection resolves (reviewed in [2, 43, 44]). Thus, it seems that two conditions need to be met for infections to trigger AAV. First, tolerance to self-antigens needs to be broken, i.e., host conditions permissive for the development of autoimmunity need to allow for the ongoing production of antibodies directed against self-antigens (ANCA). Second, the persistent specific autoantibodies (ANCA) need to have pathogenic potential for the development of tissue injury characteristic of AAV.

One concept by which infections can elicit an autoimmune response in susceptible hosts is molecular mimicry [45]. Subsequent diversification of T and B lymphocyte responses ("epitope spreading") may lead to reactivities with different epitopes on the same target molecule (intramolecular spreading) or even extend to other molecules (intermolecular spreading) [46, 47].

The only example of direct epitope mimicry leading to ANCA in patients with pauci-immune glomerulonephritis has been reported by Kain and co-workers [48]. They found ANCA targeting lysosomal membrane protein-2 (LAMP-2) in the majority of patients with pauci-immune focal necrotizing glomerulonephritis [48]. These ANCA recognized an epitope on LAMP-2 and cross-reacted with the homologous bacterial adhesin FimH [48]. Antibodies to LAMP-2 transferred to rats caused pauci-immune glomerulonephritis in the recipients [48]. Rats immunized with FimH developed antibodies to FimH that cross-reacted with human LAMP-2 and also developed pauci-immune glomerulonephritis [48]. The frequency of LAMP-2-specific ANCA has recently been confirmed in different patient cohorts from Europe [49], but not from the United States [50]. It also appears that the LAMP-2-specific ANCA disappear quickly after initiation of immunosuppressive therapy, and methods for their detection are not as robust as those used for the detection of PR3-ANCA and MPO-ANCA [49]. Consequently, the clinical relevance of this finding remains controversial.

An indirect mechanism of molecular mimicry leading to typical PR3-ANCA has been proposed by Pendergraft and colleagues [51]. According to this theory, idiotypic networks are instrumental in the formation of ANCA [52]. First, antibodies are formed against complementary peptides (antisense peptide sequence) to PR3 (cPR3) [51]. The cPR3 peptides, which may represent mimics of microbial peptide sequences, are the target of the primary immune response. Indeed, several bacterial peptide sequences including *Staphylococcus aureus* (*S. aureus*) sequences were found to have homologies with cPR3 [51]. True PR3-ANCA are subsequently the result of a secondary

immune response mounted against the idiotype of these anti-cPR3 antibodies [51]. Pendergraft et al. were able to show that mice immunized with cPR3 developed antibodies against both cPR3 and PR3, and they found antibodies against cPR3 in 7 of 34 PR3-ANCA-positive patients [51]. However, the same group was unable to demonstrate a similar scenario for MPO-ANCA [53]. Furthermore, Tadema et al. could not confirm an increased frequency of anti-cPR3 antibodies in patients with GPA compared to healthy volunteers or MPO-ANCA-positive patients [54]. Interestingly, portions of the cPR3 sequence have homology with portions of the plasminogen sequence, and anti-plasminogen antibodies were detected in some patients with AAV, potentially contributing to the well-recognized increased risk for thromboembolic events [55, 56].

Direct or indirect epitope mimicry may explain how autoantibodies are formed, but not how they escape elimination by mechanisms that preserve immune tolerance to self-antigens. A variety of mechanisms by which infections promote the autoimmune response as well as the propensity for chronic relapses in AAV have recently been studied. Nearly two thirds of patients presenting with GPA are nasal carriers of *S. aureus* [57]. This is much higher than in the general population. *S. aureus* carriers are at higher risk for relapse of GPA than noncarriers, and treatment with trimethoprim–sulfamethoxazole resulted in a significant reduction of the relapse rate [57–59]. Multiple mechanisms may contribute to the increased risk of relapse conveyed by *S. aureus*.

S. aureus produces superantigens known to be powerful nonspecific (antigen-independent) T and B lymphocyte activators able to induce significant cell proliferation and cytokine release [60, 61]. Patients colonized with superantigen-producing strains of *S. aureus* are at higher risk for disease flares than those colonized with superantigen-negative strains [62]. Compared to healthy controls, patients with GPA were found to have an expansion of T lymphocytes expressing Vbeta segments specific for *S. aureus* superantigens [63]. However, a direct association between the presence of *S. aureus* producing superantigens and the expansion of T lymphocytes reactive to these superantigens in individual patients could not be confirmed [63].

S. aureus-derived superantigens and peptidoglycans as well as fungal beta-glucans can induce the expansion of IL-17-producing CD4-positive T cells, the so-called Th17 cells, in an IL-23-dependent manner [64–66]. Th17 cells, which are now recognized as central players in the development of autoimmunity, are highly potent inflammatory cells that initiate and maintain tissue inflammation by recruiting other inflammatory cells while generating a milieu that makes them resistant to control by T-regulatory (Treg) cells [67, 68]. IL-23 produced by antigen-presenting cells appears necessary to maintain Th17 cells at the site of inflammation [68]. Both elevated IL-23 and IL-17 levels have been found in patients with active disease and remained elevated despite treatment during clinical remission [69]. An increased frequency of Th17 cells responding to staphylococcal enterotoxin B was found in GPA patients in remission compared to healthy controls, regardless of ANCA status, whereas an increased frequency of PR3-responsive Th17 cells was restricted to PR3-ANCA-positive patients [70]. IL17 can also induce IL1-beta and TNF-alpha production and release by macrophages, and these cytokines prime neutrophils and monocytes, resulting in the expression of ANCA target antigens on their surface [71]. Chronic infections may thus set the stage for chronic ongoing inflammation and the loss of self-tolerance.

Necrotizing granulomatous inflammation consisting of monocytes, macrophages, neutrophils, T cells, B cells, and plasma cells, predominantly located in the respiratory tract, sets GPA clinically apart from MPA [1]. Structures resembling germinal centers have been documented within the granulomatous inflammatory tissue of GPA, and the tissue also stained positive for PR3 [72]. The immunoglobulin (VH) gene mutational patterns obtained from granulomatous nasal tissue of patients with GPA suggest that the selection and maturation of PR3-ANCA-producing B lymphocytes may start within the granulomatous lesions [72].

Cytosine–phosphate–guanine (CPG) motifs are pathogen-associated molecular patterns (PAMPs) that are recognized by the pattern recognition receptor Toll-like receptor 9 (TLR9). Unmethylated CPG oligodeoxynucleotides (CpG-ODN) are potent immune stimulants. B lymphocytes isolated from patients with AAV with active disease as well as during remission can be induced to produce ANCA when exposed to CpG-ODN and IL-2 [73, 74]. Significantly, more PR3-ANCA patients than MPO-ANCA patients produced ANCA in vitro in response to CpG exposure [74]. These observations provide another link between *S. aureus* infection, granulomatous inflammation of the respiratory tract, and the higher relapse rate of patients with PR3-ANCA or GPA compared to patients with MPO-ANCA or MPA.

Neutrophils are key players of the innate immune defense against microorganisms. An additional antimicrobial defense mechanism of neutrophils has been described recently—a unique type of cell death distinct from apoptosis and necrosis that is associated with the formation and release of neutrophil extracellular traps (NETs) [75]. NETs are extracellular structures containing chromatin and granule proteins (including ANCA target antigens) in which invading microbes are trapped and killed. The formation of NETs depends on reactive oxygen species generated by NADPH oxidase [76]. ANCA, which are known to induce a respiratory burst in primed neutrophils, can also induce the formation of NETs, and ANCA antigens bound to NETs are accessible to ANCA [77]. NETs containing PR3 and MPO are detectable in the kidneys of patients with AAV in the absence of infection. Interestingly, *S. aureus* can rapidly and strongly induce

NETs formation even without causing neutrophil death [78]. Chromatin-immunoglobulin complexes are thought to lead to the loss of tolerance and autoantibody (ANCA) production in a TLR9-dependent manner [79]. Taken together, these observations suggest that ANCA may induce a vicious cycle of self-perpetuated NET formation and more ANCA production, particularly in the presence of *S. aureus* infection. This hypothesis is further supported by observations of increased TLR9 expression by monocytes of patients with GPA who were *S. aureus* nasal carriers [80].

Taken together, this evidence suggests that *S. aureus* and possibly other microbial organisms contribute to the pathogenesis of GPA and MPA by creating a permissive inflammatory environment by promoting T and B lymphocyte activity, which in turn can initiate and maintain ANCA production in predisposed patients. Both the chronic inflammatory milieu with ongoing cytokine release and the loss of tolerance to the ANCA target antigen appear to be required for the cascade that results in necrotizing small vessel vasculitis.

The Pathogenic Role of Lymphocytes

ANCA are high-affinity class-switched antibodies [81]. This implies that ANCA production is dependent on T lymphocyte help, that autoreactive T lymphocytes are present, and that there is insufficient counter-regulation by Tregs. Indeed, T lymphocyte abnormalities have long been suspected to be the main reason for the chronic relapsing nature of GPA [82]. Patients with GPA in remission have an increased percentage of circulating CD4-positive effector memory T lymphocytes as well as a functional defect of circulating CD4-positive CD25-positive Tregs [83–85]. Moreover, patients with reduced numbers of Tregs required more prolonged treatment to achieve remission, and their relapse rates were higher [85].

The role of B lymphocytes in the pathogenesis of AAV appears more clearly defined than that of T lymphocytes. First, B lymphocytes have been found in affected tissues including kidneys and most significantly in granulomatous lesions of the respiratory tract, where they are located in close proximity to numerous PR3-positive cells and where the selection and maturation into PR3-ANCA-producing B lymphocytes may occur [72]. Second, antigen-specific B lymphocytes are the progenitor cells of short-lived plasma cells thought to be the source of autoantibodies including ANCA [86, 87]. Third, the proportion of circulating activated B lymphocytes is increased in patients with GPA compared to healthy controls, and it is higher in patients with active disease versus those in remission and in patients with generalized disease versus those with limited disease [82]. Most importantly, the beneficial therapeutic effects of cyclophosphamide (CYC) in GPA have been attributed to its effect on B lymphocytes, and the pathogenic role of B lymphocytes explains why rituximab, which targets these cells selectively, is so successful at controlling the disease activity of GPA [88, 89].

The Pathogenic Role of ANCA

What do ANCA do once they are formed? Clinical observations and a large body of experimental data support a pathogenic role of ANCA for the development of small vessel vasculitis. The small vessel injury of AAV appears to be caused by activated leukocytes.

The pro-inflammatory pathogenic effects of ANCA are all contingent on their interactions with their target antigens expressed on the surface of primed neutrophils and monocytes. When primed with inflammatory cytokines such as tumor necrosis factor (TNF)-α[alpha] or microbial products in vitro, leukocytes express proteinase 3 (PR3) and myeloperoxidase (MPO) on their surface [90, 91]. PR3-ANCA and MPO-ANCA can activate primed neutrophils and monocytes by binding directly to their antigens expressed on the surface or by Fc-receptor engagement; these interactions initiate signal transduction cascades via multiple pathways that are similar in neutrophils and monocytes [92–96].

The activation of neutrophils and monocytes by ANCA has several pro-inflammatory effects that in aggregate cause tissue injury. Fully activated neutrophils degranulate and release toxic proteases and enzymes including elastase, PR3, MPO, and others [90]. ANCA also induce a respiratory burst resulting in the release of oxygen radical species [90, 97]. Furthermore, ANCA induce the expression of cell adhesion molecules on neutrophils and endothelial cells leading to an increased adhesion of neutrophils to endothelial cells [98–102]. Moreover, the binding of ANCA to primed leukocytes induces the production and release of chemotactic cytokines including IL-1, MCP-1, and IL-8 [103–106]. These cytokines attract more neutrophils and monocytes to the site of inflammation. Thus, when the ANCA-induced cytokine release occurs at the endothelial interface, the normal chemotactic gradient that draws neutrophils out of the vasculature into the tissues is lost. This causes further accumulation of fully activated neutrophils in the vessel wall, where they cause more injury.

The ANCA target antigens released from activated or dying neutrophils can also directly bind to endothelial cells [107]. This may result in apoptosis of endothelial cells and to localized immune-complex formation with circulating ANCA [108]. Low levels of localized immune-complex deposition, which has been documented in early vasculitic skin lesions as well as renal lesions, can in turn induce localized complement activation [109, 110].

Capillaritis of the lung in AAV is characterized by fibrinoid necrosis and leukocytoclasis (Fig. 10.2). In vitro studies have suggested that ANCA modify the clearance of apoptotic cells.

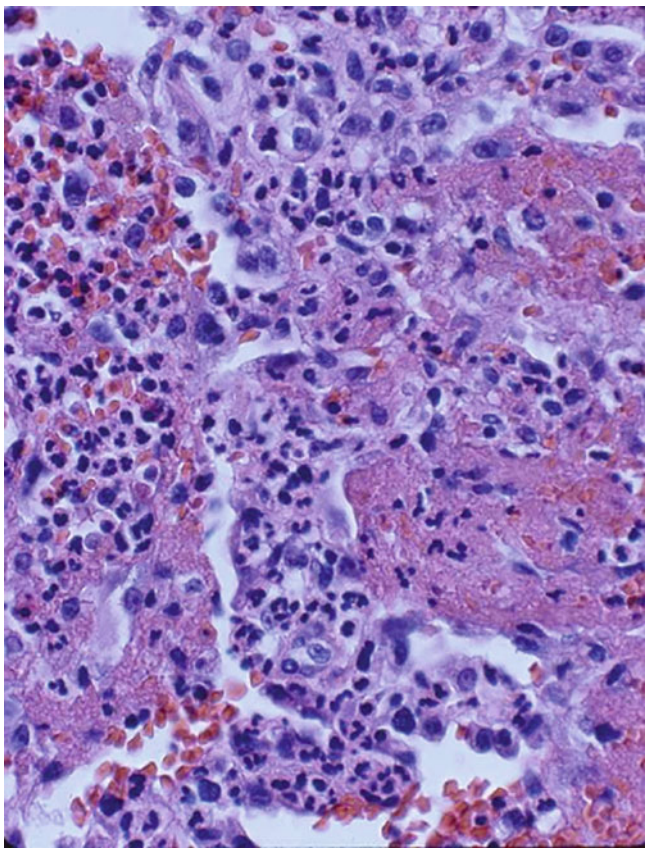

Fig. 10.2 Lung biopsy showing characteristic fibrinoid necrosis, hemorrhage, and leukocytoclasis (H & E, ×40)

Opsonization of pre-apoptotic cells by ANCA is associated with an increased production of inflammatory cytokines by phagocytozing macrophages [111]. Moreover, pre-apoptotic cells have a decreased cell surface expression of phosphatidylserine (the recognition signal for macrophages) in the presence of ANCA [112]. Consequently, in the presence of ANCA, the noninflammatory clearance of apoptotic cells by macrophages may be perturbed in favor of inflammation and necrosis.

Several in vivo animal models support the pathogenic role of ANCA. These models are based on the transfer of antibodies generated against ANCA target antigens into healthy recipient animals. The transfer of anti-MPO IgG or splenocytes obtained from MPO-knockout mice, which were immunized with murine MPO, into Rag 2 knock-out mice (lacking mature T- and B lymphocytes) as well as into wild-type mice resulted in pauci-immune crescentic necrotizing glomerulonephritis similar to that found in humans [113]. A direct augmenting effect of MPO-ANCA on neutrophil–endothelial interactions causing microvascular injury was documented in a rat anti-MPO antibody transfer model [114]. Murine anti-PR3 antibodies generated in a similar fashion only caused an increased inflammatory response at the site of tissue injury, but not a vasculitic phenotype, when transferred into wild-type mice [115].

Even though patients with active AAV have normal serum complement levels and lack significant immune-complex deposits in affected tissues, low-grade localized immune-complex formation and complement activation may play a role. It has recently been recognized that activation of the alternative complement pathway by ANCA may represent an important amplification loop of inflammation that contributes to renal (and other tissue) injury in AAV. In the murine anti-MPO antibody transfer model, the development of necrotizing glomerulonephritis is dependent on the activation of the alternative complement pathway, and the development of lesions can be prevented and treated with an antibody that inhibited complement factor 5 (C5) activation [116, 117]. Mice lacking the receptor for activated C5 on neutrophils also do not develop the renal lesions [118]. In vitro studies showed that supernatants from ANCA-activated neutrophils can cause the production of C5a in normal serum, C5a receptor-dependent priming of normal neutrophils, and the increased neutrophil membrane expression of PR3. Even though the renal lesions in humans are called "pauci-immune," the components of the alternative complement pathway can be detected in patients with AAV, but not normal controls or patients with minimal change disease [119].

Despite all this evidence, proof that ANCA alone can cause disease in humans has remained elusive. One case study in which an infant was born to a mother with active MPA is often quoted as such evidence [120]. The infant developed a pulmonary–renal syndrome 48 h after delivery and was found to have serum MPO-ANCA titers similar to the mother [120]. The child was treated with glucocorticoids and plasma exchange and recovered. However, this observation is countered by another report of a case where MPO-ANCA were also transferred from the mother to the newborn, but the newborn remained perfectly healthy despite persistence of the transferred MPO-ANCA in the newborn for several weeks [121]. The two contrasting case studies are consistent with observations made in large cohort studies. The development of severe vasculitic disease manifestations and severe flares usually do not occur in the absence of ANCA, but not all patients with persistent ANCA inevitably suffer such flares [6, 8, 57, 122].

The multiple clinical and experimental observations are best reconciled by the hypothesis that ANCA alone do not cause the disease, but in an inflammatory environment, they are conditional for the progression to systemic small vessel vasculitis. Therapeutic interventions that prevent the production of ANCA, remove them from the circulation, and interfere with their binding to target antigens or with downstream effects of this interaction all have a good scientific rationale. The clinical challenge is to achieve this in a targeted fashion with minimal adverse effects.

Management of Patients with ANCA-Associated Vasculitis

Diagnostic Approach

The patient's symptomatology at presentation is determined by the type, extent, and location of the inflammatory lesions. The initial diagnostic approach needs to be adapted according to the initial clinical presentation. However, AAV in all its flavors is a systemic disorder, and some disease manifestations may not cause overt symptoms. Therefore, every patient suspected of having a small vessel vasculitis should undergo initial testing that includes a complete blood count with differential, metabolic panel with serum creatinine and transaminases, urinalysis and microscopy, erythrocyte sedimentation rate, C-reactive protein, and ANCA determinations as well as a chest imaging study to look for clinically silent lung involvement. Tests for other autoimmune disorders (e.g., ANA, anti-GBM, anti-double-stranded DNA antibodies, antiphospholipid antibodies, and cryoglobulins) should also be part of the initial diagnostic evaluation.

The diagnostic value of ANCA testing is widely accepted. Yet, ANCA testing is not standardized, and differences in analytical sensitivity and specificity exist between different assays [123–126]. In AAV, ANCA causing a cytoplasmic immunofluorescence pattern (C-ANCA) on ethanol-fixed neutrophils are caused by antibodies reacting with PR3. A perinuclear immunofluorescence pattern (P-ANCA) can be caused by antibodies reacting with a variety of neutrophil antigens. However, only those that react with MPO are important in AAV [2]. In order to assure diagnostic accuracy of ANCA testing, both a target antigen-specific test (PR3-ANCA or MPO-ANCA) and immunofluorescence should be performed [127]. The two test results should confirm each other. Only the PR3-ANCA with C-ANCA combination and MPO-ANCA with P-ANCA are sensitive and relatively specific for AAV [124, 127]. In addition, the positive and negative predictive value of an ANCA test result depends on the pretest probability of the disease in the patient tested [124, 128–130]. PR3-ANCA are the predominant ANCA type in GPA, and only 5–10 % of patients with GPA have MPO-ANCA. It is important to note that about a quarter of patients with limited GPA have no detectable ANCA [6]. Yet, such patients may subsequently seroconvert and develop severe disease. Recently, the diagnostic interpretation of ANCA test results has been further complicated by emerging pathology associated with the widespread exposure to levamisole among habitual cocaine users [131, 132].

More invasive diagnostic tests may be necessary to fully assess the disease extent and severity in individual patients. The presence of respiratory symptoms or chest roentgenographic abnormalities should prompt complete pulmonary function testing including inspiratory and expiratory flow volume loop determinations. Respiratory symptoms that cannot be explained satisfactorily based on physical examination and roentgenographic findings, roentgenographic abnormalities suggesting the possibility of alveolar hemorrhage, and abnormalities in the shape of the flow volume loops, all represent indications for bronchoscopic evaluation to establish or rule out endobronchial disease of GPA or alveolar hemorrhage in GPA and MPA [133].

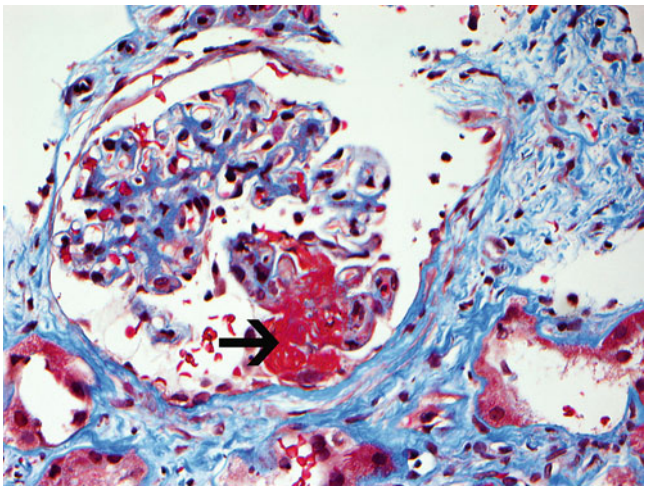

Fig. 10.3 Necrotizing glomerulonephritis with a glomerulus showing segmental fibrinoid necrosis (*black arrow* points to necrosis). Note disruption of the glomerular tuft with extension of necrosis into the Bowman's space (Trichrome stain, ×40). Contrast with thrombotic microangiopathy in which the thrombus is present within the capillary loops (Courtesy of Dr. Sanjeev Sethi, Mayo Clinic)

A renal biopsy may be necessary to establish a diagnosis, and it may provide prognostic information (Fig. 10.3). Renal outcome at 1 year can be estimated using the GFR at baseline in combination with histologic information [134]. Based on renal biopsies obtained during clinical trials performed in Europe, an international working group of renal pathologists has recently proposed a classification system for glomerular lesions that was validated to be of prognostic value for 1- and 5-year renal outcomes (Fig. 10.4) [135]. Tubular atrophy, interstitial fibrosis, and presence of intraepithelial T lymphocytes on a biopsy specimen were also found to be predictors of estimated glomerular filtration rate at 1 year, but only tubular atrophy remained an independent predictor of renal function at 2 years [136]. Despite these emerging data, the ultimate renal prognosis of an individual patient cannot be estimated conclusively based on renal biopsy findings, and treatment decisions should not be based on biopsy findings alone.

Prognostic Factors

Several studies have shown that the following factors negatively impact survival in patients with AAV and renal involvement: age at diagnosis, glomerular filtration rate at diagnosis, end-stage renal disease (ESRD) at diagnosis, and

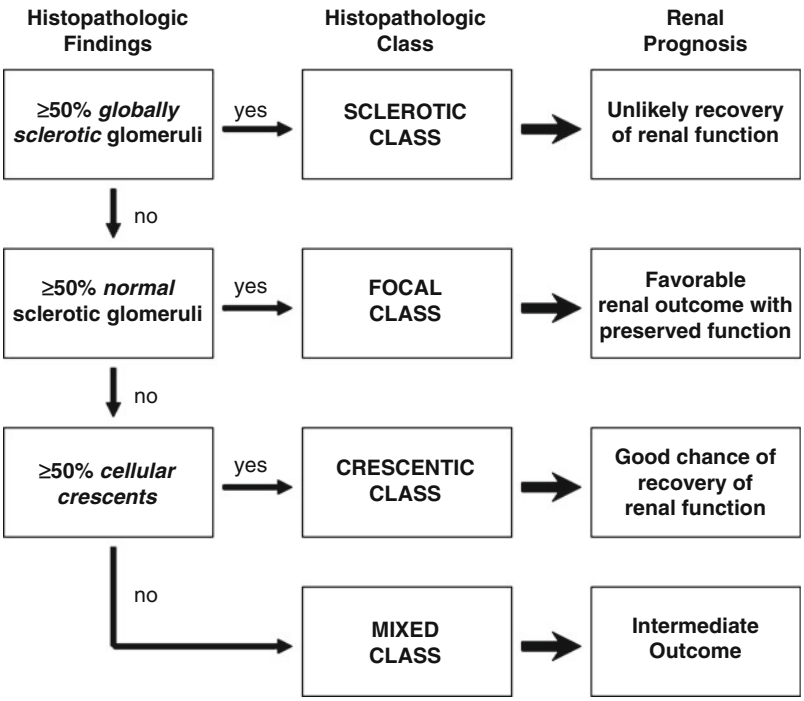

Fig. 10.4 Histologic categories and renal outcome in ANCA-associated vasculitis. Phenotypical order of categories (focal, crescentic, mixed, and sclerotic) corresponds to the order of severity of renal function impairment. Modified from Journal Am Soc Nephrol 2010; 21(10):1628–1636. With kind permission from the American Society of Nephrology

PR3-ANCA [137–140]. Consequently, to avoid irreversible damage and death, swift implementation of definitive immunosuppressive therapy is crucial.

Remission Induction Therapy for Generalized or Severe AAV

Our current treatment approach to GPA and MPA is based on the results of several randomized controlled trials (Table 10.1). Patients with GPA are categorized based on disease activity and extent into having non-severe (limited) or severe disease, whereas most disease manifestations of MPA lead to the categorization of severe disease. Severe disease refers to disease manifestations that are either life threatening or have the potential to cause irreversible damage in affected organs. Severe disease manifestations include alveolar hemorrhage, glomerulonephritis, neuropathy, scleritis, and heart and gastrointestinal involvement and are usually caused by capillaritis or small vessel vasculitis. In contrast, the non-severe disease manifestations of GPA are usually caused by necrotizing granulomatous inflammation. Sometimes the granulomatous inflammatory disease burden is so extensive or is localized such that it can be life threatening. Such patients are also considered to have severe disease for treatment purposes.

Non-severe (limited) GPA is rarely encountered by nephrologists and refers mostly to disease manifestations caused by necrotizing granulomatous inflammation affecting the respiratory tract. For remission induction in such patients, glucocorticoids in combination with methotrexate (MTX) at a dose of up to 25 mg once a week are considered the standard of care [141, 142].

CYC at a dose of 2 mg/kg/day in combination with glucocorticoids has been the standard regimen for remission induction in patients with severe GPA or MPA until recently. In contrast to the original regimen introduced by Fauci four decades ago, the duration of CYC therapy (if used at all) is limited by current consensus to the first 3–6 months of remission induction [143, 144].

Whenever CYC is used for remission induction, consideration should be given to the patient's fertility. Young men should be offered sperm banking before therapy is initiated. If time allows, ovarian protection should be offered to young women in addition to minimizing the cumulative exposure as much as possible. In women the risk of infertility is directly related to the cumulative dose of CYC received. Yet, even the limited exposure to CYC of 3–6 months duration has a substantial effect on women's fertility [145].

Intravenous pulse therapy with CYC may represent an alternative to the daily oral application. The cumulative dose of CYC is lower in a pulse regimen compared to daily oral therapy applied over similar time periods. One randomized controlled trial compared a regimen of intravenous pulse application of CYC to daily oral use [146]. The pulse regimen was non-inferior to the oral application of CYC for remission induction, and the frequency of leucopenia, but not infection, was lower. However, even though the trial was not powered to detect a difference in relapses, the relapse rate was higher following remission induction with the intermittent pulse regimen compared to the oral application of CYC.

Table 10.1 Randomized controlled trials informing the management of ANCA-associated vasculitis

Treatment investigated	Trial design	Patients randomized (randomization schedule)	Main conclusion	Trial moniker	Reference
TMP/SMX for remission maintenance in GPA	Randomized, placebo controlled	81 (1:1)	TMP/SMX reduces incidence of relapses in WG	N/A	[57]
AZA versus CYC for remission maintenance in severe AAV	Randomized, controlled, open label	144 (1:1)	AZA is as effective for remission maintenance as continuation of CYC in severe AAV	CYCAZAREM	[143]
MTX versus CYC for remission induction in early systemic AAV	Randomized, controlled, open label	100 (1:1)	MTX is not inferior to CYC in early systemic AAV	NORAM	[142]
PLEX versus intravenous methylprednisolone for AAV with severe renal disease	Randomized, controlled, open label	137 (1:1)	PLEX improves renal survival of methylprednisolone in AAV with severe renal disease	MEPEX	[153]
AZA versus MTX for remission maintenance in severe AAV following induction with CYC	Randomized, controlled, open label	126 (1:1)	There is no difference in efficacy (relapse rate) or adverse events rate between AZA and MTX	WGENT	[159]
Etanercept versus placebo added to standard therapy for limited and severe GPA	Randomized, double blind, placebo controlled	180 (1:1)	Etanercept has no benefit in addition to standard therapy	WGET	[144]
Pulse CYC versus oral daily CYC for remission induction in severe AAV	Randomized, controlled, open label	149 (1:1)	Pulse CYC is as effective as oral daily CYC for severe AAV	CYCLOPS	[146]
MMF versus AZA for remission maintenance following induction with CYC	Randomized, controlled, open label	156 (1:1)	MMF is less effective than AZA for remission maintenance	IMPROVE	[160]
RTX versus oral daily CYC followed by AZA for remission induction in severe AAV	Randomized, double blind, double placebo controlled	197 (1:1)	RTX is not inferior to CYC for remission induction in severe AAV and RTX is superior to CYC for relapsing patients	RAVE	[89, 149]
RTX versus pulse CYC followed by AZA for remission induction in severe AAV with renal disease	Randomized, controlled, open label	44 (3:1)	RTX is not inferior to CYC for induction of sustained remission	RITUXVAS	[148]

A meta-analysis of earlier cohort studies also found that intravenous pulse CYC therapy may be safer because of a lower cumulative dose, but a higher relapse rate was also observed after discontinuation [147]. In the author's experience-based opinion, intravenous CYC should be avoided in the intensive care unit setting. However, its use is preferred over oral CYC in patients with questionable compliance, in young women with fertility issues, and in patients who have gastrointestinal problems with oral CYC application.

Going forward, patients with GPA and MPA may no longer require exposure to CYC at all, since the four-decade-old standard use of CYC for remission induction in severe GPA and MPA has been challenged by the results of two randomized controlled trials [89, 148].

The RAVE (Rituximab for ANCA-Associated Vasculitis) trial was a randomized double-blind, double-placebo-controlled, multicenter trial that compared oral CYC (2 mg/kg/day) to RTX (375 mg per square meter of body surface area per week for 4 weeks) for remission induction in severe GPA or MPA in 197 patients [89]. Once remission was achieved between 3 and 6 months, patients randomized to CYC were switched to AZA for remission maintenance for 18 months, whereas patients in the RTX arm received placebo. There was no difference between the two treatment arms in rates of achieving remission at the end of 6 months and maintaining remission at 18 months. However, among the 101 patients who entered the trial with severe relapsing disease, RTX proved superior to CYC. The results of the RAVE trial led to approval by the FDA of RTX for remission

induction in severe GPA and MPA. The long-term results of the RAVE trial indicate that one course of RTX is as effective as 18 months of conventional immunosuppressive therapy [149].

Another randomized controlled open-label trial conducted in 44 patients with newly diagnosed severe AAV with active renal disease, RITUXVAS (Rituximab versus Cyclophosphamide for ANCA-Associated Renal Vasculitis), showed complementary results to the RAVE trial [148]. In this trial patients were randomized 3:1 to receive RTX (together with two pulses of CYC) compared to standard intravenous pulse CYC therapy followed by oral AZA. The primary outcome was sustained remission (of >6 month duration) at month 12. No difference between the treatment arms was found. Of note is that the patients in the RITUXVAS trial were older and had more severe renal disease than patients in the RAVE trial.

Mycophenolate mofetil (MMF) may represent an alternative to CYC or RTX for patients with MPA who have MPO-ANCA and mild renal disease (creatinine <3.5 mg/day) and no other life or organ-threatening disease manifestation. This is supported by one randomized controlled trial in 35 patients reported from China [150]. This trial compared oral MMF (1.5–2 g/day) to intravenous CYC (0.75–1.0 g/m^2 once monthly). In addition, all patients received intravenous methylprednisolone bolus therapy (0.5 g/day for 3 days) followed by oral prednisone (0.6–0.8 mg/kg for 4 weeks tapered by 5 mg/week to 10 mg/day). The efficacy of these regimens was equivalent, but MMF was better tolerated than CYC [150]. A prospective pilot trial in 17 patients conducted over 18 months at the Mayo Clinic found similar remission induction results as the Chinese trial as well as remission maintenance comparable to what can be achieved with CYC followed by AZA [151].

Remission induction therapy should be implemented even in very old patients. A multivariate analysis in octogenarians has shown that immunosuppression was associated with a lower risk of death or end-stage renal disease (ESRD) [152].

Remission Induction in Patients with Fulminant Disease

For some patients with GPA and MPA, the combination of glucocorticoids and CYC or RTX may not be sufficient to induce a remission fast enough to prevent major organ damage. Such patients have either severe diffuse alveolar hemorrhage or very rapidly progressive loss of renal function. Plasma exchange (PLEX) should be considered early in such patients. The results from two studies currently support the use of PLEX. The MEPEX (Methylprednisolone versus Plasma Exchange) trial in 156 patients who presented with a serum creatinine level of 5.5 mg/dL or greater was conducted to compare three pulses of intravenous methylprednisolone to 2 weeks of PLEX (7×60 mL/kg) in addition to standard therapy for severe disease (oral prednisone and CYC). PLEX was superior to methylprednisolone with respect to renal recovery [153]. A single-center cohort study of 20 patients presenting with alveolar hemorrhage described 100 % survival of the patients when PLEX was added to standard immunosuppressive therapy [154].

Remission Induction in Patients with "Refractory" Disease

The term *refractory disease* is commonly used to describe patients who have persistent disease activity on the maximal tolerated dose of CYC or who have contraindications for the use of CYC. A variety of agents have been proposed for use in addition to or instead of the failing regimen in such patients. Over the last decade RTX has emerged as the treatment of choice for such patients [155, 156].

Remission Maintenance in GPA and MPA

It is of note that the propensity for relapse is significantly higher in GPA than in MPA, and the same can be said for patients with PR3-ANCA versus MPO-ANCA [138, 143]. On average, patients with PR3-ANCA loose renal function faster than patients with MPO-ANCA and suffer ESRD at a higher frequency [139, 157]. Moreover, in PR3-ANCA-positive patients, long-term renal survival is determined by renal relapses rather than slow progressive renal failure without relapse [158]. Consequently, the risk–benefit balance of remission maintenance therapy may be different for patients with PR3-ANCA compared to those with MPO-ANCA. Most randomized controlled trials addressing the remission maintenance issue comprised significantly more patients with PR3-ANCA than with MPO-ANCA.

Once remission has been induced and the prednisone taper is well under way, CYC should be switched to either AZA or MTX. The first option is supported by the results of a randomized trial, which showed that AZA is as good as CYC for remission maintenance to 18 months [143]. Another randomized controlled trial has shown that MTX and AZA are equivalent for remission maintenance [159]. However, MTX should be reserved for patients with normal renal function. MMF should be considered only in patients who do not tolerate AZA because a recent randomized controlled trial that compared MMF to AZA for remission maintenance has shown that MMF is inferior to AZA for this purpose [160].

It is currently unclear what to do for remission maintenance when RTX was used for induction. The long-term results of the RAVE trial indicate that one course of RTX is as effective as 18 months of conventional immunosuppressive

therapy. RTX may also be effective and safe for long-term remission maintenance in patients who previously were classified as having refractory disease [156].

Should patients with ESRD receive immunosuppressive therapy for remission maintenance? Results from one cohort inception study indicate that the relapse rate of patients with AAV on hemodialysis was significantly lower than prior to reaching ESRD, whereas the rate of severe infection was substantially higher [161]. The authors conclude that immunosuppression should be restricted in patients with ESRD to those with active disease [161]. However, two thirds of the patients in this cohort were MPO-ANCA positive, and patients with MPO-ANCA had significantly fewer relapses than patients with PR3-ANCA in this study [161]. Consequently, remission maintenance decisions in patients on hemodialysis should be made under careful consideration of all patient-specific factors including ANCA type. To avoid extrarenal relapses, PR3-ANCA-positive patients should receive standard remission maintenance therapy.

Renal Transplantation in AAV

Renal transplant recipients have improved survival and quality of life compared to patients maintained on hemodialysis [162]. In the era of modern posttransplant immunosuppression (low-dose prednisone, mycophenolate mofetil, and tacrolimus), transplanted patients with AAV have a low risk of relapse (<10 %, about 0.02 per patient year of follow-up), and most relapses are extrarenal [163–165]. Average graft survival is in excess of 10 years, and most graft loss is caused by rejection [163–165]. In a recent multicenter study, there was no difference in graft survival or relapse rate among patients who were transplanted within 3 months of reaching clinical remission versus those receiving the graft after longer duration of remission. ANCA positivity at the time of transplant also did not affect outcomes negatively [165]. Thus, renal transplantation is a safe and effective option for patients with AAV and ESRD.

Additional Practical Considerations

All patients with AAV on immunosuppressive agents including single remission maintenance agents should receive *Pneumocystis* pneumonia prophylaxis. This should be continued for at least 9 months following RTX therapy as case reports of *Pneumocystis* pneumonia following B lymphocyte depletion are emerging. Patients receiving glucocorticoids should also receive osteoporosis prophylaxis.

Patients with tracheobronchial disease may also benefit from inhaled glucocorticoid therapy, and positive microbiologic study results should be treated with antimicrobial therapy according to susceptibility studies. The last recommendation reflects the author's personal practice patterns and does not have any support by peer-reviewed data. Yet, the rationale is based on pathogenesis considerations outlined in detail in the section on infectious triggers of AAV.

Last not least, patients with various degrees of renal insufficiency should receive all appropriate supportive measures including adaptation of diet, monitoring of electrolyte and fluid status, and supplementation of erythropoietin as needed. In addition, patients with end-stage renal disease on dialysis should be monitored carefully and regularly for disease relapses in other organs.

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Systemic Lupus Erythematosus and the Kidney

11

Brad H. Rovin, Daniel J. Birmingham, and Tibor Nadasdy

Introduction

Kidney damage in systemic lupus erythematosus (SLE) is most often due to lupus nephritis (LN). In LN glomerular immune complex accumulation leads to an inflammatory response that damages glomeruli and eventually the renal interstitium. After the kidney becomes involved in SLE, the prognosis of the lupus declines [1]. This is due in part to the development of chronic kidney disease (CKD) or end-stage renal disease (ESRD) [1–3], possibly along with the increased risk of cardiovascular disease associated with CKD [4]. The nephrologist is therefore critical to the SLE care team and requires a strong working knowledge of the treatment options available for LN.

On average about 40 % of SLE patients can be expected to develop clinically important kidney disease, although this ranges from 12 to 33 % in patients of European ancestry to 50 % or greater in patients of African, Hispanic, and Asian ancestry [5–9]. According to the United States Renal Data Service, between 1996 and 2004 there were 4.4–4.9 cases of end-stage kidney disease due to LN per million in the general adult population, but in Blacks and Hispanics, the incidence was 6–20 per million compared to 2.5 per million in Caucasians [10]. Similarly, in a UK study, 19 % of Caucasians versus 62 % of Blacks with LN progressed to ESRD [8]. The prevalence of CKD in patients with SLE is difficult to estimate, but because current therapies induce complete remission in 50 % or fewer LN patients, the prevalence of CKD is likely to be high in the lupus population.

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LN is generally treatable. Presently this requires intense, nonspecific immunosuppression, which confers considerable risk of severe infection and other morbidities. Efforts are underway to develop new LN therapies that have greater efficacy and less toxicity. These new therapies are based on our current understanding of the pathogenesis of LN.

The Pathogenesis of Lupus Nephritis

The simplest view of the pathogenesis of SLE and LN is that SLE occurs when there is a loss of tolerance to self-antigens and autoantibodies to these antigens are produced. These autoantibodies then bind to self-antigens creating immune complexes (IC) that accumulate in the kidneys, drive intrarenal inflammation, and lead to LN. Supporting players in this process include genetic variations that attenuate tolerogenic presentation of self-antigen, hyperreactive T and B lymphocytes, defective or overwhelmed clearance pathways for IC and apoptotic debris, a wide array of cytokines that affect immune cells and kidney parenchymal cells, and environmental triggers that may initiate the process in susceptible individuals. Despite considerable efforts to understand these mechanisms, a number of basic questions regarding the pathogenesis of LN remain unanswered. Among the most important is why everyone with SLE does develop clinical LN. Examination of renal tissue from SLE patients without known kidney disease has shown most patients do have renal ICs [11, 12]. This suggests that in addition to abnormalities of the immune system, there may be intrinsic properties of the kidneys in patients with SLE that predispose to LN [13]. A composite picture of the factors contributing to the development of LN is presented in Fig. 11.1, and individual components of this picture are discussed below.

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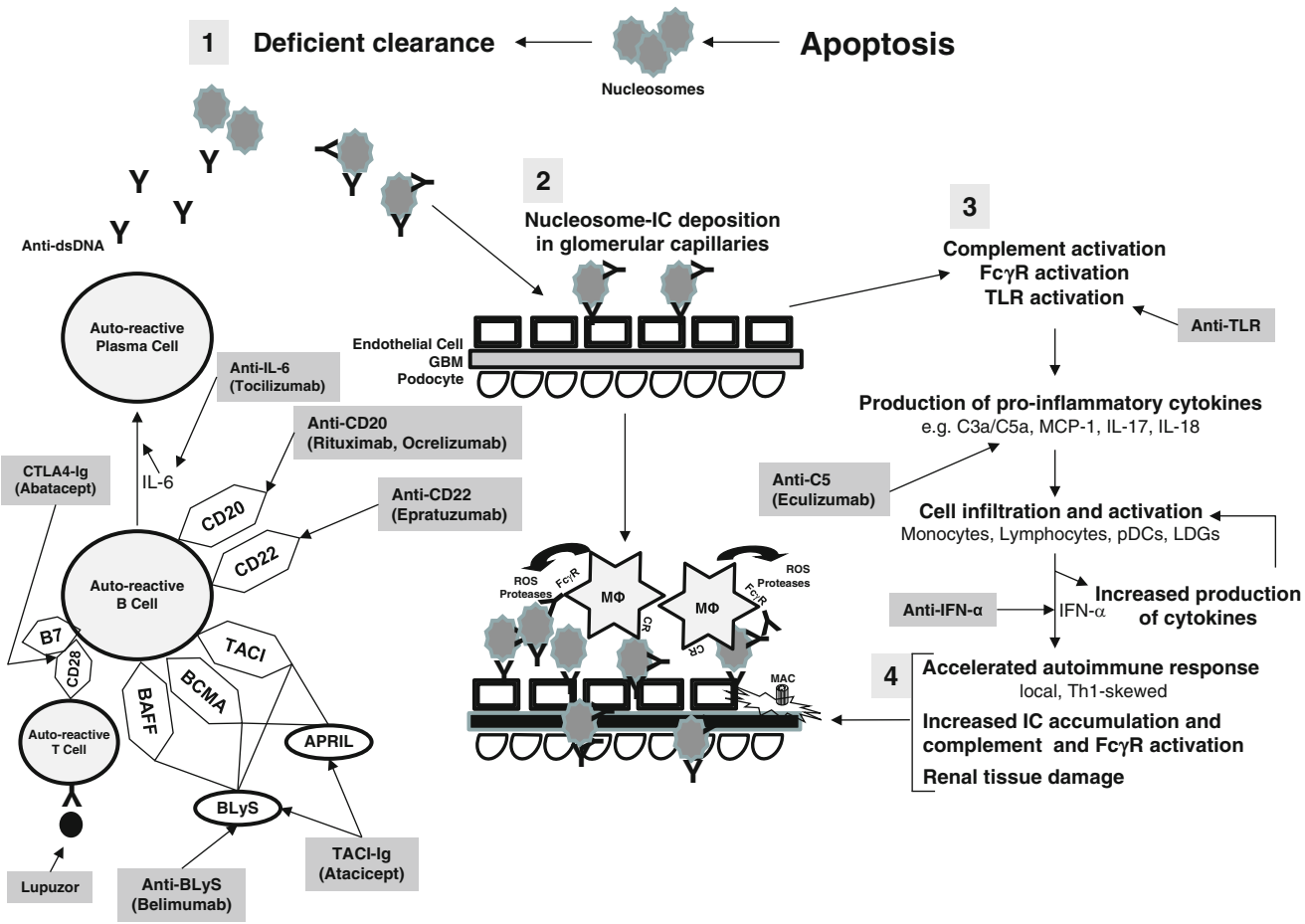

Fig. 11.1 The pathogenesis of lupus nephritis and novel therapies based on pathogenic pathways. In this model lupus nephritis begins with the accumulation of autoreactive self-antigens such as nucleosomes. The nucleosomes accumulate because of deficient clearance or overwhelming production and can drive anti-dsDNA production (Step 1). The resulting IC accumulate in the renal vascular beds (Step 2), in part because nucleosomes contain positively charged histones. After deposition, IC can activate the complement system, activate circulatory leukocytes via expressed Fc**γ**R, and activate resident cells expressing TLRs (Step 3). This establishes a cascade of inflammatory cytokine and chemokine production that recruits and activates inflammatory cells, lymphocytes, and pDCs. These infiltrating cells further amplify the intrarenal production of cytokines and chemokines. The result is a locally driven and accelerated autoimmune response with Th1 characteristics and increased IC accumulation and accompanying complement and Fc**γ**R activation. This response culminates in the production of inflammatory mediators of tissue damage (Step 4). One immediate consequence is interruption of the glomerular filtration barrier through damage to glomerular endothelial cells (EC), glomerular basement membrane (GBM), and podocytes (P), which leads to proteinuria and hematuria, the clinical hallmarks active lupus nephritis. Throughout this model, novel therapies are shown that are being tested, have been tested, or are anticipated to be tested in clinical trials of SLE and LN. These therapies are related to the pathogenic pathways, and their specific targets along these pathways are indicated. Note: *CR* complement receptor, *MAC* complement membrane attack complex

The Genetics of LN

Considerable progress has been made toward identifying the genetic basis of SLE, using genome-wide and candidate gene studies (reviewed in [14, 15]). This has resulted in the identification of over 30 genes that appear to be related to specific pathogenic pathways in SLE, including IC clearance/inflammatory pathway genes, immune response genes, and interferon alpha (IFN-α) signaling and response genes. Unfortunately, the same effort has not yet been applied to the genetics of human LN [16]. Furthermore, the studies that have been done suggest considerable heterogeneity of LN genetic susceptibility in differential racial/ethnic groups, implying that a complete picture of LN genetics will not emerge until studies are repeated in all at-risk populations [16]. For example, genome studies have identified six quantitative trait loci (QTLs) that are linked to LN [17, 18], three regions are linked in European Americans, and three are linked in African Americans. Despite these caveats, some genes bear further consideration [19] and are discussed below.

Fc receptors for IgG (known as Fcγ receptors, or Fc**γ**R) are engaged by IC and can provide either protection from

IC-mediated injury by facilitating the phagocytosis and clearance of IC or induce inflammatory responses by activating the cells expressing FcγR (reviewed in [20, 21]). Studies of polymorphic forms of FcγR have clarified which role dominates in SLE. There are three classes of FcγR (FcγRI, FcγRII, and FcγRIII), with different genes that produce full-length products for FcγRII (FcγRIIA, FcγRIIB, FcγRIIC) and FcγRIII (FcγRIIIA and FcγRIIIB). Single nucleotide polymorphisms (SNPs) that affect the peptide sequence have been identified in some of these genes that influence binding affinity for IgG, including the FcγRIIA 491G>A SNP (changing amino acid 131 from arginine to histidine) and the FcγRIIIA 559T>G SNP (changing amino acid 158 from phenylalanine to valine) [22–24]. Though not unequivocal, most studies have reported that the lower affinity forms of FcγRIIa (R131) and FcγRIIIa (F158) are associated with SLE and particularly with LN [25, 26]. The fact that the forms of these receptors that bind IC more efficiently are associated with protection against SLE suggests that their overall function is to promote IC clearance rather than drive tissue inflammation and that relative deficiencies in this function contribute to LN.

Two cytokines important for cell infiltration into the kidney, monocyte chemotactic protein-1 (MCP-1) and interleukin (IL)-18, have promoter polymorphisms that influence expression levels. MCP-1 attracts monocytes and T cells, and IL-18 attracts plasmacytoid dendritic cells (pDCs). The MCP-1 variant that results in higher expression levels is associated with LN [27]. Similarly, the IL-18 variant that causes higher expression is associated with diffuse proliferative LN [28].

Signal transducer and activator of transcription-4 (STAT4) is important for transmitting interferon (IFN)-αsignals. STAT4 has a genetic variant that is associated with increased STAT4 RNA levels and with SLE, particularly LN [29, 30].

The HLA-DR3 allele (DRB1*0301) correlates with renal disease [31, 32] and with anti-dsDNA antibodies [32], supporting a genetic contribution to a type of autoantibody that may target renal tissue.

Autoantibodies and Immune Complexes

Autoantibodies are synonymous with lupus, especially antinuclear and anti-double-stranded (ds)DNA antibodies [33, 34]. Over 100 self-antigens have been identified in SLE patients that are targets of autoantibodies, including dsDNA, single-stranded (ss)DNA, nucleoproteins, RNA–protein complexes, ribosomes, phospholipids, carbohydrates, cell cytoplasm and cell surface molecules, blood components, and endothelial cells [35]. Because autoantibodies to all of these antigens are not present in every patient, autoantibody patterns may predispose to specific organ involvement. In this regard autoantibodies to dsDNA and complement component C1q seem to be particularly relevant to LN.

Several studies have found an association of high titer anti-dsDNA with active LN (reviewed in [36]). Furthermore, anti-dsDNA antibodies can be isolated from the glomeruli of LN patients [37–39]. This may be due to the nature of the dsDNA antigen in the context of SLE. Nucleosomes are composed of DNA in association with a core of positively charged histone proteins, are released by cells undergoing apoptosis, and can be trapped in the glomeruli, perhaps facilitated by electrostatic interaction between positively charged histones and the negatively charged glomerular basement membrane [40]. Anti-dsDNA recognizes DNA in nucleosomes, and the binding of anti-dsDNA in lupus renal tissue occurs at the site of glomerular nucleosome deposition [41]. In addition, there appears to be cross-reactivity between anti-dsDNA and antigens in kidney tissue [42], in particular alpha-actinin expressed on podocytes and mesangial cells [43], and annexin II on mesangial cells [44]. Regardless of the predominant mechanism, the result is localized anti-dsDNA-containing IC with the potential to drive local tissue inflammation. Anti-dsDNA autoantibodies appear to be mainly IgG1 and IgG3 [45, 46], which are the most inflammatory IgG subtypes due to their ability to activate complement and engage Fc receptors for IgG.

Antibodies to C1q, the first component of the classical complement pathway, have been strongly associated with LN in many but not all studies [47–50]. Anti-C1q does not appear to cause an acquired deficiency of circulating C1q because anti-C1q binding requires a neoepitope formed when C1q becomes fixed to its target substrate. Rather, injury is likely related to interaction of anti-C1q with C1q already present in the kidney, such as in IC bound to nucleosomes [51, 52]. The resulting C1q/anti-C1q IC could focus an inflammatory response to the kidney, similar to anti-dsDNA, leading to nephritis. However, unlike anti-dsDNA antibodies, most anti-C1q antibodies appear to be IgG subclass 2 [53, 54], which is a poor complement activator and low-affinity antigen for Fc receptors. Other IgG subtypes (mainly IgG1) can be present in these IC, so the role of anti-C1q in LN pathogenesis may depend on the relative amounts of each anti-C1q IgG subtype.

There is some evidence suggesting that glomerular accumulation of autoantibodies and IC might not be necessary for the development of LN. An MRL/lpr mouse model of lupus with a mutant transgene that prevented B cell secretion of immunoglobulin developed interstitial nephritis and vasculitis that was similar to the wild-type MRL/lpr [55]. However, the glomerular component of the disease consisted mainly of focal glomerular atrophy and not the typical glomerulonephritis that develops in wild-type MRL/lpr. Other studies have suggested that IC are not sufficient for the full development of LN and that T cells are also required.

Subsets of T cells appear to significantly contribute to the progression of LN in the NZB/NZW mouse model of lupus, including activated CD4 T cells [56] and Th17 T cells [57]. Although caution must be taken when extrapolating these results to human LN, the role IL-17 may be particularly relevant, as discussed below.

The Complement System

The formation of IC leads to the activation of the complement cascade which can provide protective effects against SLE, mainly by promoting proper clearance of circulating IC. However, once IC are deposited in tissue, complement can drive tissue inflammation and damage, either through direct effects on tissue (complement membrane attack complex) or by activating cells to produce pro-inflammatory cytokines and toxic mediators.

Complement may provide *protection* from SLE by solubilizing IC, so they are less likely to become trapped in tissue [58, 59], clearing apoptotic debris, an immunogenic source of self-antigens, through opsonization by C1q [60, 61], and clearing IC through C4b/C3b/C3bi receptors after opsonization by the complement activation products C4b and C3b/bi [62, 63]. The type one complement receptor (CR1, CD35) binds C4b, C3b, and C3bi, is expressed in the circulation predominantly on erythrocytes (E-CR1), and mediates the binding of complement-opsonized IC to erythrocytes (a process known as immune adherence) [64]. This binding allows erythrocytes to shuttle IC through the circulation, minimizing glomerular trapping of IC and promoting IC delivery to the liver and spleen for safe removal [64]. The evidence that all of these complement functions protect against SLE includes studies showing that individuals with homozygous deficiencies of classical pathway components have an increased risk for developing SLE and SLE-like diseases [65, 66] and that E-CR1 levels are decreased in SLE and fluctuate in chronically active disease [67–69].

In contrast, there is substantial circumstantial evidence that complement-mediated inflammation and complement-mediated direct tissue damage contribute to the pathogenesis of LN: (1) Circulating levels of C3 and C4 are lower in active LN compared to inactive LN or nonrenal SLE, indicating ongoing complement activation [70–73]. A longitudinal assessment of circulating C3 and C4 levels during SLE flare showed a decrease at renal flare but not at nonrenal flare, even if the nonrenal flare occurred in patients with a history of LN [69]. (2) Complement components, including the membrane attack complex, C3, and factor B, are deposited or produced in LN kidneys [70, 74–80]. (3) The inflammatory receptors for C3a and C5a are expressed or upregulated in the glomerular endothelium, mesangium, and podocytes of LN kidneys [81, 82]. (4) The expression of regulators of complement activation, including CR1 and decay accelerating factor (CD55), is decreased in LN kidneys [83–87].

The fact that complement can both protect against the development of SLE and yet drive LN speaks to the complex and multifaceted functions of the complement system. It also suggests that the overarching role of complement in SLE pathogenesis is initially to contribute protection against SLE *onset*. However, once the disease is established, with IC accumulation in the tissue, the role of complement is reversed to that of a significant contributor to disease progression, especially as it involves the kidney.

Renal Chemokines and Cytokines

Complement activation in the kidney due to IC and IC themselves initiate further intrarenal inflammation that becomes the hallmark of kidney injury in LN. One consequence of complement activation is the generation of the membrane attack complex, which damages cell membranes through the formation of transmembrane pores [76]. Another consequence of complement activation and also Fc receptor activation by IC is the induction of pro-inflammatory chemokine and cytokine expression by renal parenchymal cells and resident leukocytes [88]. These chemokines and cytokines recruit inflammatory leukocytes to the kidney, which in turn accelerate inflammation through secretion of additional chemokines and inflammatory cytokines and, in the case of neutrophils and monocytes, induce tissue damage through release of proteolytic enzymes and pro-oxidants. Examples of upregulated chemokines and cytokines in kidneys of LN patients include MCP-1, macrophage inflammatory protein-1-alpha (MIP-1α), IL-6, IL-10, IL-12, IL-17, IL-18, IFN-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and Eta-1/osteopontin [88–95]. Deletion or inhibition of the expression of these cytokines in experimental models of SLE and LN significantly attenuates kidney injury [96–100]. In human LN intervention studies, targeting some of these cytokines is being done and if successful will verify their role in the pathogenesis of human LN.

Recent evidence is particularly strong for IL-17 playing an important role in the pathogenesis of LN. The two major cell sources of IL-17 in SLE/LN are Th17 cells and $CD4^{-}CD8^{-}$ T cells, and these have been observed in renal biopsies of LN patients [95]. Local production of IL-17 may drive inflammatory cytokine and chemokine expression by resident glomerular and tubular cells having the IL-17 receptor [101], leading to activation of infiltrating neutrophils and monocytes [102, 103]. The presence of IL-17-producing cells in the LN kidney may also represent a shift away from natural regulatory T cells capable of suppressing immune responses (see below) [104].

T and B Cells

The cytokine profile of infiltrating renal T cells suggests their role in LN. Intrarenal production of Th1 cytokines, specifically IL-12, IFN-γ, and IL-18, appears to exceed Th2 cytokines in proliferative LN and correlates with histologic activity [90, 93, 94, 105]. The Th2 cytokine IL-10 does increase in LN, but overall the Th1/Th2 ratio is higher. Th1-dominant expression can also be observed in serum, urine, and circulating T cells of LN patients [105, 106]. Th1 responses are associated with activated macrophages and with the production of IgG capable of activating complement and FcγR pathways, all implicated in the pathogenesis of kidney injury. The Th1 dominance displayed in LN patients, both locally in the kidney and systemically in the circulation, suggests that this may be an important prerequisite for developing LN. It should be noted that, while the above studies indicate a shift to a predominant Th1 profile in LN, a recent report suggests that IgE, which is a consequence of a Th2 response, is an important component of LN development [107].

Human regulatory T cells (Treg), characterized as $CD4^{+}CD25^{hi}FoxP3^{+}$, inhibit immune responses through effects on T and B cells and particularly autoantibody production [108–111]. In experimental animals there is an inverse correlation between circulating Treg numbers and circulating anti-dsDNA levels [112], and lupus-like activity, including glomerulonephritis, can be suppressed by adoptive transfer of Tregs [113, 114]. Human SLE studies generally also show fewer circulating Tregs [109, 115], but the implication of this loss of regulatory T cells for LN remains unclear [116, 117].

Infiltrating B cells have also been described in human LN kidneys. Their presence may directly target autoantibodies to the kidney, as has been shown in NZB/NZW mice [118]. B cells in renal tissue may also present kidney antigens to intrarenal T cells. Recent work has shown that intrarenal B and T cells associate with various degrees of organization, including structures resembling germinal centers with central follicular dendritic cells [119, 120]. Interestingly, these structures appear to occur mainly outside of the glomeruli and are associated with tubular basement membrane IC [120]. These may contribute specifically to tubulointerstitial inflammation in LN.

Interferon-A and Plasmacytoid Dendritic Cells

Recent work from several laboratories indicates a central role for IFN-α in the pathogenesis of SLE and LN [117]. IFN-α is produced mainly in response to nonself-stimuli, such as microorganisms. For example, it can be induced by single-stranded viral RNA and unmethylated, nonmammalian DNA [121, 122]. Although a number of cell types can produce IFN-α, pDCs appear to be a major source, and production is induced after stimulation of their intracellular Toll-like receptors 7 and 9 (TLR 7, 9) among others [121–123]. Another major source appears to be the recently described low-density granulocytes (LDGs) [124]. In addition to potentially secreting pathophysiological amounts of IFN-α, LDGs also express abnormal levels of neutrophil extracellular traps (NETs), containing autoantigens and IL-17, and induce further synthesis of IFN-α, by pDCs [125]. The effects of IFN-α on the immune response include driving maturation of conventional dendritic cells into potent antigen-presenting cells [126], inducing B cell differentiation to plasma cells [127], and contributing to the development of CD4 helper T cells [128] and CD8 memory T cells [129].

Important for SLE, TLR 7 and 9 can engage mammalian nucleic acids in the context of ICs [130, 131]. The autoantibody component of the IC allows pDC to internalize the IC via FcγRIIa, thereby bringing the nucleic acids in proximity to TLR 7 and 9 [132]. IFN-α generated in this way may facilitate an immune response that contributes to breaking tolerance to self-nucleic acids. This paradigm implies a preexisting presence of autoantibodies in patients destined to develop SLE, and in fact ANA are found in >1 % of the general population [133–135].

Supporting a role for IFN-α in SLE is the observation that patients treated with IFN-α can develop a lupus-like illness [136–140]. Furthermore, there is an increase in IFN-α-induced gene expression, known as the IFN-α signature, in many patients with active SLE [141, 142]. With respect to LN, it has been shown that during severe LN, pDC disappear from the circulation and accumulate in glomeruli, due in part to glomerular expression of IL-18 and pDC expression of the IL-18 receptor [143, 144]. It is plausible that glomerular IC containing dsDNA (e.g., nucleosomes) could drive these pDCs to produce IFN-α, amplifying the intrarenal autoimmune response and contributing to the formation of local germinal centers. Additionally, peripheral blood cell levels of IFN-α-inducible genes are associated with LN patients [142, 145], and IFN-α-inducible chemokines, including MCP-1, are associated with active LN and LN flare [146–148]. Studies in many [149] but not all [150] mouse models also support a role for IFN-α in LN pathogenesis.

The apparent importance of the IFN-α pathway in SLE pathogenesis has reinvigorated the concept that microbial pathogens may be environmental factors contributing to the initiation of SLE. The activation of TLRs that stimulate IFN-α by viral and bacterial nucleic acids may be important in breaking tolerance or in accelerating the autoimmune response.

Diagnosis of Lupus Nephritis

Most patients with LN do not present with overt signs of kidney disease but rather abnormalities of serum creatinine and/or the urine, so kidney involvement must be specifically sought. Forty to sixty percent of patients who eventually have clinical renal involvement have findings of kidney disease at the initial diagnosis of lupus [5, 6, 9]. Therefore, at a minimum, patients should be assessed for LN during the initial evaluation for SLE, whenever there is suspicion of a flare of SLE activity, and if otherwise stable, at least yearly. The rationale for close monitoring is that preservation of kidney function in patients with LN is best achieved with early diagnosis and treatment [151–157].

There are some caveats in using serum creatinine as a screening tool for LN in the lupus population. A creatinine in the normal range of the clinical laboratory may be abnormally high for a woman with small-moderate muscle mass and low rates of creatinine production. In this circumstance comparison to previous creatinine measurements would be ideal, but these are not always available. In addition, hypoalbuminemic patients with severe nephrotic syndrome may have increased tubular creatinine secretion, lowering serum creatinine, and leading to an impression of better renal function than in actuality [158]. Finally, SLE patients may develop acute renal insufficiency and an increase in serum creatinine because of infection, medications, nephrotoxins, hemolysis, thrombosis, and cardiac failure. These conditions should be excluded in order to attribute a rise in serum creatinine to LN.

A urine dipstick positive for blood and/or protein in a patient with SLE is suggestive of LN; however, a systematic study of the accuracy of the urine dipstick as a screening tool found a false-negative rate in up to 30 % of SLE patients and a false-positive rate in about 40 % of patients [159]. Therefore, the urine sediment should be evaluated for evidence of glomerulonephritis. Glomerular bleeding is suggested by acanthocytes and/or red blood cell casts. White blood cells and white blood cell casts in the absence of infection are indicative of renal inflammation and consistent with a diagnosis of glomerulonephritis.

Proteinuria is a key indicator of kidney injury in SLE and is used as a clinical biomarker of relapse, remission, and successful treatment. It also has pathogenic importance because proteinuria may injure the kidney. Therefore, accurate measurement of protein excretion is crucial to the ongoing monitoring and management of LN.

Random spot urine protein-to-creatinine (P/C) ratios can be used in addition to urine dipsticks to screen patients for proteinuria but are not accurate enough to be used to make therapeutic decisions or to follow changes in proteinuria magnitude in response to therapy. The most reliable method to quantify proteinuria is to measure the P/C ratio of a 24-h urine collection or an intended 24-h collection that is at least 50 % complete [160]. Measuring the P/C ratio reduces confounding the assessment of proteinuria by errors in collecting the 24-h urine. A 12-h overnight urine collection that includes the first morning void urine also provides an accurate measure of proteinuria magnitude and may be easier for patients to collect [161].

The Kidney Biopsy and Renal Pathology in SLE

A kidney biopsy is the gold standard for the exact diagnosis and classification of LN, but LN is not a renal biopsy diagnosis. The pathologic changes described below are characteristic, but not diagnostic of LN unless the patient also fulfills the American College of Rheumatology criteria for SLE. In the absence of a concurrent clinical diagnosis of SLE, only a diagnosis of immune complex glomerulonephritis can be made, with the suggestion that the glomerulonephritis could be associated with SLE.

A biopsy is not necessarily required if the only clinical abnormalities suggesting LN are isolated hematuria or minor proteinuria in the absence of hematuria and an active urine sediment. A biopsy should be considered when proteinuria is above 500 mg/day, because this degree of proteinuria has been associated with significant kidney injury [162–168].

The first kidney biopsy of a patient with suspected LN, while important diagnostically and therapeutically, has limited prognostic value because most of the active lesions are reversible with treatment. However, a follow-up biopsy performed after several months of treatment may provide important prognostic information in terms of progression to or lack of chronic lesions [169–172]. In addition to determining prognosis, repeat biopsies may help to decide if and how therapy should be changed if the clinical response is other than a complete remission. Thus, several groups are beginning to suggest more liberal use of the kidney biopsy in the ongoing management of patients with LN.

The clinical utility of the kidney biopsy depends on obtaining an adequate sample of renal cortex (at least 10 glomeruli) and examination by a renal pathologist [173]. In as much as every biopsy is a clinical-pathological correlation, the nephropathologist should be given all relevant clinical information in order to properly interpret the tissue and integrate the microscopic findings with the clinical data. Furthermore, it is essential that the clinician and pathologist review the findings together before initiation of therapy to ensure that specific clinical concerns are addressed and that the lesions have been appropriately contextualized.

Classification Schemes for LN

LN is currently classified by the International Society of Nephrology (ISN)/Renal Pathology Society (RPS) criteria developed in 2003 [174]. Similar to the previous WHO classification of LN, the ISN/RPS classification is based primarily on light microscopic patterns of glomerular injury. A criticism of both systems is the lack of consideration of the tubulointerstitium.

The ISN/RPS classification (Fig. 11.2) differentiates active (A) from chronic (C) and segmental (S) from global (G) glomerular lesions. Active glomerular lesions include glomerular endocapillary hypercellularity with or without leukocyte infiltration and with reduction of capillary lumina, karyorrhexis, fibrinoid necrosis, rupture of the glomerular basement membrane, cellular or fibrocellular crescents, wire loop lesions, and large intraluminal immune complexes (hyaline thrombi). Chronic glomerular lesions include glomerular sclerosis (segmental or global), fibrous adhesions, and fibrous crescents. Segmental lesions involve less than half of the glomerular capillary tuft area; global lesions involve more than 50 % of the glomerular capillary tuft area. The definition of segmental and global lesions is somewhat controversial. The degree of involvement in a given tissue section depends on the plane of the section through the glomerular tuft. Thus, depending on the level of the cut, a segmental lesion could appear to involve more or less than 50 % of the glomerular capillary surface area. Nonetheless, with these rules ISN/RPS classifies kidney injury in SLE as follows:

Class I: Minimal Mesangial LN

Glomeruli appear normal by light microscopy, but immunofluorescence and electron microscopy reveal mesangial immune complex deposits.

Clinical pathologic correlation: Usually no clinical kidney abnormalities; often normal serum complement.

Class II: Mesangial Proliferative LN

In additional to mesangial immune complexes as in Class I, there is pure mesangial hypercellularity without glomerular endocapillary hypercellularity or crescents.

Clinical pathologic correlation: Normal kidney function, mild hematuria and/or proteinuria, often normal serum complement.

Class III: Focal LN

Endocapillary and/or extracapillary (crescents) proliferative lesions are seen in fewer than 50 % of glomeruli. Glomerular lesions in focal LN are almost always segmental. By immunofluorescence and electron microscopy, mesangial immune complexes are seen, usually with segmental glomerular capillary immune complex deposits. Class III (A) shows only active lesions (focal proliferative LN).

Class III (A/C) has both active and chronic lesions (focal proliferative and sclerosing LN). In such cases, focal or segmental sclerosing glomeruli coexist with glomeruli with active proliferative/necrotizing lesions. Class III (C) shows only focal sclerosing glomerular lesions (focal sclerosing LN). Active lesions are not seen.

Clinical pathologic correlation: Normal or impaired kidney function, nephritic sediment, proteinuria (may be nephrotic), often low serum complement.

Class IV: Diffuse LN

Endocapillary and/or extracapillary (crescents) glomerular proliferative lesions are seen in more than 50 % of glomeruli. Glomerular lesions can be global or segmental, and active or chronic glomerular lesions are evaluated separately. Immunofluorescence and electron microscopy show mesangial and capillary loop immune complex deposits. The glomerular capillary loop deposits are mainly subendothelial and often large. Class IV-S(A) or G(A) indicates active diffuse segmental or global endocapillary or extracapillary proliferative glomerular lesions involving more than 50 % of the glomeruli. Class IV-S(A/C) or G(A/C) indicates segmental or global diffuse proliferative and sclerosing LN. In such biopsies, active proliferative lesions coexist with chronic sclerosing glomerular lesions.

Class IV-S(C) or G(C) indicates diffuse segmental or global sclerosing LN. In these subclasses, no active lesions are present, only inactive sclerosis or scarring.

Clinical pathologic correlation: Normal or impaired kidney function, nephritic sediment, proteinuria (may be nephrotic), often low serum complement.

It has been suggested that the pathogenesis of LN with true global lesions is different from LN with segmental glomerular lesions and that this affects outcomes and treatment choices [175–179]. Class IV LN with lesions involving more than 50 % of the glomerular tuft area (classified as Class IV-G) appears to have a worse outcome than global proliferative LN with 100 % involvement of the glomerular capillary tuft area (also classified IV-G by ISN/RPS) and Class IV-S with less than 50 % glomerular tuft involvement [177]. However, other studies did not find any difference in outcome between patients with Class IV-S and Class IV-G LN [180–184], but this may be because cases of Class IV-G with lesions involving more than 50 % of the glomerular tuft were generally not separated out from Class IV-G with 100 % tuft involvement [177–179]. At the present time, these concerns remain unresolved.

Class V: Membranous LN

Glomeruli do not reveal endocapillary hypercellularity; the mesangium may be normocellular or hypercellular. As in idiopathic membranous glomerulonephritis, spike formation on methenamine silver stain is common. Glomerular subepithelial

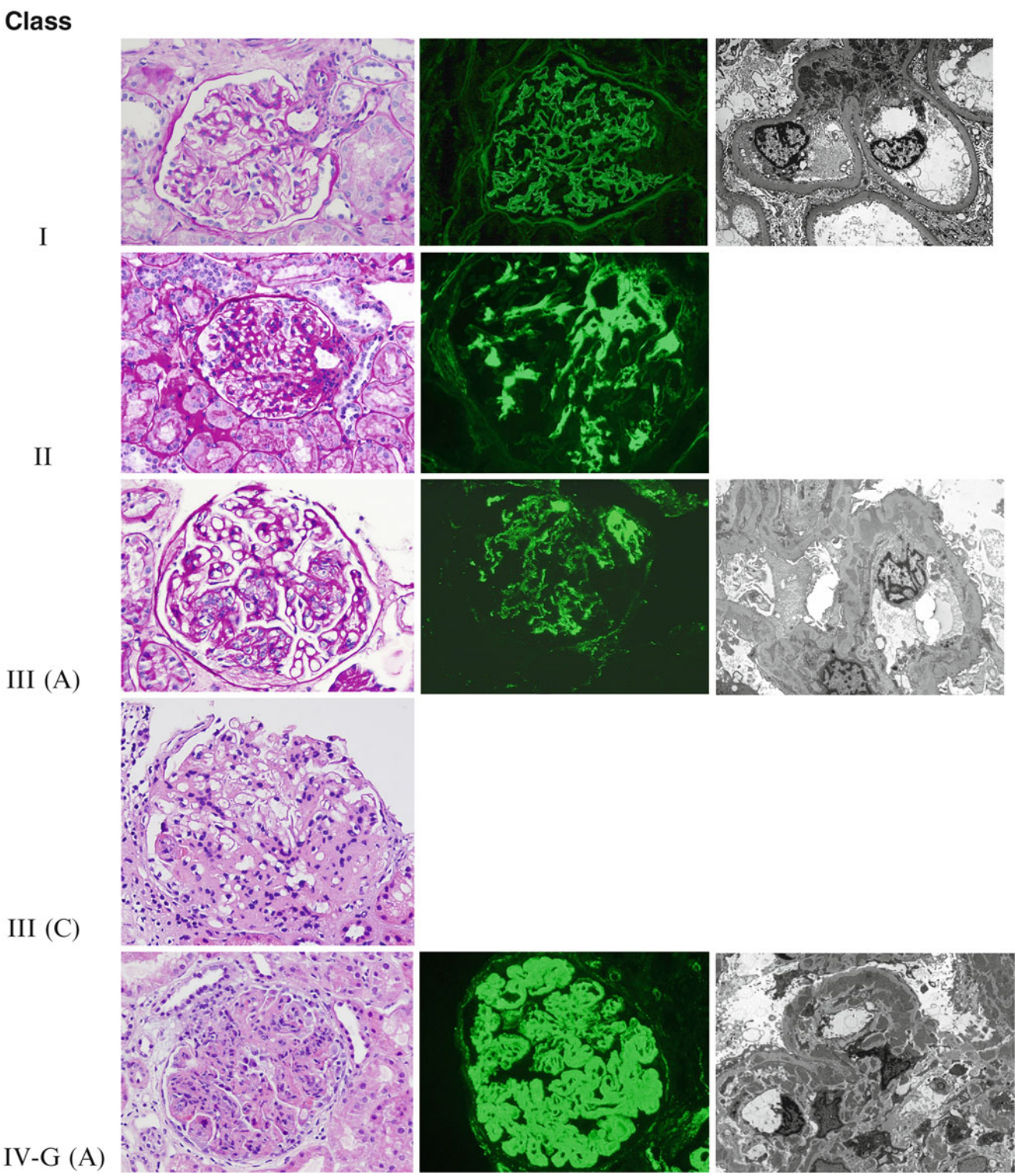

Fig. 11.2 The ISN/RPS classification of lupus nephritis. Representative photographs of the different lupus nephritis classes as seen under light, immunofluorescence, and electron microscopy. Class I: Light microscopy shows a normal-appearing glomerulus in Class I lupus nephritis (PAS ×200). Immunofluorescence demonstrates fine granular paramesangial IgG deposits (direct immunofluorescence ×400), and electron microscopy shows mesangial electron-dense immune-type deposits (uranyl acetate, lead citrate ×5,000). Class II: Mild mesangial expansion and hypercellularity are seen on light microscopy in Class II lupus nephritis (PAS ×200), while immunofluorescence shows mesangial IgG deposits (direct immunofluorescence ×400). Electron microscopy will be similar to Class I. Class III (A): Segmental glomerular intracapillary hypercellularity by light microscopy in focal proliferative lupus nephritis (PAS ×200). Granular mesangial and segmental glomerular capillary staining for C1q by direct immunofluorescence (×400). Several intramembranous, subendothelial, and occasional subepithelial

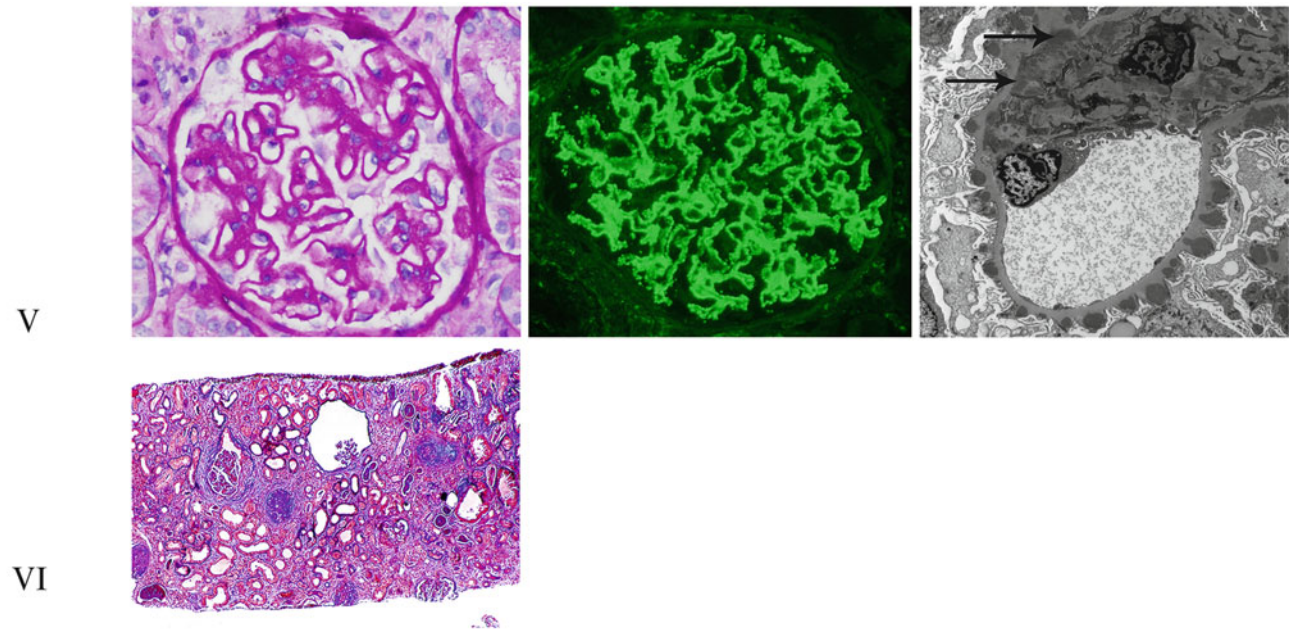

Fig. 11.2 (continued) deposits are demonstrated along the glomerular capillary loops by electron microscopy (uranyl acetate, lead citrate ×8,000). Class III (C): On light microscopy, a segmentally sclerosing glomerulus shows chronicity in focal proliferative lupus nephritis (H&E ×200). Class IV-G (A): Light microscopy shows a globally hypercellular glomerulus with segmental necrosis and apoptotic debris in a case of active diffuse proliferative lupus nephritis (H&E ×200). Immunofluorescence shows prominent widespread glomerular capillary and mesangial staining for C1q (direct immunofluorescence ×400). There are abundant large subendothelial, mesangial, and occasional subepithelial electron-dense immune-type deposits in active diffuse proliferative lupus nephritis (uranyl acetate, lead citrate ×5,000). Class V: Membranous lupus nephritis, like idiopathic membranous nephropathy, shows diffusely thickened glomerular capillary loops (PAS ×200). Direct immunofluorescence with an antibody to IgG reveals widespread granular glomerular capillary and mesangial staining (×400). Abundant subepithelial electron-dense deposits are present, often with mesangial electron-dense deposits (*arrows*; uranyl acetate, lead citrate ×8,000). Class VI: Advanced sclerosing lupus nephritis shows widespread interstitial fibrosis (*blue*) and mostly globally sclerotic glomeruli (Masson's trichrome ×40)

immune complex deposits involve over 50 % of the glomerular capillary tufts in over 50 % of the glomeruli, and in contrast to idiopathic membranous nephropathy, mesangial immune complexes are almost invariably present. Also different than idiopathic membranous glomerulonephritis, in Class V LN the IgG immune deposits contain mainly IgG1 and IGg3 as opposed to IgG2 and IgG4 [185]. Class V LN is common in combination with Class III or Class IV LN. In these combined patterns of injury, the proliferative component is listed first (such as Classes III + V or Classes IV + V).

Clinical pathologic correlation: Normal kidney function, often nephrotic syndrome, microscopic hematuria, often normal serum complement.

Class VI: Advanced Sclerosing LN

Over 90 % of the glomeruli are globally sclerosed without residual activity. There has to be clinical or morphologic evidence that the advanced glomerular sclerosis is secondary to LN. Immunofluorescence and electron microscopy still frequently reveal mild glomerular immune complex deposits in the few non-sclerotic glomeruli.

Clinical Pathologic Correlation: Chronic kidney disease.

The ISN/RPS classification, while clinically useful [184, 186, 187], is still based purely on morphologic findings and arbitrary definitions. Kidney biopsies can and should be exploited in novel ways to better inform the understanding of LN pathogenesis and the development of new therapeutics. For example, leukocyte subsets can be analyzed by specific staining in lupus kidneys and may yield new insights on renal inflammation [188]. Proteomic techniques can be used to look for patterns of protein expression in LN [189, 190]. Gene expression in biopsies can be analyzed with microarray techniques [89, 190]. As these technologies are applied to kidney biopsies, they will undoubtedly enhance the amount of information available from renal tissue and result in a new molecular/functional classification of LN kidney biopsies.

Class Transformation in LN

Follow-up biopsies of LN often show a class different from the initial biopsy [172, 191]. Successful treatment of Class

Table 11.1 Activity and chronicity indices for lupus nephritis biopsies

		Indicators of activity					Indicators of chronicity			
Score[a]	Crescents	Glomerular necrosis/ karyorrhexis	Glomerular neutrophils	Endocapillary hypercellularity	Large subendothelial immune deposits	Interstitial inflammation	Glomerular sclerosis	Fibrous crescents	Tubular atrophy	Interstitial fibrosis
None	0	0	0	0	0	0	0	0	0	0
Mild	2	2	1	1	1	1	1	1	1	1
Mod	4	4	2	2	2	2	2	2	2	2
Sev	6	6	3	3	3	3	3	3	3	3

[a]Lesions are scored on a semiquantitative scale; the maximum activity score is 24; the maximum chronicity score is 12

III or IV may result in improvement to Class II LN, whereas treatment failures may end in Class VI. Class I or II LN may evolve to any of the higher classes. Another common transformation is for Class III to become Class IV. Class III or IV may transform into Class V LN or Class V into Class III or IV. In these cases the resulting LN often shows a proliferative plus membranous pattern.

Activity and Chronicity Indices in LN

In an effort to standardize the evaluation of LN kidney biopsies, a scoring system was developed for active and chronic lesions [192–194]. The scoring criteria are described in Table 11.1. While the prognostic and therapeutic value of these activity and chronicity indices is debated [195], the score gives the clinician a readily understood estimate of the severity and acuity of the LN.

Immunofluorescence Findings in LN

Classically, glomerular immune complex deposits in LN often show a "full house" immunofluorescence pattern, meaning that all or almost all immunoreactants (IgG, IgA, IgM, kappa and lambda light chains, C1q, C3) are present. This is unusual in other forms of glomerulonephritis. Importantly, the absence of full house immunofluorescence does not exclude LN, especially in Class V. C1q staining is usually quite prominent in LN and rare in other forms of glomerulonephritis. Another characteristic immunofluorescence feature in LN biopsies is the frequent deposition of immune complexes along the tubular basement membrane, the peritubular capillary basement membrane, Bowman's capsule, and arterial and arteriolar walls. Most LN immune complexes contain IgG1 and IgG3, less IgG2, and minimal IgG4. Interestingly glomerular and extraglomerular immune complexes frequently have different IgG subclass distribution [196], suggesting different mechanisms for the deposition of glomerular and extraglomerular immune complexes.

Electron Microscopy in LN

In addition to immune complex deposits (discrete electron-dense immune-type deposits), a very common ultrastructural finding in LN is the tubuloreticular inclusion (Fig. 11.3). Tubuloreticular inclusions are seen mainly in endothelial cells and, while not diagnostic of LN, reflect high interferon levels in patients with active SLE. They are not restricted to renal endothelial cells but can be found throughout the body.

Tubulointerstitial Lesions in LN

Light microscopic lesions in the tubulointerstitium are nonspecific. Interstitial inflammatory cell infiltrates may or may not be present in biopsies with LN. They are more common in patients with proliferative LN (Class III or IV) and indicate an active disease process. Interestingly, the degree of interstitial inflammatory cell infiltrate does not correlate with the degree of tubulointerstitial immune complex deposition [196, 197]. In later stages of LN, interstitial fibrosis and tubular atrophy appear and indicate progressive chronic injury. Interstitial nephritis in the absence of LN may also occur [198]. Interstitial fibrosis and tubular atrophy may or may not be associated with active inflammatory cell infiltrate in the same biopsy specimen.

Other Kidney Lesions in Patients with SLE

Not all kidney disease in SLE patients is the classic, immune-complex-mediated glomerulonephritis known as lupus nephritis. Several non-LN glomerular diseases have been reported in SLE patients [198–203]. This literature is mostly case reports, but in a series of 252 patients, 5 % were found to have changes consistent with focal segmental glomerulosclerosis, minimal change disease, thin glomerular basement membrane disease, hypertensive nephrosclerosis, and amyloidosis [200]. The incidence of podocytopathies in lupus patients appears to be greater than in the general population, suggesting a causal link to the immune dysregulation of SLE

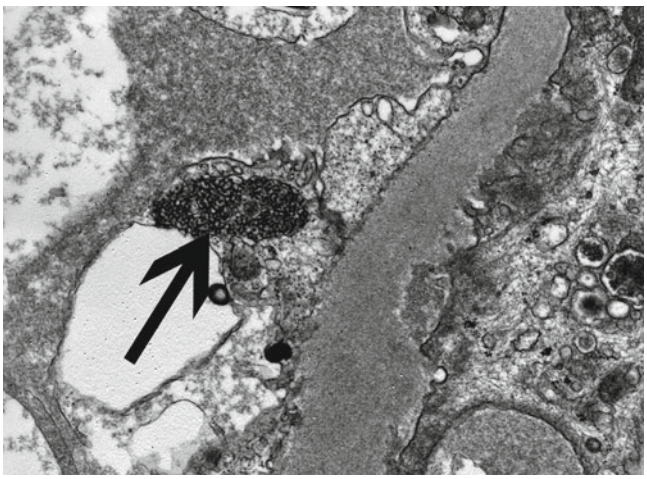

Fig. 11.3 A large tubulorecticular inclusion in a glomerular capillary endothelial cell (*arrow*). Such inclusions are common in all cases of LN and reflect increased interferon levels (Uranyl acetate, lead citrate, ×20,000)

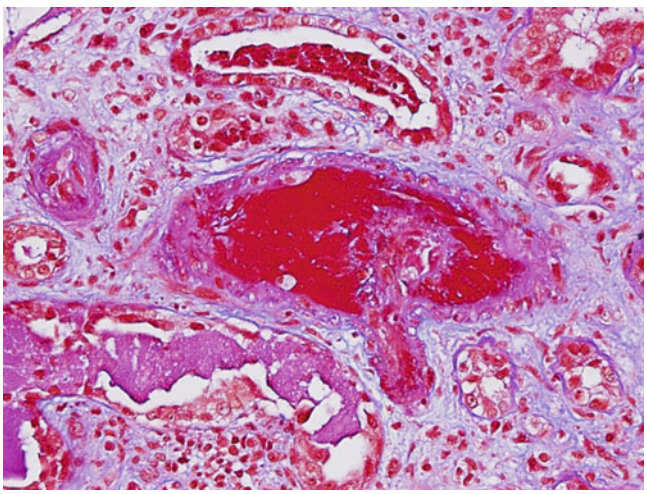

Fig. 11.4 A thrombus occluding an interlobular artery in a patient with lupus nephritis and antiphospholipid antibodies (Masson's trichrome ×200)

[204, 205]. AA amyloidosis has also been reported in some series [198, 201–203].

Thrombotic microangiopathy (TMA) with or without LN is not uncommon, particularly in patients who have circulating antiphospholipid antibodies and high d-dimer levels [206–208]. The biopsy findings (Fig. 11.4) include arterial/arteriolar fibrin thrombi with or without fibrinoid necrosis of the vessel wall, fragmented red blood cells in the fibrin thrombi or embedded in the thickened loosened arterial/arteriolar walls, and mucoid subendothelial widening of the arteries/arterioles. In more chronic stages, concentric thickening (onion skinning) of the arterial/arteriolar walls may develop. Arterial/arteriolar immune complex deposits may or may not be present. The glomerular changes include fibrin thrombi and/or prominent thickening of the glomerular capillaries, secondary to subendothelial electron lucent widening between the glomerular capillary basement membrane and the swollen endothelium (seen on electron microscopy). Because of the capillary wall thickening, the glomerular capillary lumen is narrowed and many of these glomeruli appear "bloodless." Fragmented red blood cells are not unusual in the glomerular capillaries.

Management of Lupus Nephritis

Recently three separate guidelines for the management of LN have been published. The guidelines have come from the American College of Rheumatology [209], the Kidney Disease-Improving Global Outcomes work group [210], and the Joint European League Against Rheumatism and the European Renal Association-European Dialysis and Transplant Association [211]. These groups were not totally independent, because some of the lupus experts worked with more than one of the sponsoring organizations. The major recommendations of all of the guidelines and of this chapter are evidence based and fairly consistent. A commentary comparing the LN guidelines and puts them into perspective is also available [212].

Proliferative and membranous forms of LN generally require treatment with immunosuppressive agents. However, because these therapies have considerable toxicity, their use should be informed by clinical and pathologic severity. For example, in advanced stage sclerosing Class III (C) or Class IV (C), the therapeutic strategy should shift from a focus on immunosuppression, except as needed for extrarenal SLE, to a focus on renal protection. Renal protection is also used for Class VI LN. The goal of renoprotection in inactive sclerosing LN is to prolong kidney function and avoid ESRD requiring renal replacement therapy for as long as possible. Renoprotective strategies include control of blood pressure with antiproteinuric antagonists of the renin-angiotensin-aldosterone system, sodium restriction, protein restriction, and correction of metabolic abnormalities and are discussed elsewhere in this text.

In addition to immunosuppression, all SLE patients should receive hydroxychloroquine unless they have a specific contraindication. Antimalarials have activity against TLR7 and 9, and as discussed previously TLRs are important in the pathogenesis of SLE and LN [213–215].

Hydroxychloroquine may protect against vascular thrombosis [216], kidney damage [217], renal flares [218], and ESRD [219] and has a favorable impact on lipid profiles.

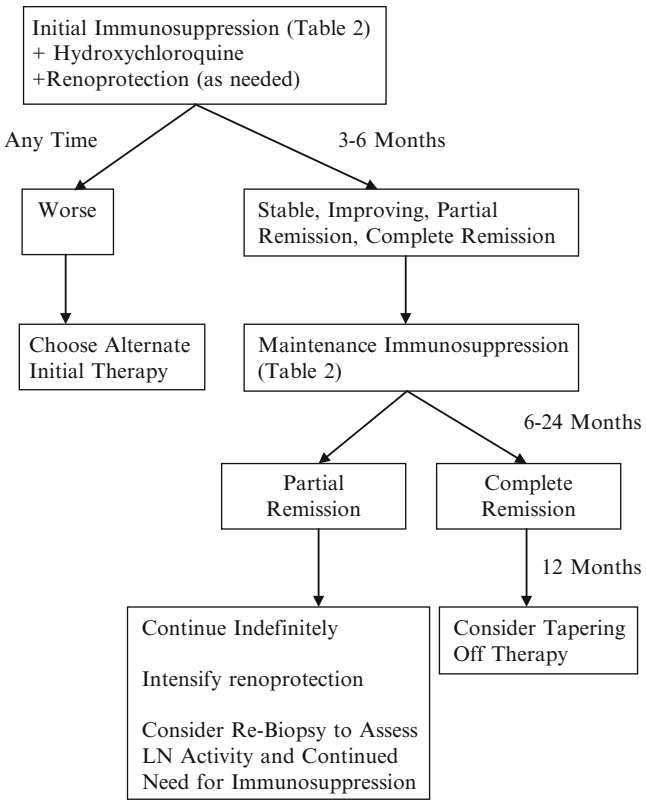

Fig. 11.5 A general strategy for the treatment of proliferative lupus nephritis

Mesangial Lupus Nephritis

Class I LN is rarely diagnosed because there are no or few clinical renal manifestations that would warrant a kidney biopsy. Patients with Class II LN may have glomerular hematuria and proteinuria (usually non-nephrotic), but kidney function is normal [220, 221]. The immunomodulatory regimens used to treat extrarenal SLE are generally sufficient for Class II (and I), along with renoprotective measures for hypertension and proteinuria as clinically indicated. If nephrotic proteinuria is present, a podocytopathy such as minimal change disease or focal segmental glomerulosclerosis should be considered and, if found, treated as for the idiopathic variants of these glomerular diseases [200, 204, 205].

Proliferative Lupus Nephritis

Proliferative LN (Class III or IV) can be an aggressive disease that requires intense therapy. A general strategy for the treatment of proliferative LN is outlined in Fig. 11.5. All current approaches initiate treatment with a cytotoxic agent and a corticosteroid. This is done because pioneering randomized clinical trials conducted by the National Institutes of Health (NIH) showed that although corticosteroids were effective in controlling proliferative LN, adding a cytotoxic agent at the beginning of treatment decreased the frequency of renal relapse and the development of future CKD or ESRD [222, 223]. Importantly, the beneficial effect of cytotoxic agents to preserve kidney function was only apparent after 3–5 years of follow-up [222–224].

Based on the early NIH trials, proliferative LN was treated with the cytotoxic agent cyclophosphamide for 18 months or more, a regimen associated with considerable morbidity. Limiting cyclophosphamide to 6 months however resulted in an increase in renal relapses [223]. The need for therapy beyond 6 months was thus apparent, but cyclophosphamide seemed too toxic. This problem was addressed in a prospective study of azathioprine (AZA), mycophenolate mofetil (MMF), or intravenous cyclophosphamide after 6 months of initial cyclophosphamide therapy [225]. Over 72 months patients treated with AZA or MMF were significantly less likely to reach the composite end point of death or CKD than the cyclophosphamide-only group and experienced fewer adverse side effects. Thus, the treatment strategy for proliferative LN evolved into an *induction* phase of high-dose corticosteroids plus cyclophosphamide for 6 months, followed by substitution of an antimetabolite, usually AZA or MMF for cyclophosphamide, for a prolonged *maintenance* phase. Although induction could imply a remission is achieved after induction, this is not generally the case with LN (see below). *Initial* phase may be a more appropriate description. Initial therapies for proliferative LN are given in Table 11.2.

The most widely used cyclophosphamide delivery format for initial LN treatment is intravenous pulses of 0.5–1.0 g/m^2 given monthly for 6–7 pulses. Oral cyclophosphamide shows comparable efficacy to intravenous cyclophosphamide, is easier to administer, and generally costs less [222, 226–231]. Oral cyclophosphamide lost favor because several studies associated it with increased toxicity, especially cystitis [222], but many of these early studies used very high doses (up to 2.5 mg/kg/day) for 6 or more months. Lower-dose, shorter-duration oral cyclophosphamide (Table 11.2) is effective, well tolerated, and results in a cumulative cyclophosphamide exposure similar to 6 months of pulse therapy [232]. In an effort to reduce cyclophosphamide exposure in LN, a true low-dose cyclophosphamide induction regimen (Table 11.2) was compared to 6 monthly pulses followed by two quarterly pulses of cyclophosphamide [233, 234]. This low-dose regimen was termed "Euro-lupus," and after 10 years of follow-up, the end points of death, ESRD, and doubling of the serum creatinine were similar in both groups, suggesting that low-dose cyclophosphamide can be used successfully in proliferative LN. Importantly, the Euro-lupus patient population was mostly Caucasian, and the proliferative LN was of mild-moderate severity.

The major morbidities with any cyclophosphamide regimen are infertility, future malignancy, and infection. To protect

Table 11.2 Treatment options for proliferative LN

Initial therapy[a]	Dose	Duration
Cyclophosphamide-modified NIH	0.5–1 g/m^2 IV monthly	6 months
Cyclophosphamide-oral	1–1.5 mg/kg/day[b]	2–4 months[c]
Cyclophosphamide-Euro-lupus	500 mg IV q2 weeks	3 months
MMF[d]	1–3 g/day	6 months
Maintenance therapy	Dose	Duration
MMF[d]	1–2 g/day	1 year beyond complete remission Indefinitely in partial remission
AZA	1.5–2.5 mg/kg/day	1 year beyond complete remission Indefinitely in partial remission
CSA	2.5–3 mg/kg/day	1 year beyond complete remission Indefinitely in partial remission

[a]Corticosteroids are added to all initial therapies. In severe disease intravenous methylprednisolone (250–500 mg) can be used for up to 3 days, followed by 1 mg/kg/day oral prednisone up to 80 mg/day. In less severe disease 0.4–0.6 mg/day oral prednisone may be started. In all cases ideal body weight should be used to determine corticosteroid dosing. Corticosteroids should be tapered to a dose of 10 mg/day by week 15 in severe disease and week 8 in moderate disease
[b]Maximum dose of 150 mg/day
[c]Duration based on response
[d]MMF dosing may be guided by mycophenolic acid levels

fertility women may be offered prophylaxis with leuprolide and men testosterone while cyclophosphamide is being given [235, 236]. Sperm banking and ovarian tissue cryopreservation are additional options. To decrease the risk of future malignancy, lifetime cumulative exposure to cyclophosphamide should be <36 g [237, 238]. To reduce infectious morbidity, the white blood cell count should be monitored weekly and the dose adjusted to keep the neutrophil count ≥2,000 cells/μ[mu]l. In patients with moderate-severe kidney insufficiency, reduce the cyclophosphamide dose by 20–30 % [239].

To completely eliminate the undesirable side effects of cyclophosphamide, The Aspreva Lupus Management Study (ALMS) prospectively compared MMF + corticosteroids to intravenous pulse cyclophosphamide + corticosteroids, looking for superiority in response at the end of a 6 month induction period [240]. This end point was not achieved; however, the ALMS induction trial showed an equivalent response to MMF and cyclophosphamide at 6 months with a similar incidence of adverse events, serious infections, and deaths for each drug. In a post hoc stratification of ALMS results by race and ethnicity, black or mixed-race patients who received intravenous cyclophosphamide did not do as well as those who received MMF, and the response rate among Hispanic patients was greater with MMF [241]. These findings suggest that Black and Hispanic patients, generally considered to have more resistant LN [242], may respond better to MMF than intravenous cyclophosphamide, but this will need to be verified in an independent prospective trial.

The ALMS study MMF target dose was 3 g/day. An increasing number of studies indicate that MMF dose and mycophenolic acid (active metabolite) levels do not correlate well, but that levels do influence therapeutic response and toxicity [243–245]. Therapeutic drug monitoring for MMF may thus prove useful in managing LN patients, especially if patients are not responding as expected and to avoid over treatment.

AZA was also prospectively compared to cyclophosphamide and showed a similar clinical outcome, but repeat biopsy after treatment showed more chronic damage in the AZA group, and those treated with AZA had a higher incidence of renal relapse and doubling of the serum creatinine [246, 247]. Because of this AZA was not placed as an initial therapy in Table 11.2, but in some areas of the world, AZA may be the only option due to cost or availability, and at least some large retrospective studies have shown long-term responses similar to initial treatment with cyclophosphamide [248].

The calcineurin inhibitors tacrolimus (TAC) and cyclosporine A (CSA) have recently been tested as an alternative to cyclophosphamide for initial therapy in proliferative and mixed proliferative plus membranous LN [249, 250]. Although these trials suggest similar complete and partial remission rates in the short term, the number of patients enrolled was small, and prospective long-term follow-up is lacking. The role for calcineurin inhibitors in treating proliferative LN remains to be determined in long-term prospective randomized trials.

Leflunomide is currently used to treat rheumatoid arthritis and works by blocking lymphocyte proliferation, T cell activation, and suppressing production of cytokines such as interleukin-2. Response rates were similar to those of cyclophosphamide when leflunomide was used to treat LN in two small trials from China [251, 252]. Repeat kidney biopsies at 6 months showed a large increase in the chronicity index in one of these studies [251], but this was not seen in repeat biopsies from the second study [252]. Thus, long-term trials

will be required to determine if leflunomide preserves kidney function over time as well as cyclophosphamide.

As will be discussed later, the overall response rates for Class III and IV LN with any of the initial therapies are only about 60 % at 6–12 months, with fewer complete remissions. With plenty of room for improvement, the anti-B cell monoclonal antibody rituximab was added onto MMF and corticosteroids in a randomized controlled trial to determine if 12-month outcomes could be improved [253]. This large trial was based on several small, open-label, uncontrolled trials that suggested rituximab may be effective in proliferative LN, either for refractory disease or as initial therapy [254–260]. However, at 12 months there were no differences between the rituximab and placebo groups in terms of complete or partial remissions. Thus, rituximab cannot be recommended as adjunctive initial therapy.

Therefore, for most patients with proliferative LN, the current choice for induction/initial therapy is between a cyclophosphamide-containing regimen and an MMF-only regimen. A number of factors must be considered in making this choice. Some investigators favor a full-dose (modified NIH or oral) cyclophosphamide-based regimen for patients with very severe LN because many patients in the two largest studies of MMF versus cyclophosphamide had less severe LN than the patients in some of the randomized clinical trials of cyclophosphamide [224, 240, 261, 262]. In contrast, others point out that in the ALMS trial, the subset of patients with severe LN did as well with either drug (presented at an American Society of Nephrology Meeting). MMF should be considered if patients have a cumulative dose of cyclophosphamide approaching 36 g. Low-dose (Euro-lupus) cyclophosphamide could be considered in Caucasians with moderate LN.

One more variable needs to be factored into treatment choice, and that is long-term preservation of kidney function. As mentioned previously, the long-term benefit of cytotoxic therapy was seen only after 3–5 years of follow-up [222–224]. There are a few studies comparing the long-term benefit of initial MMF versus initial cyclophosphamide. In a Chinese cohort, there were no significant differences in renal function between groups after a median of 64 months [231]. However, more MMF-treated patients had relapses, prolonged proteinuria >1 g/day, and persistent serum creatinine >2 mg/dL, all variables associated with deterioration of kidney function over time. The ALMS Maintenance Trial (discussed below) was a 3-year extension of the ALMS trial designed to evaluate maintenance regimens [263]. Although not designed to compare the long-term efficacy of initial therapy on kidney function, there was a (nonsignificant) trend toward fewer treatment failures in those who received cyclophosphamide as initial therapy as opposed to MMF, and this was independent of the choice of maintenance therapy. Thus, it cannot yet be stated with certainty that initial therapy of proliferative LN with MMF is equivalent to cyclophosphamide in preserving kidney function.

Several factors may be used to assess how patients are doing during initial therapy. If patients become clearly worse during the first few weeks to months of therapy, with continued loss of kidney function, increasing proteinuria, or increasing activity of the urine sediment, moving them to an alternate initial treatment protocol seems reasonable (Fig. 11.5). Post hoc analysis of the ALMS data showed that a positive renal response at 6 months could be predicted at 8 weeks if patients experienced a reduction in proteinuria of ≥25 % or a normalization of complement components C3 and/or C4 if they had low complement levels at the beginning of treatment [264]. This was not dependent on whether the patient was in the MMF or cyclophosphamide arm. The positive predictive value of these variables was about 70 %, and therefore they could be helpful in guiding treatment changes. Nonetheless, few patients reach complete remission by 6 months. In six studies of Black, White, and Hispanic patients [225, 232, 233, 240, 246, 261] treated with intravenous cyclophosphamide (modified NIH), low-dose cyclophosphamide (Euro-lupus), MMF, or AZA, the median complete plus partial remission rate 6 months after starting treatment was 54 % (range 18–85 %), and the complete remission rate was only 8.6 % (range 7.4–25 %). The median complete plus partial response rate 12 months after therapy was 60.5 % (range 32–85 %). In contrast, in four Chinese cohorts treated with MMF or cyclophosphamide [227–230], the median complete response rate at 12–24 months was 71 % (range 57–81 %), with an overall response rate of 90 % (range 73–95 %). Clinical improvement in Class III/IV LN continues well beyond 6 months and into the maintenance phase of therapy [229, 232, 233, 246, 265]. Consistent with this, kidney biopsies after 6 months of initial therapy generally show improvement in inflammation but rarely complete resolution of pathologic changes [49, 188, 266, 267]. In summary one may expect approximately half of LN patients to achieve a complete or partial response by 12 months and another 5–25 % to respond by 24 months. Considering only complete responses, half are achieved by 12 months and the other half by 20–24 months.

Maintenance therapies after the initial treatment of proliferative LN are outlined in Table 11.2. AZA and MMF are the most commonly used drugs for maintenance. To determine if AZA or MMF is superior, the ALMS trial was extended for 3 years in patients who achieved a complete or partial remission after the 6-month induction phase [263]. These patients were re-randomized to maintenance with MMF or AZA and followed prospectively. Over 3 years the composite treatment failure end point (death, ESRD, renal flare, sustained doubling of serum creatinine, or requirement for rescue therapy) was reached in 16 % of maintenance MMF-treated patients compared to 32 % of maintenance AZA-treated

patients ($P=0.003$). The superiority of MMF over AZA was not dependent on initial therapy (MMF or cyclophosphamide) or race of the patient. In contrast, the smaller MAINTAIN trial prospectively compared MMF to AZA as maintenance therapy in a predominantly Caucasian population after initial treatment with the low-dose Euro-lupus cyclophosphamide protocol, regardless of whether patients had achieved remission [268]. The primary end point was time to renal relapse, and after at least 3 years of follow-up, MMF and AZA were found to be statistically equivalent, although MMF was numerically better. These studies suggest MMF may be the first line for maintenance therapy, but the choice must be individualized to patient-specific factors such as desire for pregnancy and side effects.

CSA may also be considered for maintenance therapy. A pilot randomized clinical trial in 69 patients with Class III/IV LN suggested that 2 years of CSA may be as effective as 2 years of AZA for maintenance after initial treatment with prednisone and oral cyclophosphamide [269]. The outcomes measured in this study were relapse and proteinuria. Another randomized clinical trial showed CSA was as effective as AZA in terms of tapering maintenance corticosteroids in severe systemic lupus, but only 29 % of the patients had LN [270].

There is little evidence to guide the duration or withdrawal of maintenance therapy, but long treatment times are commonly used. For example, immunosuppression was continued for an average of 3.5 years in seven randomized clinical trials [222, 223, 225, 228, 231, 233, 234, 246]. Also supporting the need for a prolonged maintenance phase, only 40 % of proliferative LN patients showed biopsy evidence of improvement to Class II after 2 years of intense immunosuppression [247]. After complete remission is achieved and has been sustained for at least a year, or longer in patients with a history of renal flares, it is reasonable to consider slowly tapering therapy. Unfortunately there is no standard definition of complete remission for LN in the literature. Nonetheless, controlling proteinuria is important for preserving kidney function, and the target for complete remission should be a proteinuria level less than 0.5 g/day [271, 272]. Serum creatinine should improve to a patient's pre-LN baseline if known, although serum creatinine may be increased (acceptably) by renoprotective therapies. Thus, at a minimum serum creatinine should remain stable. Urine sediment should not have any RBC or WBC casts, but hematuria may persist for months [273]. Although reassuring, resolution of abnormal serologies is not necessary for remission of LN [274–280].

For patients who achieve only a partial remission, defined generally as a stable or improved serum creatinine and a reduction of proteinuria ≥50 % and to below nephrotic-range, immunosuppression should be continued indefinitely, and renoprotective therapies intensified (Fig. 11.5). Kidney biopsies done 2 or more years after initial treatment often still show activity in the presence of continued significant proteinuria and/or abnormal serum creatinine [281, 282]. Increasing corticosteroids or using alternative immunosuppressive agents to convert a partial to complete remission is not supported by evidence. In partial responders consideration should be given to repeat biopsy to determine the level of pathologic activity. Significant activity may provide a rationale for re-induction therapy, while significant sclerosis/fibrosis may provide a rationale for tapering immunosuppression other than what is needed for extrarenal SLE.

The long-term treatment objectives in proliferative LN are to prevent renal flares, ESRD, and death. Examining several studies that included Black, Hispanic, Caucasian, and Chinese patients observed for a median of 6 years (range 3–10 years), mortality and ESRD were 5 % (range 0–20 %) and 4 % (range 0–10 %), respectively [225, 227, 229, 246, 247]. Doubling of serum creatinine occurred in 7.2 % (range 0.04–18.2 %) of patients and renal flare in 23 % (range 0.04–42 %). Similarly, during 10 years of follow-up after treatment with low-dose (Euro-lupus) cyclophosphamide, 25 % of patients reached the composite end point of death, doubling of serum creatinine, or ESRD [234].

After diagnosis of proliferative LN, the question of predicting how a patient will do in the short and long term often comes up. This has been addressed in several prospective and retrospective studies, using univariate and multivariate analyses. As expected, multivariate analyses demonstrated that many of the factors identified in univariate analyses were not truly independent. A summary of independent risk factors for LN outcomes is shown in Table 11.3 [227–229, 242, 247, 265, 266, 283, 284]. From these data, two observations suggest that biomarkers currently used to predict outcomes are inadequate. First, almost none of the biomarkers are consistently identified across studies looking at the same outcome. Second, several studies identify no independent risk factors for many of the outcomes. This may be explained by study design, patient populations, variations in operational outcome definitions, and/or the strength of the current selection of biomarkers. An exception is the initial serum creatinine, which was identified across studies and outcomes as a biomarker of future remission, renal relapse, chronic kidney disease, and ESRD. It is interesting that failure to achieve a complete remission was identified by only a few investigations to be a significant risk factor for chronic kidney disease, ESRD, or mortality [157, 229, 285]; in most proteinuric kidney diseases, resolution of proteinuria is the strongest predictor of renal survival [286–288].

Membranous Lupus Nephritis

Membranous LN (Class V) is a nonproliferative glomerulopathy that can be seen in 8–20 % of LN patients [289–291].

Table 11.3 Risk factors for outcomes in proliferative LN

Variable[a]	Hazard ratio or relative risk	Retrospective study[b]	Prospective study
Ability to achieve remission			
White race	2.63	×	
Class IV LN	2.05	×	
Proteinuria	0.86 for every g/day increase	×	
Therapy delayed >3 months	0.58		
Serum Cr[c]	0.21 for every mg/dL increase	×	
Serum Cr	0.96 for every μ[mu]mol/L		×
None[d]			×
Renal flare			
Class IV LN	0.28	×	
Time to remission	1.03 per month	×	
CNS lupus	8.41	×	
Chronicity index[e]	1.22 per point	×	
Persistently + DS-DNA	2.94	×	
Activity index[f]	1.13 per point		×
CR[g] not achieved	6.2		×
None			×
Doubling serum Cr			
Serum Cr > 1 mg/dL	4.1	×	
Hispanic	3.6	×	
Poverty[h]	3.5	×	
Govt insurance[i]	3.0	×	
Nephritic flare	17.7	×	
Chronicity index	2.1 per point		×
None			×
ESRD			
Serum Cr	2.8 for every mg/dL increase	×	
No remission	6.8	×	
Anti-Ro positive	2.35	×	
Class III LN ≥ 50 %[j]	2.77	×	
None		×	
Composite: Doubling serum Cr, ESRD, death			
Serum Cr	1.26 for every mg/dL increase	×	
Chronicity index	1.18 per point	×	
Composite: ESRD, death			
Age > 50	3.3	×	
Non-White race	2.28	×	
Serum Cr	2.32	×	

[a]All clinical variables are taken at baseline before SLE LN treatment
[b]Retrospective or prospective study
[c]Serum creatinine
[d]No variables were predictive in multivariate analysis
[e]Chronicity index on kidney biopsy
[f]Activity index on kidney biopsy
[g]Complete remission not achieved
[h]Living in a neighborhood where >10 % of residents are below federal poverty line
[i]Medicare, Medicaid as opposed to private insurance
[j]Class III with active and/or necrotizing lesions in ≥50 % of non-sclerotic glomeruli

While less aggressive than proliferative LN, 20 % of Class V patients may develop CKD over time, and ESRD develops in about 8–12 % of patients [289–292]. In addition, left unchecked, the nephrotic proteinuria of Class V LN predisposes to hyperlipidemia, atherosclerosis, and thrombotic events [3, 287, 293]. Unlike idiopathic membranous nephropathy, spontaneous remission of heavy proteinuria occurs in only a minority of Class V LN patients [294, 295]. For these reasons Class V LN, especially with heavy proteinuria, does warrant therapy.

Renoprotective and antiproteinuric therapies should be used for pure membranous LN with low-level proteinuria. Class V LN patients with nephrotic-range proteinuria and/or renal insufficiency should be considered for immunosuppression. In contrast to proliferative LN, there has only been one prospective, randomized clinical trial for Class V [296]. This showed that the addition of cyclophosphamide (six intravenous pulses of 0.5–1 g/m^2 every other month) or CSA (5 mg/kg/day for 11 months) to corticosteroids improved the 12-month complete plus partial response rate from 26 % to 60–83 %, respectively. However, 40 % of the CSA group relapsed within a year of finishing treatment, but relapses were not seen in the cyclophosphamide group for 48 months. A subgroup analysis of Class V patients from the ALMS trial concluded that MMF (2–3 g/day) and cyclophosphamide were equally efficacious for Class V [297]. Consistent with this a number of small, nonrandomized, retrospective, or open-label studies also showed that MMF and AZA (1–2 mg/kg/day) are effective in Class V LN [298–301], achieving a total remission rate of 40–60 % within 4–6 months [300, 302].

Because kidney function is not likely to deteriorate rapidly in Class V, it is reasonable to try to induce remission with MMF or AZA (plus corticosteroids) and switch to the potentially more toxic cyclophosphamide or CSA only if that fails. Failure to achieve remission is predicted by an initial proteinuria level over 5 g/day [296]. Failure to achieve sustained remission is a risk factor for decline in kidney function in Class V, independent of race or ethnicity [296].

Patients with mixed membranous and proliferative LN are generally treated as for the proliferative component but may have a less favorable prognosis [290]. Alternatively, in a small randomized, controlled trial from China in patients with mixed Class IV and V LN, the combination of TAC (4 mg/day), MMF (1 g/day), and oral corticosteroids (multitarget therapy) was compared to pulse monthly IV cyclophosphamide (0.75 g/m^2 for 6 months) plus oral corticosteroids. At 6 months 90 % of patients treated with the "multi-target" therapy achieved either complete or partial remission compared to 45 % of patients treated with cyclophosphamide ($P=0.002$) [303].

Follow-Up of the LN Patient

LN is a characteristically relapsing disease. A survey of LN patients who had participated in randomized clinical trials showed flares in 40 % of complete responders within a median of 41 months of remission and 63 % of partial responders within a median of 11.5 months of response [304]. Renal flare risk factors were discussed previously (Table 11.3), but there is little consensus on what predisposes to a flare. Kidney biopsies done after successful treatment often show an increase in the chronicity index, suggesting that each flare can cause some chronic damage that may culminate in CKD or ESRD [49, 169, 170, 247, 266, 267, 303, 305].

Renal flare is diagnosed by sustained increases in activity of the urine sediment, proteinuria, or serum creatinine (reflecting a decline in GFR) and generally prompts consideration of an intensification of therapy [306, 307]. Typical criteria include an increase in serum creatinine of ≥25 %, and/or an increase in proteinuria above 1 g/day in patients who have completely responded, or a doubling of proteinuria in partial responders [306, 307], although these are generally based on expert consensus, and evidence-based criteria are just emerging [308]. Other findings that support a diagnosis of renal flare include a fall in serum complement C3 and C4 levels and a rise in anti-double-stranded DNA antibody titers. However, these serologies have low sensitivity (49–79 %) and specificity (51–74 %) for concurrent renal flare and do not reliably predict impending flare even when measured serially, with sensitivities and specificities around 50 % and 70 %, respectively [274–280]. Thus, the absence of changes in C3, C4, and anti-DNA levels does not exclude flare. Flares are less likely to occur in patients who have been highly immunosuppressed, and immunoglobulin levels may be useful in identifying such patients. However, although low serum immunoglobulin levels may indicate overt, treatment-associated immunosuppression, severe nephrotic syndrome can also cause serum immunoglobulins to be low. Non-LN causes of an increase in creatinine or proteinuria must be excluded. Increases in proteinuria can occur with pregnancy, uncontrolled hypertension, and increased sodium intake, and increases in serum creatinine can occur with drugs and sepsis.

It is reasonable to approach a relapse of LN with the same initial and maintenance therapies that were effective in inducing the original remission. If resuming the original regimen will result in the patient receiving a cumulative lifetime cyclophosphamide exposure of 36 g or more, a non-cyclophosphamide-based regimen should be used. A repeat kidney biopsy at relapse could be considered to guide therapy, especially if there is suspicion that the histologic class of LN has changed, or uncertainty as to whether a rising serum creatinine and/or increasing proteinuria is due to active disease rather than chronic scarring.

Resistant LN

Although there is no consensus definition of resistant LN, operationally the term may be applied to patients who have not responded to conventional cyclophosphamide or non-cyclophosphamide regimens that are typically used for initial therapy [309]. Often if one initial treatment regimen fails, an

alternative is tried, and if that fails the patient could be resistant. A repeat kidney biopsy confirming continued active disease should be considered before embarking on "salvage" treatment regimens.

Salvage or rescue therapies for LN have not generally been tested in large, prospective randomized clinical trials. With this limitation in mind, intravenous immunoglobulin (IVIg), low-dose calcineurin inhibitors, and rituximab have shown some promise in refractory LN. IVIg has been as effective as cyclophosphamide, but some formulations can be nephrotoxic and should be used with caution in patients with impaired kidney function [310]. CSA (2.5 mg/kg/day) and TAC (3 mg/day) have affected reductions in refractory proteinuria [311, 312]. Interestingly, although rituximab did not appear to improve initial LN therapy [253], there have been a large number of positive open-label studies in series of patients who have failed multiple other conventional treatments, suggesting it may be worth testing in a controlled study of resistant LN [257, 313, 314].

Biomarkers in LN

There is currently a major effort to identify biomarkers that can predict future LN flares, that reflect kidney pathology, and that can be used to determine the effectiveness of treatment [315]. Several urine biomarker candidates have been identified [282, 316–330], but to date none have been validated for clinical use in a large, prospectively followed lupus cohort. The rationale for developing LN biomarkers is to change management from reactive to proactive. For example, being able to anticipate an imminent renal flare and start therapy preemptively could attenuate the development of chronic kidney injury and minimize exposure to highly toxic drugs. Similarly, modification of drug dose and duration based on biomarkers that indicate how therapy is working would be expected to improve treatment efficacy and reduce toxicity. Finally, because kidney biopsies are not repeated at every flare, a noninvasive surrogate of renal pathology would be very useful in planning therapy.

Renal Replacement Therapies in SLE

Patients who reach ESRD due to LN should be offered the same renal replacement therapies as patients who have ESRD from other causes. Hemodialysis may be preferable to peritoneal dialysis, because some studies have shown a higher mortality and more infectious complications in SLE patients receiving peritoneal dialysis [331–335]. Comparison of LN patients to other types of patients who have received kidney transplants, in terms of allograft survival, patient survival, and acute rejection episodes, has generally shown fairly comparable outcome statistics or only slight disadvantages to the SLE recipients [336–339]. MMF may be a drug of choice posttransplant because MMF reduced allograft loss in lupus patients who received deceased donor kidneys and improved patient survival [337, 340]. Interestingly, it was found that SLE recipients have a higher rate of thrombotic events than non-SLE recipients [339, 341].

Extrarenal SLE activity does not necessarily stop when LN patients go on dialysis [332, 333, 342, 343], and LN can recur in 2.4–11 % of transplanted kidneys but does not appear to affect patient survival [338–341, 344]. In terms of allograft loss, some studies did not find an effect of recurrent LN [338, 339, 341], but in the largest investigation ($n=6{,}850$ recipients), recurrent LN was independently associated with allograft loss (hazard ratio 4.09; 95 % CI 3.41–4.92) [344]. Nonetheless, the attributable risk for allograft loss was low because the recurrence rate of LN was only 2.4 %.

Thrombotic Injury to the Kidney in SLE

The antiphospholipid syndrome (APS) is the most common clotting event affecting the kidneys in SLE. The incidence of renal APS is about 30 % in SLE and often occurs in conjunction with LN but can also occur alone [207, 345]. In renal APS a lupus anticoagulant is present in 30–52 % of cases and anticardiolipin antibodies in 72–95 % of patients, but up to 15 % had neither [206, 207]. Thrombi or evidence of past clotting may be found in any of the kidney blood vessels. The term APS nephropathy describes renal injury due to thrombi or their consequences in glomeruli and small intrarenal blood vessels and characteristically presents a histologic picture of a thrombotic microangiopathy [346]. While renal artery occlusion and renal vein thrombosis due to APS can be diagnosed with imaging studies, APS nephropathy requires a kidney biopsy. APS nephropathy is not treated with the immunosuppressives given for LN, because it causes noninflammatory occlusions of renal blood vessels and renal ischemia. Rather, APS nephropathy is treated with chronic anticoagulation plus hydroxychloroquine. Failure to recognize renal APS can lead to insidious CKD or ESRD.

Thrombotic thrombocytopenic purpura (TTP) may also occur in the setting of SLE and is associated with a high mortality [347]. TTP is treated with plasma exchange in addition to high-dose steroids. Because of the high associated mortality, it is important to consider this diagnosis and treat early.

Pregnancy and LN

In patients with LN, pregnancy may adversely affect the kidneys, and the LN may adversely affect the pregnancy. It should be noted that much of the reported data on pregnancy in LN is retrospective. Renal flares may be higher in LN

patients who have achieved only partial remission or continue to have proteinuria greater than 1 g/day [348–351], and flare rates of 10–69 % have been reported during or directly following pregnancy. Fetal losses of 8–25 % have been reported in patients with inactive LN [349–352] but 35–95 % in active LN [351, 352]. In several retrospective analyses the risk of fetal loss in SLE patients with LN was not higher than SLE patients with no history of LN [348, 352]. Hypocomplementemia also appears to be a risk factor for fetal loss [349]. In contrast, some studies have not found increased risk of fetal loss in SLE patients with or without LN [348, 352] and that renal flares and progressive renal dysfunction were not different between pregnant and nonpregnant patients with LN [348].

Considering all these data, it is recommended that SLE patients with kidney involvement be advised to wait at least 6 months after complete renal remission before trying to become pregnant [353, 354]. Hydroxychloroquine may be protective against SLE flares in the setting of pregnancy [352]. Low-dose aspirin may protect the fetus [349]. Cytotoxic drugs such as cyclophosphamide and MMF, and antihypertensive/renoprotective agents like ACEi and ARBs should not be used during pregnancy. Corticosteroids and AZA may be used if needed to control SLE activity.

The Future Direction of LN Treatment

Despite significant improvement in the morbidity and mortality of LN, current therapies are plagued by an undesirable side-effect profile and a low complete response rate. New therapeutics being developed are more narrowly directed against specific mediators and pathways important in the pathogenesis of SLE (Fig. 11.1). This should increase specificity and decrease side effects compared to the broad-spectrum immunosuppressives in use now. For example, antagonists of IFN-α, IL-6, complement component C5, and TLR7 and 9 have been developed and are at various stages of preclinical or clinical testing [254–256, 355].

The B cell has been the most studied therapeutic target in SLE and LN. This is likely because the B cell has such a wide array of relevant functions including autoantibody production, antigen presentation, and regulation of T and dendritic cells. As discussed, above rituximab, the anti-B cell monoclonal antibody to CD20, did not improve outcomes when added to MMF and corticosteroids as initial therapy of LN but may have a role in resistant disease [254–260]. Epratuzumab, another monoclonal anti-B cell antibody, is directed against CD22 and appears to partially deplete B cells and interfere with their proliferation and activation [254–256]. The cytokines BLyS and APRIL are B cell survival factors and are targeted by the monoclonal antibody belimumab (to BLyS) and the soluble receptor atacicept (binds BLyS and APRIL) [253–256]. Belimumab was recently approved for SLE in general but has not yet been specifically tested for LN [355, 356]. Autoreactive B cells communicate with and activate T cells through interaction of B7.1/B7.2 receptors with CD28 on T cells. Recombinant CTLA4 fused to IgG heavy chain components (abatacept) blocks the interaction of CD28 and B7.1/B7.2 and has been shown to reduce proteinuria in a rodent model of LN [254–256, 357]. Abatacept is currently approved for rheumatoid arthritis and is being tested in human LN.

To induce tolerance in autoreactive T cells, a small peptide therapeutic was developed. It is a phosphorylated analog of a portion of the 70K spliceosomal protein from nuclear RNP. This analog, called P140 (lupuzor), prevents T cell proliferation and induces secretion of the anti-inflammatory cytokine interleukin-10. Thus far, in a phase II trial in SLE patients, lupuzor showed minimal side effects and reduced anti-double-stranded DNA antibody levels by over 20 % [358].

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Tropical Infectious Diseases and the Kidney

12

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Introduction

Infectious and parasitic diseases are important morbidity factors and mortality causes, especially in underdeveloped areas of the world. Many such illnesses may be accompanied by acute or chronic kidney involvement [1, 2]. Acute kidney injury (AKI) and tubulointerstitial defects are frequently observed in the course of leptospirosis, malaria, and the several viral hemorrhagic fevers [2, 3]. All known varieties of glomerular lesions have been observed, with clinical presentations ranging from mild proteinuria or hematuria to the nephrotic syndrome [1]. Tubular dysfunction may also occur, particularly in visceral leishmaniasis and leprosy, where distal tubular acidosis may be an early clinical expression of the disease [4, 5].

Tuberculosis

Tuberculosis is a systemic disease caused by the *Mycobacterium tuberculosis* bacillus which is highly prevalent in some poor areas of the world. Yet lately it has been increasingly diagnosed in developed countries, in association with AIDS infection and among dispossessed dwellers of big cities [6, 7].

Clinical Presentation

The most prevalent clinical presentation is pulmonary cavitations, usually accompanied by productive cough, fever, night sweating, and wasting. However, following a primary respiratory inoculation, widespread seeding of bacilli may occur, and typical lesions may develop in other locations, such as the pleural cavity, lymphatic nodes, and eventually the urogenital tract [7].

Urogenital Tuberculosis

The spectrum of urogenital tuberculosis comprises kidney granuloma, interstitial nephritis, glomerular disease (including proliferative glomerulonephritis), end-stage renal disease, dialysis, and transplantation-associated tuberculosis and genital tuberculosis (most commonly affecting the epididymis and prostate) [8].

This is a frequent extrapulmonary location for *Mycobacterium tuberculosis* lesions [7]. Typically the lesions—tuberculous granulomas—initiate at the kidneys, spreading distally to the ureters, bladder, and testicles. Sometimes, a lengthy period separates the primary infection and an established diagnosis of urogenital tuberculosis. Early granulomatous kidney disease may present as proteinuria, pyuria, and loss of kidney function. Lower urinary symptoms occur whenever the disease spreads down to the ureters and bladder. Urinary symptoms suggestive of urinary infection, accompanied by pyuria and hematuria with no bacterial growth, point to urogenital tuberculosis. Advanced disease may cause obstructive uropathy, bladder defects, and loss of kidney function [6].

Images

Ultrasonography, computerized tomography, and magnetic nuclear resonance will demonstrate grossly distorted ureters, with alternating stenotic and dilated areas, reduced bladder volume, hydronephrosis, and reduced kidneys in advanced disease (Fig. 12.1) [6, 9]. Intravenous urography may show chalice distortion or cavities suggestive of tuberculosis' pelvic lesions and right kidney silence (Fig. 12.2) [6, 10].

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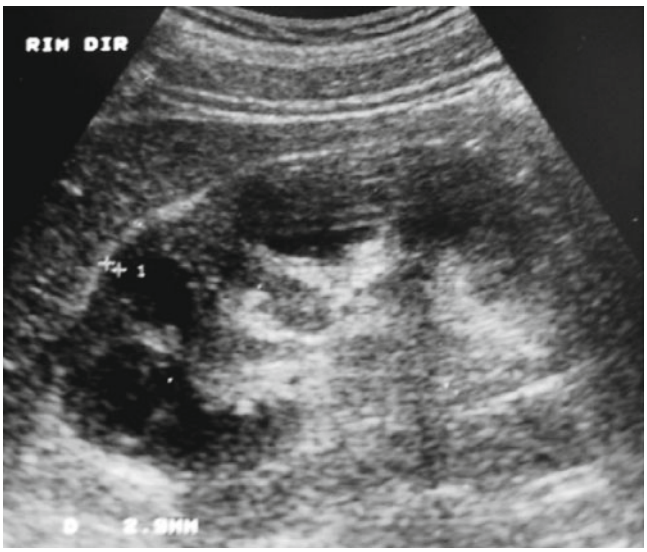

Fig. 12.1 Ultrasonography of a patient with unilateral renal tuberculosis showing dilated collecting system (caliectasis) and thinning of the renal parenchyma. From Figueiredo et al. [9]; used with permission

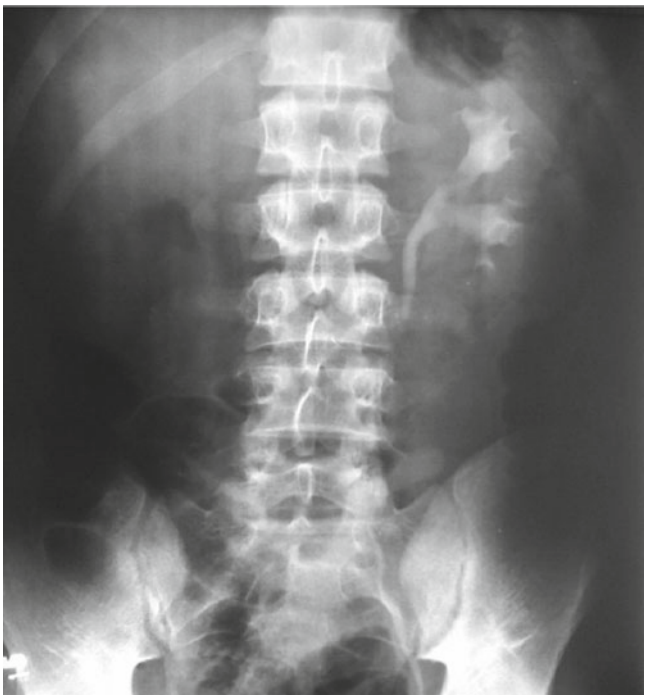

Fig. 12.2 Intravenous pyelogram showing right kidney silence in a patient with renal tuberculosis. From Oliveira et al. [10]; used with permission

Urine and Lower Urinary Tract Examination

Urinalysis may vary from mild changes, such as proteinuria and leukocyturia, to extreme pyuria, sometimes accompanied by hematuria. Urine cultures are regularly negative, unless there is severe bladder dysfunction. However, urine culture aimed at mycobacteria—using Lowenstein-Jensen solid culture medium—may be useful. Multiple samplings should be obtained in order to increase test sensitivity. Mycobacterium culture and identification results provide a specific diagnosis, yet may not be available for 2–3 weeks or longer [6]. Polymerase chain reaction (PER) techniques, such as the direct Gen-Probe MAD test, have been lately used. It is a reliable and fast-performing diagnostic test. Cystoscopy with biopsy is particularly recommended as it allows visualizing and sampling bladder lesions—this is possibly the best test to perform [6].

Pathophysiology

Urogenital tuberculosis is always secondary to a respiratory inoculation, which may be clinically unapparent [7]. Bacilli reach the renal cortex by blood or lymphatic dissemination, where they thrive, before extending to the lower urinary tract. The spreading lesions, most often bilateral, reach the pyramids, pelvis, ureters, and bladder—seminal vesicles, epididymis, and testicles may be also involved in advanced situations [6].

Pathology

Typically, the initial lesion to be found in kidney biopsies is a granuloma with an area of central caseous necrosis and tubular-interstitial inflammation (Fig. 12.3) [11, 12]. However, necrosis may not always be present, what might suggest other conditions such as sarcoidosis or granulomatous vasculitis [6]. Finding of acid-fast bacilli (which will be bright red on staining) by Ziehl-Neelsen stain inside the granuloma is clearly suggestive of tuberculosis. Yet, the finding of a diagnostic granuloma in a percutaneous kidney biopsy occurs by chance, as the disease is more often focal [6]. In more advanced disease involving the lower urinary region, cystoscopy and biopsy of bladder lesions allow reaching diagnosis [11, 12].

Treatment

Urogenital tuberculosis treatment does not diverge from pulmonary tuberculosis therapy. Scarring and development of obstructive lesions may require surgical treatment, besides the placement of endoprosthesis in some special situations. Using a combination of the following drugs, according to the WHO recommendations'—isoniazid,

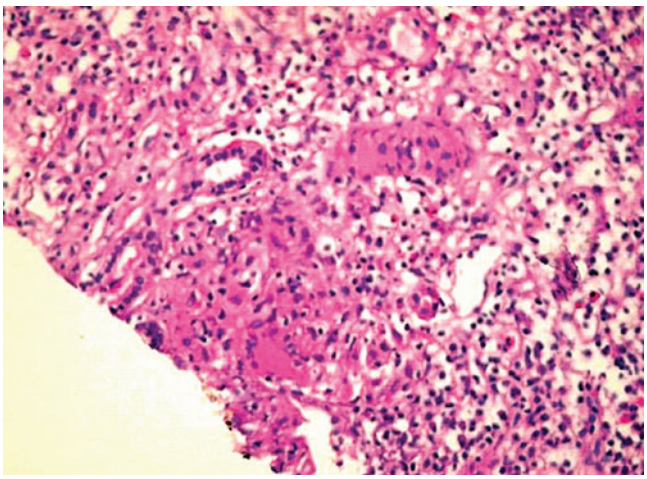

Fig. 12.3 Kidney biopsy of a patient with renal tuberculosis showing severe chronic tubular-interstitial inflammation with tubular atrophy, marked infiltration by lymphoplasmocytes, scattered medium-sized epithelioid cells with noncaseating granulomas, and Langhans giant cells, with one of the granulomas showing caseous necrosis. From Chaudhari et al. [11]; used with permission

rifampicin, pyrazinamide, streptomycin, ethambutol hydrochloride, and ethionamide—is currently recommended. Therapy usually starts with a combination of isoniazid (300 mg/day), rifampicin (600 mg/day), pyrazinamide (1,600 mg/day), and ethambutol (1,100 mg/day) ("RIPE" schedule), extending for the first 2 months, followed by isoniazid (400 mg/day) and rifampicin (600 mg/day) for the next 4 months. Such doses are suggested for individuals weighting more than 50 kg [13]. Dose adjustments are required for patients with reduced renal function (GFR < 30 ml/min). Many drugs should be administered after a dialysis session and in a thrice-weekly schedule. Antituberculosis drugs for patients presenting with renal failure should undergo the following adjustments: rifampicin, no required adjustment; isoniazid, 75–100 % of the usual dose if GFR is between 10 and 50 mL/min and 50 % if GFR < 10; pyrazinamide, no change in dose, yet in a 24-h interval schedule if GFR is between 10 and 50 mL/min and 48- to 72-h if GFR < 10 mL/min; ethambutol, 50 % of the usual dose if GFR is between 10 and 50 mL/min and 25–50 % if GFR < 10; and streptomycin, no change in dose, yet keeping 24- to 72-h intervals if GFR is between 10 and 50 mL/min and 72–96 h with GFR < 10 mL/min [13].

Antituberculosis therapy interacts with other drugs and increases the risk for hepatotoxicity. Some patients may present with asymptomatic mild elevation of liver enzymes during the first months of treatment, followed by spontaneous decrease. There is usually no need for drug adjustment in those cases. Treatment should only be withdrawn once liver enzymes are over three times the reference values. Following withdrawal, drugs should be reintroduced in the following scheme: rifampicin and ethambutol, followed by isoniazid, and then pyrazinamide. Before reintroducing a drug, liver function tests should be performed. Time for treatment should be counted from the day all drugs have been started again. If reintroducing a drug is not possible, alternative approaches should be attempted. In non-cirrhotic patients, streptomycin (1,000 mg/day) + rifampicin (600 mg/day) + ethambutol (1,100 mg/day) should be given for 2 months, followed by rifampicin (600 mg/day) + ethambutol (1,100 mg/day) for an additional 7-month period. In cirrhotic patients, treatment should be attempted with streptomycin (1,000 mg/day) + ethambutol (1,100 mg/day) + ofloxacin (800 mg/day) for 3 months, followed by ethambutol (1,100 mg/day) + ofloxacin (800 mg/day) for additional 9 months [13].

HIV/AIDS infection in tuberculosis-endemic countries has resulted in a significant increase in pulmonary tuberculosis with negative sputum smears and extrapulmonary infection. Those patients are usually more significantly immunocompromised, more frequently present adverse reactions to antituberculosis drugs, and have higher mortality rates. HIV positivity concomitant with tuberculosis is frequent. Tuberculosis treatment in HIV-infected patients follows the same recommendations as for uninfected individuals. Higher rates of treatment failure and recurrence of tuberculosis have been demonstrated, requiring special attention to the follow-up of such patients. Treatment failure recommendations for the management of recurrence and drug resistant are also similar [13]. No evidence-based, randomized, controlled trials have indicated the best drug schedule for resistant tuberculosis. Recommendations are based on general principles of microbiology and therapy for tuberculosis, observational studies, and specialist opinion. When indication for treatment change emerges, the choice should contemplate an association of the more effective drugs, with the highest probability of cure. An important point in treating patients with resistant tuberculosis is never add one single drug to an already failing regimen, as this may lead to resistance to the added drug. Instead, at least two and preferably three new drugs, to which susceptibility could be inferred, should be combined to lessen the probability of additional resistance. Empirical re-treatment regimens might include a fluoroquinolone; an injectable agent, such as streptomycin (if not used previously and the patient is not from an area of the world having high rates of resistance), amikacin, kanamycin, or capreomycin; and an additional oral agent such as *p*-aminosalicylic acid (PAS), cycloserine, or ethionamide. Once drug-susceptibility test results are available, the regimen should be adjusted according to the results. Patients having tuberculosis caused by strains of *M. tuberculosis* resistant to at least isoniazid and rifampicin (multidrug

resistant [MDR]) are at high risk for treatment failure and further acquired drug resistance [13].

Tuberculosis is an important opportunistic infection in kidney transplant recipients in endemic areas. Both randomized and nonrandomized studies support the use of isoniazid as prophylaxis in such patients. Clinicians should consider prophylaxis in all kidney transplant recipients in endemic areas or in recipients in non-endemic countries who may be at risk [14].

Leptospirosis

Leptospirosis is a zoonosis caused by organisms of the *Leptospira* genus, holding worldwide distribution. Its acute evolution, in humans, produces a variety of clinical situations, from nonspecific symptoms to profound jaundice, hemorrhages, meningeal symptoms, and acute kidney injury (AKI). It is a quite uncommon disease in developed countries. Yet, in some areas of endemic leptospirosis, as Thailand and Singapore, it has been associated with AKI in 24 % and 33 % of all cases, respectively [15]. In a 10-year period (1996–2005), 33,174 occurrences were notified in Brazil, and during a single year (2007), 1,547 new cases were notified, mostly in the southern states [13].

Clinical Presentation

Clinical syndromes associated with renal leptospirosis are summarized in Table 12.1. Leptospirosis incubation period varies from 5 to 14 days, with a median time of 10 days [7, 15]. Its clinical presentation varies, depending on the prevalent *Leptospira* serotype and the geographic area, from a febrile, almost asymptomatic condition, to a severe multisystem disease [7]. Its clinical presentation may occur as (1) a non-jaundice, febrile, auto-limited disease (in 85–90 % of instances); (2) Weil's syndrome, with jaundice, AKI, hemorrhages, and heart arrhythmias—myocarditis (in 5–10 %); (3) meningitis/encephalitis; and (4) pulmonary hemorrhages, with respiratory insufficiency [16]. It usually follows a two-phase course: the first one (3–7 days) characterized by high fever (100–102 °F), chills, and severe headaches and the second one in which anorexia, nausea, vomiting, diarrhea, and intense myalgia, particularly in lower limbs, dominate. During the first phase it is possible to isolate the *Leptospira* in blood samples. During the second phase, IgM antibodies appear. Disease severity seems to depend on the intensity of the individual's humoral immune response [15]. The severest forms of the disease may lead to hemodynamic changes secondary to acute intravascular volume decrease, or a direct toxic effect upon vessel endothelium, and diffuse mounting of capillary permeability [17]. Pulmonary hemorrhagic syndrome may appear independently of other systemic symptoms, sometimes requiring mechanical ventilation, which leads to greater mortality risk [18].

Table 12.1 Clinical syndromes in leptospirosis-associated kidney disease

Clinical presentation	Kidney biopsy
AKI, fever, jaundice, myalgia, headache, vomiting, dehydration, chills, calf pain, diarrhea, hepatomegaly, anorexia, oliguria, tachypnea, dyspnea, crackles or rhonchi, petechiae, arthralgias, hemoptysis, hematemesis, conjunctival suffusion, edema, obtundation, flapping, constipation, splenomegaly, seizure	Acute tubular necrosis, interstitial nephritis

AKI acute kidney injury

Kidney Changes

The kidneys are almost always involved in severe leptospirosis. Non-oliguric leptospirosis-associated AKI is the most frequent presentation, usually unaccompanied by hyperkalemia—hypokalemia may even occur—contrary to AKI associated with other infectious diseases, such as malaria, diphtheria, or meningococcemia. Experimental, as well as clinical, studies have demonstrated that proximal tubule injury and collecting duct vasopressin blunted response may account for such metabolic alterations [19]. Acute, severe jaundice has been linked to functional kidney changes that may encompass falling of glomerular filtration rate and reduced urinary concentration ability [19]. Severe leptospirosis is frequently accompanied by intense jaundice, which may add to the development and severity of the AKI [19]. Rhabdomyolysis and AKI association is well established. Yet, how important rhabdomyolysis may be in leptospirosis-associated AKI is less evident. Increased serum creatine phosphokinase (CPK) levels have been more often noticed in patients with severe leptospirosis-associated AKI than in those with less compromised renal function, suggesting an added risk for AKI from rhabdomyolysis [3]. Proximal tubule damage and collecting duct vasopressin resistance reduce proximal sodium reabsorption and increase free-water clearance, respectively—with resulting polyuria and enhanced natriuria [19]. Increased distal tubule potassium secretion may be induced by increased sodium delivery to distal tubules and raised aldosterone and cortisol levels [19]. Such findings point to a primary proximal tubule defect, with a comparative preservation of distal tubule functional ability to manipulate sodium and potassium. A prospective study on patients with leptospirosis-associated AKI found reduced proximal tubule sodium reabsorption, thus demonstrating the presence of a proximal tubule defect in such patients [20, 21]. Hypokalemia may occur in 45–74 % of patients at admission, potassium replacement being necessary in up to 80 % of instances [19].

Diagnosis

Tests to be used in leptospirosis diagnosis will depend on the disease's stage the patient is going through. During the initial febrile period, it is possible to visualize the *Leptospira* by direct examination of blood, to grow it by seeding blood in adequate culture media, or to recover it by laboratory animal inoculation. As it might take several weeks to get a positive result from cultures, usually only a retrospective diagnosis may be obtained in this way [7]. During the second immune phase, *Leptospira* may be found and grown from urine. Given the difficulties in obtaining a direct diagnosis, serologic tests, such as ELISA IgM macro- and microagglutination tests, have been extensively performed [7].

Pathophysiology

Several factors seem to be involved in the pathophysiology of the kidney lesion, such as a direct nephrotoxic effect by the *Leptospira*, loss of salt and water, jaundice, and rhabdomyolysis. Experimental studies have suggested that lesions are associated with the physical presence of the organism in the kidney—*Leptospira icterohaemorrhagiae* has been visualized, as soon as 3–6 h after inoculation, at the mesangium and interstitial tissue [19]. It seems that glomerular capillary *Leptospira* passage is accompanied by a transitory, moderate, mesangial proliferation [19]. Lack of hyperkalemia is remarkable, even in oligo-anuric individuals, and is a significant characteristic of AKI in such severe infectious disease. It should call the attention of an attending physician, whenever considering differential diagnosis of AKI. Leptospira outer membrane proteins (OMPs) may elicit tubular injury and inflammation through a toll-like receptor (TLR)-dependent pathway, followed by activation of nuclear transcription factor kappa B and mitogen-activated protein kinases and a differential induction of chemokines and cytokines relevant to tubular inflammation [22].

Pathology

Leptospira reach the interstitium by way of peritubular capillaries, causing an acute inflammatory response with focal interstitial edema, lymphocytes, macrophages, plasma cells, and, occasionally, eosinophils infiltrate [19]. Variable degrees of tubular necrosis are always present [19]. *Leptospira* adhesion to tubule epithelial cells occurs early in the course of the disease, and the infecting organism may be detected even by light microscopy [19]. Importantly, *Leptospira* antigens' loading of tubular cells occurs early in disease's course and may be detected by immunohistochemical staining techniques [23].

Treatment

Quick clinical recovery is the usual outcome—serum creatinine returning to normal levels by the forth to eighth day of symptomatic disease, depending on the severity of kidney involvement. Glomerular filtration rate, proximal sodium reabsorption, fractional potassium excretion, and tubular hydrogen generation complete recovery take place by the third month of follow-up [24]. Yet, a concentration defect may persist for up to 6 months and echoes the severity of AKI [24]. Penicillin seems to reduce symptoms and AKI severity; yet, its advantage has been only demonstrated once started during the first infection week. Early dialysis and treatment of *Leptospira*-associated AKI seems to be helpful in reducing mortality [25]. Leptospirosis is a disease evolving with low mortality rate. However, in the presence of AKI, mortality rate may be as high as 22 % [19]. Previous studies have examined mortality risk factors associated with leptospirosis—oliguria, old age, episodes of cardiac arrhythmias, and pulmonary involvement are associated with poorer outcome, oliguria being present in over 50 % of reported deaths [19].

Leprosy

Leprosy is a chronic disease associated with infection by *Mycobacterium leprae*—an obligatory intracellular parasite—an acid-fast bacillus that preferentially infects peripheral nervous system's Schwann cells and the skin [7]. Its global prevalence has been estimated at 10–15 million, spreading among 106 countries—leprosy world report during the year 2007 included 254,525 new cases [7]. Kidney lesions have been demonstrated in all disease presentations, yet particularly in the multibacillary leprosy form [26–28]. Recent studies show that renal involvement in leprosy is common, with proteinuria in 4.8 % and hematuria in 6.8 % of cases [28]. Risk factors for kidney disease in leprosy include reaction episode, multibacillary classification, and advanced age [28].

Clinical Presentation

Clinical syndromes associated with leprosy are summarized in Table 12.2. Skin and peripheral nervous system damages are leprosy hallmarks. Apparently, host immune response seems to be determinant on the clinical pattern. Two different immunological complications in leprosy course may occur, sporadically intensifying symptoms: (1) a so-called reversal reaction (type 1), a clinical presentation associated with paucibacillary leprosy pattern and (2) "erythema nodosum leprosum" (type 2), frequently associated with multibacillary disease [7]. Leprosy has been classified in four different

Table 12.2 Clinical syndromes in leprosy-associated kidney disease

Clinical presentation	Kidney biopsy
Polyarthritis, proteinuria, hematuria, urinary concentration and acidification defects, AKI, CKD	Diffuse proliferative lesion, amyloidosis, acute tubular necrosis, crescentic nephropathy, membranoproliferative nephropathy, membranous nephropathy, mesangial proliferative lesion, interstitial nephritis, glomerular sclerosis

AKI acute kidney injury, *CKD* chronic kidney disease

forms, according to WHO: indeterminate, tuberculoid, dimorphic, and virchowian forms [7]. Diagnosis and classification are dependent upon the clinical presentation and laboratory tests—lesion direct bacilli counting allows classifying leprosy lesions as pauci or multibacillary.

Kidney Changes

Kidney involvement reports started appearing around 1937, from autopsy studies of patients diagnosed as having died from leprosy [27]. From then on, a series of autopsy and kidney biopsy studies have attempted to elucidate kidney involvement in leprosy. Acute and chronic, nonspecific, glomerular and interstitial lesions—besides amyloid deposits—have been linked to the disease. Daher et al. found kidney changes in 35/923 patients (65 % had multibacillary leprosy) [28]. Risk factors for kidney involvement were old age, having immunological complications and being classified as multibacillary [28]. Glomerular involvement is the more prevalent structural change associated with leprosy, yet with a variable reported prevalence. Kidney biopsy studies on leprosy patients place prevalence at approximately 37 %—higher among multibacillary individuals [29]. Glomerular lesions were strongly associated with occurrence of erythema nodosum, even though lesions have been also reported with no such complication. Almost all known morphological glomerular lesions have been reported, except for focal segmental glomerular sclerosis (FSGS) [30]. Yet membranoproliferative glomerulopathy, so often associated with infectious diseases, has been reported slightly more frequently than other forms [30].

Pathophysiology

Mechanisms leading to leprosy-associated glomerular lesions have been only partly elucidated. Despite bacilli being found in glomerular lesions, no clear evidence for direct *Mycobacterium leprae* involvement in their genesis exists. Immunological mechanisms may be required: serum complement may be reduced; subendothelial immune complexes have been demonstrated by electron microscopy; IgA mesangial deposition has also been detected. Circulating immune complexes typically accompany erythema nodosum leprosum, with its conceivable deposition in vessels and tissues, including glomeruli. *Mycobacterium leprae* antigens may be freed, once antibiotics are initiated [31]. Alternatively, antibodies directed toward antigens somewhere inside the glomerulus may complex and deposit locally [31]. However, not every leprosy-associated kidney lesion relates with the concomitant development of erythema nodosum leprosum, thus suggesting glomerular lesions multifactorial origin. Significant reduction on cellular immune response occurs in virchowian leprosy with humoral immune response hyperactivation, which might facilitate immune complex formation and development of glomerular lesions [29].

Tubular dysfunction occurs with some frequency (from 25 % to 85 %), either in multi- or in paucibacillary leprosy [5]. Urine acidification defect appears in 20–32 %, whereas inability to concentrate urine may occur in up to 85 % of leprosy patients [5]. Immunohistochemical examination of kidney samples identified IgM, C3, and, less often, IgA and IgM deposits in the mesangium and capillary basal membranes [31]. Electron microscopy substantiates the presence of mesangial and subendothelial or subepithelial, granular dense deposits [29]. The complement may be reduced in some patients, supporting the idea of an immune-mediated lesion [29].

Urine Changes

Leprosy has been often associated with hematuria, especially in its virchowian form, and with erythema nodosum leprosum, even in the absence of glomerular changes. Microscopic hematuria accompanies virchowian form leprosy in 12–17 % of instances—it often disappears after a couple of months on treatment [29]. Several studies have reported the occurrence of proteinuria (from 2 to 68 %), more frequently associated with immunological complications of multibacillary disease [29]. It usually varies from 0.4 to 8.9 g/24 h, yet nephrotic syndrome is an unusual presentation. Virchowian form of leprosy is more frequently associated with proteinuria and presence of leukocytes, red blood cells, and casts in urine, being uncommon in other forms of the disease [5].

Pathology

Glomerular Lesions

Renal tissue reaction to *M. leprae* could be induced by various local immunologic or physiological factors. The great variety of lesions suggests a heterogeneous disease, though dependent on a single cause—immune complexes quantity and quality may stand for a divider [5, 31–37]. Adequate kidney biopsy was obtained from 54 cases of leprosy:

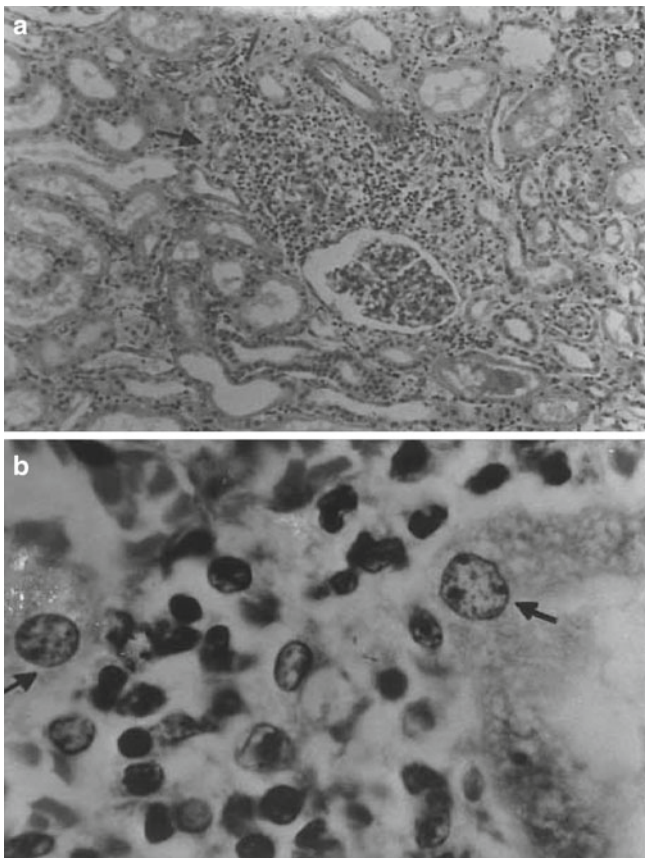

Fig. 12.4 Leprosy-specific renal lesions characterized by the presence of epithelioid granuloma in the renal parenchyma (**a**), H&E; original magnification ×40; Hansen's bacilli in the kidney (**b**); Faraco-Fite stain; original magnification ×400. From Nakayama et al. [32]; used with permission

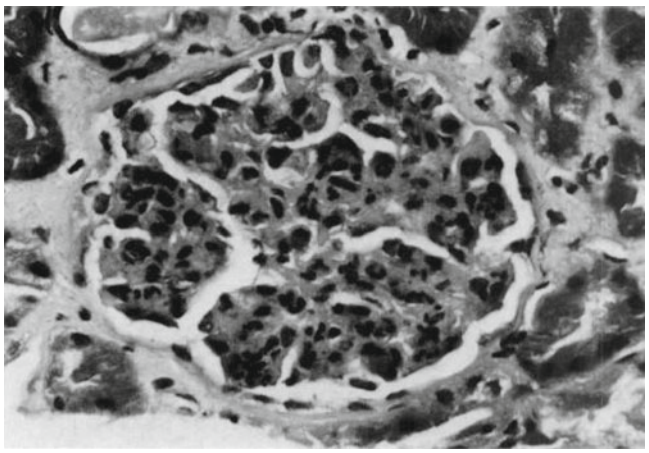

Fig. 12.5 Glomerulus from a patient with leprosy showing a diffuse, endocapillary, proliferative process with numerous neutrophils occluding the peripheral capillary loops, H&E. From Ahsan et al. [33]; used with permission

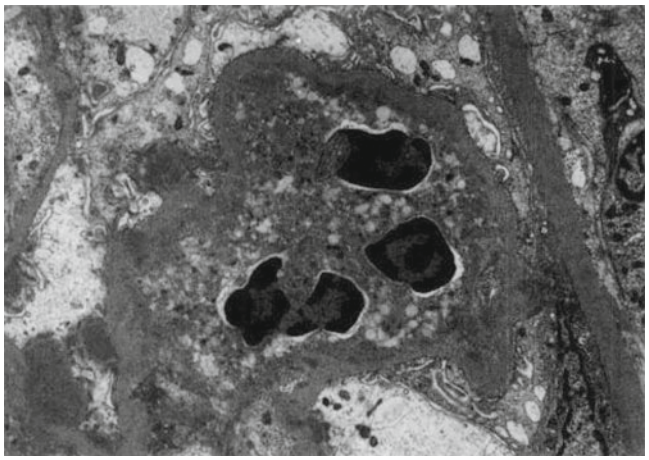

Fig. 12.6 Electron photomicrograph of a patient with leprosy showing a neutrophil occluding a peripheral capillary loop. Immune complex-type, electron-dense deposits can be seen in subendothelial areas, whereas electron-dense "humps" are noted in the subepithelial space, consistent with a post-infectious glomerulonephritis. From Ahsan et al. [33]; used with permission

45 were lepromatous form, 4 tuberculoid, and 5 belonged to borderline form of leprosy. Membranous nephropathy in 17 (32 %) was the commonest type of glomerular lesion followed by diffuse proliferative lesion in 12 (22 %) and membranoproliferative lesion in 6 (11 %); two samples presented a crescentic nephropathy. Specific glomerular lesions in leprosy include epithelioid granuloma with Hansen's bacilli in the kidney [32]. Figure 12.4 depicts some features of leprosy kidney disease [32]. Diffuse, endocapillary, proliferative process, with numerous neutrophils occluding peripheral capillary loops, can also be found in leprosy. Figures 12.5 and 12.6 demonstrate such lesions [33]. Electron microscopy may show immune complex-type, electron-dense deposits in the subendothelial area, with electron-dense *humps*. Figure 12.7 depicts such *humps* [34]. Crescent formation has also been described in leprosy. Figure 12.8 depicts a leprosy-associated crescent [37].

Tubulointerstitial Lesions

Interstitial nephritis has been reported chiefly in patients with lepromatous leprosy and seems to relate with long-time illness and extended therapy—such lesion may be the more regular histological finding in leprosy [35].

Chronic Kidney Disease and Leprosy

ESRD has been reported as cause of death in patients with leprosy [36]. ESRD in leprosy has been associated with amyloidosis, more often accompanying virchowian form leprosy. Figure 12.6 depicts amyloid kidney disease in leprosy [33]. Amyloid has been detected in as short an

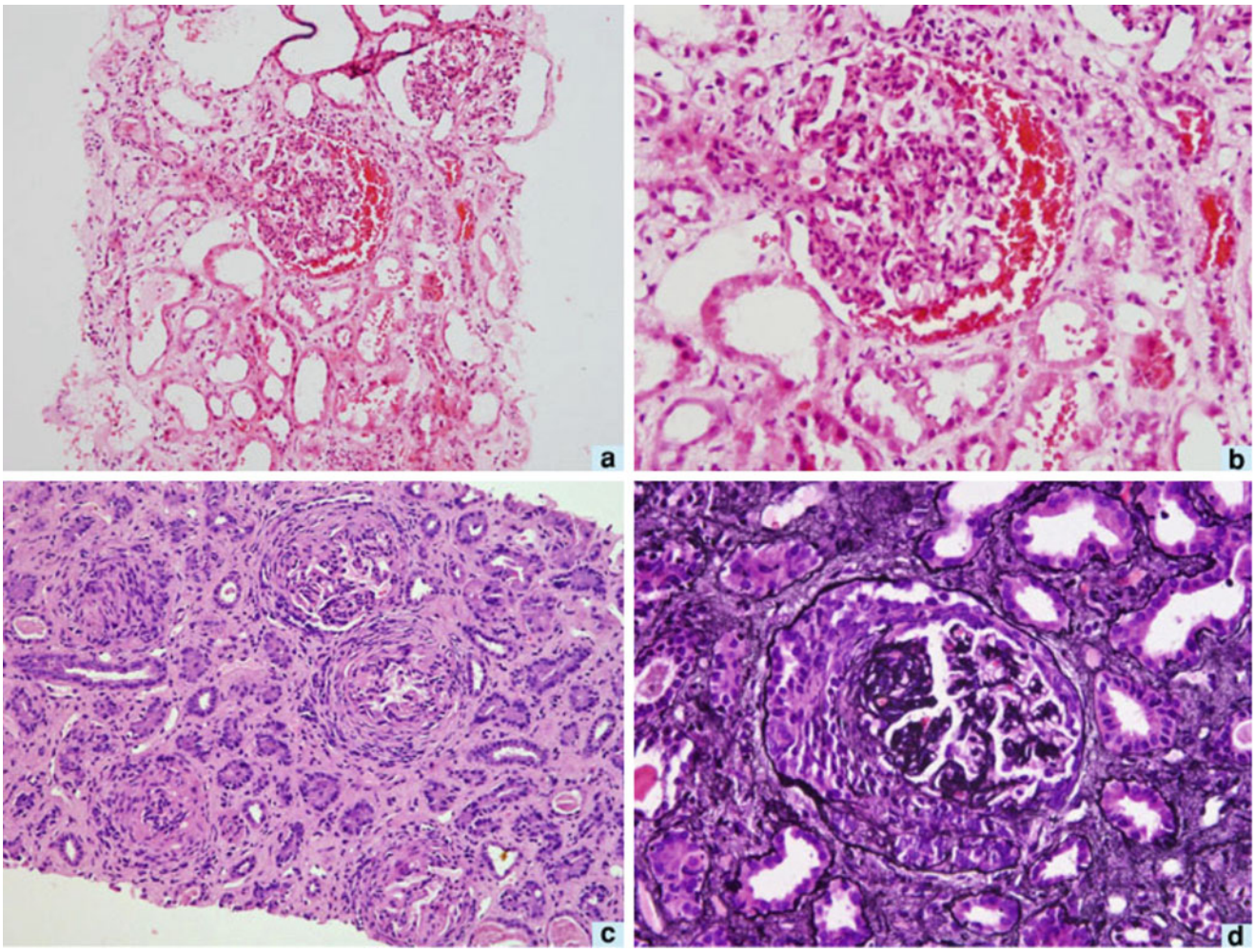

Fig. 12.7 Kidney biopsies from the same patient with leprosy. Composite panel of photomicrographs from first and second biopsies. The first biopsy shows hypercellular glomerulus with hemorrhage in Bowman's space, interstitial edema, red cell casts in the tubules, and features of acute tubular necrosis (**a**; H&E, ×100). The glomerulus shows endocapillary cell proliferation with focal neutrophil infiltration (**b**; H&E, ×200). The second biopsy shows crescent formation in several glomeruli with tubular atrophy and interstitial fibrosis (**c**; H&E, ×40). Silver-methenamine stain shows a compressed glomerular tuft and overlying cellular crescent (**d**; ×200). From Sharma et al. [34]; used with permission

evolution period as 2 years, suggesting that a long disease course may not be necessary for its development [38]. Elevated serum amyloid A levels have been shown during episodes of immunological complications, remaining elevated for several months [38]. In India, where leprosy prevalence is high, almost 50 % of patients have some renal abnormality, yet ESRD has seldom been cause of death [38].

Drug-Associated Renal Changes

Despite renal changes associated with drugs used in leprosy therapy being unusual, acute kidney injury (AKI) described as acute tubular necrosis, acute interstitial nephritis, or papillary necrosis have been reported [28]. Both rifampicin (intermittently, in high doses) and dapsone have been implicated in interstitial nephritis and intravascular hemolysis with AKI [29].

Treatment

Leprosy treatment encompasses specific therapy to overturn *M. leprae*, avoid immunological complications, and prevent physical deformities, simultaneously promoting physical and psychosocial rehabilitation. Additionally, health authority notification is mandatory [13]. WHO-standardized leprosy therapy includes rifampicin, dapsone, and clofazimine. Prednisone (1–2 mg/kg/day) and nonsteroidal anti-inflammatory drugs (NSAI) may be used to

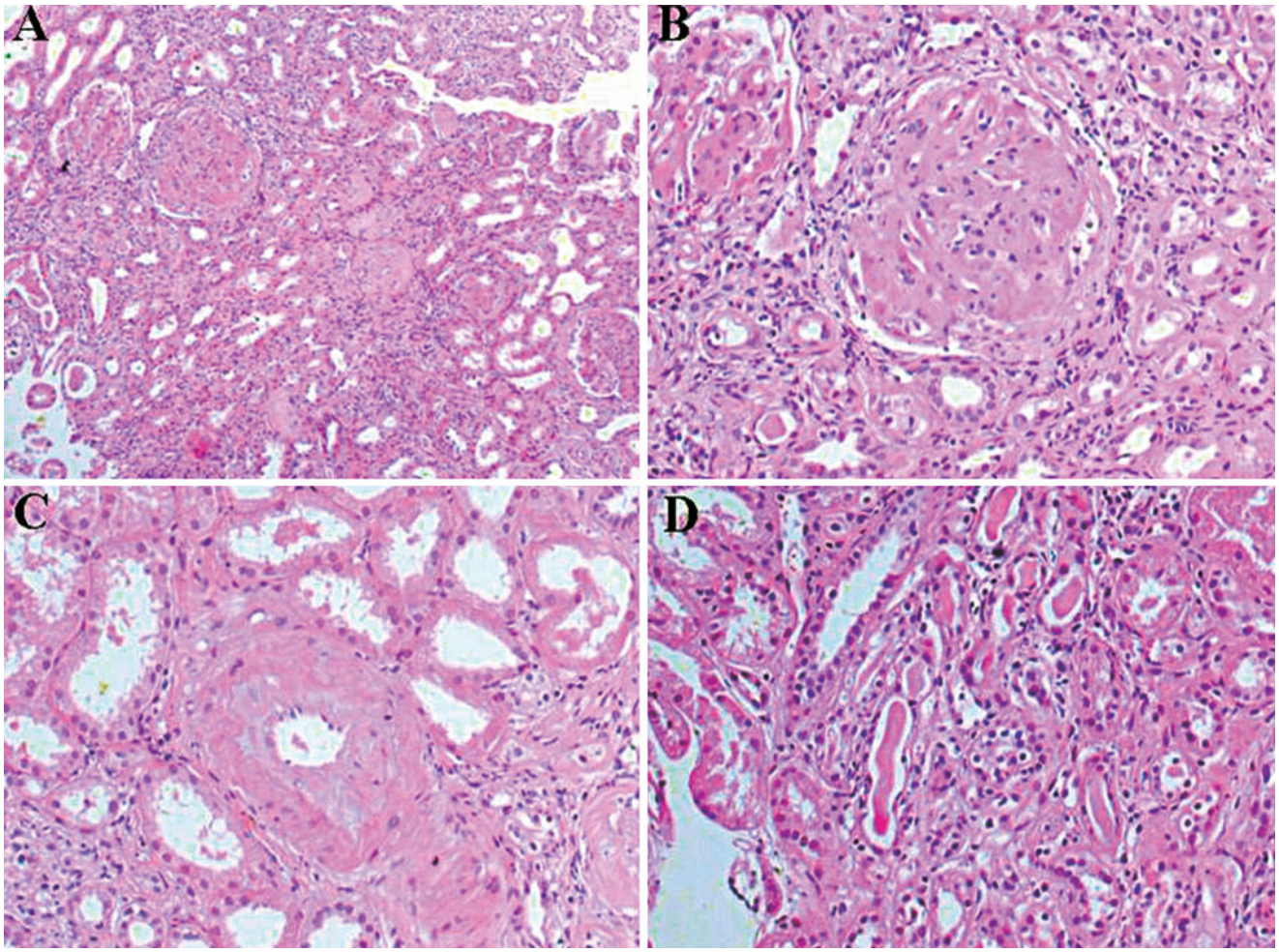

Fig. 12.8 Kidney biopsy from a patient with leprosy and chronic kidney disease showing amyloid deposits, H&E, ×200 (**a**); glomeruli without mesangial proliferation, with amyloid deposit in mesangium, H&E, ×400 (**b**); amyloid deposit, H&E ×200 (**c**); tubules without abnormalities, H&E ×200 (**d**). From Silva Junior et al. [37]; used with permission

control acute immunological episodes. Erythema nodosum leprosum (ENL) may sometimes have a protracted course (months or years) and is usually treated with NSAIDs, steroids, thalidomide, clofazimine, and pentoxifyline. Management of ENL can be handled with corticosteroids, or corticosteroids + clofazimine. The ideal corticosteroid dose has not been established, but should not exceed 1 mg/kg body weight, during no more than 12 weeks. Addition of clofazimine (100 mg thrice daily, for a maximum of 12 weeks) to corticoid is indicated when severe ENL does not respond to corticosteroid therapy. Analgesics can be used to control fever and pain. Multidrug therapy for leprosy should be continued [13].

It must be kept in mind that all are potentially nephrotoxic. Hemodialysis or kidney transplant are alternatives in treating leprosy ESRD. Posttransplant immunosuppression apparently does not modify leprosy response to drugs. Leprosy is rare in kidney transplant patients, being seldom reported from endemic areas. Treatment is not different from non-transplant patients and immunosuppressive therapy should not be discontinued [39].

Systemic Histoplasmosis

Systemic histoplasmosis is an infectious disease caused by *Histoplasma capsulatum*—a dimorphic fungus [40, 41]. Disease transmission occurs by fungus inhalation [40]. The illness has worldwide dissemination, having been found in more than 60 countries, in almost every tropical region [40, 41]. It is highly prevalent in the Americas and Africa, being endemic in certain areas of the USA, Argentina, and Brazil. Over 500,000 subclinical infectious instances have been estimated to occur yearly. However, only one among 2,000 or 5,000 instances results in severe clinical histoplasmosis [41].

Clinical Presentation

Histoplasmosis may present a variety of symptoms, ranging from an asymptomatic illness in immunocompetent individuals (90–95 % of instances) to disseminated histoplasmosis affecting several organs or systems [42]. Acute histoplasmosis is a self-limited disease, fever, shivering, headache, and cough being more often present. Disseminated histoplasmosis is more frequently seen in immunocompromised individuals, especially in AIDS patients [42]. A positive intradermal histoplasmin reaction is, many times, the only clue to the diagnosis. In up to one-third of instances, calcified spots in the lungs or in reticuloendothelial organs can be detected. Acute pulmonary, chronic pulmonary, and skin or disseminated histoplasmosis are the more frequent disease presentations, the last one specially affecting immunocompromised individuals [41]. Infants, patients undergoing leukemia or lymphoma therapy, and HIV-positive individuals are particularly vulnerable (22–85 % affected) [40, 41]. Disseminated tuberculosis, leukemia, and lymphoma should be considered for differential diagnosis. Symptoms usually include fever, wasting, diarrhea, vomiting, palpable liver and spleen, augmented lymph nodes, pulmonary infiltrates, and blood changes (anemia, leucopenia, and low platelets count) [40–42]. Skin lesions—papules with necrotic center, covered by bloody crusts—may appear. Other skin lesions may occur, such as petechiae, macules, pruriginous and squamous papules, nodules, pustules, ulcers, and vegetating lesions [41]. In 164 HIV-positive patients with disseminated histoplasmosis, fever (95 %), cough (76 %), wasting (73 %), diarrhea (61 %), weakness (56 %), anorexia (48 %), and vomiting (39 %) were the main complaints [43].

Diagnosis and Laboratory Findings

Histoplasma Identification

Diagnosis of histoplasmosis is backed by the finding of positive antigens or antibodies against the parasite, by direct fungus staining on fluids or tissues or fungus culture [41, 42]. In the acute pulmonary disease presentation, sputum or bronchoalveolar lavage yields the best materials where to look for fungus occurrence. Yet those turn not to be the best diagnostic sources in the chronic disease presentation form—lung biopsy must be usually performed to clarify the clinical picture [41]. Multiple organs are compromised in disseminated histoplasmosis. Peripheral blood, bone marrow aspirate, or some other organ biopsy may be used in search of fungus. *H. capsulatum* has been isolated in AIDS patients in up to 70 % by blood cultures, using a cell lysing technique (DuPont Isolator) [40, 41]. Additionally, bone marrow cultures have yielded positive results in excess of 60 % [40]. Tzanck's cell diagnostic test on scuffed material from molluscum-like skin lesions allows the causal agent direct microscopic identification [40]. *H. capsulatum* is a slow-growing organism: in vitro colonies take up 3 weeks to mature, sometimes up to 2 months [41].

Histoplasmin Skin Test

Histoplasmin is an antigen obtained from cultures of *Histoplasma capsulatum* that has diagnostic usefulness similar to that of tuberculin when tested by intradermal injection. The histoplasmin skin test has been used in epidemiological surveys performed in endemic areas with apparently good clinical correlation [41].

Other Laboratory Tests

In endemic areas, a significantly increased lactic dehydrogenase (LDH) serum level (usually ≥1,000 UI/l) is highly suggestive of the so-called histoplasmosis *endofagocytic syndrome* [43]. Abnormally high creatinine, urea, bilirubin, alkaline phosphatase, LDH, AST, and ALT serum levels, accompanied by low protein and albumin serum levels and hematological indexes, have been more often found in AIDS patients with histoplasmosis, compared with those without histoplasmosis—viral loads were higher and CD4 levels lower [43].

Kidney Involvement and Pathophysiology

Previous studies have demonstrated that different fungus species, e.g., *Candida*, *Aspergillus*, *Cryptococcus*, *Histoplasma,* and some species from the *Mucorales* genus, may affect the kidney, producing symptoms that may range from minor urine sediment abnormalities to advanced kidney disease and death [44]. Systemic fungus infections have become particularly significant, since the number of surviving immunocompromised patients has substantially expanded. Nephrotic range proteinuria has been demonstrated in HIV-positive patients having disseminated histoplasmosis. Mesangial immune deposits including histoplasma antigens have been shown in kidney biopsies [45]. Antifungal therapy (itraconazol) significantly reduced proteinuria [45].

Histopathology

Granulomatous interstitial nephritis has been occasionally reported. In a case report, by Ahuja et al., an HIV-positive patient with histoplasmosis presented with hematuria, mild proteinuria (365 mg/day), and granular casts. Lymphocytes, monocytes, and plasma cells interstitial infiltrates, non-caseous granulomas, mild interstitial fibrosis and tubular atrophy, and *H. capsulatum* identification were the main

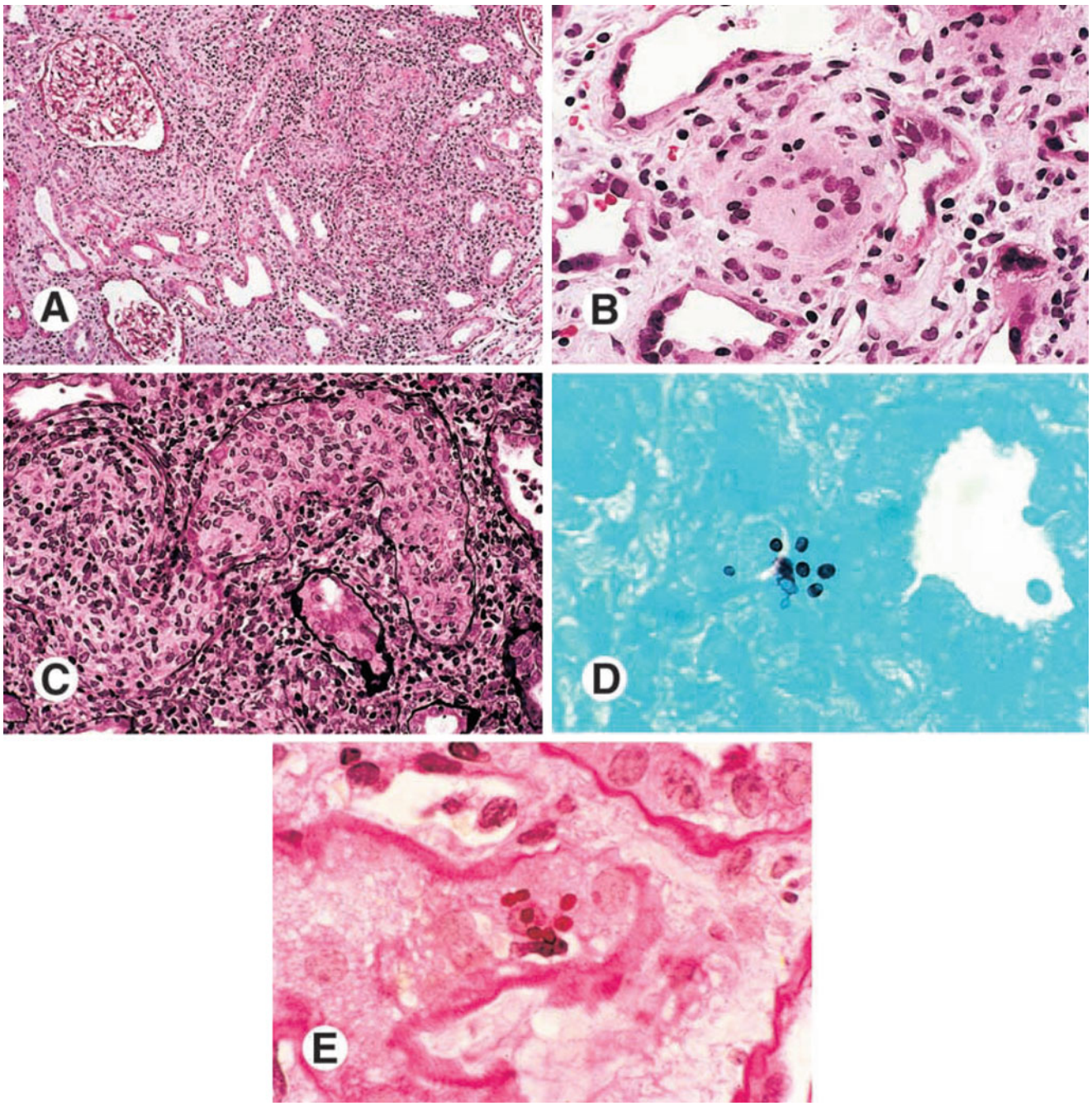

Fig. 12.9 Histopathological findings of a kidney biopsy from a patient with histoplasmosis showing (**a**) interstitial infiltrate and tubulitis (PAS, ×100), (**b**) Langhans giant cells in the interstitium (H&E, ×400), (**c**) obstruction of tubular lumen by a granuloma (Methenamine silver, ×250), (**d**) ovoid structures with 2–4 mm in the interstitium and tubular epithelium (Giemsa, ×1,000), (**e**) organisms stained by PAS, forming small groups in the peritubular interstitium (×1,000). From Nasr et al. [47]; used with permission

changes on biopsy [46]. Granulomatous interstitial nephritis and glomerular capillary fibrosis, with identified *Histoplasma* corpuscles, have been reported [47, 48]. Figures 12.9, 12.10, 12.11, 12.12, 12.13, and 12.14 show renal biopsy features in the setting of disseminated histoplasmosis [47–52], and Table 12.3 depicts kidney changes associated with the disease.

Evolution and Treatment

Amphotericin B has been the customary treatment for disseminated histoplasmosis, yet itraconazol has been successfully employed too [41]. AIDS or immunosuppressed individuals should be kept under strict control to avoid disease reactivation, in which case itraconazol or ketoconazole

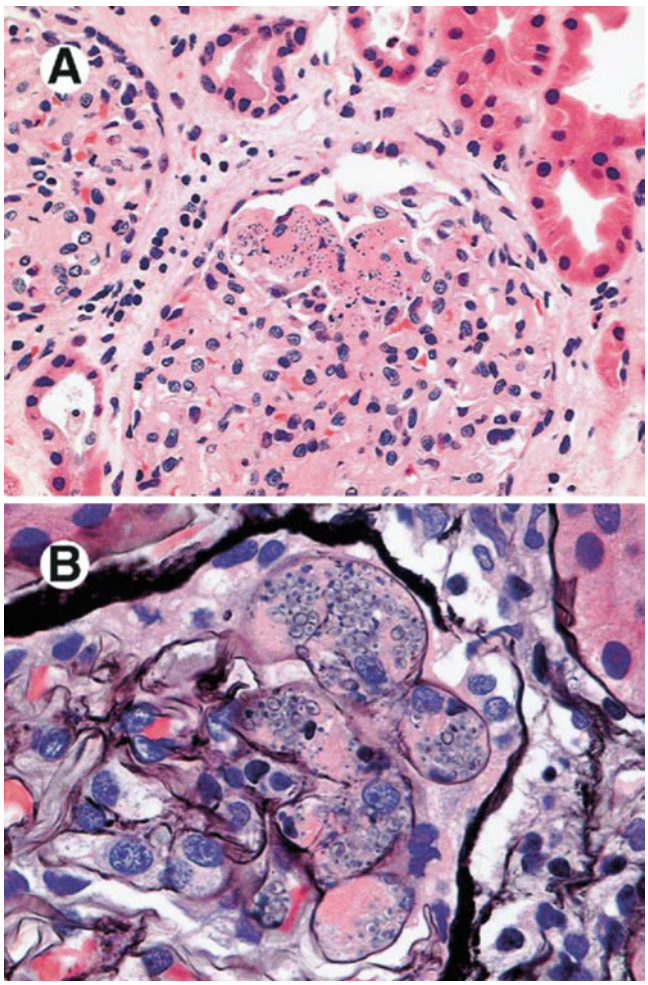

Fig. 12.10 Renal biopsy of a transplanted patient with histoplasmosis showing fibrosis on glomerular capillary vessels (**a**, H&E) and microorganisms with characteristics of fungus near glomerular capillaries (**b**, Silver). From Sethi [48]; used with permission

may be used [41]. Mortality may be as high as 90 % in immunosuppressed individuals—kidney involvement has been associated with poorer outcomes [41]. AKI is an especially dismal event in the disease course [43].

Malaria

Malaria is the global most prevalent infectious disease and, consequently, of extreme epidemiological concern. Its infective agents are *Plasmodium* genus protozoae. Four different species have been associated with human disease: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae* [7]. Kidney disease is more frequently associated with infection by *P. falciparum* and

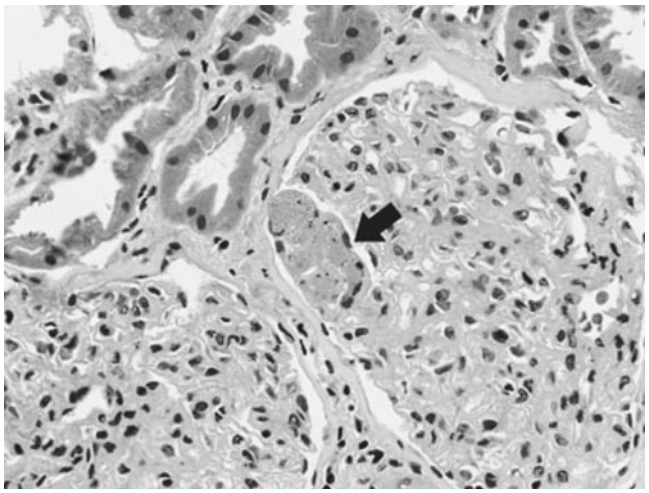

Fig. 12.12 Renal biopsy of a transplanted patient with histoplasmosis showing acute tubular necrosis with microthrombi in the capillaries (*arrow*). From Dwyre et al. [50]; used with permission

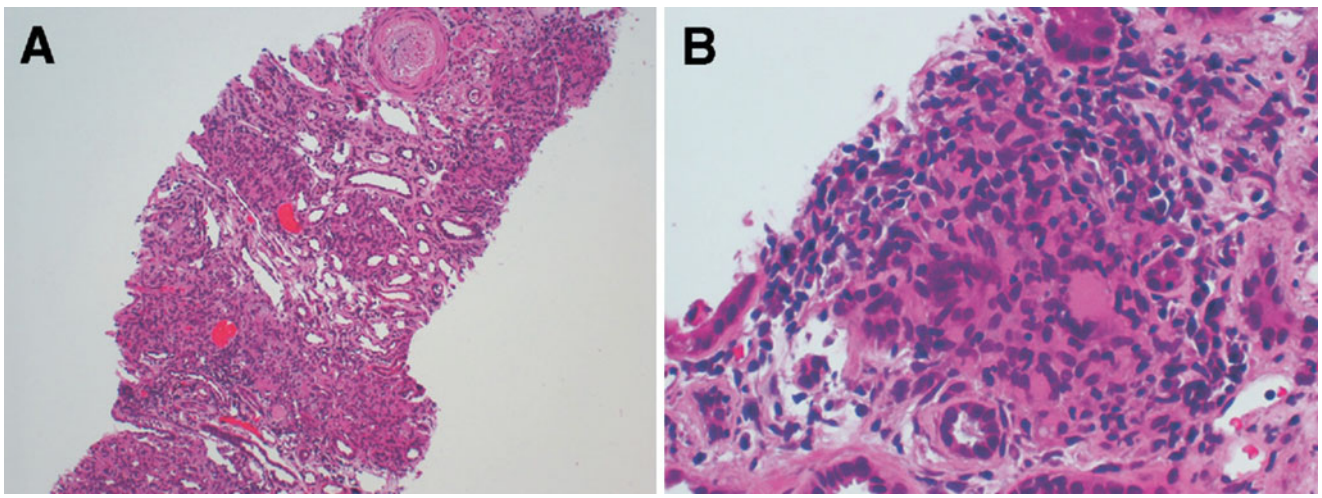

Fig. 12.11 Renal biopsy of a patient with histoplasmosis showing interstitial infiltrate (**a**) and occasional giant foreign bodies (**b**). From Adams and Cook [49]; used with permission

P. malariae [7]. *P. falciparum* is highly prevalent in tropical areas, developing progressively increased drug resistance. *P. falciparum* infection may be accompanied by AKI in 1–4 % of instances. Additionally, immune-mediated glomerular lesions have been associated with *P. falciparum* infection.

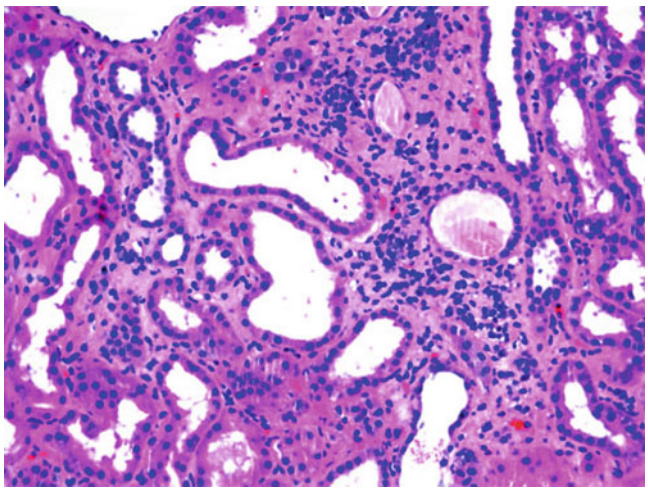

Fig. 12.13 Renal biopsy of a patient with HIV and histoplasmosis showing interstitial inflammatory infiltrate, H&E, ×100. From Bani-Hani et al. [51]; used with permission

Malaria is an endemic and widespread infectious disease in Asia, Africa, and the Americas. According to WHO surveys, its incidence varies between 0.3 and 0.5 billion new cases/year, leading to between 1.5 and 2.7 million deaths, especially among children below age 5. In Brazil, 97 % of diagnosed patients concentrate in the Amazon rural areas [13].

Clinical Presentation

Renal clinical syndromes associated with malaria are summarized in Table 12.4. Malaria transmission usually occurs when parasite sporozoite forms enter human host blood

Table 12.3 Clinical syndromes in histoplasmosis-associated kidney disease

Clinical presentation	Kidney biopsy
Diarrhea, dyspnea, fever, weakness, wasting, nausea, cough, dysphagia, abdominal pain, swollen lymph nodes, nephritic syndrome, edema, AKI	Histoplasma identification, membranoproliferative lesion, interstitial infiltrate, interstitial fibrosis, histiocytic infiltrate, tubular atrophy, granulomatous interstitial nephritis, acute tubular necrosis, thrombotic microangiopathy

AKI acute kidney injury

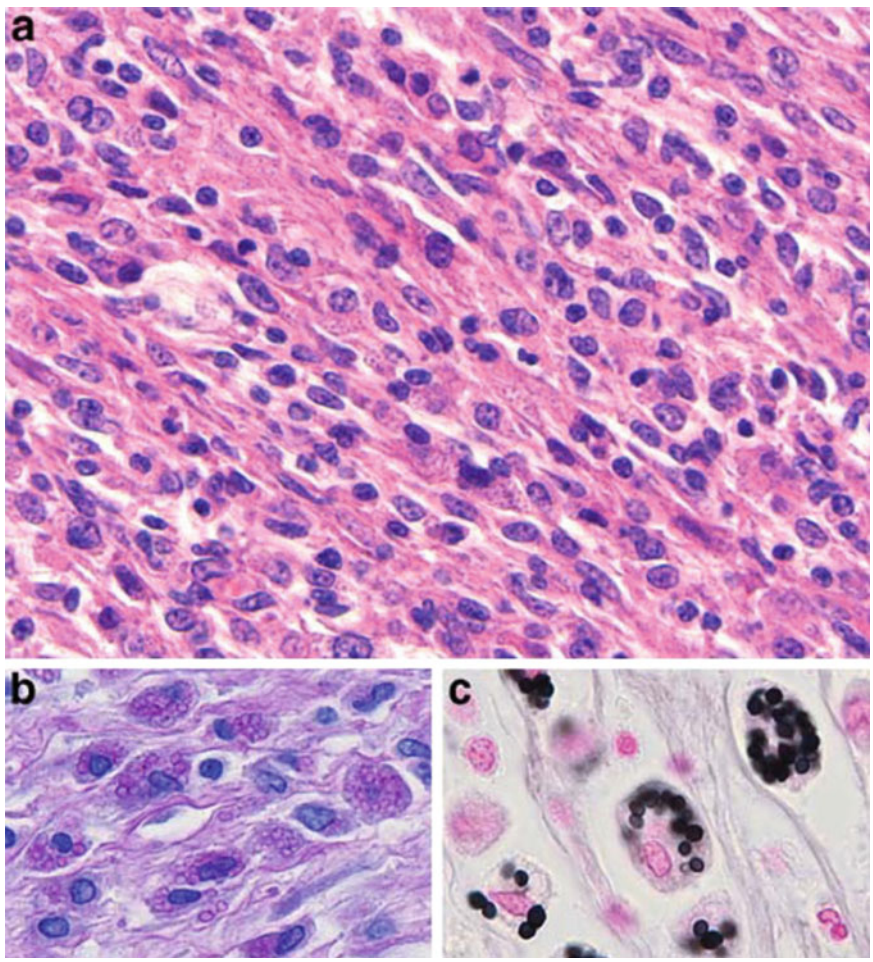

Fig. 12.14 Renal biopsy of a patient with idiopathic immunodeficiency showing histiocytic infiltrate, associated with lymphocytes (**a**), cytoplasm showing granular aspect caused by numerous intracellular organisms compatible with *Histoplasma*, PAS (**b**) and Grocott (**c**). From den Bakker et al. [52]; used with permission

Table 12.4 Histological findings in *P. falciparum* kidney disease

Structure	Pathology	Clinical presentation	Pathogenesis
Glomeruli	OM: light mononuclear infiltrate, prominent mesangial proliferation, mesangial matrix expansion, parasite-loaded red blood cell in glomerular capillaries IF: IgM and C3 mesangial and capillary wall granular deposits, glomerular endothelial cell, and medullar capillary malarial antigens detection EM: subendothelial electron-dense deposits, amorphous, fibrillar, or granular mesangial deposits	Mild proteinuria, hematuria, and hyaline casts No progression to kidney failure; remission with specific therapy Nephrotic and nephritic syndrome: rare Hypertension: seldom	Immune complex mediated
Tubules and interstitium	Patients with uncomplicated *F. malariae* disease presenting mild proteinuria: no tubular or interstitial lesion Patients with AKI: tubular cells with vacuolization or "bald tubules," hemosiderin casts, interstitial edema, mild to moderate interstitial mononuclear infiltrate	AKI in 1 % to 4 %; above 60 % in severe disease. Associated with intense parasite blood load or intravascular hemolysis (with or without G-6PD deficiency). Usually between 4 and 7 days after starting fever Oliguria (days to weeks) and increased catabolism (BUN/Cr > 15), severe hyperkalemia, hyperuricemia, and jaundice	Kidney ischemia induced by parasite-loaded red blood cells, cytokines, and acute phase inflammatory response factors
Vessels	No significant changes. Parasite-loaded red blood cells in peritubular capillaries and venules		

OM optical microscopy, *IF* immunofluorescence, *EM* electron microscopy, *BUN* blood urea nitrogen, *Cr* creatinine

through the bite of an infected female Anopheles genus mosquito; it can also occur through contact with infected blood, or transmitted from a mother to her fetus before or during delivery. After several of the parasite's evolution stages, tissue schizont and merozoite forms make appearance, infest, and multiply inside red blood cells causing their bursting. Mean incubation time for *P. falciparum* is around 12 days, for *P. vivax* is 14 days, and up to 30 days for *P. malariae*. Malaria may follow an acute—sometimes vicious—or a chronic course. Weakness, anorexia, myalgia, headache, nausea, and vomiting are frequent presentation symptoms, besides fever, chills, and sweating, that may recur daily or up to every fourth day. Anemia, with enlarged liver and spleen, soon turns up. In malaria fulminans, caused by *P. falciparum* infection, patients develop anemia, weakness, diarrhea, jaundice, coagulation defect, AKI, acute respiratory failure, and coma, accompanied by severe electrolyte disturbances. Infection by either *P. ovale* or *P. vivax* may undergo reactivation, once quiescent hypnozoite forms harbored in the liver appear. Wasting, fever, anemia, liver, and spleen enlargement follows. Jaundice almost always accompanies malaria with AKI. Nephritic or nephrotic syndrome may be the clinical depiction. However, differently from kidney involvement in *P. malariae* infection, *P. falciparum* glomerular lesions disappear between 2 and 6 weeks from parasites eradication [53].

Diagnosis

Malaria laboratory diagnosis depends upon demonstration of the parasite in blood. However, several immunological tests are currently available and useful.

Kidney Pathology and Pathophysiology

Malaria was the first parasitic infection associated with kidney involvement in tropical areas [54]. Advanced malaria may affect every kidney structure. *P. malariae*-associated rapidly progressive glomerulonephritis has been demonstrated in children of endemic areas [54]. Microscopic hematuria may occur in older patients, as well as proteinuria ranging from mild up to full-blown nephrotic syndrome; hypertension is usually a late symptom. Disease progression occurs despite parasites eradication, ESRD arising in a 3- to 5-year period [54].

Independently of age, *P. falciparum* glomerular lesions are quite uncommon in adult patients, yet less so in children [55]. Incidence of glomerular involvement in *P. falciparum* is uncertain (18 % previously reported); microalbuminuria, mild to moderate proteinuria, hyaline, and cellular casts were reported in 20–50 % of all affected individuals [55]. Nephrotic syndrome has been seldom detected. However, AKI is a frequent malaria kidney involvement presentation. Acute tubular necrosis with blood casts, diffuse interstitial infiltrates, and edema microscopically characterizes kidney lesions [54]. Tissue damage progression to AKI is complex and possibly includes mechanical and immunological factors interaction—cytokines and acute phase inflammatory response factors [54]. *P. malariae* has been associated with glomerular lesions more frequently than other species. Proteinuria has been found in 46 % of patients harboring *P. malariae*, occasionally accompanied by microscopic hematuria [55]. Complement is not usually depressed. On electron microscopy, immune deposits may be seen in association with membranoproliferative glomerular lesions [55]. Nephrotic syndrome may appear several weeks after the

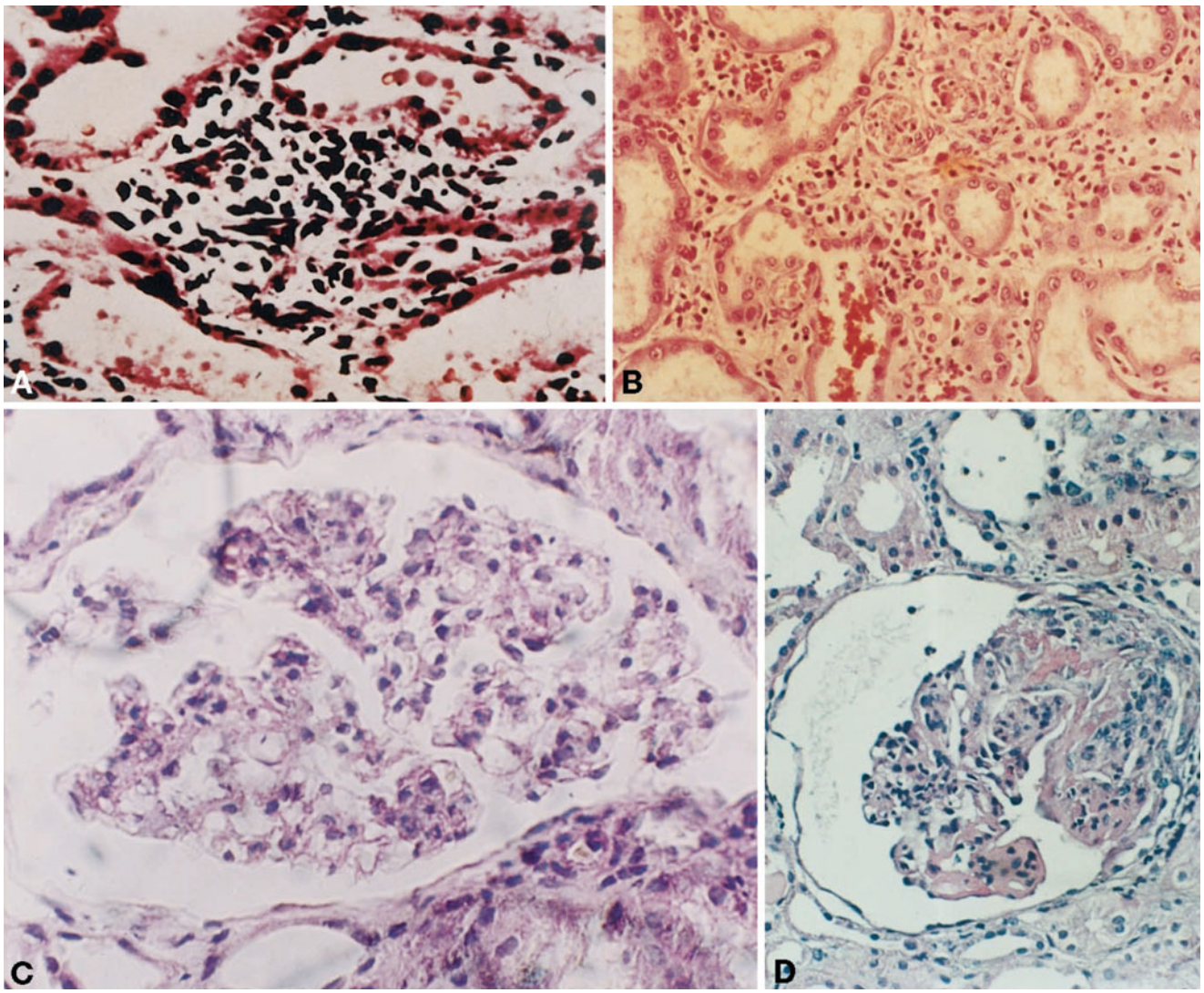

Fig. 12.15 Renal lesion associated with malaria. (**a**) Acute tubular necrosis, rupture of tubular cells, erythrocytes into the tubular lumen, interstitial edema, and inflammatory infiltrate; (**b**) acute interstitial nephritis; (**c**) proliferative glomerulonephritis; and (**d**) necrotizing segmental glomerulonephritis. From Barsoum [55]; used with permission

infection start [55]. The disease clinical course may evolve in two ways: (1) a benign course, with mild and transitory proteinuria with no loss of renal function, appearing during the second or third infection week and (2) a more severe clinical presentation, with persistently moderate to heavy proteinuria, eventually as nephrotic syndrome. Figures 12.15, 12.16, 12.17, and 12.18 depict pathology changes in malaria kidney involvement [55–57].

Malaria kidney injury is primarily owed to red blood cell changes, as well as Th1- and Th2-cell activation [54]. It has been proposed that preferential Th2-cell stimulation leads to complement cascade activation, glomerular immune deposits, and glomerular injury. Otherwise, parasitic proliferation making red blood cells to massively burst may induce AKI—as seen in *P. falciparum* infections. When Th1-cell activation predominates, acute interstitial nephritis or acute diffuse proliferative lesion may be seen [54]. Several factors may contribute to such outcomes, such as reduced circulating blood volume; generalized vasoconstriction; red blood cells lysis, with hemoglobinuria; immune complex glomerular deposits; microcirculatory dysfunction induced by parasite-modified erythrocytes; and, less often, rhabdomyolysis [53]. Hypergammaglobulinemia has been a frequent finding in malaria infection [53]. Augmented globulins production and immune complex formation and deposition relate to glomerular disease development [54]. Malaria kidney disease mechanism has been examined—TNF-α[alpha], IL-1α[alpha], IL-6, IL-10, as well as macrophage and granulocyte colony-stimulating factors seem to be part of it [54]. Few studies have evaluated glomerular

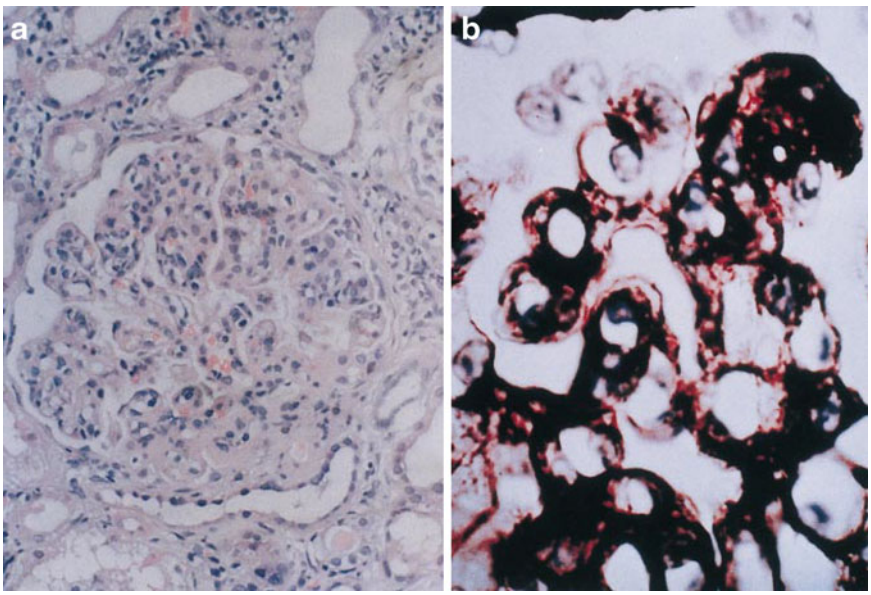

Fig. 12.16 Renal lesions associated with malaria. (**a**) Glomerulus with capillary wall thickening and mesangial hyperplasia (H&E) and (**b**) glomerular basement membrane rupture with subendothelial deposits (Silver). From Barsoum [55]; used with permission

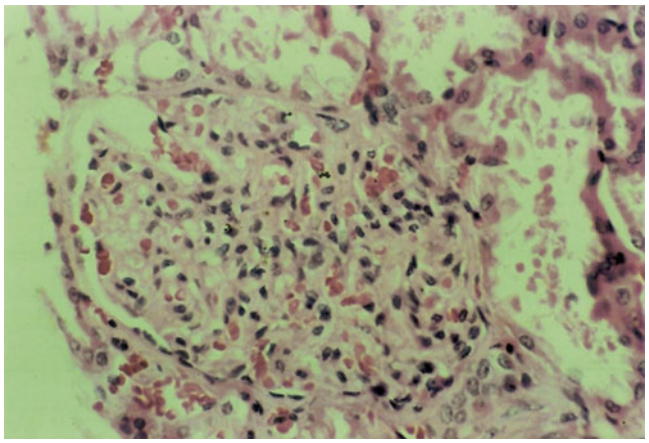

Fig. 12.17 Renal lesions associated with malaria. Glomerulus with mesangial proliferation and basement membrane thickening (PAS). From Mishra and Das [56]; used with permission

involvement in *P. falciparum* malaria. Several histological patterns can be identified, including glomerular lesions, acute tubular necrosis, and interstitial nephritis, either isolated or in association, yet basal membrane modifications have not been demonstrated; blood vessels with parasite-laden erythrocytes have been occasionally spotted [54]. Previous studies have demonstrated glomerular changes in *P. falciparum* infection: conspicuous mesangial cell proliferation and moderate mesangial matrix expansion with occasional basal membrane thickening. Capsular, endothelial, and mesangial granular eosinophilic deposits were also identified. IgM, C3, and parasitic antigens could be demonstrated as immunofluorescent deposits [54]. Subendothelial and mesangial electron-dense deposits, associated with amorphous granular or fibrillar material, were also demonstrated on electronic microscopy [54]. Tubular changes include hemosiderin granular deposits, hemoglobin casts, interstitial edema, and mononuclear cell interstitial infiltrates. Yet interstitial nephritis seems to be the pattern more frequently associated with *P. falciparum* infection, possibly dependent upon host immune responses to parasites. Complement consumption may be seen during the disease's acute phase [55]. In 1–4 % of *P. falciparum-affected* patients, acute tubular necrosis occurs [58]. It usually presents as oliguric AKI, sometimes associated with intravascular hemolysis and coagulation, or rhabdomyolysis [58]. In certain areas of Africa, malaria has been associated with AKI in more than 12 % of reported instances [58]. Membranoproliferative glomerular lesion has also been associated with *P. malariae* infection. On electron microscopy, basal membrane segmental thickening with subendothelial deposits creates a typical double contour image, accompanied by mesangial proliferation. Granular subendothelial IgG3, IgM, C3, and parasite antigens may be identified on immunofluorescence microscopy in up to 25 % of instances; sometimes fine granular IgG2 deposits may also be seen [55]. Eosinophil infiltrates have been demonstrated in rare instances of diffuse proliferative lesions associated with malaria [55]. Occasionally, capsular epithelial crescent formation may be seen, more often so in adults [54]. Progression to glomerular sclerosis may occur. Quite possibly, glomerular immune complex deposits require formation of antigen-antibody combinations involving parasite antigens [54].

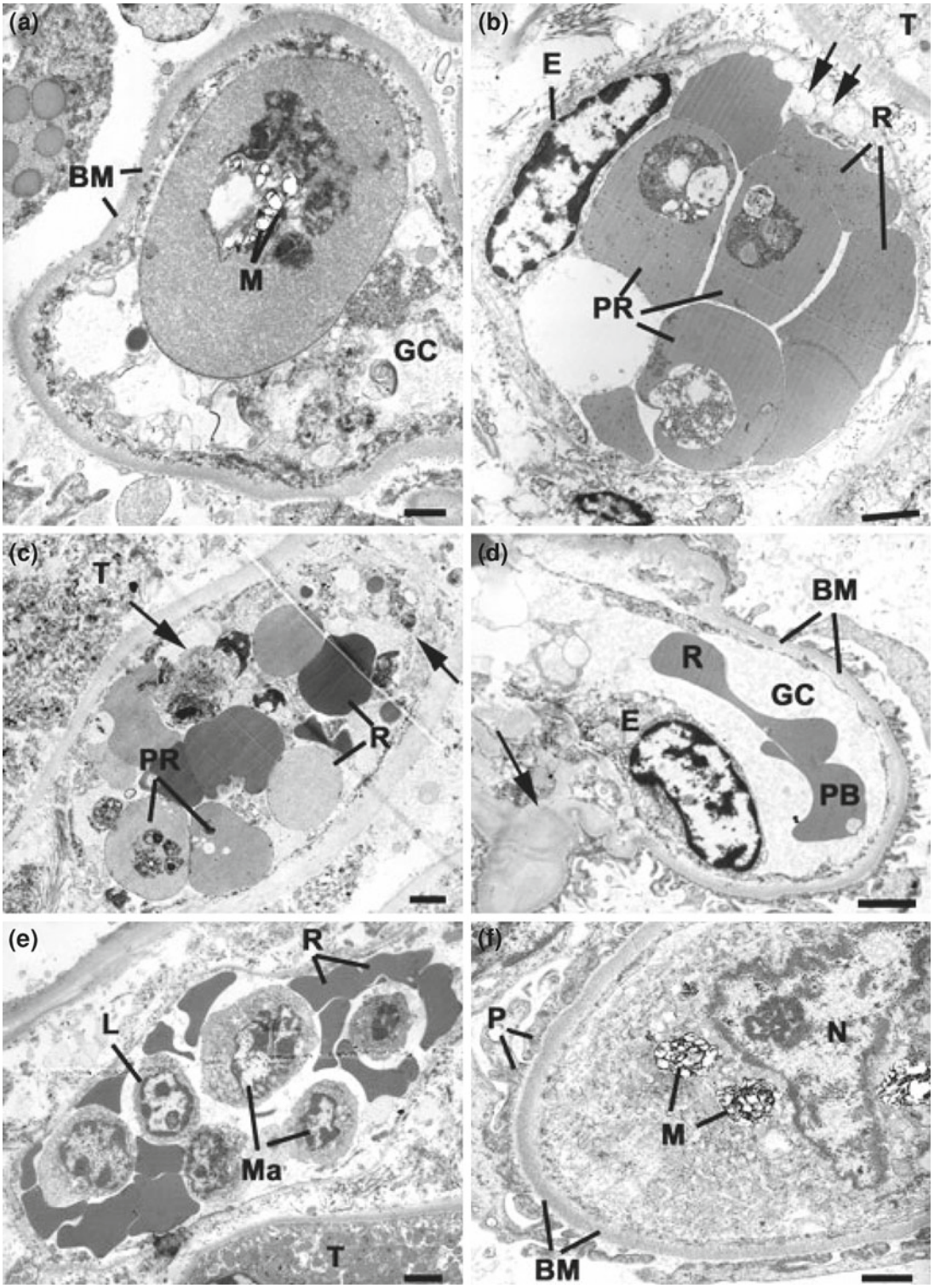

Fig. 12.18 Transmission electron micrographs from the kidney of *P. falciparum*-infected patients. (**a**) Sequestration of a parasitized red blood cell (PRBC) within a glomerular capillary (GC). The glomerular basement membrane (BM) is of normal thickness, with no evidence of immune complex deposition. *M* malarial pigment. Bar = 1 **μ**[mu]m. (**b**) Sequestration of PRBC in a peritubular capillary showing cytoadherence to the endothelial cell (E) and some uninfected red blood cells (R). Mild vacuolation of the endothelial cell cytoplasm is seen (*arrows*). *T* tubule. Bar = 2 **μ**[mu]m. (**c**) Sequestration of PRBC in a peritubular blood vessel showing PRBCs attached to each other and uninfected RBCs (R). Some amorphous and unidentified material is also seen in the vessel lumen (*arrows*). Bar = 2 **μ**[mu]m. (**d**) Endothelial cell hypertrophy (E) within a GC which also contains non-adherent ring stage PRBC (PB) and an uninfected RBC (R). The *arrow* shows mild ischemic wrinkling of the basement membrane (BM). Bar = 2 **μ**[mu]m. (**e**) A peritubular microvessel congested with red blood cells (RBC) and leukocytes including mononuclear monocyte/macrophages (Ma) and lymphocytes (L). *T* tubule. Bar = 2 **μ**[mu]m. (**f**) A monocyte, exhibiting a number of phagolysosomes containing granules of malaria pigment, located in a glomerular capillary is illustrated. Note the normal thickness of the glomerular BM and lack of fusion of podocyte foot processes on the epithelial surface (P). *N* nucleus, *M* malarial pigment. Bar = 1 **μ**[mu]m. From Nguansangian et al. [57]; used with permission

Treatment and Outcome

Chloroquine is the foremost drug used in malaria treatment. However, some strains of *P. falciparum* may be chloroquine resistant. Primaquine, quinine, and mefloquine may be used, isolated, or in association. Early dialysis has been suggested for patients presenting with AKI. As in other situations associated with AKI, outcome depends on the severity of systemic involvement; mortality may be as high as 30 % [53].

Visceral Leishmaniasis (Kala-azar)

Visceral leishmaniasis is a chronic, lethal, parasitic disease. The *Leishmania* parasite is an intracellular protozoon. A large spectrum of clinical manifestations accompanies *Leishmania* assault on reticuloendothelial tissues—liver, spleen, bone marrow, lymph nodes, and digestive system. Symptoms range from irregular and recurrent fever to pancytopenia, hemorrhagic spells, liver, and spleen enlargement [7]. Kidney involvement in chronic leishmaniasis is frequent and associated with increased mortality. It is endemic in southern Europe and in tropical and subtropical areas of the globe, with incidence reaching approximately 0.5 million/year [59]. When untreated, its mortality may reach 95 %. Among the so-called tropical diseases, Kala-azar is one of WHO priorities. Endemic in Brazil, its agent is *Leishmania chagasi*. Humans are infected through the vector insect *Lutzomyia longipalpis* [60].

Kidney Involvement

Patients presenting with chronic Kala-azar may have mild proteinuria, microscopic hematuria, and leukocyturia. Diagnosis of Kala-azar is confirmed by demonstrating the parasite in tissues using Giemsa stain, besides detection of parasite antigen K-19 [7]. Interstitial nephritis with glomerular changes may be seen. A mesangial proliferative lesion is often found, yet a membranoproliferative lesion is not rare [7]. Additionally, amyloid deposits may occur in chronic disease. Yet renal involvement is usually mild and transitory. Loss of kidney function, and urine sediment changes, has been reported in visceral leishmaniasis. Kala-azar prospective studies have demonstrated that hematuria, mild to moderate proteinuria, and increased urine leukocytes appear in over 50 % of instances [61]. A large, retrospective study demonstrated that more than 11 % of patients with chronic *Leishmania* disease has decreased filtration rate at hospital admission—with antiparasitic therapy, changes disappear. Table 12.5 depicts known kidney involvement in Kala-azar. Interestingly, hypoalbuminemia, polyclonal hypergammaglobulinemia, and leucopenia usually occur in chronic leishmaniasis [7].

Table 12.5 Clinical syndromes in visceral leishmaniasis-associated kidney disease

Clinical presentation	Kidney biopsy
AKI, proteinuria, nephritic syndrome, nephrotic syndrome, urinary concentration and acidification defect	Interstitial nephritis diffuse proliferative lesion, collapsing FSGS, necrotizing FSGS, membranoproliferative lesion, AA amyloid glomerular deposits, chronic tubulointerstitial nephritis, arteriolosclerosis, tubular atrophy, interstitial fibrosis, mononuclear infiltrate, mesangial hyperplasia, peritubular Leishmania-loaded histiocytes, moderate to severe lymphocyte, histiocytes, and plasma cells interstitial infiltrates

FSGS focal segmental glomerular sclerosis, *AKI* acute kidney injury

Pathophysiology

Most parasitic diseases evolve into chronic illness, with fluctuation in antigenemia and in host response. Several possible explanations are possible, such as low natural immune response or parasite's ability to evade host immune system attack. It has been demonstrated that development of host resistance is usually dependent upon T-CD4+ cells producing interferon γ[gamma] (IFN-γ[gamma])—a TH1-type cell. However, a mixed TH1 and TH2 response seems to be involved in extracellular parasites eradication [62]. The *Leishmania* is able to manipulate the host immune system by inducing production of growth factor b, a macrophage-inhibiting cytokine, and interleukin-10, besides interfering in IFN-γ[gamma] signaling, all affecting cellular immune response and inducing polyclonal B-cell activation, which has been associated with Kala-azar glomerular disease [62]. Antibodies produced in response to infection may be trapped in glomeruli by different mechanisms, such as immune complexes, in situ development of complexes (antibodies linked to previously implanted glomerular antigens), or directly attached to glomerular antigens. Yet recent studies demonstrated that antibodies alone do not explain the occurrence of proteinuria [62, 63]. Macrophages, granulocytes, and natural killer lymphocytes are all part of host defenses and participate on the genesis of glomerular lesions through an intricate chain of cytokines and inflammatory mediators, as evidenced experimentally [62, 63]. It is possible that reduced tubular concentration and acidification functions are caused by IgG overload of tubular cells, in patients presenting with major globulins plasma level changes [64]. A distal tubule acidification defect may occur.

Histopathology

Mesangial proliferative, membranoproliferative, and collapsing FSGS seem to be the more frequently seen patterns associated with Kala-azar nephropathy, the severity of which

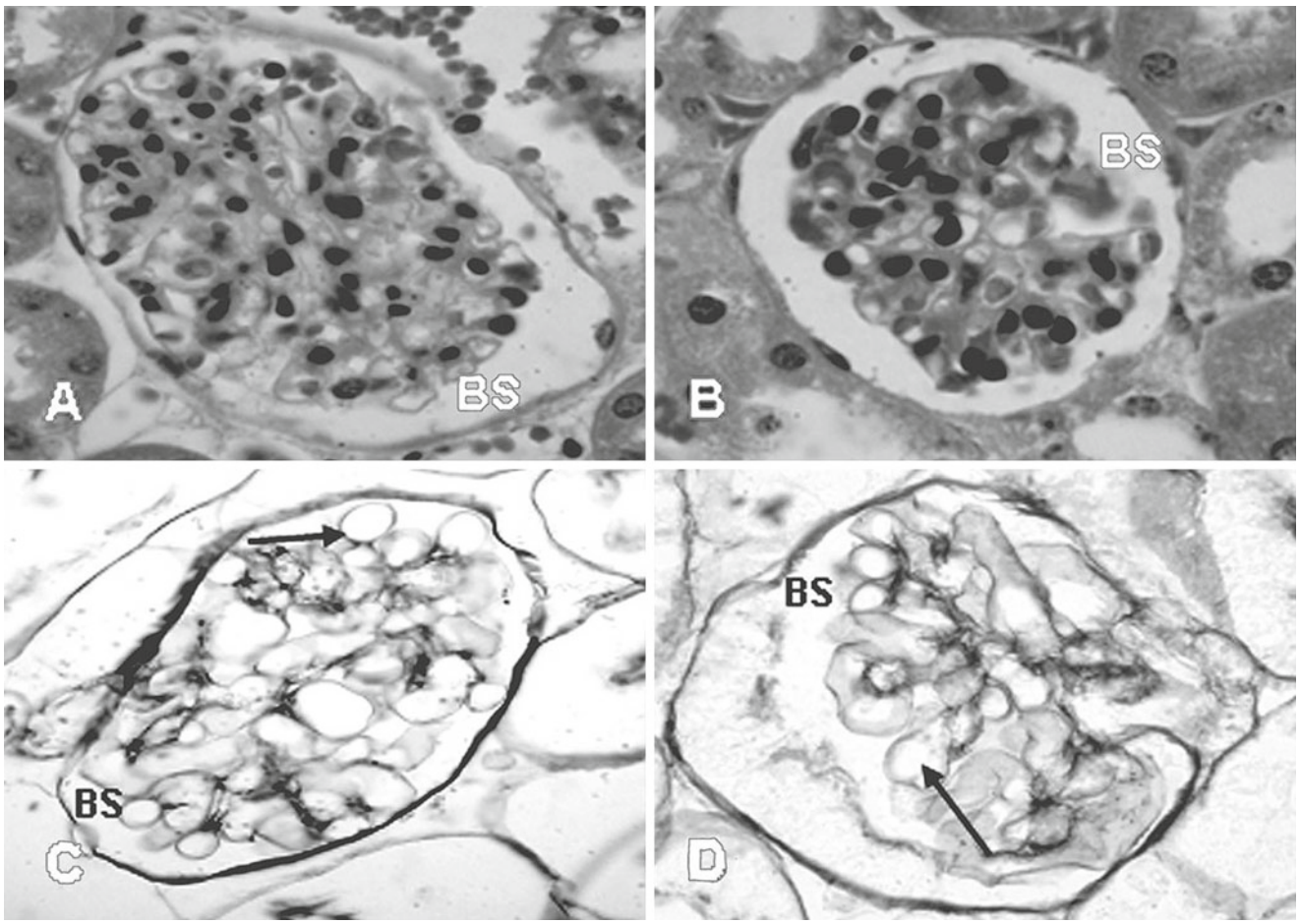

Fig. 12.19 Kidney sections stained with hematoxylin-eosin. (**a**) Glomerulonephritis in BALB/c mice infected with *Leishmania chagasi*. Hypercellularity in infected mice with glomerular tuft occupying almost the whole Bowman's space (BS) (×40). (**b**) Normal cells in glomeruli from control noninfected mice (×20). Kidney stained with periodic acid methenamine silver (×40). (**c**) Glomerulonephritis in BALB/c mice infected with *L. chagasi*. Hypercellularity in glomerulonephritis and normal glomerular capillary wall (*arrow*) in infected mice. (**d**) Normal cellularity in glomeruli of a control noninfected mouse. From Prianti et al. [63]; used with permission

may vary from mononuclear interstitial infiltration to a severe, diffuse, inflammatory infiltrate composed by macrophages, lymphocytes, and plasma cells [65]. On immunofluorescence microscopy, IgG, IgM, IGA, and C3 deposits in the mesangial matrix may be found [65]. Experimentally, tubular and interstitial lesions have been the most frequently Kala-azar-associated kidney lesions. However, amyloid deposits, rapidly progressive glomerulonephritis with the nephrotic syndrome, have been reported in human leishmaniasis [66, 67]. Experimental infection by *L. donovani* may end up with amyloid deposition, following an initially diffuse proliferative glomerular lesion [68]. The finding of *amastigote* forms in the kidney is a rare event, yet it is possible to identify *Leishmania* antigens in inflammatory infiltrates [62]. Histopathological findings in leishmaniasis-associated kidney damage are shown in Figs. 12.19, 12.20, 12.21, 12.22, 12.23, and 12.24 [63, 65, 67–69].

Treatment and Evolution

Pentavalent antimonium compounds are still the drugs of choice in treating visceral leishmaniasis. However, amphotericin B may be equally effective. Usually, the kidney changes disappear soon after infection control.

Schistosomiasis

Schistosomiasis is a parasitic disease produced by parasites of the genus *Schistosoma*. Three main species, *S. mansoni*, *S. japonicum*, and *S. haematobium*, and two other with restricted distribution, *S. mekongi* and *S. intercalatum*, are the causative agents of human disease [7]. The parasite's adult forms infest its final host mesenteric vessels. *S. mansoni* is usually found in South America and the Caribbean,

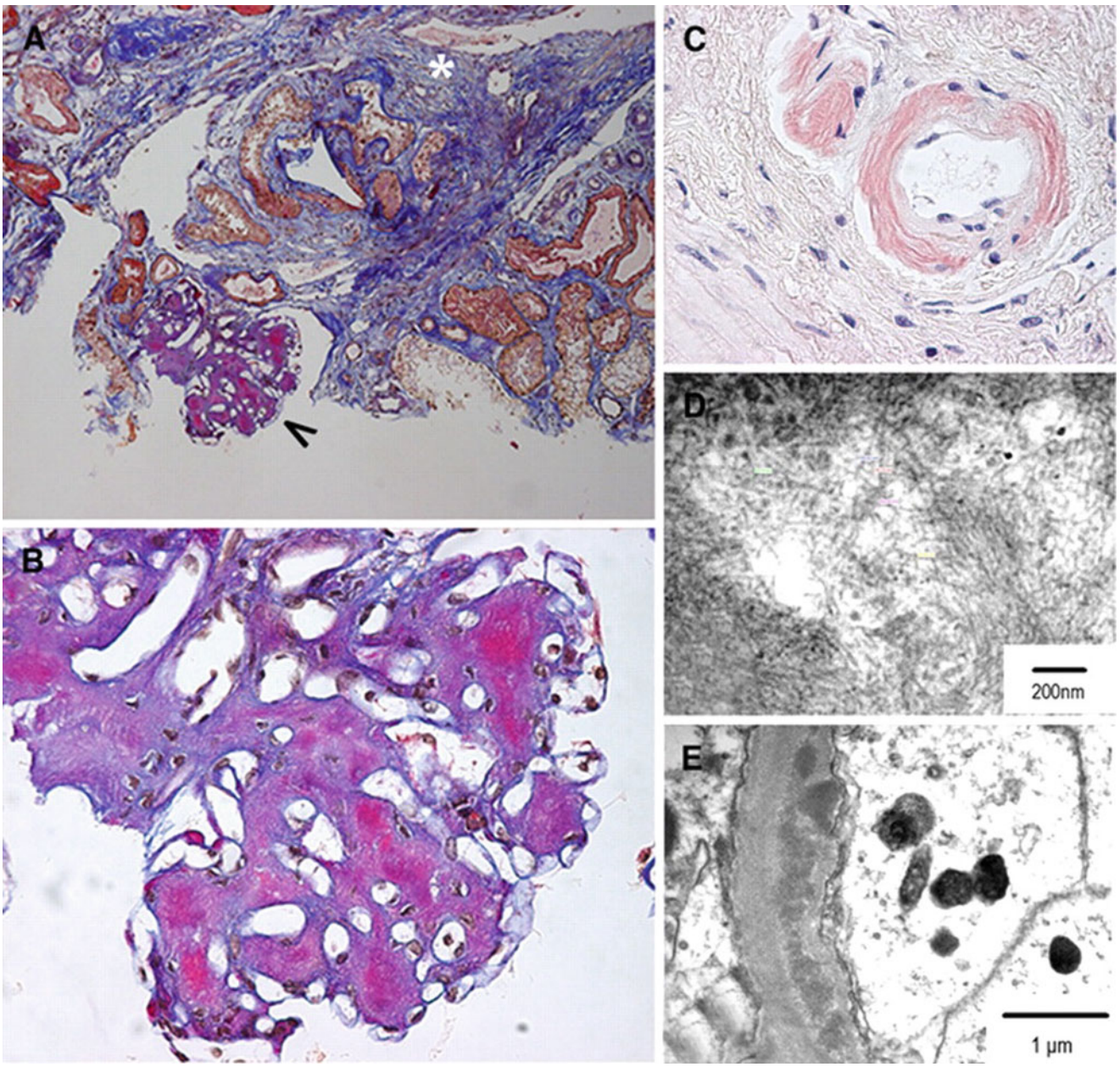

Fig. 12.20 Renal amyloidosis in a patient with visceral leishmaniasis and HIV. (**a**) Abundant mesangial amyloid deposits [*black arrowhead*; enlarged in (**b**)] and interstitial fibrosis (*white asterisk*); FAOG stain; ×100. (**b**) Almost complete obliteration of the glomerular architecture by mesangial amyloid deposits; FAOG stain; ×600. (**c**) Amyloid deposits in arteriolar wall that are congophilic and produce apple green birefringence; Congo red; ×600. (**d**) Typical ultrastructural appearance of amyloid fibrils in mesangium, transmission electron microscopy (uranyl acetate and lead citrate). (**e**) Amyloid fibrils are also seen in capillary membranes in a subendothelial location, transmission electron microscopy (uranyl acetate and lead citrate). From de Vallière et al. [67]; used with permission

S. haematobium in Africa and the Middle East, *S. intercalatum* in several areas of Southeast Asia, and *S. japonicum* in China and the Philippines [7]. The disease has been registered in 74 different countries in tropical areas, especially in Africa, East Mediterranean, and South America. Globally, more than 200 million individuals are infected, 120 million will develop symptoms, 20 million progresses to severe illness, and 100,000 die each year due to schistosomiasis [7].

Clinical Presentation

Schistosomiasis is a variably severe, chronic illness, humans being its ultimate host. The adult parasite occupies liver and spleen vessels, evolution depending upon the host's immune response [13]. Most infected individuals remain asymptomatic. Usually, the disease follows a two-phase course: (1) early infection, *cercariae* skin penetration, characterized by allergic

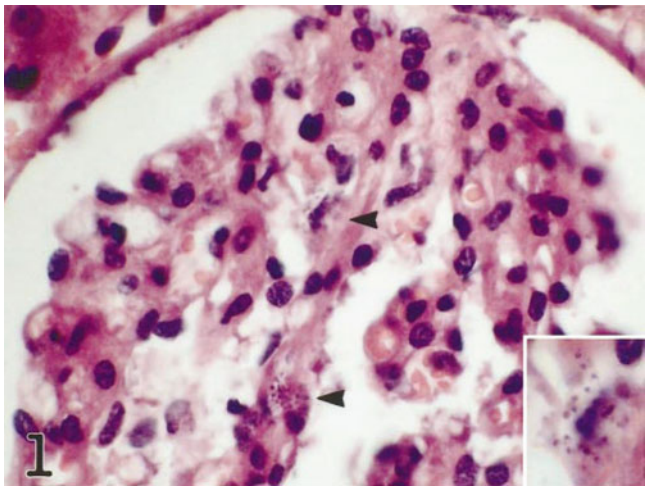

Fig. 12.21 Renal biopsy from a patient with visceral leishmaniasis. Presence of figures typical of *Leishmania* inside mesangial cells (*arrows*). From Kumar et al. [65]; used with permission

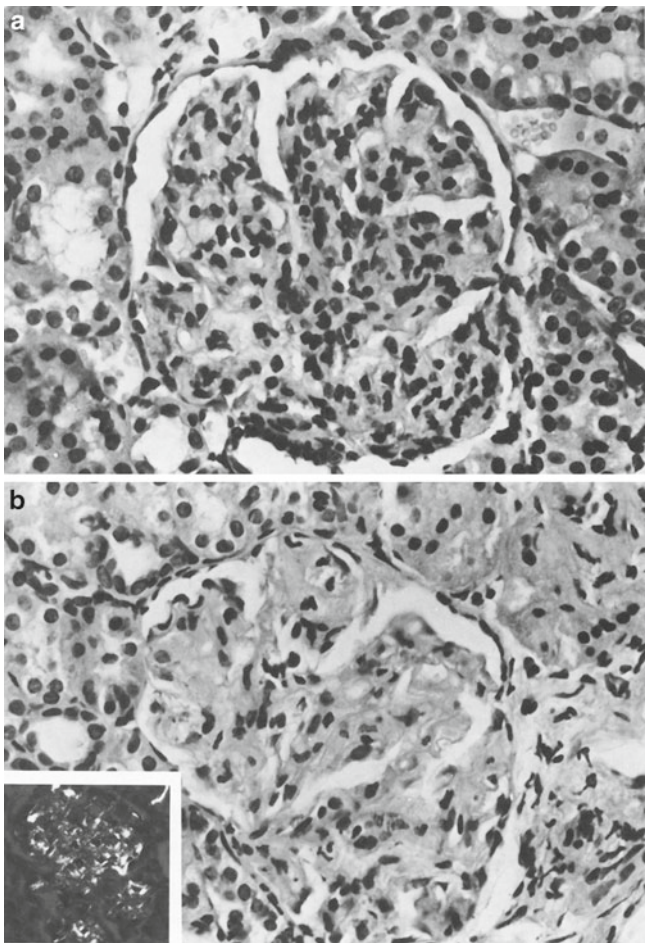

Fig. 12.22 Renal lesions in visceral leishmaniasis. (**a**) Glomerulus of a hamster at day 21 after infection showing marked mesangial hypercellularity. Discrete infiltration of mononuclear cells is also seen. (H&E, ×540); (**b**) Glomerulus of a hamster at day 42 after infection showing deposits in the mesangium and involving capillary loops (Congo red, ×540). Inset birefringence of the deposits after Congo red staining. (Polarized light, ×260). From Oliveira et al. [68]; used with permission

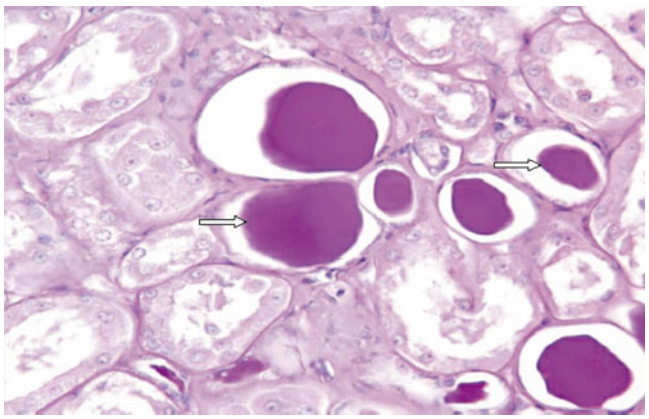

Fig. 12.23 Renal lesion in a dog naturally infected by *Leishmania chagasi*. Presence of hyaline cylinders (*arrow*) in renal tubuli; PAS, ×140. From Gomes et al. [69]; used with permission

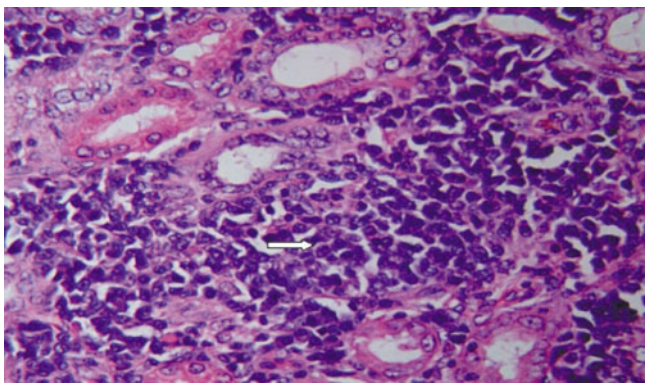

Fig. 12.24 Renal lesion in a dog naturally infected by *Leishmania chagasi*. Severe inflammatory interstitial infiltrate with mononuclear cells (*arrow*); H&E, ×140. From Gomes et al. [69]; used with permission

symptoms, including skin rash, followed by fever, headache, anorexia, abdominal pain, swollen lymph nodes, and eventually nausea, vomiting, diarrhea, and dry cough and (2) late course, usually starting after 6 months and stretching for several years, with pulmonary and portal hypertension, ascites, and esophageal varicosities [13]. Increased blood eosinophils are usually present [7]. *S. japonicum* disease seems to progress faster, leading to severe liver disease in very short time [7].

Kidney Involvement

S. mansoni

Kidney involvement in all forms of schistosomiasis has been estimated to be around 5–6 %, reaching up to 15 % when liver and spleen are compromised [70]. Glomerular lesions have been demonstrated in 10–12 % of autopsy cases. As much as 20 % of *S. mansoni*-infected patients present with proteinuria [71]. Glomerular lesions have been demonstrated in 10–12 % of autopsy cases [71]. Schistosomiasis glomeru-

lopathy may be initially symptomless, evolving into proteinuria and the nephrotic syndrome, or non-nephrotic proteinuria and microscopic hematuria. A small percentage of patients may progress to chronic kidney disease [71]. Kidney biopsy may show basal membrane deposition of immune complexes holding *Schistosoma* antigens [7]. *Schistosoma* ova deposition-associated granulomas have been reported in kidney tissue [72].

Pathophysiology

Pathophysiology of glomerular lesions holds some similarities with malaria. It seems to depend upon development of immune mechanisms. The presence of parasite antigens appears to be related with occurrence of glomerular disease, such finding having been demonstrated in experimental and clinical events of *S. mansoni* infection [73, 74]. *Schistosoma* antigens have been detected in kidney tissues in 44 % of patients with moderate proteinuria and in 63 % of those with the nephrotic syndrome and advanced kidney disease [75]. Circulating immune complexes bearing parasite antigens, as well as *Schistosoma* antigen-containing glomerular deposits, have been reported, strengthening the impression of immune-mediated lesions [76]. Anti-DNA antibodies have been found in *S. japonicum*-infected hamsters, suggesting that development of such antibodies could play a role in B-lymphocytes' activation [77]. Kidney disease unresponsiveness to the parasite's eradication and the lack of correlation between the severity of renal lesions and extent of infection render plausible an autoimmune-related mechanism [78]. Apparently, the level of proteinuria and the severity of kidney disease correlate with the intensity of liver macrophages dysfunction [78]. Finally, adult parasites and ova antigens have been identified in different body fluids during *S. mansoni* infection [70].

Pathology

Mesangial proliferative and membranoproliferative glomerular lesions have been more often seen in patients presenting with liver and spleen schistosomiasis [70]. The mesangium is the glomerular structure usually involved in kidney schistosomiasis. Mesangial matrix expansion accompanied by mesangial cell hypertrophy and hyperplasia, granular dense deposits in subendothelial and mesangial location may be seen [70]. IgM, IgG, C3, and, occasionally, IgA deposits may appear by immunofluorescence examination [70]. Experimentally, mesangial proliferative lesion is the predominant variety of glomerular lesions—*Schistosoma* antigens having been identified even without the accompanying antibodies, suggesting their being embedded in the glomerular structures [70]. Membranous disease is rarely seen, its relationship with schistosomiasis doubted on clinical and experimental data [70]. Amyloid renal disease is rarely seen in association with schistosomiasis and other parasitic diseases [70]. In a recent Brazilian study, 8/63 individuals had abnormal albuminuria. On kidney biopsy, mesangial expansion was evidenced in all, mesangial cell proliferation was visible in five, and basal membrane duplication in four. Focal and segmental glomerular sclerosis appeared in four patients [79]. Electron microscopy revealed subendothelial electron-dense deposits with predominantly IgG and IgM deposition and occasional IgA and C3, along the basal membrane. Additionally, minimal change nephropathy may occur in asymptomatic individuals; yet mesangial proliferation has prevailed [71]. Based on pathology findings, a five-category classification of schistosomiasis nephropathy has been proposed: (1) mesangial proliferative lesion, (2) membranoproliferative nephropathy, (3) FSGS, (4) exudative glomerulitis, and (5) amyloid deposit [80]. Patients presenting with mesangial proliferative lesions are usually asymptomatic or have mild proteinuria and microscopic hematuria, yet may evolve into full nephrotic syndrome and ESRD [81]. Patients displaying membranoproliferative changes usually present with low CH50, C3, and C4 levels, suggesting a classical activation of the complement system. It has been demonstrated that mesangial proliferative lesions may undergo transformation into membranoproliferative lesions [82]. Ruling out Hepatitis B and C virus infection is mandatory. FSGS was the only lesion identified in 11–38 % of infected individuals—lesions not significantly differing from the idiopathic variety [81]. It has been a matter of speculation, kidney lesions being a primary tissue response to *Schistosoma* infection—as in HIV infection—or focal and segmental scarring of prior mesangial proliferative lesions [82]. Other pathological pictures have occasionally been reported [82]. Feature of renal schistosomiasis is shown in Fig. 12.25 [72, 83].

S. haematobium

Bladder lesions are found in *S. haematobium* infection, ova of which may be observed in urine of infected individuals [82]. Microscopic or macroscopic hematuria and low urinary tract symptoms are frequently reported, as bladder inflammation and ulceration may occur sometimes after infection starts [7]. It is not unusual to find microscopic hematuria in otherwise asymptomatic African children. A variety of mucosal and structural lesions may induce ureteral or bladder dysfunction, accompanied by urinary infection and eventual renal failure [7].

Evolution and Treatment

Several previous studies suggest that schistosomiasis kidney lesions may be irreversible, possibly because lately

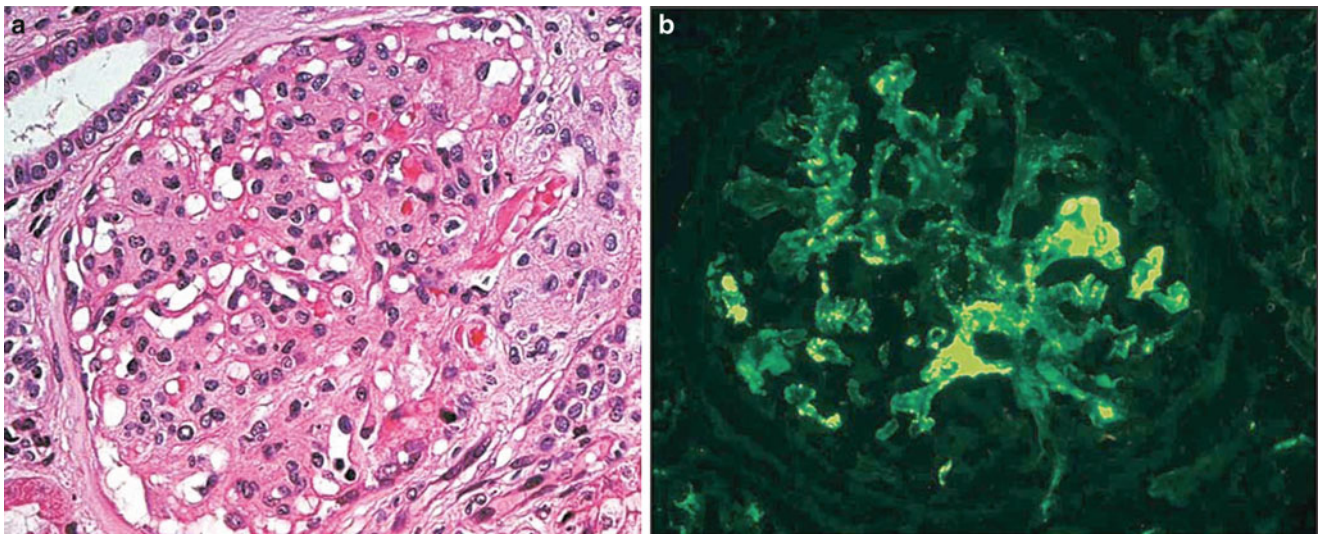

Fig. 12.25 Renal lesions in schistosomiasis—renal biopsy of a patient with schistosomiasis (*S. mansoni*) showing a type I membranoproliferative glomerulonephritis, with duplication of glomerular basement membrane and cellular proliferation (**a**) and subendothelial deposits of IgG, IgM, and C3 identified by direct immunofluorescence (**b**). From Lambertucci et al. [83]; used with permission

identified [71]. That is particularly so when proliferative lesions occur [71]. All infected patients should be treated to eradicate the parasite. Two drugs are currently available for schistosomiasis therapy: praziquantel (single dose, 50 mg/kg) and oxamniquine (single dose, 15 mg/kg). Frequent adverse effects are nausea, dizziness, and skin rash [13].

Chagas Disease (American Trypanosomiasis)

Chagas disease, or American trypanosomiasis, is a zoonosis caused by a protozoa—*Trypanosoma cruzi*. Its vector insect is a member of the *Triatominae* family [7]. Estimates put the number of infected individual at over 10 million, with more than 60 million at risk in Latin America [84]. Incidence has significantly decreased lately, as well as the death toll that has been reduced from 45,000 to 12,500/year [85]. Transmission occurs by oral ingestion, inoculation of parasites following insect bites, contaminated by its fecal contents, by blood transfusion or organ transplant [86].

Clinical Presentation

Incubation period varies from 1 to 2 weeks, being as long as 4 months in transplanted patients [87]. Chagas disease has two different clinical pictures: (1) an acute phase, usually asymptomatic—fever, edema, swollen lymph nodes, liver and spleen enlargement, acute myocarditis, or meningoencephalitis occurs in severe illness and (2) a chronic illness, mainly affecting the heart, gastrointestinal tract, or the so-called undetermined form [88].

Kidney Involvement

Kidney involvement in Chagas disease is quite uncommon. Very little has been written on the subject. In endemic areas, recipients of solid organ transplants may reactivate the disease [85]. Glomerular changes have been reported during the chronic phase of the disease, induced by either *T. cruzi* or other species [89]. Even not causing human disease, *T. brucei* subspecies induces glomerular changes in several other infected mammalians.

Pathophysiology

Kidney disease in Chagas disease seems to include autoimmune phenomena. Acute phase kidney injury occurs by the sixth day and relates with the degree of cardiovascular dysfunction: transitory renal blood flow decrease, observed in several studies, appears quite similar to ischemia-reperfusion injury [90]. No relationship with the presence or reproduction of parasites seems to exist. AKI is a possible complication of acute Chagas disease and an important prognostic marker [91]. During the chronic phase of Chagas disease, there is a fall in the number of circulating parasites, contrary to what happens in other organs, like heart, esophagus, intestines, and kidney [7]. *T. Cruzi* antigens, like B13-molecule, *cruzipain,* and *Cha* cross-react with host antigens [7].

Pathology

No significant loss of kidney function has been seen in *T. cruzi*-infected rats [90]. Yet glomerular atrophy, proximal

tubule subtle damage, and mild inflammatory infiltrates were evidenced [90]. Intracellular *amastigote* forms have been identified in infected kidney transplants, as well as in kidney grafts glomeruli and interstitial tissue of patients presenting with acute Chagas disease. IgG, IgM, and C3 mesangial deposits have been shown in chronic Chagas disease, accompanied by mesangial proliferation [92]. *T. cruzi* antigens and rheumatoid factor may be detected in serum and in glomerular deposits, along with IgG and C3 [92]. In summary, mesangial proliferative and membranoproliferative glomerular lesions may be associated with chronic Chagas disease [89]. Electron-dense deposits have been shown [92]. Kidney infarction has also been detected—a necropsy study demonstrated that 19/78 individual with chronic Chagas disease had kidney infarcts, especially among those presenting with heart failure [93].

Treatment

Benznidazole and nifurtimox have been used successfully in Chagas disease. Treatment should be started the sooner possible, in the acute phase, in order to avoid evolution into chronic disease [13].

Syphilis

Syphilis is a sexually transmitted disease, caused by a *Spirochaetum—Treponema pallidum*. Transmission occurs by sexual contact, congenitally, or by contaminated blood transfusion [94]. Currently, more than 60 % of infected individuals are male homosexuals, and many are also HIV positive [95].

Clinical Picture

Untreated syphilis follows three distinct stages: primary, secondary, and tertiary. An ulcerated, usually genital, lesion characterizes primary syphilis. Diffuse maculopapular skin lesions typically appear in secondary syphilis. Central nervous system, aorta, and other organs may be affected in tertiary syphilis [95].

Kidney Involvement

Even kidney involvement being uncommon nowadays, proteinuria may accompany the infection [96]. Albuminuria has varied from 0.3 to 8.0 % of instances in tertiary syphilis [97]. Additionally, kidney changes may range from mild to heavy proteinuria. Occasionally, proteinuria and hematuria, hypertension, and loss of kidney function may occur. Membranous nephropathy has been reported in congenital syphilis and occasionally in adults. Several different kidney lesions have been associated with syphilis, such as mesangial proliferative, epithelial (crescentic, rapidly progressive), and minimal lesion accompanying a nephrotic syndrome [98].

Diagnosis

Laboratory screening tests include non-treponemal tests: Venereal Disease Research Laboratory (VDRL) and Rapid Plasma Reagin (RPR) test. It has been recommended to follow those tests by a *Treponema*-specific test to confirm the diagnosis: fluorescent treponemal antibody absorbed (FTA-ABS) and *Treponema pallidum* microhemagglutination assay (MHA-TP) [95].

Pathophysiology

Kidney lesions display immune complexes and complement fractions along the basal membrane. Low complement levels may occur, especially in congenital syphilis but also in adult cases [96].

Pathology

Augmented glomerular tufts are a feature of congenital syphilis. Membranous nephropathy with subepithelial or membrane deposits may occur [97]. Capillary granular IgG deposits with occasional IgM deposits, as well as C3 and IgA deposits, may be seen [95].

Treatment

Penicillin is the specific treatment for syphilis. Biopsy kidney lesions apparently disappear after adequate treatment [96]. Syphilis should be always considered in young male patients with the nephrotic syndrome [96].

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Glomerular Diseases Associated with HIV, Hepatitis B, and Hepatitis C Infections

13

Jasmine Riviere Marcelin, Lynda A. Szczech, and Stacey Rizza

Introduction

Our understanding of the glomerular diseases associated with the human immunodeficiency (HIV), hepatitis B (HBV), and hepatitis C (HCV) viruses has evolved over the last decade. Therapeutic advances in the management of these viruses have resulted in treatment of the associated glomerular diseases when their renal manifestations remain subclinical. Much of the evidence for the treatment of these viruses is derived from studies in which the use of the antiviral agents was not for the dedicated treatment of the kidney disease but rather for the treatment of the systemic infection. Unfortunately, some of the antiviral therapies are nephrotoxic, which confounds the difficulty in deciding what is viral-mediated renal disease vs. drug-induced renal disease. Consequently, one must consider the context in which a patient's kidney disease is diagnosed in these infections. The epidemiology, clinical course, and responsiveness to therapy of kidney disease diagnosed *prior* to the initiation of antiviral treatment may be very different to that of kidney disease which is diagnosed *after* the initiation of therapy. This chapter reviews the clinical presentation, pathogenesis, and diagnosis of glomerular diseases associated with HIV, HBV, and HCV, as well as traditional treatment and newer management strategies.

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Human Immunodeficiency Virus (HIV) Infection

Introduction

At the end of 2011, approximately 34 million people worldwide were living with a diagnosis of HIV, with 69 % of the people living in Sub-Saharan Africa [1]. However, the number of newly infected people varies according to region, with the Caribbean and sub-Saharan Africa seeing declines of 42 % and 25 %, respectively, since 2001, but increasing by nearly 35 % in the Middle East and North Africa during the same period [1]. Since the advent and improvement of highly active antiretroviral therapy (HAART), there have been sharp declines in the numbers of acquired immunodeficiency syndrome (AIDS)-related deaths worldwide. In the USA, it is estimated that 1.1 million people are currently living with HIV/AIDS, with African Americans, Hispanics, and women disproportionately represented among persons with newly diagnosed HIV infection or living with HIV/AIDS [2]. In 2011, approximately 1.7 million people died from AIDS-related causes worldwide, a number that represents a 24 % decline since 2005 [1]. This means that adequately treated HIV-infected individuals generally have an improved life expectancy such that they are now more likely to develop non-HIV-related chronic diseases with renal complications such as cardiovascular disease, diabetes, smoking, or hypertension [3]. In fact, HIV infection has been estimated in approximately 1 % of patients with end-stage renal disease (ESRD) in the US and Europe [4]. In the pre-HAART era, the vast majority of kidney disease in HIV-infected individuals was due to HIV-associated nephropathy (HIVAN). The introduction of HAART has resulted in a decline in the percentage of HIVAN, better understanding of the pathogenesis of kidney disease in HIV, and the description of numerous other etiologies of kidney disease associated with HIV, including immune complex disease and HAART-related nephrotoxicity [5]. In addition to treating HIV with HAART,

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newer strategies have emerged to treat the associated kidney diseases, including the use of antihypertensives, immunosuppressive agents, and even renal transplant [4].

Clinical Presentation

HIV-associated kidney disease can take the form of acute kidney injury (AKI), chronic kidney disease (CKD), or rapidly progressive kidney disease (RPKD) with any of these leading to ESRD. These are diagnosed based on laboratory values including blood urea nitrogen (BUN), creatinine, urine protein, protein/creatinine ratio, and urine microscopy. It can occasionally be reversible if the offending cause is removed, but once AKI has progressed into CKD and ESRD, it is usually irreversible. In general, the clinical presentation of kidney disease in HIV is the same as that of the general population, including proteinuria (can be mild, moderate, or nephrotic range), peripheral edema, hypertension, and renal failure. Certain additional features may point more towards a particular etiology than others; for example, large, echogenic kidneys and the absence of peripheral edema in African Americans with HIV and renal failure are suggestive of HIVAN [6, 7]. The immune-mediated kidney diseases associated with HIV can be acute and rapidly progressive, presenting with the general AKI picture described above and additional features of microscopic or gross hematuria, active urine sediment including casts, thrombi, or fragmented red blood cells. The patient may have other systemic signs including coagulopathies or purpura [6]. Drug-induced nephropathies can cause symptomatic nephrolithiasis in the case of drugs like indinavir and atazanavir; alternatively they could be either asymptomatic with unknown clinical significance or symptomatic with significant mineral and bone loss in Fanconi syndrome in patients taking tenofovir [8].

Epidemiology of Kidney Disease in HIV

Far different from its early rapidly progressive days, HIV has become more of a disease of chronicity and aging. Compared to the first decade or so after HIV/AIDS was described when deaths due to opportunistic infections, dementia, or cachexia were prevalent, modern times have seen increases in chronic kidney disease (6.3–9.1 %), heart disease (4.2–6.9 %), and liver disease (4.9–11.6 %) [9], with renal disease being described as the fourth leading cause of non-AIDS-related deaths [10]. Maggi et al. [9] describe multiple studies identifying the incidence of CKD in HIV patients as approximately 1.2 per 100 person-years, with multiple risk factors including low CD4+ T cell counts, antiretroviral therapy, and diabetes [9]. Abnormalities in renal function are commonly seen in HIV-positive patients especially in those being treated with HAART [9]. Hospitalized HIV-positive patients experience acute renal failure more frequently than their non-HIV-infected counterparts, resulting in a higher mortality [9]. African Americans have greater risk for advancement of CKD to ESRD compared to their Caucasian counterparts and do so at a faster rate [9]. Among African Americans with HIV and CKD, HIV-associated nephropathy (HIVAN) is present in approximately 50–60 % of renal biopsies performed, representing the third leading cause of CKD in African Americans [6, 11, 12]. In South Africa, biopsy-proven HIVAN has been reported in a greater proportion of people with HIV, and glomerular disease is estimated at greater than 80 % [13–17].

Pathogenesis of Kidney Disease in HIV

The current literature suggests that HIVAN is likely due to direct effects of the virus on the glomerular and tubular cells, which is at least partially due to direct viral infection of these cells [18], and is facilitated by the presence of a genetic risk factor for the development of kidney disease [19, 20]. Direct viral infection is further supported by the fact that infants who have acquired HIV by vertical transmission show evidence of HIVAN in the absence of any of the adult risk factors for CKD, which is supported by animal models [21]. Additionally, aggressive treatment of HIV with HAART has resulted in the regression or at least slowed progression of HIVAN [21]. HIV messenger RNA has been found in the glomerular and tubular epithelium with the kidney acting as a latent HIV reservoir; HIVAN has been demonstrated in kidneys where systemic viral load was significantly suppressed, but just the presence of these proteins in epithelial cells was enough to result in renal injury [22]. The virus has been shown to replicate within the renal epithelium, but the method by which it gains access to these cells is unclear given that renal epithelial cells lack the CD4+ receptor and only episodically express the chemokine co-receptors necessary for HIV cell entry [22].

African-American patients in general have a higher likelihood of developing hypertension and CKD. The strong association of HIVAN with patients of African descent points to a strong genetic link. Two genes, APOL1 and MYH9 on chromosome 22 have been implicated in this genetic predilection for renal disease in HIV [23]. MYH9 encodes non-muscle myosin heavy chain IIA which is found in the podocytes of the Bowman's capsule. It is postulated that the expression of the MYH9 risk allele confers an inherent fragility to the podocytes making them more susceptible to injury and apoptosis, and subsequent detachment from the basement membrane [24]. Renal epithelial cell apoptosis may be increased due to the increased oxidative stress triggered by reactive oxygen species [21, 25].

APOL1 is located near MYH9 and encodes apolipoprotein L1 which is a component of circulating serum HDL. The exact function of this apolipoprotein is unknown, but it has been found to have the ability to lyse trypanosomes that infect a person's blood [26]. Since trypanosomes have developed the ability to avoid lysis by this protein, a dominant variant of APOL1 appeared to have been selected out which has the ability to kill the trypanosomes. These protective mechanisms are necessary in endemic regions like Africa, so the gene has conferred a selective advantage to the inhabitants of this region, much like the sickle-cell mutation [26]. However, this same variant APOL1 gene is associated with an increased risk of CKD, HIVAN, and classic FSGS, and this selective advantage explains the increased prevalence of these conditions in patients of African descent [27]. The effect appears to act in a recessive manner; age of onset of kidney disease is earlier in patients carrying two alleles for APOL1 than their heterozygote counterparts [28]. Additionally, the presence of the APOL1 variants increased the probability of developing classic FSGS or HIVAN by 20-fold compared to patients with two normal alleles [28]. The exact mechanism by which the variant APOL1 increases these risks is unknown, but the APOL1 is expressed in the glomerulus and proximal tubules, and it is speculated that overexpression can induce cell death [27].

Diagnosis of Kidney Disease in HIV

The Infectious Disease Society of America has published guidelines regarding the management of CKD in HIV [29]. It is recommended that all patients diagnosed with HIV should undergo an initial screening evaluation for the presence of CKD with urinalysis and measurement of renal function. They should then be screened annually, especially if they are at higher risk for the development of CKD, i.e., low CD4+ T cell count (<200 cells/μL), high HIV viral load (>4,000 copies/mL), African American, hypertensive, diabetic, or coinfected with hepatitis viruses B and/or C [29].

Urinalysis

Proteinuria ≥1+ on dipstick urinalysis should prompt a spot urine protein/creatinine ratio or albumin/creatinine ratio determination [29]. Glomerular pathology is suggested by an increased albumin/creatinine ratio, whereas tubular pathology is suggested by the absence of albumin [30]. Significant proteinuria should warrant further investigation.

Imaging

Ultrasound is the imaging modality of choice for renal diseases. Ultrasound can provide information about obstruction and nephrolithiasis. The size of the kidneys can also be important. Small, fibrotic kidneys (<9 cm) indicate advanced, usually irreversible renal disease [29]. Large, echogenic kidneys can be seen in HIVAN, but this feature is not pathognomonic, therefore HIVAN cannot be diagnosed based on ultrasound only [6].

Kidney Biopsy

With the description of glomerular diseases always comes the question of when to perform a kidney biopsy. In diabetic patients, the presence of chronic proteinuria and slow decline in renal function is generally assumed to be due to diabetic nephropathy. Biopsy is usually not indicated unless the clinical scenario is suspicious for nondiabetic kidney disease or evaluation reveals hematuria, rapidly progressive renal failure, or significantly worsened proteinuria in the absence of retinopathy [31]. However, this generally does not apply to HIV-related kidney disease. Unfortunately, while it is true that HIVAN is more likely to be found in hypertensive African-American patients with low CD4+ T cell counts, the clinical situation cannot always predict the histological features, and specific management strategies may depend on the histologic diagnosis. Therefore, a kidney biopsy is the only definitive way to distinguish HIVAN from other non-HIVAN kidney diseases in HIV and is highly recommended in these clinical situations if available [29, 32]. Images of lesions seen on kidney biopsy can be seen in Figs. 13.1 and 13.2.

Types of Kidney Disease in HIV

The underlying etiologies of CKD in persons with HIV can be categorized as follows: (1) HIV-associated nephropathy (HIVAN), (2) HIV-related glomerular diseases that are

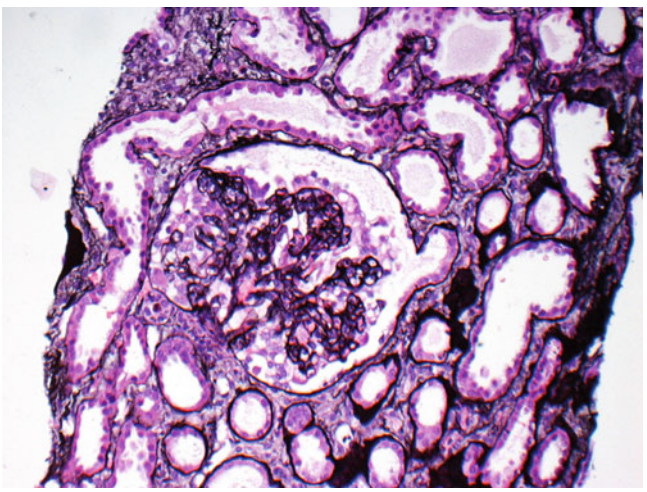

Fig. 13.1 Collapsing glomerulopathy seen in HIVAN. Jones-methenamine stain showing collapse of the capillary loops with overlying podocyte hyperplasia (×40)

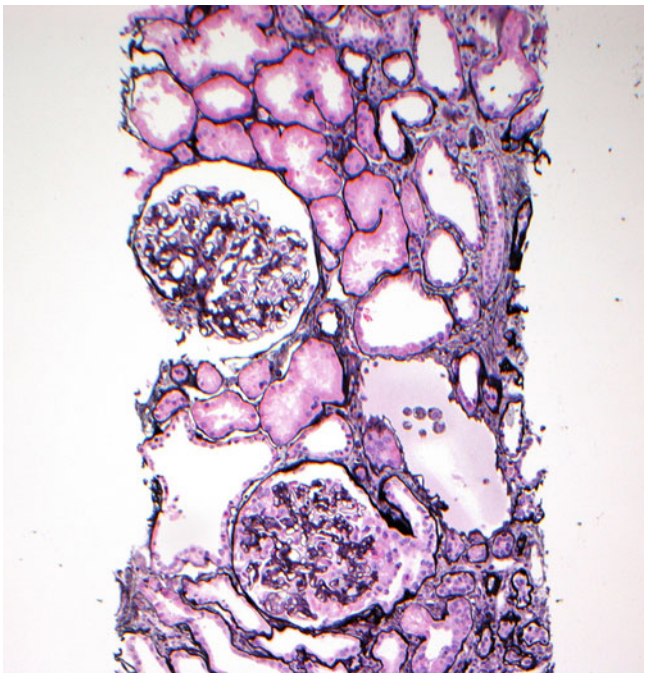

Fig. 13.2 Low-power view of collapsing glomerulopathy in HIVAN. Jones-methenamine stain showing collapse of the capillary loops with overlying podocyte hyperplasia in the lower glomerulus (×20)

not HIVAN including immunodeficiency renal diseases, (3) drug-induced nephrotoxicity, and (4) CKD related to non-HIV comorbidities such as diabetes mellitus or hypertension [33]. The first account of kidney disease associated with HIV was in 1984 as a description of what is known today as focal segmental glomerulonephritis [34, 35]. Regardless of etiology, persons with kidney disease have a greater risk of death and cardiovascular events when compared with the community-based population without CKD, a phenomena that still applies in the HIV-infected community with CKD [21, 36].

HIV-Associated Nephropathy (HIVAN)

HIVAN was first described on renal biopsy as collapsing focal glomerulosclerosis [34, 35]. However, in the largest biopsy series of HIV-infected patients of African descent, it has more recently been defined as a histological spectrum, a "constellation of glomerular, interstitial, and tubular abnormalities," albeit with a glomerular predominance [37]. Table 13.1 describes the changes that can be seen in the nephron which correspond to a histological diagnosis of HIVAN, including tubular microcysts, mesangial hyperplasia, interstitial fibrosis, and lymphocytic infiltrates [6, 37]. HIVAN has been traditionally reported as a late manifestation of HIV but has occasionally been described earlier in the disease [6]. Clinically, it is characterized by variable proteinuria, renal failure, large echogenic kidneys, and absence of peripheral edema and can rapidly progress to ESRD [6, 7]. The progression of HIVAN to ESRD can be significantly slowed but not completely prevented by HAART therapy [6]. In addition to ethnicity, risk factors for the progression of HIVAN likely include lower CD4+ T cell count (<200 cells/μL), higher HIV RNA level, and severe renal failure on presentation [5, 38]. There are also genetic associations that have been explored earlier in this chapter. In the study by Wearne et al. [37], without antiretroviral therapy, the expected median survival after biopsy confirmed HIVAN was approximately 4.5 months, with the "fetal variant" having the worst prognosis [37].

HIV-Related Glomerular Diseases That Are Not HIVAN

While biopsy studies suggest that HIVAN represents approximately one-third of the glomerular disease among African Americans with lower CD4+ T cell counts, it should be emphasized that it is uncommon in Caucasians and Asians; thus there must be other HIV-related glomerular disease seen more often in these ethnic groups and those with higher CD4+ T cell counts [11, 17, 21]. These include HIV-immune complex disease like membranoproliferative glomerulonephritis (MPGN; including hepatitis C associated), lupus-like glomerulonephritis, IgA nephropathy, membranous nephropathy (MN), and HIV-thrombotic microangiopathies [6, 11, 17]. The HIV-immune complex renal disease involves the deposition of circulating immune complexes containing HIV antigens with B cell lymphocytic infiltrates (compared to T cell infiltrates in HIVAN), inflammation, and scarring [6].

Table 13.1 Histological changes in the nephron seen with HIVAN

Glomerular changes	Interstitial changes	Tubular changes
FSGS, collapsing variant	Fibrosis	Presence of microcysts
Global sclerosis with epithelial cell involvement (fetal variant)	Lymphocytic infiltrate	Epithelial cell hyperplasia and hypertrophy
FSGS, non-collapsing variant[a]	Plasma cells within the lymphocytic infiltrate	
	Diffuse inflammatory lymphocytic syndrome	

Adapted from Wearne N, Swanepoel CR, Boulle A, Duffield MS, Rayner BL. The spectrum of renal histologies seen in HIV with outcomes, prognostic indicators and clinical correlations. Nephrol Dial Transplant. 2012 Nov;27(11):4109–18

[a]Needs additional features including parietal and/or visceral epithelial (mesangial) cell hypertrophy/hyperplasia, ± presence of pseudocrescents

Table 13.2 Described renal toxicities of selected antiretroviral medications

Antiretroviral	Renal toxicity
Tenofovir	Proximal tubulopathy resulting in Fanconi syndrome, acute tubular necrosis, allergic interstitial nephritis, nephrogenic diabetes insipidus
Atazanavir	Allergic interstitial nephritis (case report), nephrolithiasis
Indinavir	Allergic interstitial nephritis, intratubular drug precipitation and nephrolithiasis, renal papillary necrosis, renal atrophy
Stavudine/lamivudine	Renal tubular acidosis, hypophosphatemia (case report)
Didanosine	Fanconi syndrome, lactic acidosis, nephrogenic diabetes insipidus
Enfuvirtide	Membranoproliferative glomerulonephritis (case report)

Adapted from Izzedine H, Harris M, Perazella MA. The nephrotoxic effects of HAART. Nature reviews Nephrology 2009 Oct;5(10):563–73

It has been reported in up to 80 % of Caucasians with HIV and glomerular disease [5]. The benefit of HAART therapy on slowing the progression of the HIV-immune complex kidney disease is less clear compared to that seen in HIVAN. While some studies of IgA nephropathy demonstrate that HIV antigens may play an important and causative role [39] in the pathogenesis of these lesions, the definitive evidence linking HIV to these lesions is not available. The HIV-related lupus-like glomerulonephritis involves the subendothelial deposition of multiple immune complexes including IgA, IgG, IgM, C3, and C1q in the absence of serologic and clinical diagnosis of systemic lupus erythematosus (SLE) [40]. HIV-thrombotic microangiopathy (hemolytic uremic syndrome or thrombotic thrombocytopenic purpura) is rare but, when identified, predominates among Caucasians; like its non-HIV-associated counterparts, histological features include the deposition of microthrombi in the glomerular capillaries, fibrinoid necrosis, and onion skinning [6].

Anti-neutrophil cytoplasmic antibodies (ANCA) have been noted in the sera of HIV-infected individuals with varying frequency (18–41.0 %) in the absence of clinical features suggestive of a vasculitis [41–43]. A review of the literature finds only two case reports of persons with HIV who developed an active ANCA-positive vasculitis [42, 44, 45]. Similarly, only one case report of anti-GBM disease in a patient with HIV has been published [46]. The finding of these two autoantibodies in the absence of kidney disease suggests that their pathophysiologic effects may be modified in the presence of HIV infection. Positive tests for these antibodies should be interpreted with caution and should not be used to decide on empiric therapy in the absence of a kidney biopsy to confirm histology.

Drug-Induced Nephrotoxicity

While the treatment of HIV with antiretroviral medications has therapeutic benefit in HIVAN, the medications can also result in acute kidney injury (AKI) due to acute tubular necrosis, allergic interstitial nephritis, and obstructive uropathy [21, 36]. Table 13.2 lists common HIV medications and their potential renal toxic effects. Tenofovir, indinavir, and atazanavir are the most frequently described as having nephrotoxic effects. Tenofovir is a nucleoside reverse transcriptase inhibitor, and while early clinical trials did not reveal appreciable nephrotoxicity, there have been numerous post-marketing reports describing renal tubular injury with this agent [47]. Tenofovir is actively secreted into the tubules and damages the proximal tubules by a direct cytotoxic effect; this eventually causes a Fanconi syndrome and potential renal tubular acidosis [48]. These phenomena have been seen more frequently in cases where tenofovir was administered as part of a regimen containing HIV protease inhibitors such as ritonavir or lopinavir as these increase the maximum serum concentration of tenofovir by >30 % [6, 49]. Tenofovir nephrotoxicity is largely reversible with the removal of the offending drug. The HIV protease inhibitor indinavir causes nephrotoxicity by inducing crystal formation in approximately 20 % of patients receiving it; this subsequently leads to obstructive uropathy and drug-induced interstitial nephritis [6, 50]. Indinavir also reduces the production of nitric oxide which is a potent vasodilator. A reduced amount of this molecule in the kidney vessels therefore leads to vasoconstriction and subsequent ischemia, which is another proposed mechanism of indinavir-mediated renal injury [51]. Patients can be asymptomatic or present with classic symptoms of dysuria and flank pain and the urinalysis will reveal crystalluria and pyuria; renal ultrasound may reveal signs of obstruction, but indinavir stones are radiolucent and are usually not visualized on imaging [6]. Similar events have been described with atazanavir [32, 33]. Additionally, many of the antimicrobials used for prophylaxis of opportunistic infections can also exert nephrotoxic effects such as renal tubular acidosis, acute interstitial nephritis (AIN), and acute tubular necrosis (ATN) (Table 13.3). Trimethoprim-sulfamethoxazole can also cause an isolated elevation in serum creatinine without significant change to renal function [6].

Glomerular Diseases Unrelated to HIV Infection

The epidemiology of HIV infection has changed significantly with the advent of effective HIV suppression using HAART. With the improved survival come an increase in the mean

Table 13.3 Described renal toxicities of selected antimicrobials used to treat opportunistic infections in HIV-positive patients

Antimicrobial	Opportunistic infection	Renal toxicity
Foscarnet	Cytomegalovirus	Acute tubular necrosis, nephrolithiasis and intratubular obstruction, crescenteric glomerulonephritis, nephrogenic diabetes insipidus, renal tubular acidosis
Trimethoprim-sulfamethoxazole	*Pneumocystis jirovecii*	Acute tubular necrosis, interstitial nephritis, renal tubular acidosis
Rifampin	Tuberculosis	Interstitial nephritis, crescenteric glomerulonephritis
Sulfadiazine	Toxoplasmosis	Nephrolithiasis and intratubular obstruction
Pentamidine	*Pneumocystis jirovecii*	Acute tubular necrosis

Adapted from de Silva TI, Post FA, Griffin MD, Dockrell DH. HIV-1 infection and the kidney: an evolving challenge in HIV medicine. Mayo Clin Proceed. 2007 Sep;82(9):1103–16

age of those living with HIV/AIDS and an increase in the number of comorbidities such as diabetes, hypertension, and atherosclerosis [8, 11, 52]. A review of kidney tissues contained within the Manhattan HIV Brain Bank obtained from patients who had consented to postmortem organ donation for scientific study reveals that nearly one third of 89 deceased individuals had CKD [53]. Notably, while HIVAN was present in some subjects, vascular diseases including arteriolar nephrosclerosis and diabetic nephropathy were also seen, as well as pyelonephritis, interstitial nephritis, fungal infection, and amyloidosis [53]. Other non-HIV glomerular pathology include postinfectious glomerulonephritis, classic FSGS again most commonly seen in African-Americans, and hepatitis B- and C-related glomerulopathies (discussed elsewhere in this chapter) [32].

Treatment of Kidney Disease in HIV

Antiretroviral Therapy in HIVAN

Through the reduction of HIV replication with antiretroviral therapy, the clinical course of HIV infection has improved dramatically since the early 1990s. The goal of HIV therapy is maximal inhibition of HIV replication as measured by consistent plasma HIV RNA (viral load) values below the level of detection [54]. With an undetectable HIV viral load, there should be little circulating free virus and ideally prevent the development [55] or slow the progression of HIVAN [54, 56]. However, it has been shown that in patients who achieved remission of nephritic syndrome in HIVAN, the discontinuation of antiretrovirals results in increased HIV replication and subsequent progression of kidney disease [57, 58]. Additionally, in a study of patients on zidovudine (AZT) therapy with HIVAN comparing renal outcomes in compliant vs. noncompliant patients, subjects who were compliant with AZT treatment experienced a stable creatinine over 8 weeks while their noncompliant counterparts progressed to ESRD requiring dialysis during this same time period [59]. HIV protease inhibitors also provide the same renal-protective benefits; Szczech et al. [56] described a slower decline of kidney function in patients with HIVAN treated with HIV protease inhibitors compared with those not receiving protease inhibitors [56]. These observational studies strongly suggest a therapeutic role for antiretroviral medications in the treatment of HIVAN.

No randomized trials, however, have specifically examined the treatment of kidney disease in HIVAN. One of the few large randomized trials on how to administer HAART that collected renal outcomes was the Strategies for Management of Antiretroviral Therapy (SMART) Trial [60]. SMART randomized 5,472 patients with HIV who had a CD4+ T cell count >350 cells/μL to the continuous use of antiretroviral therapy (2,752 patients; viral suppression) or the episodic use of antiretroviral therapy (2,720 patients; drug conservation) and then followed them for an average of 16 months [60]. Renal events were infrequent in general in the study but slightly more common in the group who had intermittent antiretroviral therapy (drug conservation group) compared with the group who maintained constant HIV suppression (9 of 2,720 patients or 0.2 events/100 person-years vs. 2 of 2,752 patients or 0.1 events/100 person-years, respectively) [60]. Given the consistency of the association between better renal outcomes and HAART among those with HIVAN, clinical practice guidelines currently suggest the initiation of HAART for anyone with HIVAN irrespective of CD4 lymphocyte count [61].

Angiotensin II Blockade in HIVAN

In patients with both diabetic and nondiabetic CKD, antihypertensive agents that affect the renin-angiotensin axis [angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II receptor blockers (ARB)] have been proven to reduce proteinuria and slow progression of renal disease [62]. These effects have also specifically been discussed with respect to HIVAN. A retrospective analysis of 18 patients with biopsy-proven HIVAN concluded that AZT and ACEI were independently associated with a slower progression to ESRD [63]. The independent association was confirmed by Wei et al. [64] who described a study where ACEI significantly improved renal survival in HIVAN, even in the absence of HAART therapy. Others have suggested that

ACEI therapy is effective in slowing or stabilizing renal disease for up to 12 and 24 weeks in HIV-infected patients with nephrotic and non-nephrotic range proteinuria, respectively [65]. Notwithstanding these findings, others have postulated that ACEIs and ARBs, despite their benefits, are also capable of increasing hypertension and renal disease by increasing the circulating renin leading to potential continued renin-angiotensin-aldosterone system activation [66]. Therefore, it has been suggested more recently that direct renin inhibitors like aliskiren should be considered as an improved alternative to ACEIs and ARBs because they also slow the progression of renal disease without further activation of the renin-angiotensin-aldosterone system [67].

Immunosuppression in HIVAN

Before HAART became available and was documented as the treatment for HIVAN, variable success was reported with the use of corticosteroids in patients with HIVAN. The rationale behind their use lies in the anti-inflammatory effects on the nephron given the prevalence of tubulointerstitial inflammation in HIVAN [68]. Many reported on the use of corticosteroids associated with a reduction in the risk of progression to ESRD [69, 70]. However, relapses after the discontinuation of prednisone were common, often requiring retreatment; additionally, with prednisone came increased incidence of serious complications including avascular necrosis of the femoral head, mycobacterium avium-complex infection, and CMV retinitis [71, 72]. In the era of modern HAART therapy, prednisone does not have a major role in the treatment of HIVAN but can be considered in patients with HIVAN who have refractory nephrotic syndrome despite maximum HAART and ACEI therapy, although no randomized trials exist in the literature to support this [68, 73].

Kidney Transplant

Before HAART, HIV infection was an absolute contraindication for organ transplant, including renal transplant for ESRD. However, with the introduction of HAART, maximal viral suppression, and increased longevity, transplant has become an option for HIV patients with ESRD. Allograft and patient survival after transplant in HIV patients have been shown to near that of non-HIV-infected kidney transplant patients [6]. Eligible patients are those who are on regular HAART therapy for at least 6 months, with undetectable viral loads (<50 copies/μL), CD4+ T cell count >200 cells/mm^3, and without AIDS defining illness [6, 74]. Transplant is not without limitations, however, and has been shown to have increased risk of elevated posttransplant creatinine, with acute rejection being more common in HIV transplanted patients [6, 75]. Kidney transplant and antirejection immunosuppression do not increase viral replication and promote progression of HIV disease [75]. However, HIV transplant patients are at greater risk of steroid-resistant rejection, and the metabolism of immunosuppressant medications may be affected by antiretroviral drugs, thereby increasing the risk of inadequate immunosuppression as well as drug-induced toxicity [6, 75]. Nevertheless, transplant still remains a viable option for HIV patients with ESRD who can be followed by a nephrologist and infectious disease transplant teams familiar with the specifics of this special patient population.

Treatment for Glomerular Diseases Other than HIVAN

While approximately half of African Americans with HIV who undergo kidney biopsy have HIVAN, little is known about the treatment of the glomerular diseases that are demonstrated in African Americans without HIVAN and in Caucasians with glomerular diseases. Current evidence does not suggest that HAART benefits patients with HIV-associated immune complex-mediated renal diseases and thrombotic microangiopathies [17, 76, 77]. Among a cohort of 89 persons with HIV undergoing kidney biopsy, the stabilization of kidney function observed in patients with HIVAN concurrent with the use of antiretroviral therapy was not seen among those with glomerular diseases other than HIVAN [11]. While the conclusions from this cohort study are not definitive due to its limitations, one might conjecture that HAART played a role that is augmented by concurrent immunosuppressive therapies to affect patient response to antigen stimulation in the kidney. However, studies of cyclosporine and other agents are small and similarly limited in their ability to draw conclusions [78]. With respect to ACEI and ARB, among persons *without* HIV infection, a beneficial effect has been demonstrated [79]. However, the effect has not been specifically noted among those with HIV infection [80].

Summary

Renal disease associated with HIV is multifactorial and can affect any part of the nephron, from the well-described direct viral effect causing HIVAN to nephrotoxic effects of antiretroviral drugs. The presence of certain chromosomal foci may increase the risk of developing renal disease among HIV-infected patients of African descent. Due to the current effectiveness of HAART therapy, HIV-infected patients now have improved life expectancies that may lead to the development of chronic kidney disease secondary to both HIV and non-HIV-related causes. Renal biopsy could provide definitive histologic diagnosis but may not be readily available or even necessary for diagnosis and treatment. Appropriate therapy for HIV-related renal disease includes HAART initiation or adjustment and the use of ACEIs and ARBs.

If proteinuria and serum creatinine fail to improve or at least stabilize in the short term after initiation of treatment, renal biopsy should be considered at that time if not previously

performed, to further define the renal disease and allow for more aggressive and tailored therapy. Kidney transplant is a viable therapeutic option for end-stage renal disease in HIV patients and is becoming more accepted as HAART can effectively keep the HIV viral load low and CD4+ T cell count high.

Hepatitis B Virus (HBV) Infection

Introduction

Hepatitis B is a DNA virus that is transmitted by infected blood or body fluids including semen and vaginal secretions. The prevalence was increased among hemodialysis patients until the 1970s when the CDC released guidelines for prevention by vaccination of susceptible patients and healthcare workers [81]. The clinical spectrum of HBV infection is variable and includes acute hepatitis, fulminate hepatic failure, an inactive carrier state, chronic hepatitis, cirrhosis, and hepatocellular carcinoma [82]. Extrahepatic disease has been decreasing in incidence with more effective treatment and vaccination for HBV. These extrahepatic manifestations may include renal disease, vasculitides like polyarteritis nodosa, or dermatologic manifestations [83]. Immune complex renal disease represents one of the most common of these extrahepatic manifestations, with membranous glomerulonephropathy representing the major HBV-associated nephropathy (HBVAN) (analogous to HIVAN) [82]. The immune response to hepatitis B infection is correlated with the natural history of the infection and extrahepatic manifestations. Circulating "e-antigen" or HBeAg is a marker of active viral replication. Although normal transaminases can be seen during this phase due to immune tolerance, the patient remains contagious [83]. The immune response to HBV frequently also results in active hepatocyte destruction. With continued immune recognition, the immune system may be able to suppress viral replication without eliminating the virus or virally infected hepatocytes [82]. This situation results in an inactive carrier phase which is manifested as a loss of the HBeAg and development of the anti-HBe antibody [83]. HBV has been more prevalent in renal failure patients, although this association has been decreasing with the improved compliance with HBV vaccination [84]. The presence and absence of HBeAg with treatment of HBV may play an important role in understanding the response of kidney disease to treatment of the virus.

Clinical Presentation and Diagnosis of Kidney Disease in HBV

As with HIV, renal disease associated with HBV commonly presents as nephrotic syndrome. Less common presentations are asymptomatic proteinuria, peripheral edema, hematuria, or rapidly progressive glomerulonephritis (RPGN), which could eventually lead to ESRD [85–87]. The diagnosis is established by serologic evidence of HBV antigens/antibodies, presence of an immune complex glomerulonephritis on kidney biopsy, immunohistochemical localization of 1 or more HBV antigens, and pertinent clinical history, when available [88]. The natural history of HBV-associated kidney disease is not well delineated, and its course may differ depending on the population affected. In children, especially those in sub-Saharan African and Asian countries where HBV is endemic and acquired by horizontal transmission, HBVAN can have a benign course and even achieve spontaneous remission [88]. However, adults would be more likely to develop progressive proteinuria and renal failure, with >50 % of those with nephrotic syndrome and elevated transaminases requiring renal replacement therapy [82]. In developed regions like North America, HBV is less prevalent and largely acquired as a sexually transmitted disease or parenterally by healthcare workers or as a result of intravenous drug abuse [89].

HBV-Associated Nephropathy

The association between hepatitis B infection (HBV) and kidney disease was first reported in the 1970s [90]. It is estimated that one third of the world's population has had a past or present infection with HBV and approximately 350–400 million people worldwide and 1.25 million people in the US are chronic HBV carriers [83, 91]. The mode of transmission depends largely on the geographical location. In East Asia where HBV is endemic, it is often acquired through vertical transmission from mother to infant and is associated with the chronic carrier state of HBV. Chronic HBV is also endemic in Africa, but transmission is thought to be horizontal with familial clustering, though the precise mechanism of transmission is unknown. In low-prevalence areas like North America, the predominant mode of transmission is sexual or parenteral, with a large majority of cases seen in intravenous drug abusers and those who engage in risky sexual habits (52 % combined) [89]. Hepatitis B infection is the most common cause of secondary glomerulonephritis in endemic regions and occurs more frequently in chronic carriers, since it is uncommon for acute HBV infection in adulthood to become a chronic HBV infection [88, 89]. HBV has a male predilection, with chronic HBV occurring 1.5–2 times more frequently in males vs. females [89]. Efforts to immunize endemic regions for HBV infection have likely played a significant role in lowering the incidence of HBV infections and subsequently HBVAN [89].

Pathogenesis of Kidney Disease in HBV

The exact pathogenesis of HBVAN is not completely understood, but four possible mechanisms have been suggested: (1) direct cytotoxic effect of HBV, (2) immune complex (viral antigen–antibody) deposition, (3) virus-induced

immune cell-mediated host response, and (4) virus-induced cytokine-mediated tissue injury [89]. The role of immune recognition and control of the virus, marking the success of treatment, is underscored by the fact that development of anti-HBeAg antibodies and HBeAg clearance are both associated with remission of proteinuria [92, 93]. The HBeAg has been identified as the major antigen found in the subepithelial deposits of HBV membranous nephropathy and thus responsible for the immune complex-mediated injury [89]. Additionally, failure to clear or become immune reactant to HBeAg is associated with progressive loss of kidney function [87, 94, 95].

Types of Kidney Disease in HBV

The glomerular diseases that are associated with chronic hepatitis B infection include immune complex glomerulopathies such as membranous nephropathy [92], membranoproliferative glomerulonephritis (MPGN) [96, 97], focal segmental glomerulosclerosis (FSGS), minimal change disease [98], lupus nephritis [99, 100], IgA nephropathy [101], and polyarteritis nodosa (PAN) [102]. Membranous glomerulonephropathy is the most common HBVAN, and while it typically leads to a progressive nephrotic syndrome in adults, it usually resolves spontaneously in children [89]. On light microscopy, glomeruli are uniformly enlarged with thickened basement membrane and subepithelial spikes of immune complex deposits including C3, IgG, HbeAg, HbcAg, or HbsAG [85, 90]. Membranoproliferative glomerulonephritis appears as thickened capillary walls with segments of double contours and mild to moderate mesangial proliferation [85]. Immune complex deposits consisting mainly of IgG or C3 are deposited in the mesangium and subendothelial space and HBeAg and HBsAg are often implicated [89]. While IgA nephropathy may be a "complication" of HBV infection, it represents one of the most common glomerulonephritides worldwide [103, 104]; thus it can often be an incidental finding on biopsy in regions where both HBV infection and IgA nephropathy are prevalent, such as East Asian countries. Therefore, it can be difficult to ascertain if the primary kidney disease in HBV-infected patients within this population is due to the HBV or the IgA nephropathy [104]. This poses a therapeutic challenge as kidney lesions unrelated to the HBV may not be responsive to traditional anti-HBV treatment.

HBV is also strongly associated with systemic necrotizing vasculitis—polyarteritis nodosa (PAN). The vasculitis affects small to medium sized vessels, and pathology typically shows fibrinoid necrosis and perivascular infiltration [105]. Angiographic lesions typical of PAN, including microaneurysms are frequently observed. PAN due to hepatitis B infection is felt to be caused by immune complex deposition of the hepatitis B virus with excess antigen [89, 106]. Improved immunization compliance worldwide has led to an overall decrease in the frequency of hepatitis B-associated PAN (36–7 %) [106], and in HBV-infected individuals, PAN does not relapse once viral replication has stopped [107].

Treatment

Antiviral Therapy

The goals of treatment of hepatitis B are to sustainably suppress HBV replication and to prevent cirrhosis, hepatic failure, and hepatocellular carcinoma. A number of potential regimens are available for the treatment of adults with HBV all of which are effective in decreasing HBV DNA levels. The choice of a particular treatment strategy should be made based on side effects, medication interactions, presence or absence of HIV coinfection, and the potential for the development of viral resistance. Therapeutic agents can be broadly classified into nucleoside/nucleotide analogs (tenofovir, entecavir, lamivudine, adefovir, and telbivudine) and interferon alpha (conventional interferon-α[alpha]2b and longer-acting peginterferon-α[alpha]2a).

Antiviral agents may be used singly or in combination and vary in required length of therapy (e.g., interferon therapy is administered for a prescribed duration while nucleoside or nucleotide analogs may be used long term) [91]. The decision on which medication to use should be made using a multidisciplinary approach according to a standard Clinical Practice Guideline for HBV infection [83, 91].

Few studies have been performed to assess and compare the efficacy of antiviral agents on HBVAN; in a 2010 meta-analysis to evaluate the efficacy of antiviral or corticosteroid treatment, the remission of proteinuria and clearance of hepatitis B e-antigen (HBeAg) occurred more frequently in the groups receiving antiviral therapy than among those who did not [108].

Nucleoside and Nucleotide Analogs

Nucleoside and nucleotide analogs are oral agents that cause profound HBV DNA suppression. However, the resurgence of HBV DNA levels occurs after the discontinuation of the drugs, potentially leading to the development of resistance, which can be circumvented by long-term antiviral therapy [82]. This class of drugs is renally cleared and therefore requires dose adjustment among persons with decreased renal clearance.

Lamivudine

Lamivudine is a nucleoside analog, the most well studied of the antiviral agents on renal outcomes. Treatment with lamivudine is associated with significant reduction in proteinuria and increase in serum albumin, with reduction of progression to ESRD [109]. Lamivudine is inexpensive, is well

tolerated, and is not associated with renal toxicity; however, it is highly susceptible to resistance mutations in hepatitis B [82]. Consequently, current guidelines advise against its use as monotherapy unless other more potent drugs with high barriers to resistance are unavailable; it is acceptable as a second-line agent, often in combination with other antivirals [83].

Tenofovir and Entecavir

As the most potent inhibitors of HBV DNA polymerase with the highest barrier to resistance, tenofovir and entecavir both have grade A1 recommendations for use as a first-line monotherapy in the treatment of HBV [83, 110]. Tenofovir is a nucleotide analog active against lamivudine-resistant HBV and is also active against HIV, making it an appropriate treatment of HIV/HBV coinfected patients (when used in combination with a highly active antiretroviral program for HIV) [111]. While significant data exists on their efficacy in suppressing hepatitis B viral replication resulting in undetectable HBV DNA, loss of HBsAg, HBeAg seroconversion, and normalization of liver enzymes [111], little information exists on their efficacy in the remission of proteinuria in HBVAN. Additionally, tenofovir has been reported to cause renal tubular dysfunction leading to Fanconi syndrome and kidney failure after prolonged use (months to years) in patients taking this drug for HBV or HIV infection [112]. However, tenofovir-related iatrogenic renal impairment is more pronounced in HIV patients, presumably due to the influence of other antiretroviral agents on kidney function [113]. Nevertheless, some have recommended that tenofovir be avoided for these reasons.

Entecavir is a nucleoside analog like lamivudine but is more effective than lamivudine in suppressing viral replication in both HBeAg-positive and HBeAg-negative patients [114]. Cross-resistance has been reported between lamivudine and other nucleoside analogs like entecavir (seen in greater than 7 % of lamivudine-resistant patients); subsequently tenofovir still remains superior to entecavir with regard to resistance barriers [82]. To date, however, no renal toxicities have been reported with entecavir.

Adefovir

Adefovir is a nucleotide analog like tenofovir. Adefovir is more costly than tenofovir and has a higher potential for resistance than tenofovir [83]. Additionally, there is a greater potential for viral rebound after treatment discontinuation [115]. For these reasons, adefovir is not recommended for first-line monotherapy [83]. Finally, the potential for nephrotoxicity should be considered. Renal toxicity is characterized by a tubular nephropathy like that seen with tenofovir, leading to phosphate wasting, osteomalacia, and renal insufficiency [112]. Adefovir was originally FDA approved for HIV therapy at a higher dose, however, was removed from the market at the higher HIV dosing, due to renal impairment.

Telbivudine

Telbivudine is a nucleoside analog that is potent in suppressing HBV replication but associated with a high rate of resistance, making it inferior to tenofovir and entecavir [82, 83]. Given this, it has limited role as a single agent and should be used in combination therapy [116, 117]. The side effect profile includes myopathy and peripheral neuropathy when combined with interferon [82], but no data on nephrotoxicity or its effects on HBV-related glomerular disease are available.

Randomized controlled trials that compare the renal outcomes in patients with HBV-mediated kidney disease undergoing antiviral therapy are lacking; however, the success seen in the observational studies with lamivudine suggests that these regimens, particularly tenofovir and entecavir, will also successfully treat subclinical glomerular disease [83]. Finally, although lamivudine, tenofovir, and entecavir all have activity against HIV; they are contraindicated as monotherapy for HBV in HIV/HBV coinfected patients, since monotherapy for HIV would result in HIV resistance. Rather, in these patients, adefovir, telbivudine, and interferon are more appropriate as HBV monotherapy as these drugs have no activity against HIV and therefore cannot promote resistance [83]. However, current DHHS HIV treatment guidelines recommend starting HAART which includes anti-HBV agents for any HIV/HBV coinfected individual, regardless of the CD4+ T cell count [61].

Interferon (IFN)

IFN is a cytokine produced by white blood cells which has a natural antiviral function by the induction of proliferation of natural killer and CD8+ cytotoxic T cells [89]. Treatment with IFN is designed to have a finite duration; usually at least 48 weeks is recommended [82, 83, 89]. IFN potentially offers curative treatment with the goal being immune-mediated viral control and sustained suppression after treatment is concluded [82, 83]. Additionally, Interferon does not cause resistance as may be the case with nucleoside and nucleotide analogs [82, 83]. Pegylated-IFN alone or in combination with lamivudine is more effective than lamivudine alone at suppressing HBV DNA and inducing HBeAg seroconversion which is the ultimate goal of HBV treatment and is clearly associated with positive long-term outcomes [118, 119]. Treatment with IFN has been shown to suppress proteinuria and improve renal function in patients with HBVAN [120, 121]. The disadvantages of IFN treatment include the inconvenient and/or uncomfortable subcutaneous injections and frequent side effects including flu-like symptoms, increased rates of infection, and gastrointestinal effects [82]. IFN is contraindicated in patients with decompensated HBV cirrhosis, autoimmune disease, uncontrolled depression or psychosis, and in pregnancy [83]. Although combination IFN/telbivudine is highly effective at HBV suppression, it is not recommended due to the risk of severe peripheral neuropathy [83].

Immunosuppressive Therapy in HBVAN

Corticosteroid therapy is the treatment of choice for idiopathic nephrotic syndrome, but this benefit does not extend to nephrotic syndrome secondary to HBVAN [108]. In fact, the treatment of HBVAN with corticosteroids has been shown to cause an increase in HBV DNA replication, increase in HBeAg, rising transaminases, persistence of proteinuria, and worsening renal function [82, 83, 89]. Renal biopsies in patients with HBVAN obtained before and after corticosteroid therapy have demonstrated the progression of glomerulosclerosis on light microscopy and virus-like particles within the glomeruli on electron microscopy in tissue *after* corticosteroid therapy, thus confirming that corticosteroid therapy contributes to the progression of renal disease in HBV [122]. Other immunosuppressive agents like rituximab are generally not recommended for treatment of HBV or its associated renal diseases, as they generally have similar effects to corticosteroids in reactivation of HBV replication and worsening HBVAN nephritic syndrome [82, 83]. In patients who must receive these immunosuppressive agents for other comorbidities, prophylaxis with lamivudine or entecavir has been shown to be helpful in significantly reducing the reactivation of HBV replication and subsequently reducing mortality [82, 83, 110].

Transplant

Although renal transplant is a useful option to ESRD, the concern in patients with HBV infection is that of the profound immunosuppression mediated by the antirejection agents including corticosteroids. In the absence of antiviral treatment in the posttransplant period, there is a higher risk of progression of HBV and increased incidence of hepatocellular carcinoma [123]. As expected, in the presence of adequate antiviral therapy, patient and graft survival are superior to non-treated patients [123]. The only caveat is that IFN therapy, which can be very useful in non-transplant patients, is contraindicated after transplant as it has been found to have direct nephrotoxic effects on the graft and precipitate acute graft rejection [123]. Additionally, despite the nephrotoxic effects of some nucleotide/nucleoside analogs, therapy with these drugs has been shown to be beneficial in renal transplant patients in treating and preventing HBVAN; one study has even demonstrated 100 %, 97.6 %, and 88 % graft survival at 5, 10, and 20 years, respectively, in patients treated with the nucleoside and nucleotide analogs, with no graft loss related to either drug nephrotoxicity or HBVAN [123]. However, despite these promising results, frequent surveillance of renal clearance parameters is necessary to monitor for HBVAN progression posttransplant.

Prevention Strategies

The transmission of HBV has already been described earlier in this chapter. Strict screening practices at blood banks and hemodialysis centers reduce parenteral transmission, and the administration of HBV immune globulin and HBV vaccine to newborns of HBV carriers reduces vertical transmission [110]. Safer sex practices will reduce the numbers of sexually transmitted HBV, however, HBV vaccination remains the crux of HBV prevention. Increased compliance to vaccination schedules has reduced the incidence and prevalence of HBV worldwide, thereby reducing the incidence and prevalence of HBVAN and other HBV-related diseases [89].

Summary

The improvement in efficacy and availability of treatments for HBV has significantly improved the outcome of HBV-infected patients. There is a paucity of studies examining the effect of these therapies on HBVAN. The limited data however suggest that the remission of proteinuria and improvement in renal clearance occur among those who clear the HBeAg and may be more related to the success of therapy in this clearance than in the therapy chosen. In a manner similar to the disappearance of endocarditis-related glomerulonephritis after the introduction of antibiotics, the incidence and prevalence of HBV-associated glomerular diseases may eventually regress with increasingly effective and available treatments. Current available therapies have advantages and disadvantages. The recommended first-line therapy for HBV treatment is monotherapy with tenofovir or entecavir; however, combination therapy or therapy with interferon is also available. Special populations may have exceptions (i.e., renal transplant patients, pregnant patients, HIV-coinfected patients). Nephrotoxicity is a common side effect among some of the nucleoside and nucleotide analogs, but this does not prohibit their use especially in patients with renal insufficiency; the doses must simply be adjusted for renal function. Comanagement of the HBVAN with a nephrologist will always be appropriate in light of the known challenges associated with treatment.

Hepatitis C Virus (HCV)

Introduction

Hepatitis C virus (HCV) is a single-stranded RNA virus that is primarily spread by parenteral transmission. HCV is one of the most common causes of death from liver disease and is the leading indication for liver transplantation in the USA [124–126]. Approximately 3.2 million people are living with

HCV infection in the USA, with a majority of them (66 %) born in the baby-boomer generation between 1945 and 1965 [125]. The number of deaths in the US from HCV has even surpassed the number of HIV-related deaths [125]. Worldwide, it is estimated that between 130 and 170 million people are infected with HCV, with over 50,000 deaths worldwide annually as a result of HCV-related cirrhosis and hepatocellular carcinoma [127, 128]. HCV is associated with a number of different extrahepatic manifestations, including mixed cryoglobulinemia, glomerulonephritis, autoimmune diseases, porphyria cutanea tarda, non-Hodgkin lymphoma, and multiple dermatoses [129]. In addition to complications from chronic liver disease, HCV infection is associated with various glomerular diseases, and although patients with HCV infection do not have a higher overall prevalence of CKD, those who do develop CKD have an increased risk of progressing to ESRD [130, 131].

Epidemiology of Kidney Disease in HCV

The primary mode of transmission of HCV is parenteral, and subsequently renal dialysis patients represent a significant risk group for acquiring this infection, due to their exposure to blood transfusions, treatment in dialysis units, and kidney transplants [132]. Fortunately, in an effort to reduce HCV transmission, since 1992 and 1994 all donor blood and donor kidneys, respectively, are screened for HCV antibodies. Furthermore, the need for frequent blood transfusions in ESRD patients has decreased with the increased use of erythropoietin; therefore while dialysis patients are still at higher risk for HCV infection than the general population, the incidence of infection in these situations is reduced [132, 133]. However, the prevalence of HCV in dialysis patients remains approximately 7.8 % and by 2020, it is estimated that greater than 60,000 ESRD patients on dialysis will be infected with HCV [134]. Other high-risk groups include intravenous drug abusers, recipients of clotting factor concentrates made before 1987, HIV-infected patients, men who have sex with men, healthcare workers, and children born to HCV-positive mothers [124]. Six different genotypes of HCV have been described [135]. Genotypes 1 and 2 are the most common and found in a worldwide distribution, with subtypes 1a and 1b being found more frequently in American and Western European blood donors [124, 136]. The 1b subtype is thought to be the more virulent compared to genotype 2 which is associated with low viremia; southeastern Asian countries have a prevalence of subtypes 1b, 2a, and 2b [136]. Intravenous drug abusers with HCV tend to have a predominance of genotype 3, whereas genotype 4 tends to be more prevalent in Egypt and Central Africa and genotype 5a in South Africa [136]. The determination of an individual patient's HCV genotype is important as this information often guides further diagnostic testing and treatment and dictates response to therapy, as discussed later in the chapter.

The most common renal manifestation of HCV is related to mixed cryoglobulinemia, which is discussed in the further in this chapter. While only a minority of patients with mixed cryoglobulinemia (approximately 15 %) will develop end-stage renal disease ESRD [137], there is an increased mortality risk with cryoglobulinemic glomerulonephritis among these patients attributed to increased risk of cardiovascular events, liver failure, infections, and neoplasia (lymphomas) [138].

Clinical Presentation and Diagnosis of Kidney Disease in HCV

Acute HCV infection is typically asymptomatic or goes unnoticed due to nonspecific symptoms including depression, arthralgias, and fatigue [125]. Unlike HBV, where infection in adulthood rarely leads to chronic HBV, chronic HCV infection occurs in approximately 65–80 % of infected adults [129]. HCV is known to have multiple extrahepatic manifestations, with 40–75 % of HCV-infected patients exhibiting at least one [129]. HCV is the most common chronic liver disease associated with the development of kidney disease and ESRD [126, 139]. Despite the fact that the prevalence of CKD risk factors (hypertension, hyperlipidemia, diabetes) appears to be lower in HCV-infected patients, this population has been shown to have a higher incidence of rapid progression of CKD [134]. Prior to the development of CKD, the clinical symptoms of HCV renal involvement may be mild or clinically silent but when evident may include proteinuria, microscopic hematuria, acute oliguric renal failure, hypertension, or peripheral edema [126].

The most common form of HCV-associated renal disease is cryoglobulinemic glomerulonephritis. The clinical course of HCV-associated cryoglobulinemic glomerulonephritis is characterized by remissions and clinical exacerbations [138]. In these patients, cryoglobulins form and deposit in vascular endothelium of skin, nerves, and kidneys, inducing a necrotizing vasculitis in between 5 and 20 % of individuals manifesting as palpable purpura, arthritis, neuropathy, or glomerulonephritis, with the development of glomerulonephritis portending a poorer prognosis [137, 140]. The diagnosis of HCV-associated glomerulonephritis includes serologic evaluation of HCV antibodies. Renal biopsy can reveal focal capillary wall proliferation by mononuclear cell infiltration, necrotizing vasculitis with fibrinoid necrosis, and eosinophilic intracapillary thrombi, in addition to mesangial hyperplasia with subendothelial immune complex deposits of C3, IgM, and IgG [139–141]. Laboratory tests that would specifically suggest a cryoglobulinemic glomerulonephritis are the presence of low C4 complement levels and a positive

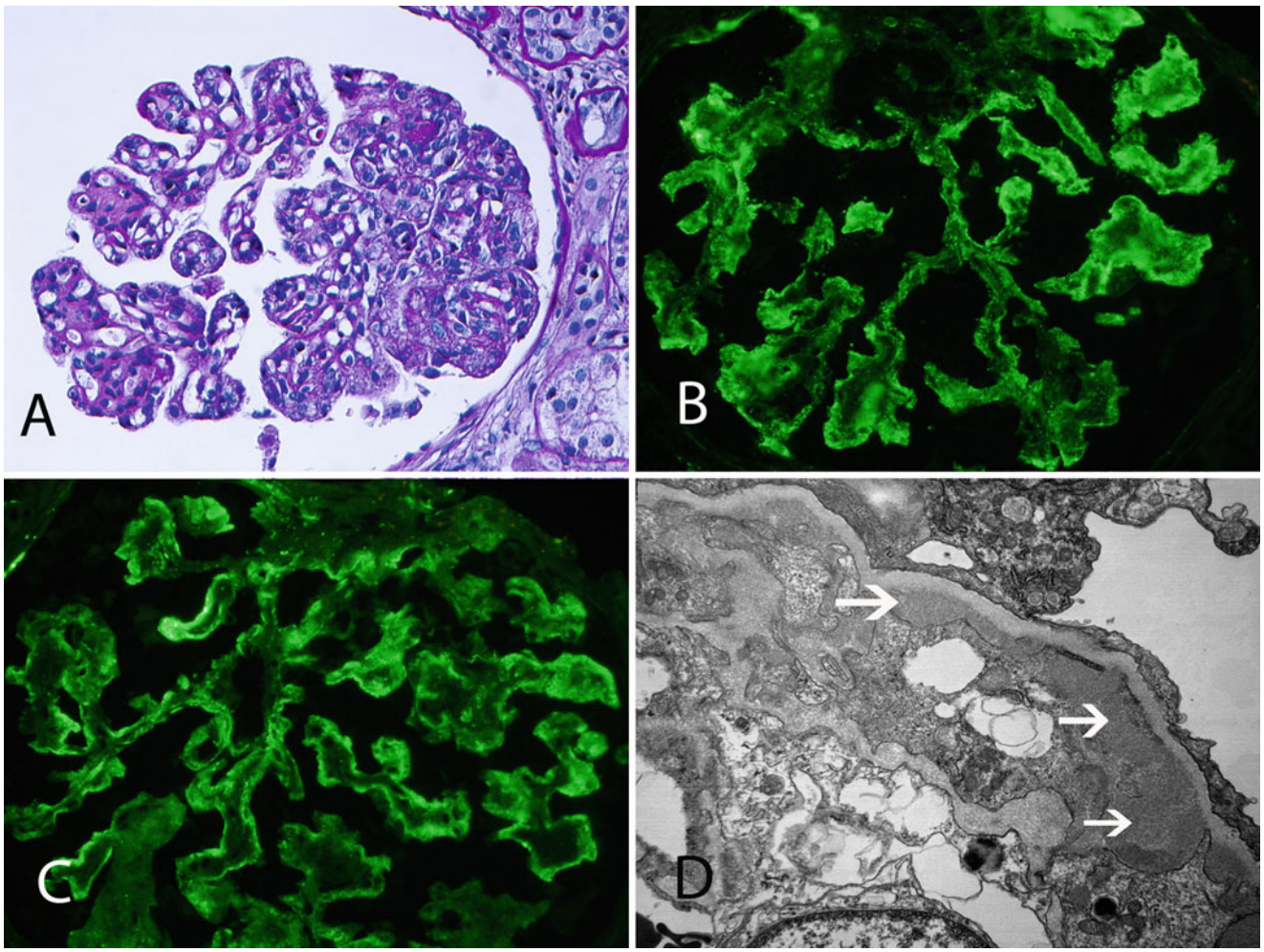

Fig. 13.3 Membranoproliferative glomerulonephritis seen with hepatitis C. (**a**) Light microscopy showing a membranoproliferative glomerulonephritis, with endocapillary proliferation, lobular accentuation of the glomerular tufts, and double contours along the capillary walls (PAS ×40), (**b**, **c**) immunofluorescence microscopy showing granular IgM (**b**) and C3 (**c**) along the capillary walls (×40), (**d**) electron microscopy showing subendothelial electron dense deposits (*white arrows*) and double contour formation (×13,500)

rheumatoid factor in an HCV-infected patient with a necrotizing membranoproliferative glomerulonephritis on renal biopsy [126, 141, 142].

Types and Pathogenesis of Kidney Disease in HCV

HCV can be associated with a variety of kidney pathology with the pathogenesis and clinical manifestations ranging from isolated proteinuria to nephritic or nephrotic syndromes. In general, HCV-related renal diseases are associated with immune complex or viral particle deposits within various parts of the glomerulus [140].

Cryoglobulinemic Glomerulonephritis

Mixed cryoglobulinemia (types II and III cryoglobulinemia) is a small and medium vessel vasculitis that is the most common form of kidney involvement due to HCV infection [126]. It is almost exclusively a membranoproliferative glomerulonephritis, as seen in Fig. 13.3, and thought to occur due to subendothelial and mesangial immune complex deposition (viral antigens, IgG, and complement), as well as deposition of the actual cryoglobulin proteins within the mesangium, glomerular capillaries, and urinary space [140, 143]. Cryoglobulins are cold-insoluble immune complexes containing IgM kappa rheumatoid factor, polyclonal IgG, HCV RNA, and/or complement. HCV has been found to be the cause of at least 80 % of mixed cryoglobulinemia [140]. Given that the majority of patients with mixed cryoglobulinemia are infected with HCV, even in the absence of evidence of liver disease, all persons with proteinuria and cryoglobulinemia should undergo screening for HCV, but it should be noted that a small (<5 %) percentage of cases may not have display antibodies against HCV [140].

Lesions Not Associated with Cryoglobulinemia

Similar to HBVAN, HCV-associated membranous nephropathy is thought to be due to the subepithelial immune complex deposits, but is not associated with cryoglobulinemia, glomerular cryoglobulin deposits, rheumatoid factor, or hypocomplementemia [129, 140]. There is a noncryoglobulinemic membranoproliferative glomerulonephritis and IgA nephropathy associated with HCV that is also due to mesangial immune complex deposition that does not appear to differ in incidence from the general population [4]. Additionally, an unusual type of glomerulonephritis called fibrillary (or immunotactoid) glomerulopathy has been described with the immune complex deposition within the mesangial and capillary walls themselves and, occasionally, crescents [4, 140]. The latter is associated with a poorer prognosis and progression to ESRD in less than 2 years [144]. Direct cytotoxic injury by the HCV has been described in HCV-associated FSGS, tubulointerstitial nephritis, and thrombotic microangiopathy [140]. It is unknown whether HCV infection causes tubulointerstitial injury via direct viral cytotoxic effect or indirectly via immune mediators, but a global glomerulosclerosis suggestive of chronic tubulointerstitial injury due to HCV has been described [145]. Through an unclear pathogenetic mechanism, chronic HVC infection is associated with insulin resistance and subsequent development of type 2 diabetes mellitus. It is thought that the viral proteins somehow interfere with insulin signaling pathways [129]. Subsequently, HCV-infected patients are at higher risk of developing diabetic nephropathy, which has the same pathogenesis and pathologic features as non-HCV-related diabetic nephropathy, including thickened basement membranes, diabetic arteriolosclerosis, hyalinosis lesions, and Kimmelstiel–Wilson nodules [129].

Kidney Disease Associated with HIV–HCV Coinfection

The association of HIV and CKD has been discussed elsewhere in this chapter. In patients with HIV, coinfection with HCV is an independent risk factor for development and progression of CKD [146]. Although the at-risk populations for HIV and HCV overlap, HCV-associated kidney disease is underreported in HIV patients because the kidney disease is similar and would typically be attributed to HIVAN [147]. Additionally, in HIV–HCV patients on HAART, the degree of immunosuppression may be adequate to suppress the formation of immune complexes, or alternatively either patients are not living long enough to develop HCV-associated disease or are developing HIVAN-mediated ESRD before HCV-associated immune complexes can form [147]. Nevertheless, coinfected patients with kidney disease typically present with nephrotic syndrome and renal insufficiency [147]. Patients with both HIV and hepatitis C infection are slightly less likely to have HIVAN and more likely to have lesions such as immune complex glomerulonephritis or membranous nephropathy [13], and coinfected patients are also at greater risk for progression of their kidney disease in spite of therapy of the HIV infection [38, 131].

Treatment of HCV-Associated Glomerular Disease

Goals of Therapy

The goals of antiviral therapy in patients with HCV are viral particle elimination and decreasing production of HCV antibodies and immune complexes [148], thereby reducing morbidity and mortality from HCV. Sustained virological response (SVR) is defined as the absence of HCV RNA from serum by a sensitive PCR assay for a period of at least 6 months following the discontinuation of therapy [148] and, if sustained, usually represents a cure of HCV infection [126, 149]. It is a class IA recommendation to perform HCV genotyping prior to initiation of anti-HCV therapy (particularly interferon therapy) because this information helps to plan type and duration of therapy as well as predict potential response [124]. Additionally, as with other causes of CKD, the supportive treatment of hypertension, edema, and proteinuria with diuretics and ACEIs or ARBs is strongly recommended [126, 135].

Pegylated-IFN-α[Alpha] and Ribavirin

IFN-α[alpha], a natural cytokine produced by immune cells in response to foreign antigens, is typically used in treatment of HCV infection. Pegylated-IFN (Peg-IFN) has a longer half-life, the same effects as interferon and exists as two formulations, PEG-IFN-α[alpha]2a and PEG-IFN-α[alpha]2b. Pegylated interferon is administered once a week instead of daily or three times a week injections required for interferon alpha [148]. Ribavirin (RBV) is an oral guanosine analog that interrupts HCV RNA replication. PEG-IFN-α[alpha] and RBV are used in combination for HCV therapy and are both excreted by the kidney, therefore both have impaired clearance in patients with decreased kidney function [148]. Subsequently, doses of both frequently need to be adjusted in patients with renal insufficiency. Common side effects of PEG-IFN include influenza-like myalgias and fatigue, myelosuppression, anorexia, diarrhea, dermatitis, alopecia, increased infection rate, and depression. The most significant side effect of RBV is a reversible hemolytic anemia which is more likely in patients with renal insufficiency [148]. Side effects are more common in general with combination therapy than monotherapy [136]. RBV is not removed by dialysis so close monitoring for adverse effects is warranted in ESRD patients on this medication, and in fact, its use is discouraged among patients with creatinine clearance <50 mL/min [135, 150]. Pregnancy and lactation are absolute contraindications to the use of this combination therapy

due to significant teratogenicity, and relative contraindications include decompensated liver disease, active psychiatric disease, and previous kidney transplantation, among others [135]. Additionally, the treatment with IFN-α[alpha] has also been reported to exacerbate cryoglobulinemic vasculitis and should be started only after an acute flare of vasculitis has been controlled with immunosuppressive agents [151]. As mentioned previously, HCV genotyping is important prior to initiation of this combination therapy to determine length of treatment and predict response rates. The best response to the PEG-IFN/RBV combination is seen among genotypes 2 and 3 HCV with at least 80 % SVR in most cases after 6 months of combination therapy [126, 149]. Genotypes 1 and 4 do not have as favorable a response to PEG-IFN/RBV, with SVR ranging from 40 to 50 % [149], but alternative therapies have been developed to address this limitation, and will be discussed further in the chapter.

Early studies of monotherapy with IFN-α[alpha] in patients with cryoglobulinemic glomerulonephritis demonstrated complete clearance of HCV RNA and improved creatinine clearance; however, the resurgence of viremia and relapse of the kidney disease were universally observed after discontinuation of IFN [152]. Subsequent studies demonstrated more favorable outcomes with PEG-IFN combination therapy with SVR rates of 60–70 % and resolution of proteinuria, hematuria, and stabilization of renal function after a 2-year observation period [153–155].

Protease Inhibitors

Newer direct-acting antiviral agents have been developed and are beginning to address the issue of suboptimal response of genotype 1 to combination PEG-IFN/RBV therapy; boceprevir and telaprevir are the only HCV protease inhibitors approved by the FDA for the treatment of HCV genotype 1 disease. They are used in combination with PEG-IFN/RBV as the new stand of care triple therapy and are equally beneficial in treatment-naïve and treatment-experienced HCV genotype 1 patients [149]. These oral drugs target the viral serine protease NS3/4A, thereby inhibiting HCV genotype 1 viral replication. Subsequently as triple therapy, they result in a significantly improved SVR compared with the traditional combination PEG-IFN/RBV therapy [149]. Barritt and Fried summarized five phase three clinical trials comparing telaprevir or boceprevir to standard of care combination therapy where the SVR was doubled in the HCV protease inhibitor group across all populations (treatment-naïve or treatment-experienced) [156].

While there are limited phase 2 data suggesting telaprevir and boceprevir activity against genotypes 2 and 3, neither is approved for use other than for HCV genotype 1 [149]. Additionally, neither drug should be used as monotherapy as it results in rapid development of viral resistance [149, 156]. HCV protease inhibitors have many advantages including shorter treatment duration for patients infected with genotype 1, thus decreasing the potential for adverse effects. Commonly reported adverse effects of the HCV protease inhibitors include the exacerbation of anemia seen with combination therapy, rash (telaprevir), dysgeusia, and anorectal symptoms [156]. Neither of the protease inhibitors require dose adjustment and can be safely used in patients with renal insufficiency and ESRD [157]. More studies are required to determine the exact effect the HCV protease inhibitors have on HCV-associated glomerular disease, but the success in achieving SVR should theoretically result in the regression of proteinuria, hematuria, and improvement of renal function, as seen in combination PEG-IFN/RBV.

A single nucleotide polymorphism (SNP) of the interleukin 28b (IL28B) gene is associated with an increased response to triple therapy and comparable response on dual oral therapy with protease inhibitor/RBV [156]. Efforts are underway to identify a serum assay testing for this SNP prior to initiation of triple therapy [156]. Additionally, second-generation HCV protease inhibitors and HCV polymerase inhibitors are being investigated in the hope of developing a completely oral, simple regimen for treatment of HCV [158]. However, until more simplified regimens are approved, in order to prevent development of HCV resistance, providers must emphasize compliance to triple therapy.

Corticosteroids and Other Immunosuppressants

The ideal treatment of HCV glomerular diseases remains combination antiviral therapy. However, cryoglobulinemic HCV patients with marked proteinuria and evidence of progressive kidney disease and/or an acute flare of cryoglobulinemic vasculitis have been treated with immunosuppressive agents. Although no randomized trials exist to support the benefits, cyclophosphamide, corticosteroids or rituximab, and occasionally plasma exchange may be required initially to treat the vasculitis and clear the cryoglobulins from the serum [126]. The goal of the course of immunosuppressive therapy is to gain control of the manifestations of the vasculitis, after which the focus should return to treating the HCV infection with combination PEG-IFN/RBV/DAA therapy [135]. While high-dose corticosteroids have been demonstrated to be helpful in patients with cryoglobulinemic vasculitis, they have significant side effects and may lead to paradoxical increases in HCV viral load [159].

Rituximab is a monoclonal antibody against the CD20 receptor on B cells that has been identified as beneficial in autoimmune diseases including membranous nephropathy and ANCA-associated vasculitis [160, 161]. The depletion of B cells producing rheumatoid factor may stop the production of cryoglobulins; therefore rituximab has shown particular utility in patients with hepatitis C and mixed cryoglobulinemia [126, 162] and has been gradually replacing cytotoxic drug therapy in such patients [163].

Rituximab is a relatively safe and well-tolerated drug, and although patients receiving it may have modest increase in HCV viremia, for others it may remain unchanged [162]. Given that symptoms may reappear with reconstitution of peripheral B cells, patients may require additional courses, but the safety profile in retreated patients is unknown [126]. Rituximab is however significantly more expensive than cyclophosphamide, and no study has directly compared rituximab with cyclophosphamide in these patients. Cyclophosphamide can still be considered a therapeutic option in patients with mixed cryoglobulinemia, especially in life-threatening situations [163].

Summary

Despite the prevalence of hepatitis C infection worldwide, few studies have been performed which assess the frequency and risk factors for kidney disease. In light of our limited understanding of the epidemiology of the glomerulonephritides other than MPGN, it is not surprising that little is known about the effect of treatment of hepatitis C on its associated kidney diseases. Given the limited safety and efficacy data for anti-HCV agents, further work is needed to determine minimally effective doses, retreatment, and viral load as a result of treatment, but particularly for the immunosuppressive therapies of plasma exchange and rituximab. Investigations on treatment of HCV and associated glomerular diseases are still evolving. Fortunately, the early data on second-generation HCV protease inhibitors and oral drug therapy is promising. The treatment of patients with HCV infection and glomerulonephritis should therefore take a multidisciplinary approach according to standard clinical practice guidelines (CPG) for HCV infection. The current standard of care is combination therapy with PEG-IFN/RBV ± telaprevir or boceprevir depending on HCV genotype. Limitations to HCV treatment remain; RBV is not recommended in patients with creatinine clearance <50 mL/min, PEG-IFN is contraindicated in pregnancy and renal transplant patients, and triple therapy must be utilized in genotype 1 patients. Treatment decisions should therefore be individualized and balance the severity of the disease and the likelihood of response to therapy with the presence of comorbid conditions and potential for serious side effects related to the treatment.

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Cytomegalovirus, BK, and Other Viral Infections of the Kidney

14

Nathan W. Cummins and M. Rizwan Sohail

Introduction

Viral infections are rare but important cause of renal disease in immunocompromised patients and can be associated with significant morbidity and mortality. Of particular interest are cytomegalovirus (CMV), polyomaviruses (BK and JC viruses), and adenovirus due to their ability to replicate in renal parenchyma. Symptomatic renal disease with these viruses is mostly seen in immunocompromised patients, such as renal allograft recipients, and is usually due to reactivation of the latent virus. Clinical manifestations are variable, depending on the degree of immune suppression, and range from mild tubulointerstitial nephritis to severe glomerulopathy resulting in transient or permanent renal dysfunction. While urinalysis may provide important diagnostic clues, the confirmation of viral infection of the kidney usually requires renal biopsy coupled with nuclear acid amplification tests, in situ hybridization, or electron microscopy. Management of renal viral infections usually involves reduction in immunosuppression with or without targeted antiviral therapy.

In this chapter, we will review some of the important viral causes of viral nephritis and provide overview of the pathogenesis, clinical manifestations, diagnostic techniques, and management strategies.

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Cytomegalovirus

Cytomegalovirus (CMV), otherwise known as human herpes virus-5 (HHV-5), is one of the eight herpes viruses known to cause disease in humans [1]. CMV contains a double-stranded DNA genome enclosed in an icosahedral nucleocapsid, surrounded by an outer membrane derived from host cells that express viral glycoproteins. Clinical presentation and severity of CMV infection is largely dependent on the competence of the host immune response. CMV infection in an immunocompetent host is typically a self-limited mononucleosis-like syndrome and rarely causes parenchymal kidney disease. However, in the immunocompromised host, such as the solid organ or hematologic transplant recipient, CMV infection can be life threatening and may involve the kidney parenchyma, potentially leading to temporary or irreversible kidney dysfunction.

Life Cycle and Pathogenesis

CMV exhibits both lytic and latent phases of infection. During lytic infection, active viral replication results in host cell death and release of viral progeny. Infectious CMV particles attach to cell surface receptors, including PDGFRα[alpha], $\alpha[alpha]_2\beta[beta]_1$, $\alpha[alpha]_6\beta[beta]_1$, and $\alpha[alpha]_v\beta[beta]_3$ on the host cell membrane, and are internalized either by endocytosis or by direct membrane fusion. The viral nucleocapsid is then transported to the nucleus, where the linear viral DNA circularizes and viral replication begins. Viral genes are transcribed in the nucleus, and proteins are produced in the cytoplasm. Finally, the progeny nucleocapsids are transported from the nucleus to the cell membrane and then exit the cell. During latent phase, when virus primarily resides in monocytes, macrophages, and CD34+ cells, little or no viral protein expression occurs. Reactivation of latent viral genomes in immunocompromised host leads to the active production of infectious virions.

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Organ- and tissue-specific diseases occur when CMV-infected cells undergo the lytic phase of infection. Because CMV can infect a wide range of cells in the body, CMV can be associated with encephalitis, myelitis, retinitis, esophagitis, pneumonitis, gastritis, colitis, pancreatitis, hepatitis, bone marrow suppression, as well as nephritis. Based on in vitro studies, it is likely that multiple cell types within the kidney can be infected. In one study, CMV virions were detected in glomerular epithelial cells, mesangial cells, tubular epithelial cells, and endothelial cells [2]. While multiple cell types in kidney parenchyma can get infected with CMV, viral replication is not supported by all cell types. In an in vitro study, human glomerular epithelial cells infected with CMV permitted viral adsorption, penetration, nuclear translocation, and restricted viral transcription, but not productive infection, while tubular epithelial cells alone permitted productive infection [3]. In a different in vitro model, mixed fetal kidney cortex cell cultures supported productive CMV infection. Immunofluorescence and in situ hybridization techniques identified mesangial cells as the specific cell type supporting CMV infection in that model [4]. While these in vitro studies indicate that CMV has the potential to infect multiple cell types within the kidney, this replicative ability may be context dependent. This may help to explain why in biopsies of in vivo infected kidney tissue, tubular epithelial cells are the most frequently involved.

Human kidney cells infected with CMV in vitro are relatively resistant to virus-induced cell death, with cell death not occurring up to 55 days after infection despite active viral replication in the tubular epithelial cells [3]. CMV infection of cultured proximal tubular epithelial cells results in increased PTEC expression of ICAM-1, in addition to MHC class I antigen [5]. As a result, the tissue damage that occurs during CMV infection of the kidney may be more immune mediated than viral mediated.

Incidence

The majority of population is exposed to CMV during infancy, and primary infection is often asymptomatic, or associated with a mild, nonspecific, febrile illness with or without a rash. CMV is shed from oral mucosa, often by asymptomatic carriers, and transmission is usually through saliva. However, primary CMV infection can also be acquired through sexual contact, vertical transmission, organ transplantation, and blood transfusions. Once the acute infection subsides, latency is established, and the only manifestation of ongoing latent infection is the presence of specific anti-CMV antibodies. While the seroprevalence of CMV in children ranges from 10 to 30 %, seropositivity in adults ranges from 40 to 80 % and is even higher in the developing world.

Reactivation of latent CMV generally occurs in the setting of immunosuppression or significant physiologic stress, such as critical illness [6]. Reports of kidney involvement during primary or reactivation CMV infection are, in large part, limited to case reports and case series. The reactivation of CMV disease in immunocompromised individuals is not a reportable disease. Therefore, incidence of CMV-induced renal disease—while likely quite low—is difficult to estimate.

Clinical Manifestation and Diagnosis

The clinical, laboratory, and pathologic manifestations of CMV infection of the kidney are highly variable and largely dependent on the immune competence of the infected host.

Immunocompetent Hosts

Most commonly reported renal manifestation of CMV infection in an immunocompetent host is that of acute interstitial nephritis. Patients with renal disease may not necessarily have other recognizable symptoms of CMV infection, such as fever, fatigue, anorexia, lymphadenopathy, and leucopenia. Even in immunocompetent patients, severity of CMV renal disease is highly variable. For instance, Platt et al. reported kidney biopsy results from two infants with neonatal CMV infection [7]. Both patients had positive urine viral cultures for CMV, but serum creatinine was normal. Interstitial nephritis, characterized by focal mononuclear cell infiltrates and mild mesangial hypercellularity, but not glomerulopathy, was seen on light and electron microscopy. The characterization of the infiltrate revealed mainly CD3+ and CD8+ cells. Intranuclear and intracytoplasmic inclusion bodies were seen in one but not both patients. In another report, Kaminska et al. described a case of a healthy 15-year-old girl who presented with mildly elevated serum creatinine (1.1 mg/dl), anemia, hypergammaglobulinemia, and elevated serum inflammatory markers. Kidney biopsy revealed interstitial nephritis, and CMV antigens were detected in tubular epithelial cells on biopsy [8]. Matsukura described a previously healthy 14-year-old boy who presented with mild acute renal dysfunction [9] and kidney biopsy revealed lymphocytic tubulointerstitial nephritis with tubular epithelial lesions and intratubular hyaline casts. However, glomeruli and blood vessels were normal. In another case report, focal segmental glomerulosclerosis with viral inclusion bodies, consistent with CMV infection, was described by Chirumamilla et al. in a 15-year-old girl with ulcerative colitis, who presented with acute renal failure [10].

Kidney Transplant Recipients

Renal allograft recipients represent the most commonly reported host with CMV nephritis. However, its true incidence is unknown and the clinical manifestations are highly variable.

CMV infection of the renal allograft usually occurs within the first 6 months after transplantation but has been reported to occur up to 21 months after transplantation [11]. In a single-center study of 100 consecutive renal allograft biopsies, CMV DNA was detected by in situ hybridization in 41 cases [12]. CMV was predominately present in the proximal tubular epithelial cells, and only one patient had CMV in the glomerular cells. Typical owl-eye inclusions were not present in any of the cases. Presence of CMV in biopsy specimens had no correlation with clinically active infection at the time of biopsy. In another single-center autopsy series of 80 renal transplant patients over a 17 year period, 19 patients had CMV infection [13]. Six of these cases had CMV infection of the kidney, and infection was limited to the kidney in two cases, whereas other four cases had evidence of other organ involvement. Four of these six cases had evidence of acute tubulointerstitial nephritis, while one patient had CMV inclusions in the glomeruli. In the largest single-center study to date [14], 540 needle biopsies in 280 renal transplant recipients and 23 (8 %) patients were found to have acute tubulointerstitial nephritis (TIN). However, TIN was attributed to CMV infection in only three of these patients. All three of these patients lost their grafts by 31 ± 3.1 months.

Besides tubulointerstitial disease [15, 16], other pathologic manifestations of CMV nephritis include vasculopathy and glomerulopathy. In many cases, intranuclear inclusion bodies and intracytoplasmic herpes-type viral particles are seen on renal biopsy (Figs. 14.1 and 14.2). Castro et al. observed frequent glomerular pathology in 20 allograft recipients with poor renal function (serum creatinine >2.0 mg/dl) in the setting of CMV infection [17]. All patients showed some degree of glomerular alteration, and vascular changes were predominating in seven cases. Glomerulopathy was associated with IgM and C3 deposits in five of the six cases. On the contrary, in the next published series to address the presence of CMV glomerulopathy [18], Herrera et al. found no evidence of CMV in the kidneys (by light or electron microscopy and immunofluorescence studies) from seven renal transplant patients who had clinical parameters suggestive of CMV infection and concern for CMV glomerulopathy. Instead, six of these patients were found to have acute cellular rejection. Only two of the seven patients in this report had proven CMV nephritis. However, glomerulopathy was not observed in either of these patients. Similarly, lack of glomerular disease was reported in a series of 32 biopsies of kidney transplant patients with active or inactive CMV infection by Battegay et al. [19]. Instead, the presence of glomerulopathy was associated with rejection. However, some patients may have concomitant acute rejection and CMV nephritis, and this has been reported in the literature [20].

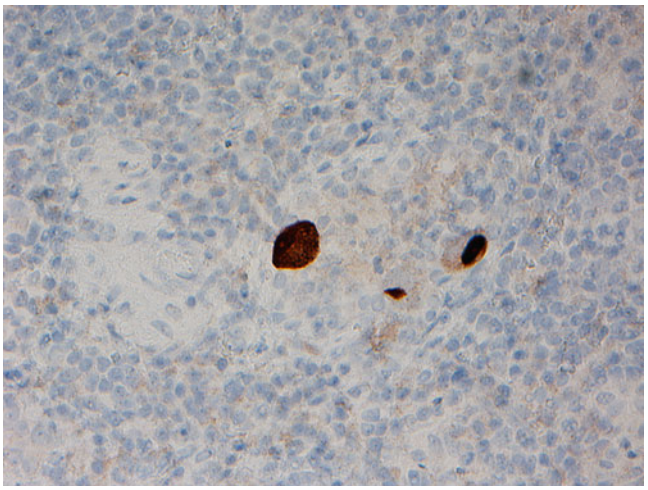

Fig. 14.2 Immunohistochemistry (immunoperoxidase staining) showing CMV-infected cells

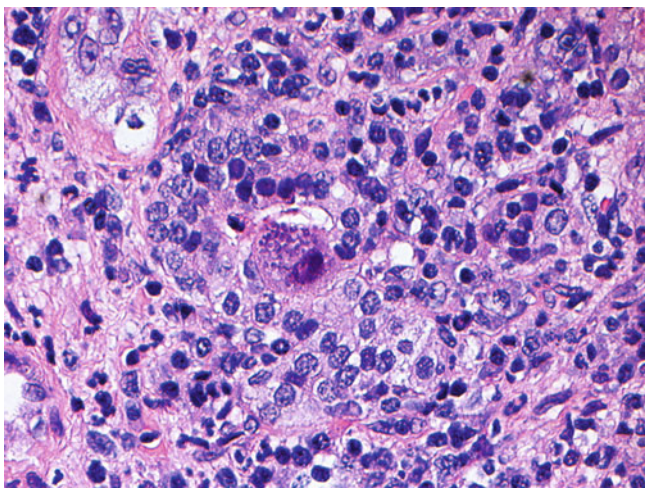

Fig. 14.1 Renal biopsy showing a large cell with CMV inclusions and surrounding interstitial inflammation (H&E, 60×)

Other Immunocompromised Hosts

Besides renal transplant recipients, CMV-associated tubulointerstitial nephritis has been reported in an allogeneic stem cell transplant patients. These patients typically present with fever and hematuria, and kidney biopsy usually reveals focal necrosis of the tubules, with acute and chronic inflammatory infiltrates around the tubules. Intracellular inclusions can be seen in the tubular cells, and immunofluorescent staining for CMV antigens is frequently positive in the degenerating tubular epithelium [21] (Figs. 14.1 and 14.2). However, the frequency of CMV nephritis in stem cell transplant recipients, even in the setting of active CMV infection, is unlikely to be high [18]. In an autopsy series of 33 immunocompromised patients with manifest CMV infection at the time of death [19], CMV was detected in the kidney in only eight patients. Moreover, characteristic "owl's eyes" cells were present in three cases only. All the patients in this study had evidence of CMV involvement of other organs aside from the kidney.

Treatment and Outcomes

The prognosis of CMV nephritis depends on multiple factors, including but not limited to the immune competence of the host, the presence of preexisting kidney disease, concomitant CMV disease involving other organs, and whether the infection is in a native kidney or renal allograft. It is well known that CMV disease, even if outside of the kidney in renal transplant recipients, is associated with an increased risk of allograft rejection. Moreover, active CMV disease is associated with increased risk of other opportunistic infections. Some renal transplant recipients with CMV-infected allografts may eventually lose all allograft function. Besides, CMV infection of the renal allograft may be accompanied by allograft vasculopathy.

Based on above considerations, we believe it is prudent to treat CMV infection of the kidney similar to other end-organ CMV disease in the immunocompromised hosts. The cornerstone of CMV treatment is antiviral agents with good anti-CMV activity, including ganciclovir, valganciclovir (the valyl ester prodrug of ganciclovir), cidofovir, and foscarnet [22]. However, drug-induced bone marrow suppression and nephrotoxicity are major limiting factors in using these antiviral agents in CMV disease. Therefore, CMV nephritis is best managed in consultation with an infectious diseases specialist.

Treatment benefit of CMV therapy in immunocompetent hosts is questionable. In the few reported cases of CMV-associated interstitial nephritis in immunocompetent hosts, the renal dysfunction is usually self-limited. Therefore, antiviral agents are usually not prescribed and are unlikely to be beneficial in the majority of these cases.

BK Virus

BK is a small (30–45 mm), nonenveloped, double-stranded DNA polyomavirus belonging to Papovaviridae virus family. BK virus was first isolated from the urine of a kidney transplant recipient in 1971 [23]. Polyomaviruses are ubiquitous and primary infection is usually acquired in the early childhood or adolescence via oral or respiratory exposure. Majority of the primary infections are asymptomatic in children. However, mild upper respiratory tract infection symptoms have been reported in up to 30 % of the immunocompetent patients with primary BK virus infection. By adulthood, 60–80 % of the population in the USA is seropositive for BK virus. However, BK virus can remain latent in lymphoid cells, renal tubular cells, and epithelial cells and can reactivate in immunocompromised host to cause symptomatic disease.

BK virus has tropism for urinary epithelium and has been implicated in three distinct genitourinary diseases in immunocompromised patients [24]. These include tubulointerstitial nephritis, ureteral stenosis, and hemorrhagic cystitis. While tubulointerstitial nephritis and ureteral stenosis are mostly seen in renal transplant recipients, hemorrhagic cystitis is typically described in bone marrow transplant patients. Non-renal infections attributed to BK virus in immunocompromised hosts include rare cases of hepatitis, pneumonitis, meningitis, vasculitis, and retinitis [25].

Risk of BK virus reactivation and symptomatic disease depends on degree of immunosuppression [26]. Patients with allogenic stem cell transplant are higher risk of reactivation disease and hemorrhagic cystitis compared to patients with autologous stem cell transplant (50 % vs. 15 %). Reported rate of BK nephritis in kidney transplant patients ranges from 1 to 10 % [27]. Infection of native kidneys in patients with other organ transplants, such as liver or lung, is rare.

Risk factors for BK virus nephropathy [28] are summarized in Table 14.1.

JC virus, another DNA polyomavirus belonging to Papovaviridae virus family, can cause a similar illness in immunocompromised patients [29]. However, chronic inflammation and fibrosis are more pronounced in renal disease due to JC virus [30].

Clinical Manifestations

The clinical presentation of BK virus reactivation disease involving kidneys is nonspecific. Typically patients with BK allograft infection present 10–13 months after transplant

Table 14.1 Risk factors for BK virus nephropathy

Age (young children or elderly)
Male gender
White ethnicity
Diabetes mellitus
Degree of immunosuppression (allogenic stem cell transplant recipients are at higher risk compare to patients with autologous bone marrow transplant. Similarly, patients with kidney transplant who are receiving combination therapy with multiple agents such as mycophenolate mofetil, tacrolimus, cyclosporine, or azathioprine are at higher risk)
Ureteral trauma
Organ donor with BK virus seropositivity
Absence of HLA-C7 in donor and recipient

(range 6 days to 5 years) [31]. Asymptomatic viruria and viremia usually precede symptomatic BK nephropathy. Patients usually present with fever and subacute renal failure due to interstitial nephritis. BK virus is clinically indistinguishable from allograft rejection. BK virus related hemorrhagic cystitis in bone marrow transplant patients usually presents with fever and microscopic or macroscopic hematuria.

Diagnosis

Differentiating BK virus nephropathy from allograft rejection in kidney transplant patients can be quite difficult. While ureteral stenosis can be identified on ultrasound or computed tomography (CT) imaging, the diagnosis of interstitial nephritis requires a combination of urinalysis, molecular testing, and frequently renal biopsy.

Seropositivity to BK virus is very prevalent in the general population, and therefore, serology is not helpful in establishing the diagnosis of reactivation disease in transplant patients [32]. However, seropositive individuals tend to have less severe disease [33].

Urinalysis typically reveals pyuria, microscopic hematuria, cellular casts, and renal tubular cells, suggestive of interstitial nephritis. Cytology may be helpful in detecting viral shedding in the urine. Decoy cells, termed due to their resemblance to renal carcinoma cells, may be seen on urine microscopy [31]. These are characterized by enlarged nucleus with a single large basophilic intranuclear inclusion. Although decoy cells may be observed with cytomegalovirus (CMV) infection, viral inclusions are cytoplasmic and not intranuclear in CMV-infected cells [34]. Presence of white blood cells in urine and >10/cytospin decoy cells has high correlation (up to 70 %) with biopsy-proven BK interstitial nephritis [35].

Urine electron microscopy (EM) may reveal cast-like three-dimensional polyomavirus aggregates, termed Haufen. Presence of Haufen has high correlation with BK virus nephropathy (99 %) [36]. Although EM may help to differentiate between asymptomatic viral shedding and active BK infection, it is not readily available at most centers, is labor intensive, and needs experienced eyes for appropriate interpretation.

Polymerase chain reaction (PCR) testing for BK virus in plasma and urine is perhaps the most helpful molecular diagnostic tool available in routine clinical practice. Prolonged and high-grade viremia usually precedes and has good correlation with BK virus nephropathy. Therefore, plasma PCR to detect BK viremia has high sensitivity (100 %) and specificity (88 %) with renal disease [37]. With 98 % negative predictive value, absence of sustained DNAemia makes BK nephropathy highly unlikely [38].

Quantitative blood PCR is also useful for monitoring response to therapy in transplant patients. However, it should be noted that 1–2 log change in viral load is not significant and trends, rather than single values, are important in making critical treatment decisions. PCR on kidney biopsy specimens is not helpful because it can detect latent virus in asymptotic individuals and is not helpful in making treatment decisions [39].

Considering that most patients with BK nephritis have viral shedding in the urine, one practical approach to make the diagnosis is to start from BK virus PCR from urine sample. If urine PCR reveals >10 [7] cop/mL, one should proceed with plasma testing. In patients with blood BK viral load of >10 [4] cop/mL and evidence of allograft dysfunction, it is reasonable to make a presumptive diagnosis of BK virus nephritis and treat accordingly. However, renal biopsy is frequently needed to accurately differentiate between BK nephritis and allograft rejection [40].

Viral cultures are cumbersome and only performed in research setting. Their role in clinical practice is yet unproven [41].

Histologic features of BK nephropathy are quite variable and range from minimal changes to patchy tubulointerstitial inflammation and graft sclerosis [42]. Although BK nephritis has certain characteristic features (Table 14.2) [24, 26, 35, 40, 42–44], these are not pathognomonic and can be found in other viral infections such as adenovirus, CMV, and HSV. However, location of viral inclusions may provide important diagnostic clues towards viral etiology of underling nephritis. While cells infected with BK virus have intranuclear inclusions, CMV-infected cells have cytoplasmic and HSV results in both intranuclear and cytoplasmic inclusions (Fig. 14.3).

Both BK virus and allograft rejection can cause severe tubular inflammation. In general, the presence of endarteritis,

Table 14.2 Characteristic histologic features of BK virus nephropathy

Interstitial, mononuclear or neutrophilic, infiltrate at the site of infection
Evidence of tubulitis with lymphocyte permeation of tubular basement membrane. Other features indicative of tubular injury include tubular cell apoptosis, cell dropout, desquamation, and flattened epithelial lining
Variation in nuclear size (anisonucleosis), hyperchromasia, and chromatic clumping of infected cells
Intranuclear basophilic viral inclusions without a surrounding halo
Electron microscopy may reveal BK viral particles

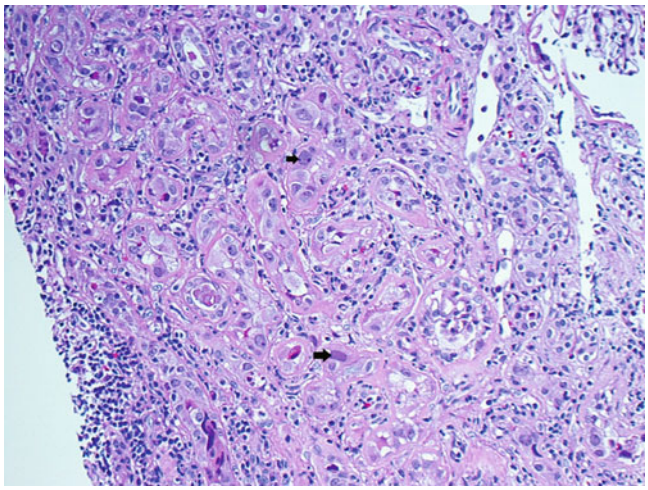

Fig. 14.3 Renal biopsy revealing BK virus (*black arrows*)-infected cells (H&E, 40×)

fibrinoid vascular necrosis, glomerulonephritis, and C4d deposits along peritubular capillaries is more suggestive of allograft rejection [26]. However, vasculopathy has been occasionally reported with BK infection and does not exclude BK nephritis.

Due to focal nature of the disease, diagnosis of BK nephropathy may be missed in one-third of renal biopsy specimens. Therefore, examination of two core biopsy samples (including medulla) may help to increase the yield. In situ hybridization for BK virus can improve the diagnostic yield of renal biopsy (Fig. 14.4). Repeat biopsy may be needed where pathologic findings on first biopsy are inconclusive.

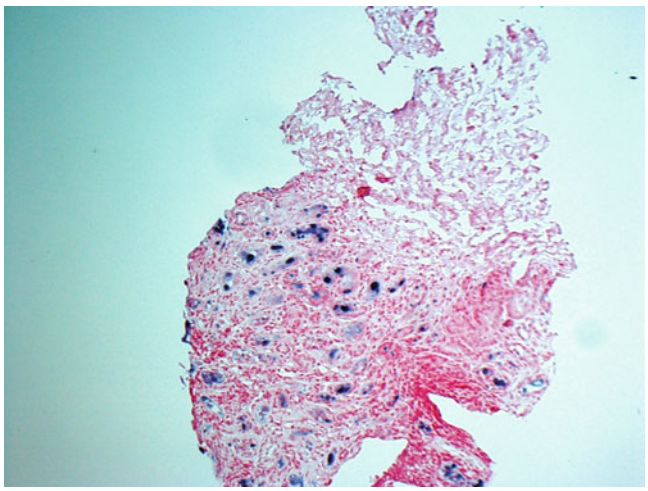

Fig. 14.4 Renal biopsy with in situ hybridization for BK virus showing infected cells (40×)

Treatment

BK nephritis is associated with significant morbidity and leads to allograft failure in 15–50 % of the infected patients [28, 31]. The key to appropriate management of BK nephritis is to accurately differentiate between allograft rejection and BK nephritis, as treatment is diametrically opposite. While increasing immunosuppression is the warranted in acute rejection, reduction in immunosuppressive therapy is the cornerstone of BK nephritis.

Treatment of BK virus nephritis in renal transplant patients can be approached in two different ways [45, 46]. First option is periodic monitoring for BK viremia and preemptive treatment based on rising BK viral load. Optimal interval and duration of screening is unclear but checking BK viral load every 3 months for the first 2 years after renal transplant may be a reasonable approach [26]. Some experts recommend closer monitoring (monthly BK-PCR) for the first 3 months as majority of the patients with BK renal disease develop viremia in the first 3–4 months after renal transplant [47]. The alternative approach is to reserve therapy for patients who develop allograft dysfunction and have biopsy-proven BK nephritis. There is some evidence to suggest that periodic surveillance and preemptive treatment may be a more effective approach [48].

Treatment of BK nephritis includes (a) reduction in immunosuppression, (b) use of intravenous immunoglobulins (IVIG), and (c) antiviral drugs. In patients who have increasing level of BK viremia without allograft dysfunction, reduction in immunosuppression alone may be adequate. This usually involves stopping the antimetabolic agent (such as azathioprine or mycophenolate mofetil) and reducing the dose of calcineurin inhibitor (cyclosporine). However, for patients who have biopsy-proven BK nephritis that is unresponsive to maximal reduction in immunosuppressive therapy, antiviral therapy may be necessary [47].

Unfortunately, antiviral drugs have limited activity and efficacy against BK virus. Drugs with known activity against BK virus include quinolones, leflunomide, and cidofovir [49]. Quinolone therapy has been shown to reduce BK viremia in transplant patients [50]. Considering that quinolones are fairly nontoxic, these should be tried first, with or without IVIG [51]. Pooled IVIG have neutralizing antibodies against BK virus and can be helpful, especially in patients who have hypogammaglobulinemia. Quinolones and IVIG can be used in combination. Use of IVIG therapy has the additional benefit of treating concomitant allograft rejection [52]. This is particularly important in cases where it may be difficult to differentiate between cellular rejection and BK nephritis.

Antiviral drug, leflunomide, inhibits BK virus replication in vitro and had limited success in the treatment of BK nephritis in a small study in transplant patients [53]. However, more data is needed before it can be used in routine clinical practice. Cidofovir, approved for CMV retinitis, is also active against BK virus. There is limited data to support its benefit in BK nephritis with improved allograft survival [54]. However, cidofovir is quite nephrotoxic [55] and its use should be limited to refractory cases. In cases where treatment is strongly indicated, use of lower doses (1 mg/kg every other day or three times per week) instead of standard dose of 5 mg/kg weekly should be considered to reduce risk of nephrotoxicity. Patients should be aggressively hydrated before and after each infusion. (Please see the section on treatment of adenoviruses for details.)

Hantavirus

Hantavirus is an enveloped, negative-sense, RNA virus that belongs to the Bunyaviridae family of viruses. Twenty species of hantavirus exist in nature and 11 have been reported to cause disease in humans. Among these, most human infections are cause by Hantaan, Puumala, Seoul, Dobrava, Sin Nombre, and Andes viruses.

Hantaviruses have worldwide distribution. Humans usually acquire hantavirus infection by inhalation or contact with secretions, urine or feces, of infected rodents [56]. Activities that may predispose to such exposures include farming, forestry, animal trapping, military activity, crisis conditions, and camping. Mice and rats are the most commonly implicated rodents in human cases. In the USA, most cases are reported from the southwestern states including New Mexico, California, Washington, and Texas and are due to Sin Nombre virus, which is usually associated with cardiopulmonary disease [57]. However, cases of hemorrhagic fever with renal syndrome have also been reported in the USA [58]. Epidemics of hantavirus infection from laboratory exposure have also been reported.

Clinical Manifestations

Typical incubation period of hantaviruses ranges from 2 to 4 weeks. Fever, malaise, and abdominal or back pain are the first signs of illness in most cases. However, infection can rapidly progress from fever to hypotensive shock and acute renal failure. Severity of illness partly depends on the causative species of hantavirus [59]. Severe renal disease is mostly caused by Hantaan and Dobrava viruses [60]. This was previously termed as nephropathia epidemica, epidemic hemorrhagic fever, and hemorrhagic nephrosonephritis. However, these cases are now collectively referred to as *hemorrhagic fever with renal syndrome* or HFRS based on World Health Organization (WHO) classification [56]. Puumala virus related renal disease is usually mild and carries good long-term prognosis [61].

Sin Nombre virus, the most common species of hantavirus in the USA, usually begins with fever, flushing, and pharyngitis but can quickly progress to acute respiratory distress and heart failure [62]. Respiratory failure in acute hantavirus infection is caused by noncardiogenic pulmonary edema due to diffuse capillary leak syndrome. Severe disease with this viral strain is termed *hantavirus-related cardiopulmonary syndrome* (HCPS). However, organ involvement is not limited to heart or lungs and renal dysfunction is frequently reported. Low-grade proteinuria and hematuria are found in up to 50 % of the cases and are usually associated with moderate increase in serum creatinine value [63]. Severe renal disease is less common with this particular species of hantavirus.

The clinical presentation of severe hantavirus infection closely mimics septic shock with fever, hypotension, and acute renal failure. These manifestations are primarily caused by increased vascular permeability leading to decreased peripheral vascular resistance and increased cardiac output [64]. As a result, there is poor renal perfusion and abrupt decline in glomerular filtration rate (GFR). Disseminated intravascular coagulation (DIC) is a complication of severe hantavirus infection and manifests as hemorrhage, petechiae, ecchymoses, hemoptysis, hematemesis, and melena.

Besides poor perfusion, hantavirus infection may also result in direct renal injury. Tubular and interstitial damage can be caused by cytokines and other humoral factors including tumor necrosis factor (TNF) [65]. Renal manifestations of hantavirus infection may vary based on genetic predisposition. Published data suggest that HLA-B8 and DR3 alleles are associated with severe renal failure [66], frequently requiring hemodialysis, where patients with HLA-B27 are more likely to have milder disease [67].

Differential diagnosis for hantavirus-related renal disease is broad and includes leptospirosis, renal failure to nonsteroidal anti-inflammatory drugs (NSAIDs), and vasculitis (Wegener's granulomatosis, Goodpasture's disease, etc.).

Diagnosis

Laboratory findings in acute hantavirus infection are quite nonspecific and may include leukocytosis, thrombocytopenia, high C-reactive protein (CRP), increased LDH, elevated liver enzymes, and elevation in serum creatinine [68]. Chest X-ray may reveal pleural effusion, atelectasis, and interstitial infiltrates in patients with HCPS. Abnormal EKG and echocardiographic

findings are frequently reported in severe cases [69, 70]. Abdominal ultrasound may reveal an increase in the length of kidneys, restrictive indices, and peritoneal fluid collection [71].

Urinalysis typically reveals marked proteinuria (mean 2.6 g/day) [72]. Up to 25 % of the patients may present with nephrotic-range proteinuria. Although microscopic hematuria is present in 50–85 % of the cases [73], depending on the infecting strain, macroscopic hematuria is uncommon and is usually seen in the setting of severe thrombocytopenia.

Diagnosis is usually confirmed by serologic testing. Available serologic tests include enzyme-linked immunosorbent assay (ELISA), strip immunoblast test (SIA), Western blot, indirect immunofluorescence (IFA), complement fixation, hemagglutinin inhibition, and plaque reduction neutralization assays. IgM antibodies, to nucleocapsid or N antigen, are usually positive by the time of admission [74]. Many patients will also have positive IgG at the time of initial presentation. Acute infection can be confirmed by either presence of IgM antibodies or four field rise in IgG titer in convalescent sample 3–4 weeks later.

Sin Nombre virus can also be detected by RT-PCR (reverse transcription-polymerase chain reaction) using peripheral blood mononuclear cells (PMBC) or serum during early stages [75]. However, viremia is typically short lived and a negative test does not exclude the diagnosis.

Kidney biopsy is generally not necessary in making the diagnosis of acute hantavirus infection. Majority of reports describing histopathologic findings in acute hantavirus infection are from autopsy series. Typical histologic features include tubulointerstitial nephritis with mononuclear cells and CD8 lymphocytes infiltration. Congestion and dilatation of the medullary vessels is frequently observed [76]. Hantavirus typically localizes in the glomerular capillary endothelium, and its presence in the renal tissues can be confirmed by immunohistochemistry using antibodies to viral N antigen [77]. The N antigen stains in punctate pattern in the cytoplasm. Nested RT-PCR is another methods to detect the virus in frozen or fixed, paraffin-embedded tissues [78].

Treatment

Treatment for hantavirus infection is mostly supportive care. Platelet transfusion may be necessary in severe hemorrhagic shock. Also, patients with several renal failure may require hemodialysis. NSAIDs should be avoided as they can exacerbate the renal injury. Patient should be closely monitored in critical care for electrolyte imbalance, heart failure, and risk of cardiac arrhythmias. With improvements in supportive care, mortality in HFRS has reduced from 15 % in 1960s to <5 % in developed countries.

There is no proven, effective, and specific antiviral therapy for hantavirus infection. Although intravenous ribavirin use has been studied in the clinical trials, results have been variable and are not considered the standard of care at present [79].

Prognosis

The severity of hantavirus-related illness at initial presentation does not predict long-term sequelae [80]. The majority of the patients who survive the acute phase recover completely, and renal function returns to the baseline over time [61]. Acute infection is frequently followed by a diuretic phase that may last for 10–14 days. Thereafter, convalescent period may last for several weeks. However, a minority of patients may develop chronic renal insufficiency [81].

Adenovirus

Adenoviruses are medium-sized (90–100 nm), nonenveloped viruses that belong to family Adenoviridae. Adenoviruses were first isolated in 1953 from human adenoids (hence the name). These viruses contain a linear, non-segmented, double-stranded DNA genome.

Adenoviruses have worldwide distribution. Over 50 serogroups of adenovirus are known to cause disease in humans. These viruses primarily spread by respiratory droplets. However, virus can survive in environment and surfaces for prolonged periods of time. Young children usually acquire infection at day care centers or schools. Adults may contract infection from children at home. Adenoviruses may stay latent in liver or kidneys and infection can be occasionally transmitted by organ transplantation as well [82].

Clinical Manifestations

Respiratory tract is the most common site of infection with adenoviruses, and manifestation may include pharyngitis, croup, bronchitis, pneumonia, and otitis media [83]. However, infection of other organs is also well described and includes conjunctivitis, gastroenteritis, and viral exanthem. Renal involvement is a rare manifestation of adenovirus infection and is usually reported in immunocompromised children and adults. Specifically, hemorrhagic cystitis and tubulointerstitial nephritis have been reported in bone marrow and solid organ transplant recipients [84, 85]. Other manifestations of adenovirus infection in immunocompromised individuals include pneumonia, colitis, hepatitis, and encephalitis [86].

Adenovirus disease in immunocompromised hosts may result from primary infection, reactivation of latent infection, or transmission from the allograft in transplant recipients.

Majority of infections in transplant patients are reported 1.5–2 months following transplant, suggesting reactivation disease [87].

Adenovirus has been implicated in the occasional cases of necrotizing, tubulointerstitial nephritis in bone marrow, and solid organ transplant patients [84]. Presence of concomitant hemorrhagic cystitis in many bone marrow transplant patients suggests that renal infection may occur by ascending route from the bladder in this group of patients. This particular syndrome is almost exclusively associated with subgroup B types 11, 34, and 35. Renal transplant patients typically present with allograft dysfunction [84].

Diagnosis

A number of diagnostic tests are available to confirm adenoviral infection, and these include serologic tests, viral antigen detection assays, PCR, and viral cultures. Choice of a particular test depends on the site of the infection and the infected host.

Due to high prevalence of anti-adenovirus antibodies in the general population and numerous cross-reactions between related serotypes, it is important to document a fourfold or greater rise in antibody titer between acute and convalescent sera to confirm the diagnosis of acute adenoviral infection. However, serologic tests are of limited value in immunocompromised patients as majority of infections in this population are due to reactivation disease.

Quantitative blood PCR is probably the most helpful diagnostic tool for confirming adenoviral infection in immunosuppressed patients [88]. Besides detecting active viral replication, rising viremia indicates invasive disease and is usually associated with higher mortality [89]. Viral load may also be helpful in monitor response to therapy. In one study, a greater than tenfold drop in the viral load after 1 week of cidofovir treatment was associated with favorable outcome, whereas lack of significant reduction in the viral load was associated with increased mortality [90].

Kidney biopsy in patients with allograft infection due to adenovirus may reveal characteristic intranuclear inclusion bodies (Fig. 14.5) [91]. Occasionally, adenovirus inclusions may be confused with CMV. However, unlike CMV, adenovirus infection does not result in intracytoplasmic inclusions or in multinucleated giant cell formation. The presence of adenovirus in biopsy specimen can be confirmed by immunofluorescent examination using an anti-adenovirus antibody or in situ hybridization (Fig. 14.6). Electron microscopy findings include characteristics icosahedral visions that typically form large paracrystalline aggregates with the nuclei of infected cells [92].

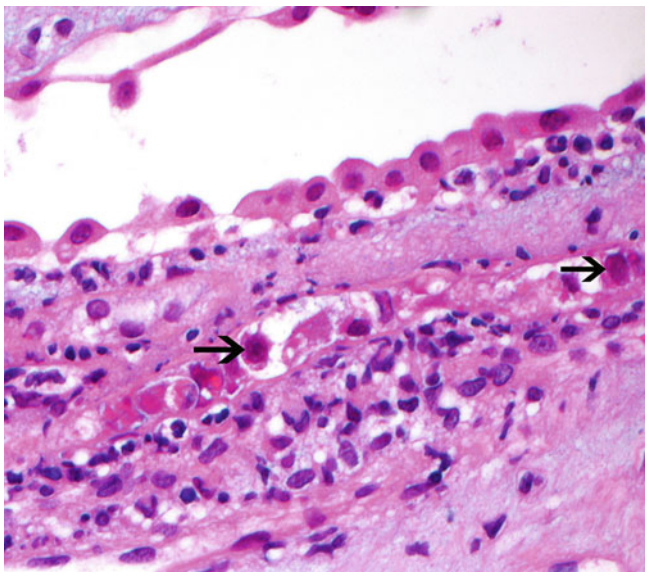

Fig. 14.5 Tubular epithelial cells with large intranuclear inclusions (*black arrows*) of adenovirus (H&E, 40×)

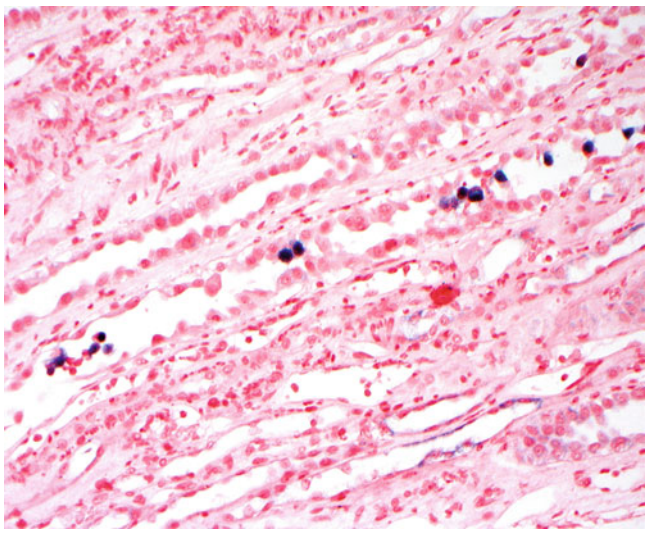

Fig. 14.6 Renal biopsy with in situ hybridization for adenovirus showing infected cells (40×)

Treatment

Treatment options for adenoviral infection in immunocompromised patients are limited and include cidofovir and intravenous immunoglobulins (IVIG). Cidofovir appears to be more active against adenovirus in vitro than other antiviral drugs such as ganciclovir. Moreover, in vivo activity of cidofovir has been demonstrated by reduction in viral load by real-time PCR [93]. Indeed, treatment with cidofovir has been associated with clinical improvement and decreased mortality in hematopoietic stem cell recipients and lung transplant patients [94].

Nephrotoxicity, however, is a major limiting factor for use of cidofovir in renal transplant patients. Besides reduction in glomerular filtration rate, cidofovir use may also result in a Fanconi-type syndrome [95]. The manifestation of this particular toxicity may include proteinuria, bicarbonate wasting, and glucosuria. Therefore, in cases where treatment is strongly indicated, use of lower doses (1 mg/kg every other day or three times per week) instead of standard dose of 5 mg/kg weekly should be considered to reduce risk of nephrotoxicity [96]. Other additional measures to reduce cidofovir-related renal toxicity include aggressive hydration and use of probenecid. One liter of normal saline (0.9 % NaCl) should be administered 1–2 h prior to cidofovir infusion, and another 1 L should be given after 1–3 h after cidofovir infusion. Probenecid interferes with cidofovir excretion in renal tubules and has been shown to reduce cidofovir-associated nephrotoxicity. Usually recommended dose of probenecid is 3 g, 3 h before cidofovir infusion, another 1 g 2 h after the cidofovir, and a final 1 g after 8 h of cidofovir administration.

Intravenous immunoglobulins (IVIG) may be a useful adjunctive therapy in management of adenoviral disease in immunocompromised patients [93, 97]. Pooled IVIG contains high levels of neutralizing antibodies against common serotypes of adenoviruses. IVIG may be used alone or in combination with cidofovir in patients with severe, disseminated adenoviral infection [92].

Prognosis

Although patients with localized adenoviral disease generally do well, disseminated infection in immunocompromised patients is associated with high (>70 %) mortality [98]. Mortality tends to be higher in patients with allogenic stem cell transplant compared to solid organ recipients.

Coxsackie B Virus

Coxsackie viruses are nonenveloped, single-stranded, RNA viruses that belong to *Picornaviridae* family of enteroviruses. They are named after a small town, Coxsackie in New York state where they were first isolated in 1948 [99].

Coxsackie viruses have a worldwide distribution. These viruses replicate in enterocytes and spread by fecal-oral transmission. They are broadly categorized into group A and group B (CVA and CVB). Most human infections are caused by CVB type 1–6. Clinical presentation with CVB infection is quite variable and the type of illness varies from a benign viral exanthem to more severe manifestations such as myocarditis and meningitis.

Renal involvement is rare and is usually reported in young children or immunocompromised adults [100–102]. Coxsackie viruses are capable of infecting mesangial cells and can cause acute or chronic focal interstitial glomerulonephritis [103]. Clinical presentation may include proteinuria, pyuria, and microscopic hematuria. Rare cases of allograft dysfunction have been reported in kidney transplant recipients [104].

Histopathologic findings of Coxsackie renal infection closely resemble mesangioproliferative glomerulonephritis and IgA nephropathy [105]. Viral cytopathic effects and lysis of infected cells can been seen on microscopy [103].

Treatment is supportive and should include reduction in immunosuppression, if feasible.

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Thrombotic Microangiopathies: Thrombus Formation Due to Common or Related Mechanisms?

15

Peter F. Zipfel and Christine Skerka

Introduction

Thrombotic microangiopathies (TMA) combine two major disorders in form of hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). Both diseases are rare and have frequencies of approximately 1–4 cases for a population of 1,000,000 individuals. The disorders affect both young children and also adults and when left untreated have a poor prognosis, often leading to kidney failure and death within months after the onset of symptoms [1–5].

Both TTP and HUS have in common the presence of thrombocytopenia and hemolytic anemia. However, each form has an organ preference, neurological manifestations in TTP, and renal manifestations in HUS. TTP is characterized by a pentad of symptoms, which include fever, thrombocytopenia, microangiopathic hemolytic anemia, neurological and renal involvement [3]. HUS is characterized by the triad thrombocytopenia, microangiopathic hemolytic anemia, and acute renal failure. In some cases neurological involvement, as well as protracted seizures and stuporous coma and heart involvement, is also reported (Fig.15.1) [5, 6]. HUS represents a syndrome and encompasses three subtypes. The most common form D+HUS (also termed typical HUS) is associated with diarrhea and is frequently caused by infections with enterohemorrhagic *Escherichia coli* (EHEC). The second form, D-HUS, also termed atypical HUS is less frequent and can develop spontaneously—but is also familial. In general terms, this form has genetic causes and for about 60 % of cases underlying genetic causes are described [4]. A third form is now termed DEAP–HUS (deficient for CFHR proteins and autoantibody-positive form of HUS, or autoantibody-associated HUS). This autoimmune disease is characterized by the presence of autoantibodies, which bind to the C-terminal recognition region of Factor H. In the majority of cases, the presence of autoantibodies correlates with a deletion of a 84 kbp chromosomal segment on human chromosome 1q32 that harbors the genes *CFHR1* and *CFHR3* [5, 7, 8].

TMA presents as a spectrum of related disorders with hemolytic anemia and thrombocytopenia. Multiple factors have been described which are related and may even sometimes cause identical pathological effects leading to thrombus formation. Thus, the two subtypes cause prominent damage of the endothelial lining and exposure of the subendothelial matrix that induces thrombus formation (Fig. 15.1b, c). The molecular causes for these defects are often linked to the defective regulation or inappropriate activation of the complement—as well as of the coagulation cascade. Both disease subtypes may have distinct initial triggers, and the affected modified molecules ultimately cause thrombus formation and endothelial damage. Despite similar presentations, the identification of the disease etiology, i.e., genetic or autoimmune, is important in order to tailor appropriate therapy. For example, the EHEC–HUS outbreak in Germany 2011 showed that deep knowledge of disease mechanisms although highly relevant may not apply to each individual case. In this chapter, we present recent scientific developments in the field of TMA and summarize the emerging common features of these complex disorders. TMA is an important example of translational medicine. Novel genetic causes have been linked to new pathological concepts and successfully transferred into clinical practice.

Thrombotic Thrombocytopenic Purpura

TTP is a severe life-threatening disease that exhibits symptoms like fever, microangiopathic hemolytic anemia, thrombocytopenia, and renal and neurological involvement and mainly affects women between 10 and 40 years of life [9–11]. In TTP, microthrombi rich in von Willebrand factor (vWF) develop in the arterioles and capillaries of the brain as well

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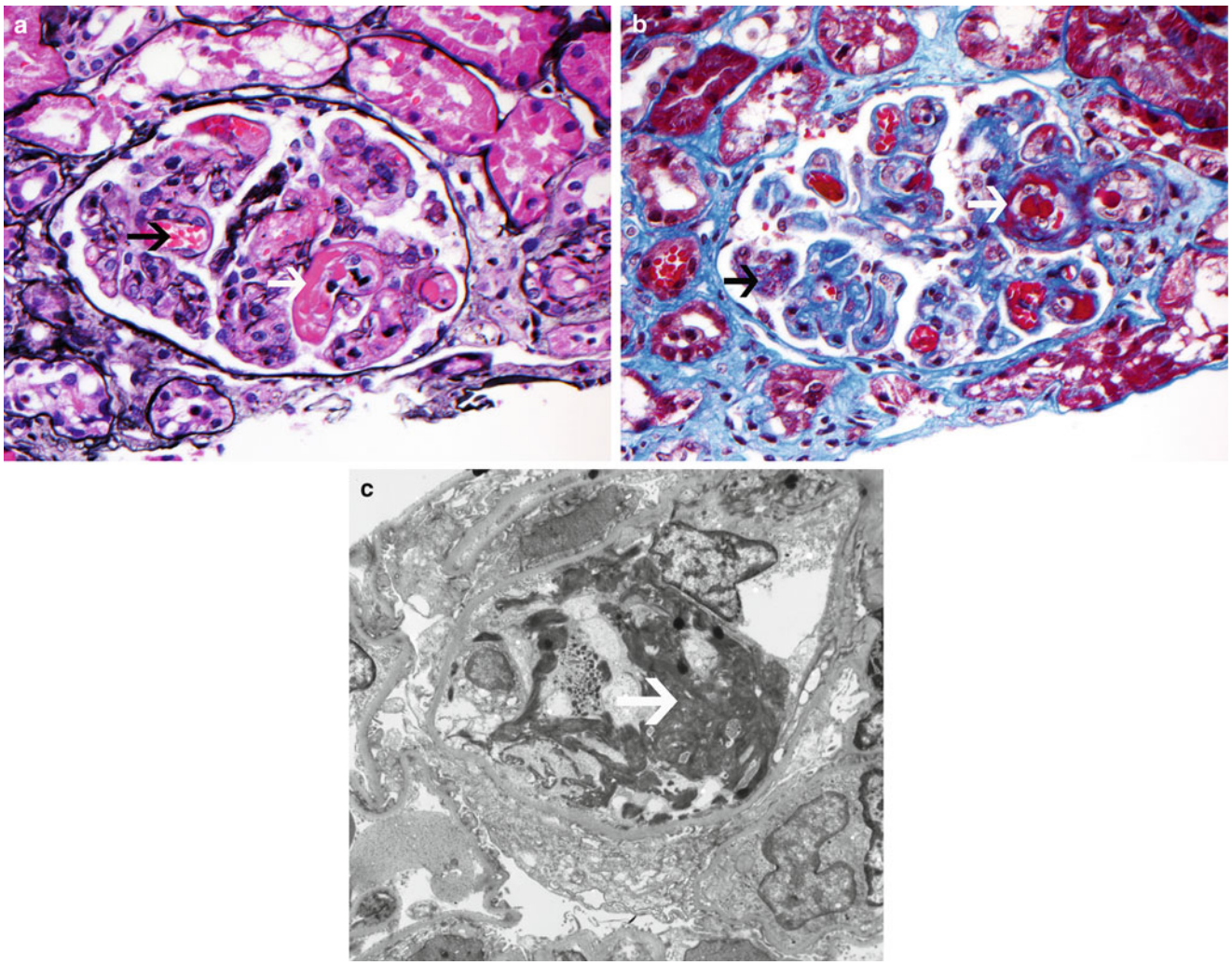

Fig. 15.1 Thrombus formation in the kidney of aHUS. (**a**) Silver-methenamine stained section of a glomerulus of an aHUS patient with microthrombi (*white arrow*). *Black arrow* points at RBCs (×40). (**b**) Thrombus at vascular pole. Light microscopy: trichrome stain showing a thrombus at the vascular pole (*white arrow*). Note fragmented red cells in capillary lumen at tubular pole (*black arrow*) (trichrome ×40). (**c**) Intracapillary thrombus. Electron microscopy: fibrinoid material within glomerular capillary completely occluding the capillary lumen (×4700). Photos kindly provided by Sanjeev Sethi, MD, PhD, Mayo Clinic, Rochester, USA

as other organs [9]. Both genetic and acquired autoimmune forms of TTP including hereditary mutations of the *ADAMTS13* gene and autoantibodies that bind to ADAMTS 13 have been described [11, 12]. Both forms lead to platelet aggregation and microvascular thrombosis. A direct link between HUS and TTP is provided with the *Escherichia coli* Shiga toxin Stx1B and Stx2B, which induce cell signaling of human umbilical vein endothelial cells and secretion of von Willebrand factor [13].

The genetic form of TTP is associated with mutations in the coding region of the plasma metalloprotease *ADAMTS13* (a disintegrin-like and metalloprotease with thrombospondin type 1 repeats) gene [12, 14] (Fig.15.2a). The gene is primarily expressed in the liver, and the encoded plasma protease ADAMTS13 is a multidomain protein, which forms complex interactions with its substrates, cleaves large multimers of vWF, and thereby affects vWF function. The large vWF multimers cause platelet adhesion, platelet aggregation, and tissue infarction [12]. Endothelial and renal tubular cells also express ADAMTS13, and at the cell surface, the secreted protein is responsible for maintaining the cellular surface free of adhesive vWF [15, 16].

Single-molecule experiments show that elongation forces in the vasculature unfold the A2 domain of vWF and that ADAMTS13 cleaves specifically the unfolded accessible A2 domain of vWF [17]. The structure of ADAMTS 13 and vWF provides important insights into the domain organization and allows us to map and define the interface of the two proteins. ADAMTS13 cleaves the Tyr1605–Met1606 bond in the central A2 domain of vWF. However, leukocyte proteases

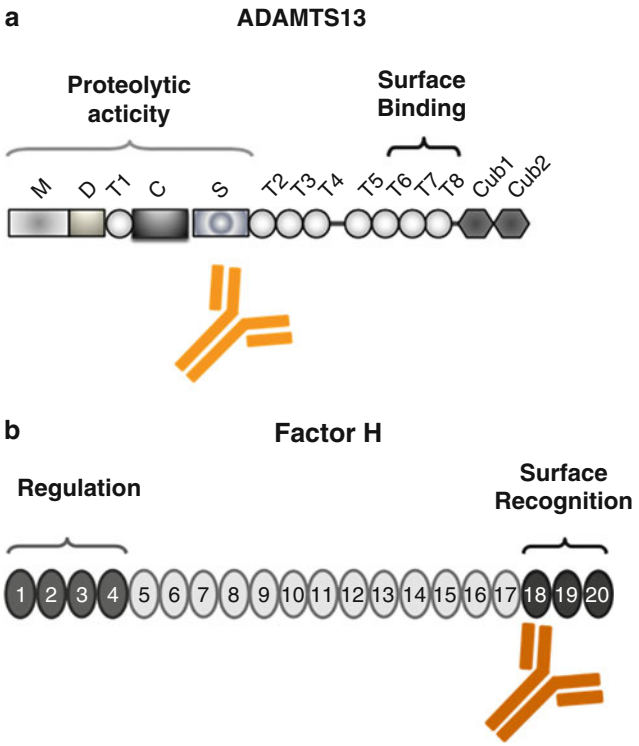

Fig. 15.2 Domain structure of ADAMTS13 and Factor H. (**a**) ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type 1 repeats) has a modular composition being formed of M: metalloprotease domains, D: disintegrin-like domain, T1: thrombospondin type 1 repeat, C: Cys-rich region, S: spacer region, and CUB-like domains 1 and 2. The position of the two major functional regions that form the proteolytic and surface-binding regions is indicated. The binding sites for the autoantibody are also shown. (**b**) Factor H is composed of 20 modules, termed short consensus repeats (SCRs). The central functional domains are located in the N terminus (SCR domain 1–4) forming the regulatory domain that controls complement activation at the level of the alternative pathway convertase C3bBb and in the C terminus (SCRs 18–20) that forms the surface binding and attachment region of the protein. The majority of aHUS-associated mutations in the Factor H gene are positioned in the C-terminal surface and attachment region and are mostly heterozygous. In DEAP–HUS which represents the autoimmune form (deficient for CFHR1 and CFHR3 proteins and autoantibody-positive form of HUS), autoantibodies to Factor H develop which bind to the C terminus and block C-terminal attachment to cell surfaces and biomembranes

like elastin and protease 3 also cleave vWF close to ADAMTS13 cleavage site [11, 18]. The contact between ADAMTS13 and vWF is quite complex and several sites of interaction occur. The terminal linked sialic residues enhance proteolysis by ADAMTS13 [19, 20]. Both the thrombospondin1 Cys-rich region and the spacer domain of ADAMTS13 bind to the A2 domain of vWF [21]. A second ADAMTS13 binding site spanning residues 1874–2813 is located in the constitutively exposed D4 region of vWF [22]. In addition N-glycans are further relevant for proteolytic cleavage and for secretion [23].

ADAMTS13 gene mutations are spread over the complete gene and include several protein domains. Mutations, which result in intravascular platelet aggregation and thrombocytopenia, may result in defective substrate processing and deregulated stability of ADAMTS13 [24]. To illustrate the complexity, factor H gene mutations have linked TTP and complement. In two sisters from one family with similar mutations in the Factor H gene, one girl developed TTP but the other HUS [25].

The autoimmune form of TTP is characterized by the presence of autoantibodies that bind to ADAMTS13 and thereby block proteolytic degradation of the ultra large forms of vWF [26, 27]. The majority of autoantibodies bind within the spacer domain of ADAMTS13 (Fig. 15.2a). In addition the various IgG and IgM antibodies apparently influence the stability of the protease or inhibit ADAMTS13 binding to the surface of endothelial cells [28, 29]. IgG_4 is the most frequent immunoglobulin subclass and is identified in about 90 % of the autoimmune-positive patients. The other subclasses include IgG_1 (52 %), IgG_2 (50 %), and also IgG_3 (33 %). IgG_4 autoantibodies may also occur in combination with other immunoglobulin subclasses. Whether and how the IgG subclasses are useful to predict disease recurrence requires further research [30].

Therapy: Genetic or acquired ADAMTS13 deficiency can be treated by plasma infusion or exchange. In addition hematopoietic progenitor cell-mediated gene therapy in ADAMTS13-deficient knockout mice was evaluated as novel therapeutic concept. This new model may form a basis for new therapy to treat genetic forms of human TTP [31].

Hemolytic Uremic Syndrome

HUS manifests primarily in the kidney and leads to acute renal failure together with microangiopathic hemolytic anemia and thrombocytopenia. At present three major forms of HUS are recognized: the classical also termed typical HUS, aHUS, the atypical form, and the autoimmune form DEAP–HUS [4, 5]. Thrombi are particularly formed in the kidney and are often associated with fragmented red blood cells. The concept of common pathogenetic mechanisms will be discussed as overlapping features, and combined factors are becoming evident between the disease subforms.

Typical HUS, also termed (D+HUS), is associated with diarrhea and is the most frequent form. This form occurs in approximately 80 % of HUS patients and is frequent in children. D+HUS is often caused by infections with *EHEC*, Shiga toxin-producing *E. coli*, *Shigella dysenteriae* type 1, and also with *S. pneumoniae*. Evidence is emerging that D+HUS can also be triggered by other infectious agents including a wide range of enterohemorrhagic *E. coli*,

Bordetella pertussis, and viral infections like H1N1 influenza and Varicella zoster [32–34].

A role for complement in the pathogenesis of EHEC-induced typical HUS is demonstrated by the fact that Shiga toxin binds the central soluble complement inhibitor Factor H modulating the alternative complement cascade at local sites [35]. Similarly a role for the alternative pathway complement in typical HUS/D+HUS is demonstrated from a cohort study with 17 children showing increased levels of the complement activation product Bb and of the terminal complex SC5b-9 in patient's plasma [36].

Organs affected and damaged by HUS are not limited to the kidney and include neurological, gastrointestinal, cardiac, and pulmonary manifestations in patients with typical, diarrhea-associated HUS. For patients with neurological symptoms, seizures, protracted severe seizures, as well as coma have been reported [6].

The EHEC outbreak in Germany during May to July 2011 was caused by enterohemorrhagic *E. coli* of the serotype O104:H4 that was distributed via contaminated sprouts. The incubation period for this *E. coli* strain was about 8 days and thus longer than the period of 4 days for the strain *E. coli* O157:H7. The infection rates were different between local states in Germany with the northern part of the country more affected. For some states the incidence for HUS was as high as 100 cases per one million populations. The majority of the infected individuals were adult females and neurological symptoms were frequently reported. A total of 2,987 infected individuals developed gastroenteritis and 18 patients (0.6 %) of this group died. In addition, 35 (4.1 %) patients of the 855 patients who developed HUS died (Robert Koch Institute report [37–40]). As typical HUS is caused by infections, the role of host predisposing genetic factors as well as determinants of infection susceptibility is an area for future research.

The second form of HUS, atypical (aHUS), also called non-diarrhea form of HUS (D-HUS), represents about 10–15 % of patients. The disease is more frequent in adults, but children are also affected. In addition to renal involvement, other organ and tissue involvements have been reported including cardiovascular ischemic events, stenosis of cranial arteries, as well as pulmonary and coronary arteries in patient with aHUS [4, 5].

aHUS is due to mutations in genes that code for complement regulators or complement components that affect the activity of C3bBb convertase of the alternative complement pathway. Most mutations in atypical HUS show incomplete penetrance, are heterozygous in nature, and affect one allele. Consequently patients have a second intact allele [41].

Regulators modulated in aHUS include the central fluid phase complement inhibitor Factor H (20–30 %) (Fig. 15.2b), the membrane-bound regulator MCP/CD46 (5–15 %), the soluble serine protease Factor I (4–10 %), and the two major components that form the alternative pathway convertase C3bBb, i.e., C3 (2–10 %) and Factor B (1–4 %). In addition, thrombomodulin, which is a regulator of the coagulation cascade and a likely effector of complement action and of the C3 convertase, has been also linked to aHUS [42, 43]. Which complement gene shows the mutation is an important parameter for the prediction of disease outcome, as well as for posttransplant recurrence.

Based on the strong involvement of complement gene mutations in more than 60 % of aHUS patients, this type of HUS is sometimes referred to as complement-HUS [41, 42, 44, 45]. In general terms heterozygous mutations in complement genes result in defective control of the alternative pathway convertase C3bBb and in widespread activation of the complement cascade. The defective regulation is thought to occur at specific sites, e.g., at the surface of renal endothelial cells, exposed subendothelial matrix, or on platelets. In addition a role for the terminal pathway of complement in aHUS is emerging. CFHR1, the gene that is deleted in DEAP–HUS, is a terminal pathway regulator that blocks the C5 convertase as well as assembly and membrane insertion of the terminal complement complex (TCC) [45]. Similarly, a mutation in the gene coding for the terminal complement inhibitor clusterin (A1298C—Q433P) has been identified in a child with typical HUS [46].

The third group of HUS, the autoimmune form DEAP–HUS (deficient of CFHR1 and CFHR3 proteins and autoantibody-positive HUS) [47, 48], is characterized by the presence of autoantibodies. Autoantibodies to Factor H are detected in about 10–15 % of all HUS patients, particularly in children age 4–17 years [47]. Autoantibodies to Factor H are of the IgG_1 and IgG_3 subtype [49]. In most cases these autoantibodies develop on a specific genetic background of homozygous deletion of a 84 kb chromosomal segment on human chromosome 1q32, which encodes the two genes *CFHR1* (complement Factor H-related protein 1) and *CFHR3*. Consequently the two complement proteins CFHR1 and CFHR3 are absent in the plasma of these patients [8, 48]. Most IgG autoantibodies of DEAP–HUS patients bind to the C-terminal surface attachment region of Factor H, i.e., SCRs 18–20, and block Factor H binding to heparin and glycosaminoglycans and also inhibit Factor H recruitment to the surface of endothelial cells. The result is reduction in the local regulatory and anti-inflammatory functions of Factor H at modified cellular surfaces. Autoantibody-positive plasma derived from aHUS patients causes enhanced lysis of sheep erythrocytes, thus demonstrating directly that autoantibodies mediate complement regulation at cellular surfaces by influencing surface binding of Factor H [50, 51] (Fig. 15.2b). Importantly, the C-terminally binding autoantibodies and mAbs do not block or influence the complement regulatory activity of Factor H in fluid phase in plasma, which is mediated by the N-terminal region.

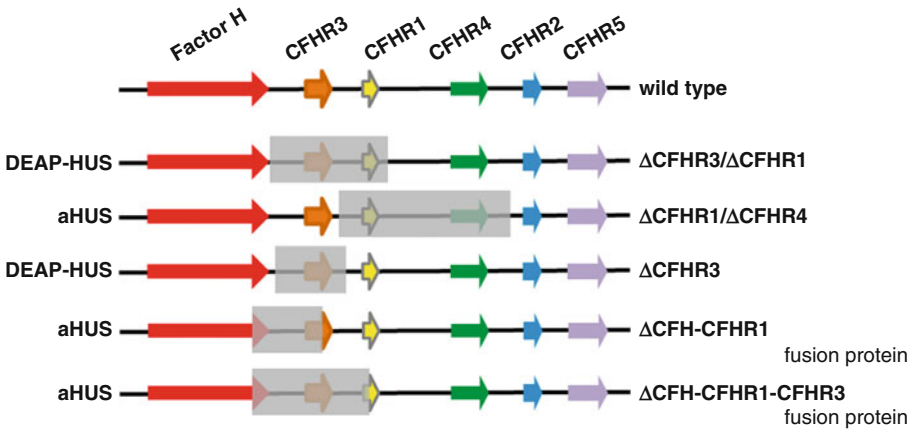

Fig. 15.3 Deletion in the *Factor H* gene cluster in aHUS and DEAP–HUS. The Factor H gene cluster is located on human chromosome 1 region 1q32 and includes the genes *Factor H*, *CFHR3*, *CFHR1*, *CFHR4*, *CFHR2*, *CFHR5*, and *Factor XIIIb*. The wild-type organization is shown in the top panel (wild type). The first rearrangement that forms a fusion gene between *Factor H* and *CFHR1* is shown in the second line (conversion type). Homozygous deletion of a 84 kbp chromosomal fragment that includes the gene coding for CFHR1 and CFHR3 is frequently associated with the autoimmune form DEAP–HUS and in HUS correlates with the presence of autoantibodies to Factor H. However, this deletion is also observed in healthy persons, but is then not associated with autoantibodies. A new deletion was reported which leaves the *CFHR3* locus intact and which results in deletion of the *CFHR1* and *CFHR4* genes (*bottom line*) [36]

The frequency of autoantibodies and the correlation of autoantibodies with CFHR1–CFHR3 deficiency have been shown in the Jena-HUS, the Paris, the Newcastle, and in the Spanish HUS cohorts [52, 53]. In all four cohorts, the frequency for DEAP–HUS was between 6 and 14 %, and more than 90 % of the DEAP–HUS patients had a combination of autoantibodies together with homozygous chromosomal CFHR1–CFHR3 or CFHR1.CFHR4 deletion. In addition, new genomic rearrangements in the CFHR gene cluster were reported in various DEAP–HUS patients. Also in aHUS patients, chromosomal deletions that results in hybrid genes, e.g., a hybrid Factor H-CFHR1 gene as well as a hybrid Factor H-CFHR3 genes and hybrid Factor H-CFHR proteins have also been reported [54, 55]. Human chromosome 1 region 1q32, which includes several long repetitive regions, is frequently involved in recombination processes, resulting in novel chromosomal rearrangements. Furthermore genetic deletion variants have been identified in the genome of both aHUS and DEAP–HUS patients (Fig. 15.3) [48, 53]. Novel breakpoints such as the deletion of the CFHR1 gene combined with the presence of CFHR3 were described, and in a large pedigree with three aHUS patients, a hybrid Factor H-CFHR3 protein was identified (Fig. 15.3). This hybrid protein consists of 19 domains of Factor H (i.e., SCRs 1–19) and five SCR domains of CFHR3 (i.e., SCRs 1–5) and shows severely reduced complement regulation and increased heparin binding. The genetic cause was microhomology-mediated end joining [55].

CFHR1 and CFHR3 are both complement regulators; however, their mode of action and level of control are different from each other and also distinct regarding the regulation of Factor H. CFHR1 is a complement regulator that inhibits the C5 convertase, as well as assembly and membrane insertion of the terminal complement complex. CFHR3 is a complement regulator that acts as cofactor for factor I and allows degradation of C3b. Both CFHR1 and CFHR3 share similar and overlapping binding sites on the C3b protein with Factor H. As a consequence both CFHR proteins compete with Factor H for C3b binding as well as for attachment to human cell surfaces. By competing with Factor H ligand binding, each CFHR protein modulates the local activity of Factor H (Fig. 15.3). The coordinated binding suggests that at a cellular surface CFHR1 and Factor H (and likely CFHR3 and Factor H also) act in concert and control complement convertase and thus local amplification of the complement cascade in a sequential fashion [48, 54].

A polymorphism of the CFHR1 gene that was reported with the original cloning of the CFHR1 cDNAs is also associated with HUS. Two major CFHR1 variants differ by three amino acids in SCR 3. The CFHR1 variant encoded by cDNA H36.1 represents CFHR1-HLE and clone H36.2 encodes the CFHR1-YVQ variant. These isoforms appear to have different electrophoretic mobilities. The basic variant with residues H157, L159, and E175 (CFHR1-HLE) corresponds to the sequence of SCR 18 of Factor H. The sequence of the acidic CFHR1-YVQ variant differs from the sequence of Factor H (Fig. 15.4). The mostly C-terminal SCR domain, i.e., SCR 5, is identical for the two CFHR1 variants. However, between CFHR1 and Factor H this most C-terminal SCR differs by two residues. CFHR1–SCR 5 uses L290 and A296 and Factor H-SCR 20—the corresponding residues are S1191 and V1197 [53] (Fig. 15.4). Thus, the C-terminal surface-binding region of the original CFHR1 cDNA (H36.1) encodes the CFHR1-YVQ variant [54] and differs by only

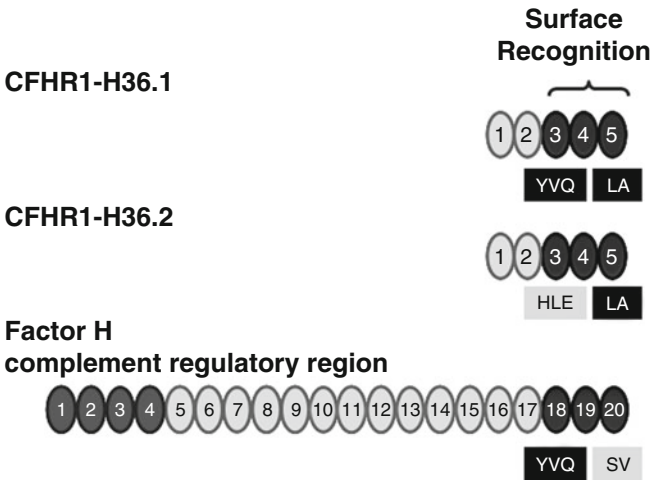

Fig. 15.4 Domain structure of CFHR1 and Factor H and sequence variation in the conserved C-terminal recognition region of the two proteins. CFHR1 (complement Factor H-related protein 1) is composed of five SCR domains and the three C-terminal domains (SCRs 3–5) show a high degree of sequence identity to the surface attachment region of Factor H (SCRs 18–20). Two polymorphic variants of CFHR1 exist, i.e., CFHR1-H36.1 with the motif YVQ in SCR 3 and CFHR1-H36.2 with HLE. Both CFHR1 variants have the LA sequence in the most C-terminal SCR. CFHR1 differs in two residues (L290 and A296) with the corresponding region in Factor H. Factor H fused to the third most C-terminal domain i.e. SCRs18-20 the motif YVQ corresponding to the CFHR1-H36.1 variant

two residues (i.e., L290 and A296) with the corresponding C-terminal surface binding of Factor H. The allele frequency of the acidic variant CFHR1-HLE is 0.57 in a Spanish AMD cohort and is higher as in the control group (0.39). It will be of interest to define whether these three residues in SCR 3 affect the function and ligand interaction of the C-terminal surface recognition region.

Diagnosis and Therapy

Given the multiple genetic as well as autoimmune causes for TMA, genetic screening for all susceptibility factors, as well as screening for autoantibodies, is crucial in order to allow a precise diagnosis as well as to plan the most appropriate therapy. Prognosis will depend on the cause of the disease, e.g., the mutated gene. Patients with Factor H gene mutations have in general a poor prognosis, while a better prognosis can be expected for patients with MCP gene mutations. Based on information recently accumulated, new and precise clinical practice guidelines for the management of patients with the atypical HUS have been proposed [56–58].

In the year 2011, both the US Food and Drug Administration (FDA) and the European Commission (EC) have approved the complement inhibitor Eculizumab (Soliris) for the treatment of adult and pediatric patients with atypical hemolytic uremic syndrome (aHUS). The treatment of aHUS patients with Eculizumab is a new, very promising, and exciting step in the treatment of this rare disorder and for the TMA story. The lack of response or unfavorable outcome to this form of therapy has raised the issue of inappropriate dosing [59, 60]. Eculizumab is extremely costly, a serious consideration in a patient who may need long-term therapy.

Transplantation

Precise genetic profiling of HUS patients has resulted in the ability to perform successful renal transplantations in several patients. For the autoimmune-positive DEAP–HUS patients, immunosuppressive therapy combined with reduction of autoantibody levels via plasma therapy in combination with the CD20-targeting therapeutic antibody Rituximab leads to successful kidney transplantations [61–63]. As more complement inhibitors are being developed, it is expected that in the future additional therapeutic options will become available.

Conclusions

TTP and HUS are rare disorders that represent striking examples for translational medicine. An additional and important highlight of the development in the field of TMA is the fact that the concepts initially established for these rare disorders have extrapolated for other diseases, including membranoproliferative glomerulonephritis and the common disorder age-related macular degeneration (AMD) [63–66]. AMD, a common form of blindness in the Western world, is also complement associated, and the same genes as identified in aHUS and DEAP–HUS, i.e., Factor H, CFHR1, CFHR3, C3, and Factor B, are all linked to this common form of blindness. Why in AMD pathology manifests in the eye along the retinal pigment epithelial cells and the Bruch's membrane and in HUS the disease involves primarily the endothelial lining of the kidney remains to be answered by future studies.

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Thrombotic Microangiopathy Not Associated with the Classic/Idiopathic TTP-HUS

16

Qi Qian

Introduction

Thrombotic microangiopathy (TMA) is, strictly speaking, a pathologic term, describing platelet thrombi in the microcirculation associated with intimal swelling and fibrinoid necrosis of the vessel wall. Clinically, TMA can occur as a classic, also known as idiopathic, TTP-HUS or in a variety of clinical settings associated with hallmark features of acute thrombocytopenia, microangiopathic hemolytic anemia, and visceral organ injury due to ischemia. Of all TMA cases, only a third of the cases are classic TTP-HUS. The remaining two-thirds are nonclassic and are considered as secondary TMA that occurs in patients with microangiopathic antiphospholipid syndrome (MAPS), systemic lupus erythematosus (SLE) and other connective tissue diseases, a wide range of systemic infections, pregnancy, drug ingestion, disseminated malignancy, solid organ transplantation, bone marrow and hematopoietic stem cell transplantations, and, rarely, severe pancreatitis and surgery (Table 16.1). This chapter focuses on entities of secondary TMA, which differ from the classic TTP-HUS in their clinical courses and therapy.

Microangiopathic Antiphospholipid-Associated Syndrome-Associated TMA

Antiphospholipid syndrome (APS) is an autoimmune disorder characterized by the persistent presence of circulating antiphospholipid antibodies (aPL) and vascular (arterial and/or venous) thrombosis and/or pregnancy-related complications. Antiphospholipid antibodies are a group of antibodies directed against plasma proteins that possess ionic phospholipids, primarily β2-glycoprotein I (β2GPI), prothrombin, and phosphatidylserine–prothrombin complex (PS/PT). In 2006, the definition of APS was updated, and thrombotic conditions affecting mainly microvasculatures (without large-vessel thrombosis or occlusions), i.e., TMA-associated conditions in patients with aPL positivity, were included in the disease spectrum and, hence, microangiopathic APS (MAPS) [1, 2].

The precise pathogenesis of TMA in MAPS has not been fully elucidated. Clinically, despite the persistent presence of circulating antiphospholipid antibodies, thrombotic events occur only occasionally, suggesting other factors are needed to trigger thrombotic events. There has been a proposal of a "two-hit hypothesis" in which aPL, representing the first hit, causes thrombotic events in the presence of another thrombophilic stimulus, the second hit. The two-hit hypothesis is supported by an animal model of APS in which aPL injection to rats resulted in thrombus formation only when the animals were also pretreated with lipopolysaccharide, not with normal saline [3].

Although not fully elucidated, aPL are thrombogenic via three potential mechanisms: (1) diminished effects of nitric oxide (NO); (2) abnormal interactions of aPL with coagulation factors and cells associated with the regulation of thrombosis, mainly monocytes, endothelial cells, and platelets; and (3) aberrant complement activation.

Nitric oxide, generated by the enzymatic action of endothelial nitric oxide synthase (eNOS), is a key regulatory molecule in maintaining vascular health. Nitric oxide regulates the expression of adhesion molecules and inhibits platelet aggregation by augmenting cGMP production in platelets. There are a number of studies in both mouse models and humans suggesting a role for impaired NO production and activity in the genesis of TMA. aPL binding to β2GPI is shown to inhibit eNOS activation with a resultant deficiency of NO production which in turn predisposes to a full-scale leukocyte (monocyte)-endothelium adhesion and thrombus formation [4], especially when there is a second hit.

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Table 16.1 A list of clinical conditions associated with TMA

1	Antiphospholipid syndrome
2	Systemic lupus erythematosus
3	Systemic scleroderma
4	Malignant hypertension
5	Pregnancy-HELLP syndrome
6	Drugs
7	Malignancy
8	Solid organ transplant
9	Allogeneic stem cell transplant—ionizing radiation
10	Infections: Sepsis, HIV
11	Pancreatitis
12	Surgeries

aPL is directed against plasma protein β2GPI; binding of aPL and β2GPI increases the affinity of aPL to membrane phospholipids. The interaction of aPL and membrane of platelets, monocytes, and endothelial cells can activate prothrombogenic cellular elements, promoting thrombogenesis. Studies have shown that the interaction of aPL and cell membrane requires co-activation of specific receptors. A number of candidate receptors have been implicated, including annexin A2, glycoprotein Ibα and apolipoprotein E receptor 2, toll-like receptors (TLR-2 and TLR-4), VLDL receptor, and possibly other receptor proteins [5–7].

Under physiological condition, β2GPI binds to the membrane of activated platelets and inhibits the generation of activated factor X. The binding of aPL and β2GPI interferes with such inhibition and promotes platelet aggregation and thrombus formation [8]. The binding of aPL to the membrane of monocytes induces upregulation of tissue factors, a hallmark of monocyte activation, also seen in patients with APS [9]. The binding also activates signaling cascades including, but not limited to, P38 MAPK-mediated activation of tumor necrosis factor (TNF) alpha, IL-1b and macrophage inflammatory cytokine 3b, NFκB, and upregulation of procoagulant substances and adhesion molecules [10, 11]. In the endothelium, pathogenic binding of aPL causes an upregulation of myeloid differentiation protein (MyD)88-dependent signaling, adhesion molecules, tissue factors, and endothelin-1, resulting in a pro-inflammatory and prothrombogenic endothelial cell phenotype [12]. The binding also stimulates endothelial cells to release microparticles that likely promote a thrombogenic endothelial cell phenotype [10, 11, 13].

Recently, complement activation has emerged as one of the important mechanisms related to the development of TMA in patients with MAPS [14–16]. Hypocomplementemia, caused by complement activation and consumption, is a commonly observed feature of MAPS. Activation of complement pathway leads to C3 activation, which induces the production of pro-inflammatory fragment of C3a (also called anaphylatoxin) and subsequent C5 activation and production of anaphylatoxin C5a, another strong inflammatory toxin. C5b, another fragment of activated C5, forms complex with C6, C7, C8, and C9 leading to the formation of a multicomplex membrane attack protein (MAC) and ultimately causing disruption of cell membrane and cell death. Thus, activation of complements produces anaphylatoxins and fragments that amplify the activation of monocytes, endothelial cells, and platelets. The complement activation also induces expression of tissue factor as well as adhesion molecules, further promoting platelet aggregation [17, 18]. The anaphylatoxin 5a is especially important in the genesis of placental damage in MAPS [19]. Obstetrical complications associated with MAPS can be markedly suppressed in animal models when complement is made deficient or anticomplement antibody is administered [20].

Histopathologically, TMA-associated MAPS shows thrombi in the capillary lumina in the kidney biopsy. It can be indistinguishable from TMA in the classic form of TTP-HUS. There could also be MAPS-related changes including fibrous intimal fibroplasia, focal cortical atrophy, and artery and/or arterial occlusions. Figure 16.1 shows biopsy images from a 42-year-old woman with MAPS.

Treatment modalities for the majority of patients with MAPS include chronic anticoagulation. In cases with TMA, depending on the severity and the degrees of organ involvement, high-dose steroids, IVIG, and/or plasmapheresis may be tried [21]. Case reports also suggest a beneficial effect of rituximab, a B-cell depletion antibody, for patients with severe MAPS [22].

In patients who develop end-stage renal failure, a positive titer of circulating aPL is not a contraindication for kidney transplant. However, posttransplant patients should be on chronic anticoagulation, and calcineurin inhibitors should be avoided. When necessary, rituximab can be tried. Recently, eculizumab has been used successfully for several kidney transplant recipients with positive aPL to prevent and to treat TMA [23, 24]. Eculizumab is a humanized monoclonal antibody that inhibits C5 activation. It has shown efficacy in treating patients with paroxysmal nocturnal hemoglobinuria (PNH). One limitation with this drug is its cost, running approximately $15,000 (September 2012 USD) for a maintenance weekly dose (900 mg) and $400,000 for one year's maintenance supply. Eculizumab is also known to increase the risk of meningococcal infections. Meningococcal vaccine is therefore required for all patients at least 2 weeks prior to beginning eculizumab therapy. Moreover, patients on eculizumab should have ongoing monitoring for early symptoms and signs of meningococcal infections to allow the start of treatment for the infections in a timely fashion. Eculizumab was recently approved in the USA as a first-line drug for atypical HUS (aHUS). However, its long-term use requires further evaluation. In patients with refractory MAPS-associated TMA, eculizumab may be tried.

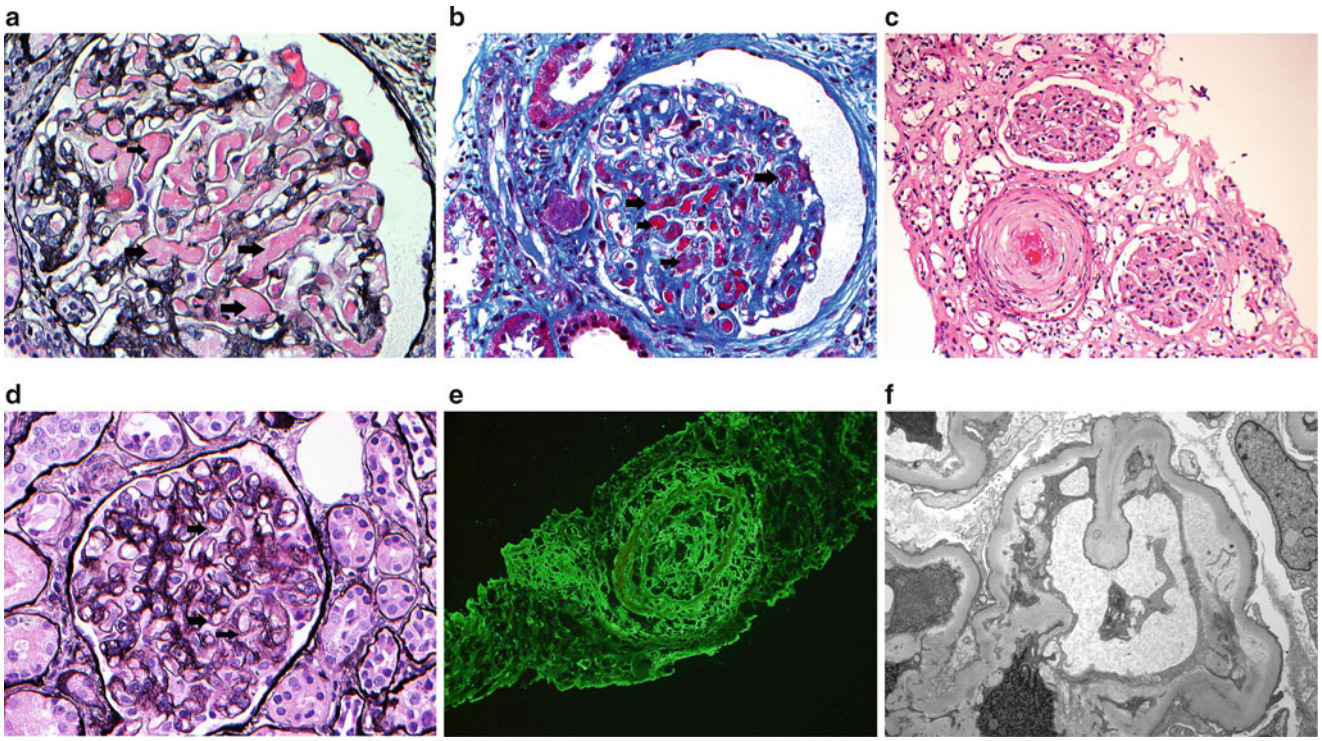

Fig. 16.1 Thrombotic microangiopathy. (**a–c**) Light microscopy showing thrombus within small arteries (**a**: silver methenamine; **b**: trichrome; **c**: hematoxylin and eosin). In **c**, note ischemic glomeruli around artery with thrombosis. (**d**) Light microscopy showing features of chronic thrombotic microangiopathy with thickened peripheral capillary walls and double contour formation (*arrows*) (silver methenamine). (**e**) Immunofluorescence microscopy showing fibrinogen within an artery. (**f**) Electron microscopy showing chronic thrombotic microangiopathy with subendothelial expansion with fluffy granular material and cellular elements. Note endothelial injury as manifested by loss of fenestrations

Systemic Lupus Erythematosus-Associated TMA

TMA occurs in patients with a variety of connective tissue diseases. Matsuyama et al. [25] examined 127 patients with connective tissue disease-associated TMA. Among the subjects, 64 were patients with SLE, 42 with systemic sclerosis [excluded patients in scleroderma renal crisis (SRC)], 11 with polymyositis/dermatomyositis, and ten with rheumatoid arthritis. Most of the 127 patients demonstrated a normal or mild degree of deficiency in ADAMTS13 activity, which was consistent with the existing literature and would not explain the development of TMA. Only a small fraction of this group of TMA patients had severe deficiency of ADAMTS13 activity which was mostly associated with circulating neutralizing antibodies. Here, we will focus on TMA in patients with SLE.

SLE, when active, is often associated with a mild degree of anemia and thrombocytopenia. Thrombocytopenia in SLE can occur through a variety of mechanisms including antiplatelet antibody, ITP- or TTP-like syndrome, and TMA with or without aPL. Although clinical presentation of SLE-associated TMA can be indistinguishable from TMA in idiopathic/classic TTP-HUS, there are differences in its incidence, its relation to ADAMTS13 activity, and its response to plasma exchange. The incidence of SLE-associated TMA is dramatically higher than idiopathic TTP-HUS, approximately 1–4 % of SLE patients and 3.7 per million residents, respectively [26]. In 2009, a single-center study showed an incidence rate of TMA in SLE of approximately 2.2 % [26]. Moreover, studies have demonstrated that TMA in SLE is not associated with an overall trend of reduction in ADAMTS13 activity, nor with dominant neutralizing antibodies against ADAMTS13, as seen in idiopathic TTP-HUS [26]. Consequently, TMA in SLE is quite resistant to plasmapheresis, unlike the majority of idiopathic TTP that are highly responsive to such treatment. Thus, TMA in SLE likely differs etiologically, at least in part, from TMA in idiopathic TTP-HUS.

Multiple etiological factors are speculated to be in play in SLE-associated TMA, including the involvement of autoantibodies or inhibitory antibodies targeting ADAMTS13 activity (in minority of the cases), D-dimer elevation which can promote microvascular thrombus formation, reduction in NO-mediated vasodilatory effects through the binding of hemoglobin to NO and potentially ADAMTS13 inhibitory effects exerted by hemoglobin, general procoagulant state in SLE patients with and without the presence of aPL, increased activity of plasminogen activator inhibitor (PAI) leading to

insufficient antithrombin III activity, underlying inflammatory state in SLE-causing resistance of vWF multimer to ADAMTS13-mediated cleavage, and direct endothelial cell activation due to multiple antibodies produced in the setting of SLE. A Scottish epidemiologic study showed that severe sepsis/infections can also be associated with the onset of TMA in patients with SLE, suggesting infection and sepsis could be a triggering event for TMA in lupus. Another study by Kwok et al. [27] showed that SLE disease activity can sometimes be a trigger for the onset of TMA in lupus. Similar results were reported by Chen et al. in 2011 [28]. Although all of the above have been proposed as contributing elements in the development of TMA in patients with SLE, the precise mechanisms are far from certain.

SLE affects multiple organ systems including the central nervous system, kidneys, bone marrow, blood elements, and aggregate cytokine release which can all mimic the presentation of idiopathic TTP, making the diagnosis of SLE-associated TMA a challenge. Providers should be on high alert for this condition, especially in patients with new onset thrombocytopenia and microscopic hemolytic anemia with a background SLE. Future standardization of tests for ADAMTS13 antigen, anti-ADAMTS13 antibody, and inhibitors of ADAMTS13 could potentially help with clarifying some of the differential diagnosis.

SLE-associated TMA carries a grim prognosis. Mortality rate in SLE patients with TMA is 34–62 % [29, 30], much higher than TMA patients without SLE, <10 % [31]. Older age, renal failure, and infections can all contribute to the higher mortality rate.

Treatment for SLE patients with TMA remains a real challenge. Strategies employed include steroids, cyclophosphamide, azathioprine, IVIG, and recently rituximab. An initial trial of plasmapheresis may be considered in cases when there is a suspicion for idiopathic TTP-HUS. In the setting of infection, the dosage of immunosuppressants should be reduced. Although neutralizing antibody does not seem to be important in SLE-associated TMA, there have been case reports of using rituximab, an inhibitor of B-cell-mediated antibody production, for treating patients with SLE-associated TMA and showing beneficial effects [32, 33]. These strategies have not been universally accepted. Better understanding the underlying mechanisms could help with development of novel approaches to its treatment.

In summary, SLE patients have a relatively high incidence of TMA compared to idiopathic TTP-HUS. Although the precise pathogenesis is not well understood, TMA in SLE does not appear to be associated with reduction in ADAMTS13 activity. TMA in SLE patients signifies poor prognosis and high mortality rate. There is no specific protocol for the treatment of TMA in patients with SLE. Future studies should be focused on clarifying the underlying pathogenesis which could potentially lead to improvement in treatment strategies and reduced mortality.

Systemic Scleroderma Renal Crisis-Associated TMA

TMA associated with systemic scleroderma (SSc) occurs typically in patients with scleroderma renal crisis (SRC) which is defined as a rapid decline in kidney function associated with hypertension without an alternative explanation. SRC develops in ~5 % of patients with systemic scleroderma, 12–19 % in diffuse systemic sclerosis and 2 % in limited systemic sclerosis [34–36]. Approximately half of the patients with SRC develop TMA.

Clinical features of SRC include (1) progressive renal impairment and often accelerated hypertension, (2) microangiopathic hemolytic anemia occurring in ~50 % of SRC patients, and (3) urinalysis showing non-nephrotic range proteinuria, hematuria, and often granular casts. Renal failure in SRC often develops over weeks rather than days. Accelerated hypertension is present in most cases but is not universal. Several reports have noted ~10–20 % normotensive SRC which usually portends a poor prognosis and high rate of irreversible renal failure and mortality. Rarely, SRC can present in association with diffuse alveolar hemorrhage along with TMA [37].

Several risk factors for SRC have been identified. Early phases of diffuse sclerosis (within months of a diagnosis of SSc) are at the greatest risk for SRC. An estimated 66 % of patients with SSc develop SRC within 1 year of the diagnosis [36, 38]. Other risk factors include rapid progressive skin lesions, recent exposure to glucocorticoids, hormone replacement therapy, and presence of SSc-specific anti-RNA polymerase antibodies. The presence of anticentromere antibody reduces the SRC risk.

In a recent multicenter retrospective study by Guillevin et al. [39] of patients with SRC, hypertension occurred in 85.7 % and hypertensive encephalopathy was found in about 60 %. More than half (56 %) of the SRC patients showed evidence of TMA. Overall, the outcome of SRC was poor. 53.8 % of patients required dialysis and 40.7 % of the patients died compared to 10.8 % in control (SSc patients without SRC). Normotensive SRC patients had worse outcomes; none of them recovered kidney function and all remained dialysis dependent. Dialysis patients showed a higher mortality rate. There was no mention of the impact of TMA on the disease presentation and outcomes in this specific study, although in the literature SRC patients with TMA typically fare worse with higher mortality rate than SRC patients without TMA.

The routine use of angiotensin-converting enzyme inhibitor (ACEi) has improved the outcomes of SRC with a reduction in 12-month mortality rate from 76 % to less than 15 % in the USA [40]. Treatment for SRC patients with or without TMA is to immediately institute ACEi and/or ARB as tolerated. Immunosuppressive regimen and/or biotherapy have not

been shown to improve clinical outcomes. Further studies are needed to clarify the usefulness of these treatments.

Malignant Hypertension-Associated TMA

TMA can develop in patients with malignant hypertension which occurs in ~1 % of hypertensive patients [41]. Malignant hypertension is a clinical syndrome featuring a severely elevated blood pressure (>180/120 mmHg), encephalopathy, congestive heart failure, acute papilledema, and funduscopic hemorrhage with exudate, change of mental status, acute kidney failure with microscopic hematuria and proteinuria. TMA associated with thrombocytopenia and microscopic hemolytic anemia can be found in about a third of patients with malignant hypertension [42].

TMA in malignant hypertension is caused by fibrinoid necrosis of small arteries and arterioles. The fibrinoid necrosis leads to a narrowing of the vessel lumen resulting in red blood cell fragmentation as the cells pass through the vessels. A study by van de Born et al. shows a mild reduction of ADAMTS13 in patients with TMA associated with malignant hypertension [43]. It is suggested that reduced ADAMTS13, endothelial activation and release of endothelial VWF can work in concert in the TMA genesis. Pathologically, intrarenal TMA in patients with malignant hypertension is similar to TMA resulting from other causes. Specific features of fibrinoid necrosis in the arterioles with fine subendothelial lipid droplets and hyalin thrombi formation are prominent.

Immediate control of blood pressure is the only effective method for reversing the process of hypertensive vascular injury and rescuing kidney function. With effective blood pressure control, platelet count typically normalizes within a week [44]. Parenteral therapy using antihypertensive agents with a rapid onset of action and short drug half-life in a monitored setting is necessary to ensure a precise and optimal blood pressure control. Agents that are being used for hypertensive emergency include nitroprusside, beta-blockers, labetalol, calcium-channel antagonists (including clevidipine, a recently introduced third-generation dihydropyridine calcium-channel antagonist) [45], hydralazine, ACEi, and fenoldopam. Depending on the nature and severity of target-organ injury, one drug could be preferred over another. For instance, clevidipine is an ultra short-acting calcium-channel antagonist with a rapid onset and half-life of <1 min. It is metabolized by red blood cell esterases, independent of renal and hepatic function. Clevidipine reduces blood pressure by a direct effect on arterioles, reducing afterload without affecting cardiac filling pressure or causing reflex tachycardia. Fenoldopam is another short-acting vasodilator. It is a dopamine 1-receptor agonist that increases renal blood flow and natriuresis. Labetalol is also a rapid onset and short-acting antihypertensive that blocks alpha-1 and (nonselective) beta-adrenergic receptors. It reduces afterload without reducing cerebral, coronary, and renal blood flow. Labetalol is safe in patients with acute coronary syndrome and is one of the most commonly used intravenous antihypertensive for patients with hypertensive emergency. However, there is no standard protocol and no evidence from large randomized controlled trials to support the use of one therapeutic agent over another with regard to its efficacy [46]. Consequently, practices vary considerably.

Overall outcomes improve with control of blood pressure [41, 47]. Without effective blood pressure control, the mortality rate can be as high as 90 %. With effective blood pressure control, the kidney function can be stabilized and improved in patients with a mild degree of kidney dysfunction (baseline serum creatinine <2.0 mg/dL). In patients with advanced baseline chronic kidney dysfunction, serum creatinine level >4 mg/dL, end-stage renal failure typically ensues and permanent renal replacement therapy is necessary [48].

Pregnancy-Associated TMA

Since the first report of pregnancy-associated TMA in 1955 by Miner et al. [49], there have been a number of case reports and case series showing that pregnancy is rarely associated with the occurrence of TMA with an estimated incidence of 1:25,000 birth [50, 51]. It is important to recognize the studies predate the era of testing for ADAMTS13 activity; thus, pregnancy-associated TMA was not distinguished from the classic TTP-HUS.

Pregnancy is a risk factor for the development of TMA. This is because even under physiological condition, pregnancy is associated with increasing concentrations of coagulating factors, decreased fibrinolytic activity, and decreased activity of ADAMTS13 [52–55]. Mannucci et al. [55] showed the mean value of ADAMTS13 activity in women during the second and third trimesters was significantly lower than during the first trimester, 64 % versus 94 %. Patients heterozygous for ADAMTS13 deficiency can potentially become more severely deficient of this enzyme during pregnancy, triggering the development of TMA. In patients with underlying genetic and procoagulant defects, such as the presence of factor V Leiden, methylenetetrahydrofolate reductase (MTHFR) 667T, factor II 20 210A, and plasminogen activator inhibitor (PAI-1) 4G, and deficiencies in protein S, protein C, and antithrombin III, pregnancy can serve as a precipitating factor to trigger acute thrombotic events [56]. These underlying genetic or acquired abnormalities become progressively more severe in the course of pregnancy with the maximum abnormalities occurring at near term and immediately postpartum.

It is not surprising that pregnancy-associated TMA tends to occur at term or shortly following delivery. The onset of the TMA tends to accompany symptoms of preeclampsia,

hypertension, and often prominent gastrointestinal symptoms. Severe neurological impairment and renal failure typically ensue, and death can be imminent without a timely diagnosis and treatment [50].

No firm treatment guidelines have been devised for pregnancy-associated TMA. Hayward et al. reviewed 67 episodes of TMA in 52 consecutive pregnant patients over a 12-year-period and showed that a plasma exchange-based therapy was associated with >95 % (65 of 67 cases) hematologic response. Despite the high response rate, two patients in remission were brain-dead and 8 % of the patients were dead in a median follow-up duration of 1.1 years. Persisting renal impairment, hepatitis, and transfusion-associated acquired immunodeficiency syndrome were seen in association with the disease and the treatment [57]. Other studies have also reported a substantial morbidity and maternal and fetal mortality despite treatment [58, 59].

Due to the poor prognosis of TMA during pregnancy and the potential confusion in the diagnosis of severe preeclampsia versus idiopathic TTP-HUS versus pregnancy-associated TMA, plasma infusion or plasma exchange and corticosteroid therapy are usually tried [57–59]. Although no standard treatment guidelines have been devised for pregnancy-associated TMA, plasma exchange or infusion is considered beneficial. The role of steroids for this condition is, however, unclear. Supportive measures, including blood pressure control and symptomatic management, should be optimized.

Although pregnancy likely acts as a precipitating event for acute TMA episodes, the risk for TMA in the future pregnancies in women who have experienced an episode of pregnancy-associated TMA is unclear. Clinical research is needed to evaluate the risk of subsequent pregnancies for women who have recovered from pregnancy-associated TMA, especially for those without genetic risk factors.

Drug-Associated TMA

A number of United States Food and Drug Administration (FDA)-approved drugs are known to be associated with TMA. Several herbal remedies including morning cupressus funebris and echinacea pallida and a diet remedy called chromium picolinate have also been reported to induce TMA-like symptoms including hemolytic anemia, thrombocytopenia, and acute kidney failure [60].

The pathogeneses of drug-associated TMA are multiple. Drugs can cause TMA via immune-mediated mechanism, via drug-dosage-dependent toxicity, or via unknown (yet to be elucidated) mechanisms. The only drug that has been proven to cause immune-mediated TMA is quinine. Quinine is a commonly used medication for leg cramps. Common beverages such as tonic water, Dubonnet Aperitif, and Schweppes bitter lemon also contain quinine. Quinine has been shown to induce production of specific antibodies against platelets, neutrophils, lymphocytes, and endothelial cells [61]. These antibodies are capable of inciting immune-mediated reaction, causing TMA and acute kidney failure. Additionally, they can cause neutropenia, disseminated intravascular coagulation, and liver dysfunction [62, 63].

Another group of drug-associated TMA is related to drug dosage. Dosage-dependent development of TMA has been observed mostly in chemotherapeutic agents including (in descending order) mitomycin C, gemcitabine, calcineurin inhibitors (cyclosporine and tacrolimus), carmustine, and pentostatin [64–68]. Dose reduction has been shown to ameliorate or eliminate the TMA.

There are a number of medications that have been reported to induce TMA due to unclear mechanism(s). For instance, ticlopidine and clopidogrel have been shown to occasionally induce TMA, although the underlying pathogenesis is unclear [69, 70]. Other medications in this category include deoxycoformycin, oxaliplatin, trimethoprim-sulfamethoxazole, and recently VEGF inhibitors [71–75]. Estrogen is another group of drugs that are known to trigger TMA. Oral contraceptives, hormonal replacement regimens, and tamoxifen have all been reported to induce TMA [76–78]. The underlying mechanisms might be related to a reduced production of prostacyclin [79].

Diagnosis of drug-associated TMA relies on a detailed drug-intake history and chronological association and the absence of other potential causes of TMA. Immune-mediated reaction associated with quinine tends to have acute onset and be severe, while nonimmune-mediated and drug-dosage-associated TMA tends to show a more gradual onset and presents with increasing TMA severity with cumulating drug exposure.

Treatment should focus on correct diagnosis and discontinuation of offending agents. A trial of plasma exchange may be considered in view of the potentially life-threatening nature of TMA, diagnostic confusion in differentiating drug-induced TMA from idiopathic TTP-HUS, and lack of other treatment options. Glucocorticoids are in general not used as their efficacy in drug-associated TMA has not been tested. Several strategies have been instituted for treating calcineurin-inhibitor-associated TMA with some success. These include cessation of calcineurin inhibitor, dose reduction, and switching one calcineurin inhibitor for another or replacing calcineurin inhibitor with sirolimus or belatacept. Unfortunately, sirolimus has been reported to induce post-transplant TMA itself. Some patients with cyclosporine-associated TMA can be rechallenged with tacrolimus without developing recurrent TMA. The combination of sirolimus with either cyclosporine or tacrolimus is associated with a higher incidence of TMA [80] and should be avoided. Plasma exchange has frequently been used as a part of the management for calcineurin-inhibitor-associated TMA especially

for patients with multisystemic involvement of TMA. In one report, the recovery of the kidney graft function reached 80 % (23 of 29 patients) by a combination treatment of plasma exchange and discontinuation of calcineurin inhibitors [81]. Mean duration of plasma exchange therapy was 8.5 (range 5–23) days in that study. IVIG has also been used either alone or with plasma exchange or as rescue therapy and the success rate has been variable. An optimal therapy for calcineurin-associated posttransplant TMA requires randomized trials which are not currently available. For VEGF-inhibitor TMA, a recent nonrandomized study showed that plasmapheresis demonstrated no outcome advantage over conservative measures and is associated with more adverse effects [82]. Recognition of potential drug etiology as a cause of TMA is critical and foremost to avoiding future exposure to the offending drug.

Cancer-Associated TMA

TMA can be associated with virtually any type of malignancy especially disseminated cancers [64]. Most patients with cancer-associated TMA exhibit overt clinical evidence of cancer. However, in a small fraction of the patients, TMA can be the presenting manifestation of cancer or cancer recurrence [83] and, consequently, be misdiagnosed as having the classic TTP-HUS and be treated inappropriately with plasma exchange.

Postulated pathogeneses for cancer-associated TMA include microvascular obstruction due to tumor emboli, activation of coagulation, and tumor-stimulated intimal proliferation. Cancer-associated TMA does not seem to be mediated by a deficiency of ADAMTS13 activity [84, 85].

Cancer-associated TMA is often accompanied by clinical features atypical for the classic TTP-HUS. Patients with cancer-associated TMA tend to be older, have previously been diagnosed with cancer, and present with a smoldering progression of symptoms over several months rather than several weeks as in the classic TTP-HUS. These patients also experience gradual onset of weight loss, generalized weakness, pain, and respiratory symptoms such as cough and dyspnea which are rare in patients with the classic TTP-HUS. Besides the characteristic biochemical changes associated with TMA, patients with cancer tend to show a much higher level of circulating LDH compared to that in the classic TTP-HUS [84, 86]. Severe ADAMTS13 deficiency (<5 %) and/or inhibitory ADAMTS13 antibodies are typically absent [85]. Identifying these features in TMA patients should raise suspicion for potential cancer-associated TMA.

The treatment should focus on the underlying malignancy. Plasma exchange has not been beneficial in patients with cancer-mediated TMA and is not indicated. In general, prognosis for cancer-associated TMA is poor as most of the patients have late-stage malignancy. Median survival after the diagnosis of tumor-associated TMA is typically in days or weeks [84, 86].

Solid Organ Transplant-Associated TMA

TMA is a recognized complication following solid organ transplantation (SOT), with an occurrence rate of roughly 0.5–15 %. Proposed risk factors for the development of TMA include HLA incompatibility, longer duration of the transplant surgery, lack of perioperative use of fresh frozen plasma, the use of calcineurin inhibitors, and underlying preexisting hypercoagulable abnormalities. The onset of post SOT-associated TMA is usually within the first few weeks after transplantation. Clinical features include anemia, reticulocytosis, LDH elevation, positive schistocytes on peripheral blood smear, and varying degrees of kidney dysfunction. The severity of this entity varies and may range from minimally symptomatic to fulminant and widespread multiorgan dysfunction with little chance of survival.

The pathogenesis of post SOT-associated TMA is incompletely understood, although direct endothelial injury related to immunosuppressive drugs, specifically calcineurin inhibitors and sirolimus; vascular rejection of the allograft; infections specifically with CMV reactivation; and other insults occurring in the course of posttransplant surgery have all been implicated in its pathogenesis (reviewed in [87]).

TMA may complicate ~1–15 % of kidney transplants among recipients on calcineurin-inhibitor-based immunosuppressive regimen [88, 89]. Most of the posttransplant TMA cases occur during the initial period of transplantation, although occasional cases have been reported to occur for up to 6 years. About a third of the posttransplant TMA cases is limited to the kidney graft and detected by allograft biopsy [89]. The remaining cases of TMA are associated with a varying degree of systemic manifestations including thrombocytopenia, microangiopathic hemolytic anemia, and organ dysfunctions. The use of calcineurin inhibitors is thought to induce vasoconstriction, endothelial injury, and hypercoagulability, potentially predisposing to TMA. Coexisting allograft ischemia-reperfusion injury presents another risk for the potential development of TMA [90]. Although long-term pathologic evolution of acute renal TMA has not been well described, the overall allograft survival is reduced with kidney transplant-associated TMA.

Studies have shown that TMA can occur in ~8 % adult recipients of living-donor liver transplant. The underlying mechanisms are also thought to be multifactorial causing direct vascular injury. In a study of 393 liver transplant recipients, 30 patients (7.6 %) developed TMA. TMA in this group of patients was associated with a reduced survival with 1-year survival rates of 60.6 % [91].

The optimal treatment strategy for post SOT-associated TMA has not been developed. The general approach has been to eliminate all possible causative conditions and potential offending medications such as cyclosporine, tacrolimus, and sirolimus. Aggressive treatment of potential opportunistic infections is critical as death in this condition is often associated with infections and other transplantation-related complications.

The outcome of SOT-associated TMA is poor in general despite the treatment, especially in patients with multiorgan involvement. Because of the grim outcome and, sometimes, uncertainty in its initial diagnosis, an urgent trial of plasma exchange as a part of the initial management may be justified.

Bone Marrow Transplant-Associated TMA

TMA is a known complication of bone marrow transplant. The incidence of TMA in bone marrow transplant recipients is approximately 7 %, 13.6 % in allograft recipients and 6.8 % in autograft recipients [92]. Risk factors for post-bone marrow transplant TMA are multiple, including intensive pre-transplant conditioning with chemotherapy and total body irradiation, posttransplant immunosuppression, and especially with toxic levels of calcineurin inhibitors, graft-versus-host disease, and infections including CMV and other opportunistic infections [93].

The etiology for the development of TMA in patients with bone marrow transplant has yet to be elucidated. Direct endothelial injury, toxic effects from radiation, and medications plus infections causing systemic inflammation are all thought to contribute to the development of TMA [92, 93]. TMA in this group of patients does not appear to be mediated by the deficiency of ADAMTS13 activity [94].

Prognosis for this condition is generally poor with mortality rate estimated between 20 % and 30 %. No standard treatment strategy has been established. In mild forms of TMA with restricted presentation of thrombocytopenia, anemia, and renal dysfunction associated with a high circulating level of calcineurin inhibitor, the conversion or discontinuation of calcineurin inhibitor plus plasma exchange has been shown to be associated with good outcome. In cases of severe TMA with multiorgan involvement, all treatment modalities including plasma exchange, discontinuation or conversion of calcineurin inhibitors, and treatment of infection have not been shown to be effective.

Hematopoietic Stem Cell Transplantation-Associated TMA

Hematopoietic stem cell transplant (HSCT) has been used as a curative therapy for a number of malignant and nonmalignant diseases. Indications for stem cell transplant are continuously being broadened, and HSCT will likely be used as a new curative therapy for more diseases in the years to come. With broadened indications for HSCT, HSCT-related complications have become a major factor relating to posttransplant morbidity and mortality.

TMA is an infrequent but potentially lethal complication of HSCT. The incidence of HSCT-associated TMA following allogeneic and autologous stem cell transplantation is approximately 0.5–15 % and 0.1–0.25 %, respectively. HSCT patients develop TMA mostly within 100 days after the HSCT with manifestations including thrombocytopenia, microangiopathic hemolytic anemia, and acute renal dysfunction with varying degrees of hypertension, hemoglobinuria, and proteinuria [95]. HSCT-associated TMA seems mostly restricted to the kidneys.

The etiology of HSCT-related TMA is thought to be multifactorial and independent of ADAMTS13 activity, including pre-transplant conditioning therapy involving high-dose chemotherapy and total body irradiation, graft-versus-host disease, viral reactivations including CMV viremia, and immunosuppressive drugs (cyclosporine and tacrolimus). Total body irradiation can cause vascular endothelial injury, contributing to the development of HSCT-associated TMA. Cyclosporine is cytotoxic to cultured endothelial cells at concentrations similar to plasma cyclosporine concentrations achieved in patients [96]. Tacrolimus, sirolimus, and VEGF inhibitors have all been associated with the occurrence of HSCT-associated TMA [67, 74, 97].

Some of the prominent risk factors for the development of HSCT-associated TMA include old age, HLA-mismatch, graft-versus-host disease, and CMV infection. It is important to point out that none of these risk factors have been consistently supported by case series and retrospective studies. The lack of reproducibility could be due to the lack of consensus criteria for the diagnosis of HSCT-associated TMA when the studies were conducted.

The renal pathology shows thickened capillary walls, occlusion of vascular lumen, fibrin deposition, and endothelial separation with expansion of subendothelial area.

Diagnosis of HSCT-associated TMA should exclude alternative causes for the TMA. A set of consensus diagnostic criteria for HSCT-associated TMA was recently validated [95]. The diagnosis can be made when all of the following are present:

1. Schistocytosis: schistocytes >2/high-powered field in peripheral blood smear.
2. Thrombocytopenia: platelet count <50,000/mm^3 or >50 % reduction from the baseline.
3. Increase in lactate dehydrogenase (LDH).
4. Decrease in hemoglobin.
5. Decrease in serum haptoglobin.
6. Negative Coombs' test.
7. Normal coagulation studies: normal prothrombin time and partial prothrombin time.

8. Concurrent kidney and/or neurological dysfunction without an alternative explanation (the kidney dysfunction is defined as doubling of serum creatinine without evidence of dehydration or 50 % decrease in creatinine clearance from the patient's baseline).

There is no standard treatment for HSCT-associated TMA. Discontinuation of potentially offending agents and aggressive treatment of graft-versus-host disease and infections constitute the initial treatment approach. Plasmapheresis, defibrotide, and intravenous immunoglobulin (IVIG) have all been tried with an unclear rate of success. Rituximab has been used in several cases with reported beneficial effects, although further evaluation of this treatment is required. Trials with C5a complement inhibitor such as eculizumab are currently underway.

The outcome of TMA in patients with stem cell transplants is generally poor. Overall mortality rate is estimated to be in excess of 60 % [98]. Death of these patients is mainly due to infection and other HSCT-related complications, indirectly related to TMA. Increased awareness of the potential risk factors, maximizing preventive measures, timely diagnosis, and early interventions will likely improve its outcome.

Acute Pancreatitis-Associated TMA

Pancreatitis has been shown to occasionally trigger acute TMA episodes. Swisher et al. reported five cases of TMA occurring shortly after episodes of acute pancreatitis. An extensive literature review added another 16 cases of TMA that occurred shortly following episodes of acute pancreatitis. All but two patients had normal ADAMTS13 activity [99].

The systemic inflammation is a hypothesized trigger of the acute TMA episode. This hypothesis was supported by the observation that even with severe ADAMTS13 deficiency due to genetic defects or inhibitory antibody or neutralizing antibody, acute episodes of TMA are generally triggered by infection and other modifying factors [100–102]. The severe systemic inflammatory responses in acute pancreatitis, presumably mediated by IL6, IL8, TNF-α, and other cytokines, have been postulated to incite TMA [103].

TMA in patients with acute pancreatitis tends to occur 2–15 days following the diagnosis of pancreatitis with medium onset of TMA 3 days after the onset of pancreatitis. All except one patient in the study by Swisher et al. [99] were treated with plasma exchange or plasma infusion, one treated with splenectomy and one with supportive care including dialysis. A few of these patients also received steroids. Notably, two patients in this report developed recurrent TMA following repeat episodes of pancreatitis. The other two patients with evidence of ADAMTS13 deficiency showed a strong inhibitor of ADAMTS13 and the inhibitor was IgG immunoglobulin. All five patients regained kidney function and 16 cases from the literature also regained kidney function without needing long-term dialysis. Although many of the patients in the report [99] showed no evidence of severe ADAMTS13 deficiency, nearly all of them received treatment with plasmapheresis. The necessity for plasmapheresis in patients without evidence of ADAMTS13 deficiency is unclear, but given the circumstances of TMA and potential lethality of delay in treating idiopathic TTP-HUS, a trial of plasmapheresis seems reasonable.

Infection-Associated TMA

A broad spectrum of infectious disease can incite clinical symptoms mimicking TTP with a complete pentad presentation. Booth et al. [104] described 31 patients who were diagnosed with idiopathic TTP and treated with plasma exchange but were subsequently found to have systemic infection; the plasma exchange was discontinued. The source of the patients was the Oklahoma TTP-HUS registry (from 1989 to 2010) in which patients were enrolled at the time of their initial diagnosis and request for plasma exchange treatment. Among these patients, four had severe ADAMTS13 deficiency. Infection in these patients was thought to trigger the onset of acute episodes of TMA. From the same registry, 68 consecutive patients with idiopathic TTP (ADAMTS13 activity <10 %) served as controls. Comparison between the infection-associated TMA and controls showed that patients with infection-associated TMA were older and tended to present with more severe neurological symptoms, such as coma, and had a high mortality rate, 68 % (21 of 31 patients) during their acute TMA episode. None of the survivors with systemic infection had relapsed.

It is important to recognize that the presence of ADAMTS13 deficiency or the presence of ADAMTS13 inhibitors should not automatically exclude the presence of systemic infection as a potential cause of TMA. Moreover, of the patients with proven systemic infections, no single infectious etiology turned out to be a major mimic of TTP. Thus, any severe bacterial, viral, or fungal infection could potentially incite TTP. HIV infection has been reported repeatedly as a cause of TMA; it is discussed below.

Human Immunodeficiency Virus-Associated TMA

HIV-associated TMA mostly occur in patients with heavy viral load and may be an AIDS-defining manifestation [105, 106]. TMA occurring in well-controlled or at the early-stage HIV-infected patients should raise suspicion of the possibility of coincidental concurrent of HIV infection and the classic TTP-HUS.

Pathogenesis of HIV-associated TMA is likely heterogeneous. HIV infection can cause endothelial dysfunction, increased production of cytokines and expression of adhesion molecules, increased neutrophil/mononuclear cell-endothelial cell adhesion [107–110] and ultimately engender a predilection of vessel wall thrombus formation. HIV patients are often coinfected by other viruses, i.e., human herpes virus-8 that is known to cause vascular endothelium dysfunction [111] and malignancy which can contribute to the development of TMA. HIV-infected patients have also shown to develop TTP with profound deficiency of ADAMTS13 activity, especially in those of African descent [112]. The connection between HIV infection and the genesis of severe ADAMTS13 deficiency is not understood. These patients tend to have a less advanced HIV disease and are highly responsive to plasma exchange and highly active antiretroviral therapy (HAART) [113, 114].

Treatment of HIV-associated TMA should focus on treatment of HIV. This is supported by evidence showing that the introduction of HAART has dramatically reduced the frequency of HIV-associated TMA from 1.4–7 % to 0.3 % [115, 116]. Plasma exchange may be used initially in HIV-TMA patients with severely reduced ADAMTS13. Hart et al. [117] studied 30 episodes of TMA in 24 HIV patients. The patients were retrospectively collected from the UK and Southeast England TTP registry. All patients were black, had typical TMA clinical features, and were referred for plasma exchange. Sixteen of the 24 patients had critically reduced ADAMTS13 level, <5 %, and the level in the remaining patients was <50 %. All patients received a combination of plasma exchange and HAART, and most of them also received steroids. The treatment led to complete TMA remission.

In practice, given the life-threatening nature of TTP and a known connection between the HIV infection and the development of ADAMTS13 deficiency, concern for withholding a potentially effective treatment (plasma exchange) often overrides the diagnostic uncertainty. However, plasma exchange should always be instituted in conjunction with HARRT. Once the plasma exchange has begun, the appropriateness of continued pheresis should be reassessed. A continuing search for other potential triggering factors especially infection or malignancy in this patient population is essential to guide treatment decisions and to avoid unnecessary plasma exchange which may be ineffective and can potentially cause harm [118].

Surgery-Associated TMA

Postoperative TMA is a rare but life-threatening complication. It was initially observed in patients undergoing cardiac and vascular surgeries [119, 120]. Subsequently, postoperative TMA was observed in patients with abdominal and orthopedic surgeries [121]. The number of reported cases, however, is too small to estimate an incidence. Patients typically develop thrombocytopenia and microangiopathic hemolytic anemia 5–9 days post surgery. As in idiopathic TTP-HUS, fever, kidney dysfunction and altered mental status can all be variably present.

Proposed mechanisms of surgery-associated TMA include direct endothelial injury occurring during the surgery and release of high-molecular weight vWF multimers [121]. Theoretically, patients with marginal levels of ADAMTS13 are at higher risk for postoperative TMA. A recent five-case series examined the ADAMTS13 activities and showed that the activity ranged between 29 and 66 % in four of the five patients with available data. None had any detectable ADAMTS13 antibody [122]. At present, diagnosis of this condition must first exclude other potential causes of TMA.

Treatment for surgery-associated TMA has not been established. Reported cases have received a combination of plasmapheresis and corticosteroids until the platelet level normalizes. Plasmapheresis theoretically removes high-molecular weight vWF multimer and replenishes the plasma with normal-sized vWF, thereby arresting the disease progression. Although with some success, the real efficacy of such strategy is unclear. The overall prognosis of postoperative TMA seems poor especially when there is a delay in making the diagnosis [121]. Such delay in reported cases has invariably resulted in mortality.

In summary, postoperative TMA is rare but potentially fatal. An increased awareness and timely diagnosis and interventions might improve its outcome although experience of this entity is thus far limited to case reports.

Conclusion

TMA is characterized by thrombocytopenia, microangiopathic hemolytic anemia and functional impairment of multiple organs, especially the kidney and brain. In addition to the classic idiopathic TTP-HUS, TMA is seen in association with many disorders and in diverse clinical settings as discussed in this chapter. It is important to recognize that, in contrast to idiopathic TTP, severe deficiency of ADAMTS13 activity rarely occurs in TMA associated with bone marrow transplantation, drugs, disseminated cancer, connective and autoimmune disorders and severe infections [123]. Pathological findings of TMA are typically endothelial cell injury with intimal hyperplasia, luminal narrowing in arteries and arterioles, endothelial cell swelling with subendothelial widening in glomerular capillary and luminal thrombi. Prognosis for TMA (excluding the classic TTP-HUS) in general is poor. Whenever possible, the potentially inciting factor should be corrected. Better understanding of the underlying pathogenesis in each clinical setting will undoubtedly lead to more effective therapy in the future.

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Renal Involvement by IgG4-Related Disease

17

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IgG4-related disease (IgG4-RD) is a recently recognized systemic immune-mediated disease, typically characterized by mass-forming fibroinflammatory lesions that may affect nearly any organ. This systemic disease was first recognized in the pancreas. In 1961, Sarles et al. described sclerosing pancreatitis [1]; this pattern of organ involvement is now called autoimmune pancreatitis type I (AIP). Sarles et al. speculated on the autoimmune nature of this disease because of the absence of evidence of an infection and the presence of hypergammaglobulinemia in some patients. The involvement of other organs has been recognized more recently [2]. The lesions in different organs often show striking histologic similarity.

The link between IgG4 and AIP was elucidated in 2001 by Hamano et al., who found increased serum IgG4 levels in patients with AIP [3]. Hamano and colleagues later demonstrated tissue infiltration by IgG4+ plasma cells in the pancreas in AIP [4]. Kamisawa et al. made the leap to systemic disease by showing increased IgG4+ plasma cells in AIP patients compared to controls not only in the pancreas but also in other organs and tissues [5]. These findings, with the aid of IgG4 tissue immunostaining, paved the way to identify other organs involved by IgG4-RD.

Some form of IgG4-RD, usually in the form of an inflammatory mass, has been described in nearly every organ system, including the liver, gallbladder, other gastrointestinal sites, salivary and lacrimal glands, orbit, breast, lung, kidney, retroperitoneum, aorta, lymph nodes, skin, pituitary gland, and prostate [5–11]. The usual clinical presentation of a mass, along with a history of unintentional weight loss and fatigue, raises the clinical suspicion of a malignancy.

IgG4-RD in the kidney may also present as an inflammatory mass evident on radiographic studies. Tissue samples of these lesions reveal tubulointerstitial nephritis [12]. Takahashi et al. found radiographic evidence of renal parenchymal involvement in 30 % of patients with established AIP [13], likely representing inflammatory masses due to the distinctive radiographic appearance of the lesions. However, renal mass lesions are not always present when the kidney is involved by IgG4-RD. Renal involvement by this disease also commonly presents as acute or progressive chronic renal failure (with or without mass lesions); in one series, most of these cases also showed radiographic abnormalities by MRI, CT, or ultrasound examination [14]. Other presenting features are proteinuria due to associated glomerular disease or obstruction related to retroperitoneal fibrosis. Renal involvement by this disease is collectively referred to as "IgG4-related kidney disease," as proposed in a nomenclature consensus statement by the International Symposium on IgG4-Related Disease, held in Boston, Massachusetts, in October 2011.

IgG4-Related Tubulointerstitial Nephritis

The most common pattern of involvement by IgG4-RD in the kidney is tubulointerstitial nephritis (TIN). This histologic pattern in general refers to interstitial inflammation accompanied by tubulitis and tubular injury, with or without interstitial fibrosis. TIN and tubulointerstitial nephropathy are classified according to the cause and can be divided into broad categories of drug-related, hereditary/toxic/metabolic, infection (direct or reactive to a distant infection), autoimmune, and idiopathic/other [15]. Some overlap exists between the different categories.

The cause of TIN can be determined by biopsy features by light microscopy, immunofluorescence (IF), and electron microscopy (EM) in conjunction with clinical history and clinical laboratory results and radiographic studies. TIN that occurs as part of IgG4-RD, referred to as "IgG4-related tubulointerstitial nephritis" (IgG4-TIN), is a specific type of

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autoimmune TIN [15]. Saeki et al. and Raissian et al. have collected data on the two largest biopsy series of IgG4-TIN, at 23 and 35 cases, respectively [14, 16]. Both of these series showed many clinical and histologic features in the kidney that have also been encountered in the pancreas: presence of radiographic abnormalities, plasma cell-rich inflammatory infiltrates with increased IgG4+ plasma cells, elevated total IgG or IgG4 levels in the serum, presence of other organ involvement, and rapid response to steroid therapy. Features specific to the kidney are detailed below.

Clinical Features of IgG4-TIN

Similar to AIP, most patients with IgG4-TIN are men (~80 %), with a mean age of 65 years. Japanese studies were composed of all Japanese patients; the Raissian et al. series from North America contained patients from other racial and ethnic groups, mostly Caucasian. Most patients (57 % and 76 %, respectively, in the Saeki and Raissian series) have acute or progressive chronic renal failure at the time of renal biopsy, while the primary indication for biopsy or nephrectomy in other patients usually is a renal radiographic lesion. In a radiographic series, in which renal lesions were incidentally found during evaluation for AIP, patients did not have renal-specific symptoms [13]. In this study, renal function was normal or mildly diminished, with a serum creatinine range of 0.9–1.6, which was not different from those with AIP without radiographic evidence of renal involvement.

Over 80 % of patients in the Raissian et al. biopsy series had other organ involvement, either concurrent or prior to the recognized renal involvement. The most common extrarenal sites affected are the pancreas and liver; other involved organs described include the salivary or lacrimal glands, lung, gallbladder, aorta (inflammatory abdominal aortic aneurysm), heart (pericarditis), skin (leukocytoclastic vasculitis or pseudolymphomatous infiltrate) [17, 18], retroperitoneum and/or ureter (retroperitoneal fibrosis), sinuses, lymph nodes, joints (inflammatory arthritis), prostate (prostatitis), pituitary, thyroid, and colon (inflammatory bowel disease) and pseudotumors in the orbit, paraspinal soft tissue, and testis.

Laboratory Features of IgG4-TIN

Elevated serum total IgG and IgG4 subclass levels has been observed in ~70–80 % of AIP patients [19] and can be a useful indicator of IgG4-RD in patients who have a positive serologic finding in the appropriate clinical setting. Similarly, in IgG4-TIN, Raissian et al. found that almost 80 % of patients with measurements available in a series of IgG4-TIN had elevated serum total IgG or IgG4 levels and 92 % had an elevated serum IgG4 level; some additional patients without IgG or IgG4 levels available had hypergammaglobulinemia [14]. However, serologic testing for IgG4 levels is not diagnostic. In AIP, which has been better characterized, only 70–80 % of patients have an elevated serum IgG4 level [19]. The finding of an elevated serum IgG4 level alone is also not specific for IgG4-RD, as 5 % of the normal population and 10 % of pancreatic cancer patients have an elevated serum IgG4 level [20].

Other common laboratory features are hypocomplementemia (decreased serum C3 and/or C4 levels), seen in 56–78 % of IgG4-TIN patients, and peripheral blood eosinophilia, seen in 33–48 % of IgG4-TIN patients [14, 16]. Approximately 30 % of patients have a positive ANA, mostly low titer [14].

Radiographic Features of IgG4-TIN

Renal involvement in patients with AIP is not uncommon and has been reported in 14–39 % of patients based on CT or MR findings [13, 21–23]. Renal lesions are commonly bilateral and multiple and predominantly involve the renal cortex; they are best visualized on contrast-enhanced CT scan. Renal parenchymal lesions can be classified as small peripheral cortical nodules, round or wedge-shaped lesions, or diffuse patchy involvement [13]. Sometimes, a renal lesion may manifest as a large solitary mass that mimics a neoplasm. The differential diagnosis of renal parenchymal lesions includes lymphoma, metastases, pyelonephritis, and vasculitis.

In a histopathologic study of patients with IgG4-TIN on a tissue specimen, 78 % with available radiographic data showed radiographic lesions [14]. Overall, 77 % of these patients had renal insufficiency, which was in most cases the reason for renal biopsy. As would be expected, the mean serum creatinine was lower in patients with renal tissue specimens obtained primarily for mass lesions compared to those biopsied for renal failure (1.4 mg/dl versus 4.2 mg/dl respectively) [14, 24].

Biopsy Features of IgG4-TIN

By light microscopy, IgG4-TIN shows a plasma cell-rich interstitial inflammatory infiltrate. There is a range of histologic appearances, from an acute tubulointerstitial nephritis with minimal fibrosis, to an intermediate pattern with some interstitial fibrosis but still a marked inflammatory infiltrate, to a densely fibrotic, pauci-cellular pattern with extensive tubular destruction and atrophy (Fig. 17.1). The fibrosis is expansile and pushes apart the tubules and often has a “storiform” pattern as seen in other organs involved by IgG4-RD. All cases by definition show TIN with increased plasma cells, as well as mononuclear cells. Some cases show numerous eosinophils.

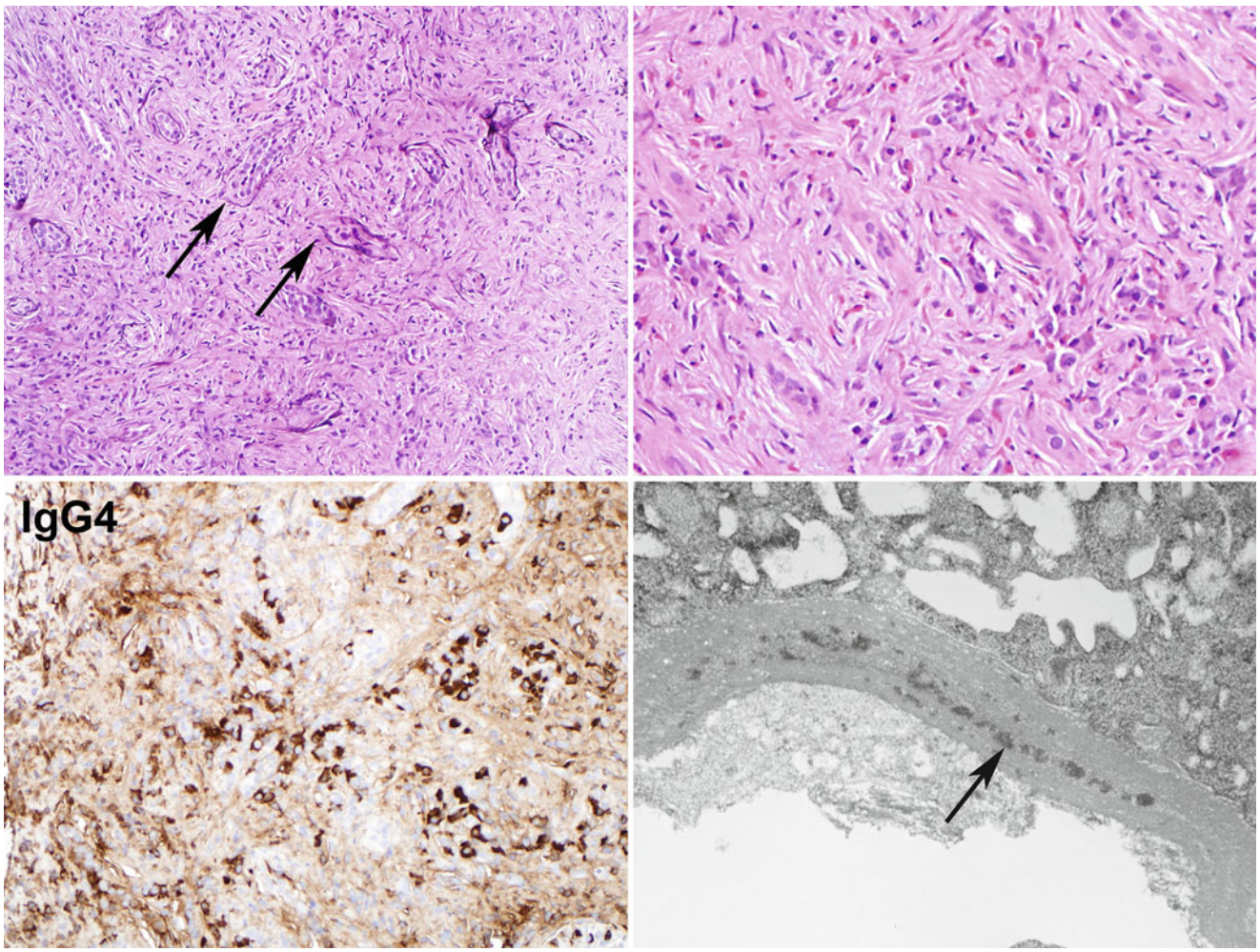

Fig. 17.1 A biopsy of a mass lesion in a 62-year-old man shows an expansile "storiform" interstitial fibrosis with marked interstitial inflammation. A silver stain (*upper left panel*) highlights tubular basement membranes (*arrows*) of tubules that have been pushed apart by the interstitial fibrosis. On higher magnification (*upper right panel*), the inflammatory infiltrate is seen to be composed of numerous eosinophils and plasma cells, as well as mononuclear cells. An immunoperoxidase stain for IgG4 (*lower left panel*) shows markedly increased IgG4+ plasma cells. As this biopsy was performed for a mass, no tissue was submitted for immunofluorescence staining, but deparaffinized tissue processed for electron microscopy reveals tubular basement membrane immune complex deposits (*arrow*; *lower right panel*)

Focal mild mononuclear cell tubulitis is seen in most cases, and eosinophilic or plasma cell tubulitis may also be seen. In some cases, tubules are destroyed, and only fragments of TBMs can be seen on PAS or silver-stained sections.

Glomeruli generally appear normal or show mild mesangial matrix expansion or hypercellularity. If there is a concurrent membranous glomerulonephritis (MGN), then glomeruli may show thickened glomerular capillary loops, glomerular basement membrane "spikes" on silver or PAS stains, or subepithelial immune deposits on a trichrome stain. Arteries show no specific features in IgG4-TIN.

By immunofluorescence, >80 % of cases show TBM immune complex deposits, which stain for IgG and kappa and lambda light chains and usually stain for C3, and occasionally for C1q [14, 24]. Biopsies can show focal or diffuse TBM granular staining. Several cases stained by immunofluorescence for IgG subclasses show IgG4-dominant staining of TBMs, although other IgG subclasses are also variably present (LD Cornell, unpublished data). TBM deposits are found more frequently in cases with fibrosis than in cases with a pattern of acute interstitial nephritis [14, 24] and are found only in areas of the fibroinflammatory process and not in adjacent unaffected areas. Glomeruli are usually negative by immunofluorescence unless there is a concurrent membranous glomerulonephritis, in which case glomeruli show granular subepithelial glomerular basement membrane staining for IgG, C3, and kappa and lambda light chains. Some cases have been described to show mesangial immune deposits without a more specific glomerular disease assigned [12, 16].

Biopsies with deposits seen by immunofluorescence show corresponding electron-dense deposits by electron microscopy. TBM deposits are present in areas with inflammation or

fibrosis and are not present in the unaffected areas of the kidney. The deposits appear finely granular and do not show substructure. Occasional interstitial deposits may also be seen within areas of fibrosis. Glomeruli typically are free of deposits, unless there is a concurrent membranous glomerulonephritis, in which case there are numerous subepithelial electron-dense deposits. Formalin-fixed, paraffin-embedded tissue from biopsy or nephrectomy samples done for a mass lesion may be deparaffinized for electron microscopy in order to visualize immune complex deposits.

Value of IgG4 Staining

Zhang et al. and others have found that IgG4 staining in pancreas for increased IgG4+ plasma cells is useful to distinguish AIP from other forms of pancreatic inflammation, including chronic alcoholic pancreatitis and inflammatory infiltrates surrounding pancreatic cancers [25]. In the kidney, more types and causes of inflammatory infiltrates are recognized that give a pattern of TIN. Raissian et al. examined the concentration of IgG4+ plasma cells in IgG4-TIN and in a variety of other forms of TIN that could mimic IgG4-TIN clinically and histologically [14, 24]. The authors found a sensitivity of 100 % [95 % confidence interval (CI), 0.9–1] and specificity of 92 % (CI 0.86–0.95) using a cutoff of focal moderate (11–30 IgG4+ cells/40× field) to marked (>30 IgG4+ cells/40× field) increase in IgG4+ plasma cells for distinguishing IgG4-TIN from other forms of TIN, with the exception of inflammatory infiltrates in pauci-immune necrotizing and crescentic glomerulonephritis. In pauci-immune necrotizing and crescentic glomerulonephritis, >30 % of cases showed a moderate to marked increase in IgG4+ plasma cells. Similar findings have been noted in granulomatosis with polyangiitis (Wegener's) affecting other organs [26]. The absence of a serum ANCA (or anti-myeloperoxidase or -proteinase 3 antibodies) and a necrotizing or crescentic glomerulonephritis on the tissue specimen helps to exclude pauci-immune glomerulonephritis as a cause of the interstitial inflammation in these cases. A few other causes of interstitial inflammation could also give focally increased IgG4+ plasma cells, including chronic pyelonephritis; these other causes usually can be distinguished by other clinical and histopathologic features. Notably, nearly all cases of Sjögren syndrome-related TIN did not show increased IgG4+ plasma cells. Clinicians and pathologists should keep in mind that IgG4 staining alone is not diagnostic of IgG4-related disease.

Diagnosis of IgG4-TIN

Two papers, from Japan and from North America, have proposed similar diagnostic criteria for IgG4-TIN that include clinical, histopathologic, serologic, and radiographic features [14, 27] (Table 17.1).

Table 17.1 Proposed diagnostic criteria for IgG4-TIN [14]

Histology	Plasma cell-rich tubulointerstitial nephritis with >10 IgG4+ plasma cells/hpf field in the most concentrated field[a]
	Tubular basement membrane immune complex deposits by immunofluorescence, immunohistochemistry, and/or electron microscopy[b]
Imaging	Small peripheral low-attenuation cortical nodules, round or wedge-shaped lesions, or diffuse patchy involvement
	Diffuse marked enlargement of kidneys
Serology	Elevated serum IgG4 or total IgG level
Other organ involvement	Includes autoimmune pancreatitis, sclerosing cholangitis, inflammatory masses in any organ, sialadenitis, inflammatory aortic aneurysm, lung involvement, retroperitoneal fibrosis

[a]Mandatory criterion

[b]Supportive criterion, present in >80 % of cases

Diagnosis of IgG4-TIN requires the histologic feature of plasma cell-rich TIN with increased IgG4+ plasma cells and at least one other feature from the Imaging, Serology, or Other organ involvement categories.

Response to Therapy

Similar to AIP, IgG4-TIN also usually shows a rapid response to steroid therapy. In both the Saeki and Raissian series, 90 % of patients with elevated serum creatinine at presentation who were treated with steroids showed decreased creatinine at follow-up, from 1 to 36 months, including 90 % at 1 month follow-up in the Saeki series. While TIN of different causes may respond to steroid therapy, IgG4-TIN tends to show a more brisk response, even in cases with severe interstitial fibrosis on the biopsy sample.

On imaging, renal lesions improve or resolve after steroid treatment. Focal cortical parenchymal loss (scars) may be present after treatment. Similar to involvement of other organs by IgG4-RD, relapse of renal lesions may occur after cessation of steroid treatment. Without steroid treatment, renal lesions may progress.

Other Renal Involvement

Glomerular Disease

Glomerular diseases have also been seen in patients with IgG4-RD. Membranous glomerulonephritis (MGN) is most commonly observed, present in approximately 7 % of IgG4-RD patients in two biopsy series of renal parenchymal

involvement by TIN, and has been noted in case reports [14, 16, 24, 28, 29]. This glomerular disease may also occur in patients without TIN but with other features of IgG4-RD: one series of MGN in IgG4-RD patients showed that 50 % lacked concurrent TIN on the biopsy [30].

Other glomerular diseases have been variably reported in IgG4-RD, including IgA nephropathy and membranoproliferative glomerulonephritis [16, 31] and minimal change disease (Takako Saeki, personal communication). Although common in patients without AIP, diabetes mellitus may be a manifestation of AIP due to pancreatic endocrine insufficiency, and this may also affect the glomeruli in the form of diabetic glomerulosclerosis.

Obstruction Related to Retroperitoneal Fibrosis

The extrarenal manifestation of retroperitoneal fibrosis or ureteral inflammatory mass(es) may give rise to hydronephrosis, with or without accompanying renal parenchymal involvement. Histologic sections reveal storiform fibrosis with scattered areas of plasma cell-rich inflammation, similar to what is described in other organs involved by IgG4-RD.

Pathogenesis of IgG4-RD

IgG4 is an unusual immunoglobulin molecule. Compared to IgG1, IgG4 has weaker interchain bonds, resulting in a high rate of dissociation of immunoglobulin half-molecules. In this way, the IgG4 molecule cannot fix complement by the classical pathway. IgG4 may also bind and thereby block antigen from access by the more pathogenic IgG1 or IgE.

Despite being thought of as an "anti-inflammatory" immunoglobulin, IgG4 nevertheless is often found in high levels in IgG4-RD, in both the serum and in the tissue as infiltrating plasma cells. IgG4-RD shows evidence of a T helper 2-dominant immune response, both in examination of peripheral blood mononuclear cells and in affected tissue in this disease [32–36]. IL-10 has an effect on IgG4 versus IgE class switching and may be required for IgG4 class-switched B cells to differentiate into IgG4-secreting plasma cells [36]. In IgG4-RD, one may speculate that an initial insult occurs, with resulting production of anti-inflammatory cytokines, including IL-10 and tumor necrosis factor alpha, along with fibrogenic IL-13. These cytokines may drive increased fibrosis, induction of IgG4 class-switched B cells, and production and massive expansion of IgG4-secreting plasma cells. Details of a specific mechanism of this disease and its relationship to IgG4 and unusual histopathologic and radiographic features, however, remain to be elucidated.

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The Collagen IV Nephropathies

18

Clifford E. Kashtan

Alport Syndrome

Studies of the molecular biology of Alport syndrome (AS) have yielded important insights into the roles played by collagen IV networks in maintaining the structure and function of the glomerular basement membrane (GBM). The clinical features of AS represent the phenotypic consequences of mutations that alter the basement membrane expression of a particular collagen IV network composed of α(alpha)3, α(alpha)4, and α(alpha)5(IV) chains. X-linked, autosomal recessive, and autosomal dominant forms of AS have been characterized at the gene and protein levels. Absence of three specific isomers of collagen IV, the α(alpha)3, α(alpha)4, and α(alpha)5(IV) chains, from Alport GBM results early in life in GBM attenuation, manifesting clinically as hematuria. Over time, GBM deficient in these chains undergoes progressive thickening and disorganization, likely secondary to transcriptional changes in glomerular endothelial and epithelial cells, leading to the development of proteinuria and, ultimately, renal fibrosis and end-stage kidney disease.

Clinical Features

Renal Findings

Hematuria is the cardinal finding of AS. Affected males have persistent microscopic hematuria that is often first detected during infancy [1, 2]. Many boys also have episodic gross hematuria during the first two decades of life, and some have nearly continuous gross hematuria [2]. Boys who are free of hematuria during the first 10 years of life are unlikely to be affected [2].

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Females who are heterozygous for X-linked AS (XLAS) have persistent or intermittent microscopic hematuria, although about 7% of obligate heterozygotes never manifest hematuria [3]. Like affected boys, girls with XLAS may have episodic or continuous gross hematuria. Hematuria appears to be persistent in both males and females with autosomal recessive AS (ARAS).

Proteinuria is usually absent in the first few years of life but develops eventually in all XLAS males, in many XLAS females, and in both males and females with recessive disease [2–4]. Proteinuria increases progressively with age and may ultimately result in the nephrotic syndrome [2, 5].

Hypertension also increases in incidence and severity with age. Like proteinuria, hypertension is more likely to occur in affected males than in affected females with XLAS, but there are no gender differences in the autosomal recessive form.

End-stage renal disease (ESRD) develops in virtually all affected males with XLAS, but the rate of progression shows significant interkindred variability [6]. About 50 % of affected males reach ESRD by age 25, 90 % by age 40, and nearly 100 % by age 60 [4]. Several authors have observed that the rate of progression to renal failure is fairly constant among affected males within a particular family [6, 7], although significant intrakindred variability in the rate of progression to renal failure has occasionally been reported [8]. The timing of ESRD in males with XLAS is strongly influenced by the nature of the underlying mutation, as discussed in detail later in this chapter (see "Phenotype–Genotype Correlations in Alport Syndrome").

Progression to ESRD in females with XLAS was, until recently, considered an unusual event. However, in a landmark study of several hundred XLAS females, Jais and colleagues found that about 12 % developed ESRD before age 40 (compared with 90 % of XLAS males), increasing to 30 % by age 60 and 40 % by age 80 [3]. Although this study may have overestimated the incidence of ESRD among XLAS heterozygotes due to ascertainment bias, the fact remains that XLAS females are at significant risk for progression

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to chronic kidney disease and end-stage kidney disease. Proteinuria, gross hematuria, hearing loss, and extensive ultrastructural changes in GBM are risk factors for renal disease progression in Alport females [9]. Both males and females with ARAS appear likely to progress to ESRD during the second or third decade of life.

Hearing Deficit

About 50 % of XLAS males display sensorineural deafness by age 25, and by age 40 approximately 90 % of XLAS males have deafness [4]. Although it has been clear for some time that the hearing defect in AS localizes to the cochlea, histological correlates of deafness in AS patients have only recently been described. Merchant and colleagues found unique and consistent abnormalities in the cochleae of AS patients, consisting of a zone of separation between the basilar membrane and the cells of the organ of Corti, associated with a cellular infiltrate in the tunnel of Corti and extracellular spaces of Nuel [10]. Electron microscopy showed that the zone of separation occurred between the basement membrane of the organ of Corti and the basilar membrane. These authors speculated that abnormal adhesion of the organ of Corti to the basilar membrane might disrupt the mechanical relationships between outer hair cells and the basilar membrane, resulting in hearing impairment. Similar histological abnormalities have not been seen in animals with AS, however.

Hearing loss in AS is never congenital, is always bilateral, and usually becomes detectable by audiogram by late childhood to early adolescence in boys with XLAS [2]. Hearing impairment in members of families with AS is always accompanied by evidence of renal involvement [2]. There is no convincing evidence that deaf males lacking renal disease can transmit AS to their offspring. In females with XLAS, hearing loss is less frequent, tends to be less severe, and occurs later in life [3]. There do not appear to be gender differences in the incidence or course of deafness in autosomal disease.

In its early stages, the hearing deficit is detectable only by audiometry, with bilateral reduction in sensitivity to tones in the 2,000–8,000 Hz range. In affected males the deficit is progressive and eventually extends to other frequencies, including those of conversational speech. Although these males frequently require hearing aids, their deafness tends to stabilize once they develop ESRD. In most AS patients, speech discrimination is preserved and hearing aids are effective.

Ocular Defects

Ocular defects occur frequently in patients with AS, affecting at least 15 to 30 % of subjects [4, 11–13]. Anterior lenticonus, the conical protrusion of the central portion of the lens into the anterior chamber of the eye, is pathognomonic of AS. Nielsen [14] found that all reported patients with anterior lenticonus who had been adequately examined exhibited evidence of chronic nephritis and sensorineural deafness. Anterior lenticonus is almost entirely restricted to AS families with rapid progression to ESRD (i.e., before age 30) and deafness. Anterior lenticonus is absent at birth, usually appearing during the second to third decade of life [15]. Marked attenuation and fractures of the central portion of the anterior lens capsule have been observed [16, 17].

Various other ocular lesions have been reported in patients with AS. Perhaps the most commonly occurring abnormalities are pigmentary changes in the perimacular region, consisting of whitish or yellowish granulations surrounding the foveal area [11, 18–20]. These retinal flecks are frequently accompanied by anterior lenticonus but may also occur in the absence of lenticonus. These lesions may arise as a result of abnormalities of Bruch's membrane, the basement membrane that supports the retinal pigment epithelium [21].

Corneal endothelial vesicles (posterior polymorphous dystrophy) have been observed in AS patients by several investigators [19, 22, 23] and may be indicative of abnormalities in Descemet's membrane, the basement membrane underlying the corneal endothelium. Rhys et al. described recurrent corneal erosion in AS patients, which they ascribed to abnormalities of the corneal epithelial basement membrane [24].

Diffuse Leiomyomatosis

The association of XLAS with leiomyomas of the esophagus and tracheobronchial tree has been reported in several dozen families [25–27]. Affected females in these kindreds typically exhibit genital leiomyomas as well, with clitoral hypertrophy and variable involvement of the labia majora and uterus. Symptoms usually appear in late childhood and include dysphagia, postprandial vomiting, retrosternal or epigastric pain, recurrent bronchitis, dyspnea, cough, and stridor. Leiomyomatosis may be suspected by chest X-ray or barium swallow and confirmed by computed tomography or magnetic resonance imaging.

Arterial Disease

There have been several reports of arterial disease in relatively young males with Alport syndrome, including aneurysms of the thoracic and abdominal aorta and the cerebral arterial system [28–31].

Pathology

Although there are no pathognomonic lesions by light microscopy or direct immunofluorescence in AS, electron microscopy frequently reveals diagnostic abnormalities. The cardinal ultrastructural feature of the kidney in AS is diffuse thickening of the GBM associated with splitting of the lamina densa into multiple, apparently interwoven strands (the so-called basket-weave transformation) (Fig. 18.1). Identical

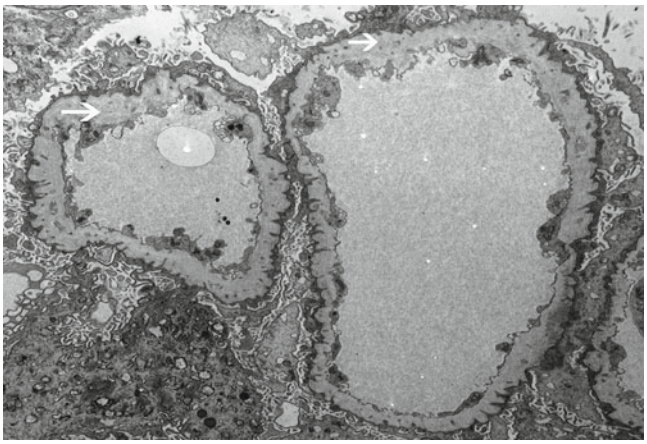

Fig. 18.1 Glomerular capillary wall showing thickening and widening (*arrows*) with basement membrane splitting and lamellations (*basket-weave appearance*). Note extensive foot process effacement. (x6000). Courtesy Dr. Sanjeev Sethi, Mayo Clinic

morphologic features are apparent in some patients without a family history of nephritis. Such patients may represent new *COL4A5* mutations; they may be offspring of asymptomatic female carriers, or may have autosomal recessive disease.

Ultrastructural alterations of the GBM were noted in several reports of hereditary nephritis in the 1960s and 1970s [1, 32–36]. The most prominent abnormality is the irregular appearance of the lamina densa, which demonstrates zones of thickening, thinning, and splitting that may be interposed with segments of GBM that appear normal. The abnormalities can affect a portion of a capillary loop, an entire loop, and some or all loops and can even appear to spare individual glomeruli.

The thick segments measure up to 1,200 nm, usually have irregular outer and inner contours, and are found more commonly in males than in females. The lamina densa is transformed into a heterogeneous network of membranous strands, which enclose clear electron-lucent areas. The electron-lucent zones may be entirely clear or contain round granules of variable density that measure between 20 and 90 nm in diameter. The origin of these granules is unknown, but Rumpelt has hypothesized that they represent degenerating islands of visceral epithelial cell cytoplasm [37]. Young children and females, and adult males on occasion [38], may have diffusely attenuated segments of GBM measuring as little as 100 nm or less; in some instances, discontinuities of the GBM have been observed [2, 39].

The specificity of the GBM changes has been questioned [40]. Focal lamina densa splitting and lamellation may be observed in renal biopsies from patients with a variety of glomerular disorders. Clinical correlation and immunofluorescence microscopic examination of all biopsies are necessary when AS is the suspected diagnosis. Diffuse thickening and splitting of the GBM are strongly suggestive of AS. In the absence of immune deposits, these changes are pathognomonic. Occasional patients have an immune complex nephritis such as IgA nephropathy superimposed on AS [41].

Not all Alport kindreds demonstrate these characteristic ultrastructural features [42–44]. Thick, thin, normal, and nonspecifically altered GBMs have all been described. Although diffuse attenuation of GBM has been considered the hallmark of "benign familial hematuria" or thin basement membrane nephropathy (TBMN) [45–47], some patients with this abnormality are members of kindreds with a history of progression to renal failure [48, 49]. Thus, the significance of an ultrastructural finding of thin GBMs must be considered in the context of the family history, basement membrane expression of collagen IV collagen α(alpha) chains (see below), and genetic information.

Rumpelt described a correlation between the percent of GBM showing splitting of the lamina densa and the degree of proteinuria in AS patients [37], suggesting that impaired permselectivity may be a functional consequence of the GBM alteration. Kim and colleagues observed that morphometric parameters such as mesangial volume fraction, cortical interstitial volume fraction, and percent global glomerular sclerosis were inversely correlated with creatinine clearance in AS, while creatinine clearance and surface density of peripheral GBM were directly correlated [50]. Similar relationships have been observed in other diseases, such as diabetic nephropathy and membranoproliferative glomerulonephritis (MPGN). However, patients with AS showed significantly greater impairment of filtration for any degree of structural change, in comparison with patients with diabetic nephropathy or MPGN. This observation suggests that these morphologic abnormalities only partially account for reductions in creatinine clearance in AS. Decreased conductivity of water across the altered glomerular capillary wall could contribute to the decrement in filtration.

Kidney biopsies of patients with advanced AS show nonspecific changes such as glomerulosclerosis, tubular atrophy, and interstitial fibrosis, potentially obscuring the diagnosis. Collagen IV immunohistochemistry and molecular genetic studies are often diagnostic in these ambiguous cases, as they are in patients with GBM thinning without characteristic lamina densa splitting.

Genetics and Pathophysiology

Genetics and Biochemistry of Collagen IV

The GBM is composed of several major constituents, including collagen IV, laminin, nidogen, and heparan sulfate proteoglycan. The collagen IV family of proteins comprises six isomeric chains, designated α(alpha)1 to α(alpha)6(IV) [51, 52].

These chains show extensive sequence homology and share basic structural features, including (1) a major collagenous domain of about 1,400 residues containing the repetitive triplet sequence glycine (Gly)-X-Y, in which X and Y represent a variety of other amino acids; (2) a carboxyterminal noncollagenous (NC1) domain of about 230 residues; and (3) a noncollagenous aminoterminal sequence of 15–20 residues. The major collagenous domain of each chain contains about 20 interruptions of the collagenous triplet sequence, while each noncollagenous domain contains 12 completely conserved cysteine residues that form critical disulfide bonds.

Each collagen IV molecule is a trimer composed of three α(alpha) chains. Collagen IV α(alpha) chains form trimers through associations between their carboxyterminal NC1 domains, accompanied by folding of the collagenous domains into triple helices [53–55]. Variable residues within the NC1 domains confer specificity upon chain-chain associations [56]. Collagen IV triple helices form networks through several types of intermolecular interaction: end-to-end linkages between the carboxyterminal domains of two collagen IV triple helices, covalent interactions between four triple helices at their aminoterminal ends, and lateral associations mediated by binding of the carboxyterminal domains of one trimer to the collagenous region of another trimer [57, 58]. Disulfide bonds formed by conserved cysteine residues are critical to the interactions between NC1 domains. These linkages between collagen IV molecules produce a nonfibrillar, open-network assembly that associates with laminin through interactions mediated by nidogen [59].

Various integrins mediate cell attachment to collagen IV. Attachment of glomerular epithelial cells to the α(alpha)3(IV) chain appears to involve the α(alpha)3β(beta)1 integrin [60].

The six collagen IV genes are arranged in pairs on three different chromosomes. The human α(alpha)1 and α(alpha)2(IV) chains are encoded by the genes *COL4A1* and *COL4A2*, respectively, on chromosome 13 [61]. *COL4A3* and *COL4A4* are located on chromosome 2 and encode the α(alpha)3(IV) and α(alpha)4(IV) chains of type IV collagen, respectively [62], while the α(alpha)5(IV) and α(alpha)6(IV) chains are encoded by the *COL4A5* and *COL4A6* genes on the X chromosome [63–65]. The 5′ ends of each gene pair are adjacent to each other, separated by sequences of varying length containing motifs involved in the regulation of transcriptional activity [66–68].

Evidence from a variety of sources indicates the existence of at least three collagen IV networks in mammalian basement membranes, and studies of the distribution of these networks in basement membranes using monospecific antibodies have revealed a high degree of tissue specificity. A network composed of heterotrimers with the composition α(alpha)1(IV)$_2$α(alpha)2(IV) is found in all basement membranes, although it is a relatively minor component of mature GBM. A network comprising α(alpha)3(IV)-α(alpha)4(IV)-α(alpha)5(IV) heterotrimers is the predominant collagen in mature GBM and also occurs in Bowman's capsules and the basement membranes of distal and collecting tubules. A hybrid network consisting of α(alpha)1(IV)$_2$α(alpha)2(IV) and α(alpha)5(IV)$_2$α(alpha)6(IV) heterotrimers is present in Bowman's capsule and distal and collecting tubule basement membranes, but is not present in GBM. The epidermal basement membrane and basement membranes surrounding aortic smooth muscles cells contain α(alpha)1(IV)$_2$ α(alpha)2(IV)/α(alpha)5(IV)$_2$α(alpha)6(IV) networks, but not the α(alpha)3(IV)-α(alpha)4(IV)-α(alpha)5(IV) network [69, 70].

The α(alpha)3(IV) chain has been identified as the target of anti-GBM autoantibodies in Goodpasture syndrome [71–73]. The Goodpasture epitope resides in the carboxyterminal NC1 domain of α(alpha)3(IV) [74].

Abnormalities of Collagen IV Genes and Proteins in Alport Syndrome

There are three genetic forms of AS [75]. The X-linked form (XLAS), resulting from mutations in *COL4A5*, accounts for about 80 % of patients with the disease. About 15 % of patients have autosomal recessive Alport syndrome (ARAS), which arises from mutations affecting both alleles of *COL4A3* or *COL4A4*. The heterozygous parents of children with ARAS often have asymptomatic hematuria, although some have normal urinalyses. Finally, approximately 5 % of patients have autosomal dominant Alport syndrome (ADAS), due to heterozygous mutations in *COL4A3* or *COL4A4*. Heterozygous mutations in *COL4A3* or *COL4A4* have also been found in families with TBMN (see below). It is not yet clear why many, perhaps most, individuals with heterozygous mutations in these genes have asymptomatic hematuria, while others have progressive disease.

XLAS

Over 600 *COL4A5* mutations have been found in XLAS families. These mutations are distributed throughout the gene, and with few exceptions each mutation is unique. In the USA, three missense mutations associated with relatively late onset of ESRD account for a disproportionate number of affected families [76, 77]. About 15 % of *COL4A5* mutations are large rearrangements, predominantly deletions [75]. Missense mutations account for about 40 %, about 15 % are splice-site mutations, and 25–30 % are nonsense mutations or small frame-shifting deletions or insertions that result in premature stop codons [75]. About 10 to 15 % of *COL4A5* mutations occur as spontaneous events in the proband, explaining why some patients with XLAS lack a family history of the disease.

The association of XLAS with leiomyomatosis of the esophagus and tracheobronchial tree has been reported in several dozen families [78]. Affected members of these families exhibit large deletions that span the adjacent 5′ ends of the *COL4A5* and *COL4A6* genes [79, 80]. These deletions involve varying lengths of *COL4A5*, but the *COL4A6* breakpoint is located in the second intron of the gene [81–83]. Leiomyomatosis does not occur in patients with deletions of *COL4A5* and *COL4A6* that extend beyond intron 2 of *COL4A6*. Mutations of *COL4A6* alone do not appear to cause Alport syndrome, consistent with the absence of the α(alpha)6(IV) chain from normal human GBM [84].

Two families have been described in which *COL4A5* deletions that extend beyond the 3′ end of the gene cause XLAS, mental retardation, and midface hypoplasia [85, 86]. In one of these families, affected individuals also displayed elliptocytosis.

The great majority (about 85 %) of missense *COL4A5* mutations are guanine substitutions in the first or second position of glycine codons that result in the replacement of a glycine residue in the collagenous domain of α(alpha)5(IV) by another amino acid [75]. Such mutations are thought to interfere with the normal folding of the mutant α(alpha)5(IV) chain into triple helices with other collagen IV α(alpha) chains [87]. Glycine lacks a side chain, making it the least bulky of amino acids and small enough to allow three glycine residues to fit into the interior of a tightly wound triple helix [88]. The presence of a bulkier amino acid in a glycine position presumably creates a kink or an unfolding in the triple helix. Glycine substitutions in the α(alpha)1 chain of type I collagen account for the majority of mutations causing osteogenesis imperfecta and are common in other genetic disorders of collagen [89, 90]. Abnormally folded collagen triple helices exhibit increased susceptibility to proteolytic degradation [89]. The position of the substituted glycine, or the substituting amino acid itself, may influence the effect of the mutation on triple helical folding and ultimately the impact of the mutation on the severity of the clinical phenotype [77, 91].

ARAS

To date, mutations causing ARAS have been found in the *COL4A3* or *COL4A4* gene in over 100 patients [75]. Some of these patients are homozygous for their mutations and others are compound heterozygotes. As with *COL4A5*, there appear to be no mutation hot spots in *COL4A3* or *COL4A4*. Although it is possible that ARAS could result from the combination of a mutation in one allele of *COL4A3* and a mutation in one allele of *COL4A4*, no such example has been described. It is worth noting that the mating of two individuals with asymptomatic hematuria due to heterozygous *COL4A3* or *COL4A4* mutations can result in a child who has mutations in both alleles of *COL4A3* or *COL4A4* and, as a result, ARAS.

The reported *COL4A3* and *COLA*4 mutations in ARAS include nonsense (10–15 %), frameshift (about 25 %), splicing (about 15 %), and missense mutations (about 50 %) [75]. As in XLAS, the majority (about 80 %) of missense mutations produce a glycine substitution in the collagenous domain of α(alpha)3(IV) or α(alpha)4(IV).

ADAS

Heterozygous *COL4A3* and *COL4A4* mutations have been described in several families transmitting AS as an autosomal dominant disorder [92–94]. It is not clear why some individuals with heterozygous *COL4A3* or *COL4A4* mutations are asymptomatic or exhibit only isolated microhematuria while others have a progressive nephropathy. Several possibilities can be proposed: the type and/or site of the mutation may be critical, the presence of certain polymorphisms in these genes may influence the effect of a pathogenic mutation, or a polymorphism or mutation in another gene may modify the effect of the mutation. In some cases, a heterozygous missense mutation in *COL4A3* or *COL4A4* might be more detrimental than a deletion or nonsense mutation, because the mutant chain can then induce the degradation of normal chains with which it forms abnormal trimers.

Phenotype–Genotype Correlations in Alport Syndrome

Male patients with *COL4A5* deletions consistently exhibit sensorineural deafness and progression to ESRD during the second or third decade of life [4]. Most of the missense, nonsense, and splicing *COL4A5* mutations described thus far have also been associated with early progression to ESRD in the second or third decade of life and sensorineural deafness. Single amino acid substitutions that alter an mRNA splice site, leading to exon skipping, or small deletions or insertions that shift the transcriptional reading frame, can clearly produce such aberrant protein products that a severe phenotype results. Several *COL4A5* mutations have been associated with late-onset (after the third decade) ESRD and deafness [76].

Large rearrangements, nonsense mutations, and frameshift mutations of the *COL4A5* gene confer a 90 % probability of ESRD before age 30 years, with 50 % reaching ESRD by age 20 years [4, 77, 91]. Splice-site mutations are associated with a probability of ESRD before age 30 years of 70 %, with 50 % reaching ESRD by age 25 years. Patients with missense mutations have a 50 % probability of ESRD before age 30 years. Non-glycine missense mutations and glycine mutations in the 3′ portion of *COL4A5* are associated with earlier development of ESRD, compared with glycine missense mutations in the 5′ portion of the gene [77, 91].

The severity of disease in a female heterozygous for a *COL4A5* mutation is probably influenced to some extent by the nature of the mutation, but the extent of inactivation of the X chromosome carrying the normal *COL4A5* allele is likely a more important factor. Jais et al. were unable to demonstrate a genotype effect on outcome in XLAS heterozygotes,

presumably due to the stronger influence of X inactivation [3]. Guo et al. described a woman with a severe Alport phenotype (ESRD at age 30), one of whose *COL4A5* alleles carried two missense mutations [95]. Analysis of DNA isolated from the patient's kidney and leukocytes showed inactivation of greater than 90 % of the X chromosomes carrying the normal *COL4A5* allele. Other investigators, however, have not been able to confirm that X-inactivation pattern of leukocytes predicts phenotype in XLAS heterozygotes [96]. In a mouse model of X-linked Alport syndrome [97], the relative activity of the X chromosome carrying the mutant *col4a5* allele determines the outcome of heterozygous females [98].

Phenotypic information on patients with ARAS is as yet somewhat sparse. Available data indicate that patients with ARAS often progress to ESRD before age 30 and have sensorineural deafness, regardless of gender [99–101].

Collagen IV in Alport Basement Membranes

Several studies in the 1980s established that the native kidneys of male AS patients failed to bind anti-GBM antibodies from patients with Goodpasture syndrome or from AS patients with posttransplant anti-GBM nephritis [102–106]. These early studies, combined with the observation by Hudson and colleagues that collagen IV was the target of Goodpasture antibodies [72], provided the first indication that AS might represent a primary disorder of type IV collagen. The availability of chain-specific antibodies has more recently allowed detailed investigation of the expression of the six collagen IV α(alpha) chains in AS basement membranes.

GBM, distal and collecting TBM, and Bowman's capsules of males with XLAS usually lack expression of the α(alpha)3(IV), α(alpha)4(IV), and α(alpha)5(IV) chains but do express the α(alpha)1(IV) and α(alpha)2(IV) chains [107, 108]. The α(alpha)6(IV) chain is not expressed in Bowman's capsule or distal TBM of XLAS males whose basement membranes lack α(alpha)5(IV) expression [109, 110]. Women who are heterozygous for XLAS mutations frequently exhibit mosaicism of GBM expression of the α(alpha)3(IV), α(alpha)4(IV), and α(alpha)5(IV) chains [108]. The epidermal basement membrane (EBM) normally expresses the α(alpha)1(IV), α(alpha)2(IV), α(alpha)5(IV), and α(alpha)6(IV) chains, but not the α(alpha)3(IV) or α(alpha)4(IV) chains [103, 109, 111]. Most males with XLAS show no EBM expression of α(alpha)5(IV) or α(alpha)6(IV), while female heterozygotes frequently display mosaicism [111]. Lens capsules of some males with XLAS do not express the α(alpha)3(IV), α(alpha)4(IV), α(alpha)5(IV), or α(alpha)6(IV) chains [112, 113].

In many patients with ARAS, GBMs show no expression of the α(alpha)3(IV), α(alpha)4(IV), or α(alpha)5(IV) chains, but α(alpha)5(IV) and α(alpha)6(IV) are expressed in Bowman's capsule, distal TBM, and EBM [114]. Therefore, XLAS and ARAS may be distinguishable by immunohistochemical analysis of renal biopsy specimens.

It is important to note that immunostaining for collagen IV is normal in some patients with XLAS and ARAS. Consequently, normal expression of α(alpha)3(IV), α(alpha)4(IV), and α(alpha)5(IV) chains in basement membranes does not exclude a diagnosis of AS.

The abnormalities of collagen IV expression observed in AS indicate that a mutation affecting one of the chains involved in the α(alpha)3-α(alpha)4-α(alpha)5(IV) network can prevent GBM deposition of the entire network. Similarly, a mutation involving the α(alpha)5(IV) chain can interfere with basement membrane expression of the α(alpha)5(IV)-α(alpha)6(IV) network. Several lines of evidence suggest that the effects of a collagen IV α(alpha) chain mutation on the basement membrane expression of its partner chains are mediated by posttranslational events. Substitution of glycine residues in the α(alpha)1 chain of collagen I comprises the majority of mutations causing osteogenesis imperfecta [115]. These mutations impair the folding of α(alpha)1(I) chains with α(alpha)2(I) chains into trimers, and the resulting trimers exhibit enhanced susceptibility to extracellular proteolytic degradation [89]. By similar means, an α(alpha)5(IV) chain carrying a glycine substitution could bring about the destruction of normal α(alpha)3(IV) and α(alpha)4(IV) chains. Studies of mutations in the α(alpha)1 chain of collagen IV in *C. elegans* suggest another possible mechanism. Whether the α(alpha)1(IV) mutation results in a null allele (deletion or nonsense mutation) or a glycine substitution, the α(alpha)2(IV) chain accumulates intracellularly and never reaches the basement membrane [116]. Thus, mutations producing null COL4A5 alleles, or α(alpha)5(IV) proteins that cannot form trimers (due to alterations of the carboxy-terminal domain), or some glycine substitutions may simply prevent secretion of α(alpha)3(IV) and α(alpha)4(IV) chains, which are eventually degraded. Results of in vitro studies support the hypothesis that α(alpha)5(IV) mutations impair the assembly of α(alpha)3-α(alpha)4-α(alpha)5(IV) heterotrimers [87].

Pathogenesis of Basement Membrane Lesions in Alport Syndrome

The tissue pathology of AS arises from abnormal basement membrane expression of the collagen IV network composed of α(alpha)3-α(alpha)4-α(alpha)5(IV) chains. This network is usually absent from or under-expressed in the basement membranes of AS patients, although in the basement membranes of some patients, the networks are present but defective in structure and function. Anterior lenticonus may represent the most straightforward demonstration of the consequences of the absence of this network from basement membranes. Absence or defective expression of these chains

results in thinning and mechanical weakness of the lens capsule, interfering with its capacity to maintain the normal conformation of the lens [17, 112, 113]. Microhematuria, the earliest renal manifestation of AS, may likewise reflect GBM thinning and a tendency to develop focal ruptures due to absence or defective expression of the α(alpha)3-α(alpha)4-α(alpha)5(IV) network. The abnormal composition of AS GBM may increase its susceptibility to proteolysis, perhaps explaining episodes of gross hematuria precipitated by infections, which are not uncommon during the first two decades of life in AS patients [117]. On the other hand, the resolution of gross hematuria, which typically occurs by adolescence, may be a function of progressive GBM thickening [2, 5].

The relentless thickening and lamellation of the AS GBM feature the accumulation of extracellular matrix proteins that are normally absent from GBM or present in only small amounts. The α(alpha)1(IV)-α(alpha)2(IV) network is highly expressed in the basement membranes of primordial glomeruli but is normally replaced by the α(alpha)3-α(alpha)4-α(alpha)5(IV) network at the capillary loop stage of glomerular maturation [118]. In mature glomeruli the α(alpha)1(IV)-α(alpha)2(IV) network is confined to the mesangium and the subendothelial region of GBM [119]. This developmental switch fails to occur in AS glomeruli [117, 120]. Instead, the α(alpha)1(IV)-α(alpha)2(IV) network persists in mature AS glomeruli and accumulates, spreading from its normal subendothelial location to occupy the full width of GBM [107, 114]. Similarly, collagen V and collagen VI, which are normally found in the mesangium and in small quantities in the subendothelial GBM, are markedly overexpressed in AS GBM [107]. In addition, laminin chains that are normally confined to the mesangium are expressed in AS GBM [121, 122]. The molecular events that underlie these anomalies in protein expression may represent maladaptive responses to biomechanical events in glomeruli [123].

AS resembles other chronic glomerulopathies in that deterioration of glomerular filtration rate is closely correlated with fibrosis of the renal interstitium [124]. Measurable increases in cortical interstitial volume are unusual in males with XLAS before the age of 10, but progressive expansion of the interstitium is common during the second decade of life [124]. Renal tubular epithelial cell toxicity associated with reabsorption of filtered proteins may contribute to tubular injury [125]. At least some of the factors driving interstitial fibrosis in AS are nonspecific, such as increased TGF-β1 activity, monocytic infiltration, epithelial-mesenchymal transformation, and enhanced metalloproteinase expression [126–130]. This suggests that therapies that nonspecifically interfere with interstitial fibrosis may be of benefit to AS patients, without correcting the primary abnormalities of collagen IV expression [131, 132].

Diagnosis

AS should be considered in the differential diagnosis of patients with persistent microhematuria. Electron microscopic examination of renal tissue remains the most widely available and applied means for diagnosing AS. The presence of diffuse thickening and multilamellation of the GBM predicts a progressive nephropathy, regardless of family history. Unfortunately, ultrastructural information alone does not establish the mode of transmission in a particular family. In a patient with a negative family history, electron microscopy cannot distinguish de novo X-linked disease from autosomal recessive disease. In some patients the biopsy findings may be ambiguous, particularly females and young patients of either sex. Furthermore, rare families with progressive nephritis and COL4A5 mutations in association with thin GBMs have been described, indicating that the classic GBM lesion is not present in all AS kindreds.

In families with a firm diagnosis of AS, evaluation of individuals with newly recognized hematuria can be limited to ultrasound of the kidneys and urinary tract in most instances. In the absence of tumor or structural anomalies of the urinary tract, a diagnosis other than AS is unlikely.

When AS is suspected, confirmation of the diagnosis may be achieved through immunohistochemical methods and molecular genetic analysis. Monospecific antibodies directed against collagen IV α(alpha) chains are available making it possible to reliably evaluate renal basement membranes for expression of α(alpha)3(IV), α(alpha)4(IV), and α(alpha)5(IV) chains [133, 134]. Since the α(alpha)5(IV) chain is normally expressed in the epidermal basement membrane (EBM), examination of skin biopsies by immunofluorescence for expression of α(alpha)5(IV) is an additional tool for making a diagnosis of AS. Absence of the α(alpha)5(IV) chain from EBM is diagnostic of XLAS [135]. Given a male patient with a positive family history and clinical features characteristic of AS, examination of skin for α(alpha)5(IV) expression may obviate the necessity for kidney biopsy. However, a normal result does not exclude the diagnosis of AS, since in about 20 % of XLAS kindreds affected males express α(alpha)5(IV) in their renal and epidermal basement membranes. Females with XLAS frequently express α(alpha)5(IV) mosaically in the skin. While clearly mosaic expression of α(alpha)5(IV) is diagnostic of XLAS in a female, a normal result does not exclude the diagnosis.

Renal expression of type IV collagen α(alpha) chains can serve to confirm a diagnosis of AS and can in addition differentiate XLAS and ARAS. In most males with XLAS, renal basement membranes are devoid of the α(alpha)3, α(alpha)4, and α(alpha)5(IV) chains, while females frequently show mosaic expression of these chains. In most males and females with ARAS, the GBM, Bowman's capsule,

and distal TBM show no expression of the α(alpha)3(IV) and α(alpha)4(IV) chains, while α5(IV) is expressed in Bowman's capsule and distal TBM but not GBM [114].

Genetic analysis provides the only means for reliably diagnosing the carrier state in asymptomatic female members of XLAS kindreds and for making a prenatal diagnosis of AS. There are also clinical situations in which a firm diagnosis of AS cannot be established or in which it is not possible to determine the mode of transmission, despite careful evaluation of the pedigree and application of the full range of histological methods. In these situations genetic analysis has the potential to provide information essential for determining prognosis and guiding genetic counseling. Genetic counseling based upon erroneous determination of the mode of inheritance may have unintended consequences [136].

Molecular genetic analysis of the *COL4A3*, *COL4A4*, and *COL4A5* genes has become widely but not universally available, and the expense of such testing may limit access. Direct sequencing of these genes offers mutation detection rates of about 90 %. When clinical suspicion of XLAS is high, direct sequencing of *COL4A5* can obviate the need for renal or skin biopsy. If clinical features and family history are not particularly suggestive of AS, kidney biopsy may be the most informative diagnostic study.

Treatment

Transplantation

Since AS does not recur in renal allografts, kidney transplantation corrects the AS nephropathy. Graft survival rates in patients with familial nephritis are comparable to those in patients with other diagnoses [137]. However, some AS patients develop anti-GBM nephritis in the renal allograft, usually resulting in graft loss. Data from several transplant centers indicates an incidence of 3–4 % in transplanted AS patients [138–140]. These patients are usually male, always deaf, and likely to have reached ESRD before the age of 30 [141]. This profile describes the majority of AS patients presenting for renal transplantation, so its predictive value is rather limited. However, AS patients with normal hearing or late progression to ESRD appear to be at very low risk for the development of posttransplant anti-GBM nephritis. Females with XLAS also appear to be in a low-risk category.

The onset of posttransplant anti-GBM nephritis was within the first year following transplantation in approximately 75 % of cases. Three-quarters of the allografts failed irreversibly, usually within a few weeks to months after diagnosis. Treatment with plasmapheresis and cyclophosphamide has been of limited benefit. Anti-GBM nephritis has recurred in at least 7 of 8 patients who underwent retransplantation. Posttransplant anti-GBM nephritis may recur despite an interval of many years between transplants and in the absence of detectable circulating anti-GBM antibodies prior to retransplantation.

The target(s) of anti-GBM antibodies in some of these patients has been determined, with variable results. Most patients with XLAS exhibit antibodies that target the carboxyterminal noncollagenous domain of the α(alpha)5(IV) chain, but anti-α(alpha)3(IV) antibodies have also been described [103, 138, 142–145]. Antibodies against α(alpha)3(IV) have been observed in ARAS patients with posttransplant anti-GBM nephritis [144].

It has been proposed that mutations in the *COL4A5* gene that prevent expression of an immunogenic gene product, thereby preventing the establishment of tolerance for α(alpha)5(IV), might be associated with an increased risk for the development of posttransplant anti-GBM nephritis [146]. Of 14 AS patients with posttransplant anti-GBM nephritis examined for a *COL4A5* mutation, seven, or 50 %, had complete or partial deletions, compared with a deletion frequency of about 10 % in the general AS population (discussed in [141]). Although it is possible that *COL4A5* deletions confer an increased risk for posttransplant anti-GBM nephritis, AS males with *COL4A5* deletions have been transplanted successfully [147], indicating that other factors, presently unknown, must influence the initiation and elaboration of the immune response to the allograft. At this time it appears that the only way to determine whether a previously untransplanted AS patient will develop posttransplant anti-GBM nephritis is to perform the transplant, although as noted previously certain patients are at very low risk.

Females who are heterozygous for *COL4A5* mutations would not be expected to be at risk for the development of posttransplant anti-GBM nephritis, since the product of the normal *COL4A5* allele would allow establishment of immunologic tolerance for α(alpha)5(IV). Nevertheless, posttransplant anti-GBM nephritis has been reported in two females with AS [100, 138]. Both of these women were found to have ARAS, due to *COL4A3* mutations [99, 100].

Renoprotective Therapies

Interventions aimed at preventing or slowing the inexorable decline in renal function typical of male AS are currently under investigation. Ramipril therapy initiated prior to the development of proteinuria delayed the onset of proteinuria and renal failure and lengthened survival in a murine model of ARAS [131, 132]. Early angiotensin-converting enzyme inhibition in dogs with XLAS had no effect on the onset of proteinuria, but did delay ESRD [148]. Prospective studies of angiotensin inhibition in AS have involved small numbers of patients with proteinuria serving as their own controls, with relatively short follow-up, and have produced mixed results [149–152]. Recently, retrospective analysis of data in the European Alport Registry showed that early angiotensin blockade was associated with delayed onset of ESRD, with a

median interval of 18 years compared to patients who received no therapy [153].

In an uncontrolled study of eight AS males, cyclosporine appeared to suppress proteinuria and stabilize renal function, over several years of observation [154, 155]. However, other investigators found that patient responses to cyclosporine are variable and that cyclosporine may accelerate interstitial fibrosis [156, 157]. In dogs with XLAS, cyclosporine failed to suppress proteinuria, but treated animals did exhibit a delay in progression to ESRD [158]. At this time, it is the author's view that the efficacy of cyclosporine as a treatment for AS has not been demonstrated with the degree of certainty needed to overcome concerns about its nephrotoxicity.

There has been interest in gene transfer approaches aimed at delivering a normal copy of the *COL4A5* gene into glomeruli as a treatment for AS [159–162]. While attractive in theory, it has yet to be demonstrated that such treatment can be accomplished in an experimental model, let alone patients.

A number of other therapies have been shown to improve outcomes in animal models of AS, including inhibitors of TGF-β1 [126], matrix metalloproteinases [163], vasopeptidase A [164], or HMG-CoA reductase [165]. chemokine receptor 1 blockade [166], BMP-7 [129], stem cells [167–170], and irradiation [171]. None of these approaches has been prospectively studied in human AS populations. In comparison with angiotensin blockade, which has been examined extensively in children with chronic renal disease [172, 173], these therapeutic approaches have little or no track record in pediatric populations and would be costlier, less accessible, and probably riskier forms of treatment.

Thin Basement Membrane Nephropathy

TBMN, like AS, is an inherited disorder of glomerular basement membranes that is characterized clinically by persistent microscopic hematuria and episodic gross hematuria. TBMN differs clinically from AS in several important respects: (1) it is only rarely associated with extrarenal abnormalities; (2) proteinuria, hypertension, and progression to ESRD are unusual; (3) gender differences in expression of TBMN are not apparent; and (4) transmission is autosomal dominant in nature. TBMN and early AS may be difficult to distinguish histologically, since diffuse GBM attenuation is characteristic of both. However, in TBMN the GBM remains attenuated for the patient's lifetime, rather than undergoing the progressive thickening and multilamellation of the lamina densa that are pathognomonic of AS.

Clinical Features

Individuals with TBMN typically exhibit persistent microhematuria that is first detected in childhood. In some patients the microhematuria is intermittent and may not be detected until adulthood. Episodic gross hematuria, often in association with upper respiratory infections, is not unusual. The hematuria of TBMN appears to be lifelong. It has been estimated that 20–25 % of patients referred to a pediatric nephrologist for evaluation of persistent hematuria will prove to have thin GBM on renal biopsy [174].

Overt proteinuria and hypertension are unusual in TBMN but have been described [48, 175–177]. Some of these patients exhibit focal global glomerulosclerosis on renal biopsy [48, 49]. In occasional adult patients with Alport syndrome, the predominant GBM abnormality is attenuation rather than thickening and multilamellation. Other glomerular disorders such as IgA nephropathy may occur in patients with TBMN, altering the expected natural history and histopathology of the condition.

Pathology

Light and immunofluorescence microscopies are unremarkable in typical cases of TBMN. Most patients exhibit diffuse thinning of lamina densa and of the GBM as a whole. GBM thickness is age and sex dependent in the normal population. Both the lamina densa and the GBM increase rapidly in thickness between birth and age 2 years, followed by gradual thickening throughout childhood, adolescence, and into adulthood [178]. GBM thickness of adult men (373 + 42 nm) exceeds that of adult women (326 + 45 nm) [179]. Thus, the age and sex of the patient must be taken into account when evaluating GBM width.

The definition of "thin" GBM in the literature is imprecise, in part because of the use of different techniques to measure GBM width. When an EM laboratory's normal values for GBM width are similar to those of Steffes et al. [179], a cutoff value of 250 nm will accurately separate adults with normal GBM from those with thin GBM. Where the normal values are significantly higher, a cutoff value of 330 nm is appropriate [180]. For children, the cutoff is in the range of 200–250 nm (250 nm is within 2SD of the mean at age 11) [178, 181]. The intraglomerular variability in GBM width is small in thin GBM disease [176]. In a patient with persistent hematuria, marked variability in GBM width within a glomerulus should raise suspicion for Alport syndrome, although focal lamina densa splitting has been described in TBMN.

Genetics and Pathophysiology

TBMN is usually transmitted as an autosomal dominant condition. A negative family history may not be reliable, since patients eventually diagnosed as having TBMN are frequently unaware that they have relatives with hematuria [182]. The first clue to the genetic basis of TBMN was pro-

vided by Lemmink and coworkers, who described a large Dutch TBMN kindred in which the disease locus was first mapped to chromosome 2 in the region of the *COL4A3* and *COL4A4* genes, and then affected individuals were found to be heterozygous for a missense mutation in *COL4A4* [183]. Since this landmark work, heterozygous mutations in *COL4A3* or *COL4A4* have been described in numerous TBMN families [94, 184–186]. However, linkage to the *COL4A3* and *COL4A4* genes has been excluded in other TBMN families, indicating that TBMN is a genetically heterogeneous condition [187]. No other genetic loci for TBMN have been identified thus far.

To date, immunohistologic studies of collagen IV in GBM of patients with TBMN have failed to reveal any abnormalities in the distribution of any of the six chains. However, it is tempting to speculate that in those kindreds with TBMN due to a heterozygous *COL4A3* or *COL4A4* mutation, a 50 % reduction in the GBM α(alpha)3(IV)-α(alpha)4(IV)-α(alpha)5(IV) network results in lamina densa and GBM thinning and fragility. This reduction is presumably insufficient to trigger the pathophysiologic events that produce the lamina densa splitting and GBM thickening characteristic of Alport syndrome. Immunohistologic evaluation of GBM collagen IV may be useful in the differentiation of TBMN and Alport syndrome.

Diagnosis

Patients with persistent, isolated microhematuria are usually candidates for kidney biopsy, once structural urinary tract abnormalities, urinary tract stones, and tumors are excluded. If the patient's family history indicates autosomal dominant transmission of hematuria, and there is no history of chronic renal failure, a presumptive diagnosis of TBMN can often be made without kidney biopsy. When family history is negative or unknown, or there are atypical coexisting features such as proteinuria or deafness, renal biopsy may be extremely informative. A finding of thin GBM may be further characterized by examining the distribution of collagen IV α(alpha) chains in the kidney. Normal distribution of these chains provides supportive, although not conclusive, evidence for a diagnosis of TBMN (see also preceding section on Alport syndrome).

Treatment

Patients who are given a diagnosis of TBMN should be reassured but not lost to follow-up examination. The risk of chronic renal insufficiency appears to be small but real [48, 49, 175–177]. Reasonable follow-up would include urinalysis and measurement of blood pressure and renal function every one to two years and updating of the family history.

Hereditary Angiopathy with Nephropathy, Aneurysms, and Cramps (HANAC Syndrome)

Clinical Features

Missense mutations in the *COL4A1* gene were found in three families displaying an autosomal dominant **h**ereditary **a**ngiopathy associated with **n**ephropathy, **a**neurysms, and **m**uscle cramps (HANAC) [188]. Retinal arteriolar tortuosity and retinal hemorrhages were observed in affected individuals in all three families, as were elevated creatine kinase levels. In two families, affected individuals had muscle cramps. Disease of both large and small cerebral arteries has been found in the majority of affected individuals studies by magnetic resonance angiography, including aneurysms of the carotid siphon, white matter changes in subcortical, periventricular or pontine regions, dilated perivascular spaces, and lacunar infarcts [189]. In two families, affected individuals had muscle cramps. Renal findings in affected individuals included microscopic and gross hematuria in one family, mild renal insufficiency in two families, and renal cysts in all three families.

Pathology

Renal biopsy in affected individuals with hematuria showed no abnormalities of GBM structure or collagen IV chain expression (Fig. 18.2). However, basement membranes of Bowman's

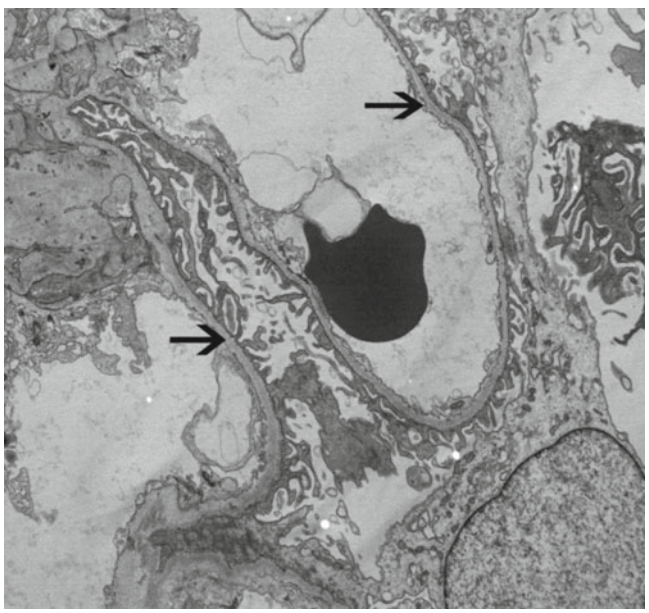

Fig. 18.2 Electron microscopy showing thin glomerular basements (*arrow point at glomerular basement membrane*). Note absence of basement membrane splitting and lamellations. In this case, the harmonic mean thickness of the glomerular basement membranes is 166 nm. (x 4200). Courtesy Dr. Sanjeev Sethi, Mayo Clinic

capsules, tubules, and interstitial capillaries exhibited irregular thickening, splitting into multiple layers, and focal interruptions [188]. Abnormalities of epidermal basement membranes and dermal arteries have also been observed [188].

Genetics

Reported mutations affect highly conserved glycine residues in the collagenous domain of the α(alpha)1(IV) chain [190]. These glycine residues are located in a region of the collagenous domain that includes major integrin-binding sites, suggesting that the HANAC phenotype may result from altered interactions between cells and collagen IV [190].

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Other Glomerular Diseases

19

Richard J. Glassock

Introduction

Human glomerular diseases represent an extremely heterogeneous collection of clinical-pathological entities. Some are limited to the kidney (primary glomerular diseases), whilst others are a part of systemic disease process, affecting many organs (secondary glomerular diseases). Most of these disorders are discussed elsewhere in this book. Herein will be described the core features and concepts relating a diverse group of rather uncommon disorders, having in common only their tendency to affect glomerular structure and function. Some are considered primary glomerular disease, but some also may have systemic features as well [1].

Collagenofibrotic Glomerulopathy

This is a quite rare glomerular disease occurring mostly in sporadic form [1–3]. Rare cases have been identified in families (with a pattern of inheritance suggesting an autosomal recessive condition), but a specific gene or genetic locus remains elusive [2]. Most cases have been described in Asians, but Caucasians can be affected as well [1–4]. The disorder affects all ages and genders and principally manifests as proteinuria, frequently in the nephrotic range. Hypertension and anemia are common. There are no specific clinical features that distinguish the disease, except for raised levels of type III procollagen peptide in serum [5]. Serum complement component levels are typically normal.

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The diagnosis is usually made upon examination of renal biopsy material [1–5]. Light microscopy shows an expanded mesangial zones with eosinophilic and weakly PAS-positive deposits often evoking a "mesangio-capillary" pattern with "double contours" and sometimes intercapillary nodules, but proliferation and cell infiltration is usually absent (Fig. 19.1). The deposits are congo red and thioflavin T negative. Routine immunofluorescence microscopy reveals scanty IgM and C3 deposits in sclerosed areas only. Special stains are strikingly positive for collagen type III in the expanded mesangial and subendothelial zones [1–5]. Electron microscopy shows the mesangial and subendothelial spaces to be filled with accumulations of typical type III collagen having a banded, 60 nM fibrillar structure, often given a lucent or moth-eaten appearance. These morphological features are pathognomonic of the disease [1–5].

The mechanisms responsible for the deposition of type III collagen in glomeruli are uncertain [1–5]. Elevated circulating levels of procollagen type III peptides suggest a systemic disease; but, no similar deposits have been detected in other organs. Hepatic fibrosis can occasionally be observed. On the other hand, mesangial cells and podocyte might acquire the capacity to synthesize and to secrete type III collagen under the influence of an abnormal cytokine milieu (such as excess Interleukin 4) or a genetic mutation [5].

The disorder usually slowly progresses to end-stage renal disease (ESRD) often after many years. The renal survival rate is about 50 % at 10 years from diagnosis. No specific treatment is available, but control of hypertension and use of angiotensin inhibition may delay ESRD [1–5]. A role for steroid treatment has been suggested by anecdotal improvement in proteinuria and procollagen type III levels. Experience with renal transplantation is limited, but levels of procollagen type III peptides increase rather than decrease posttransplantation. No recurrent disease has yet been reported, but follow-up times are short (<5 years) [1–5].

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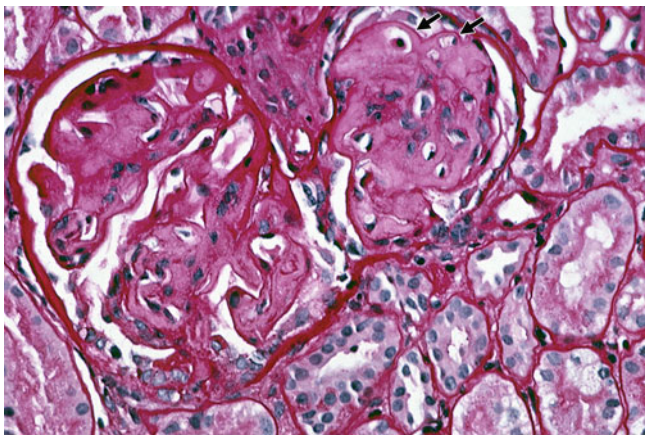

Fig. 19.1 Collagenofibrotic glomerulopathy (PAS stain). From Ponticelli and Glassock [1]; by permission of Oxford University Press

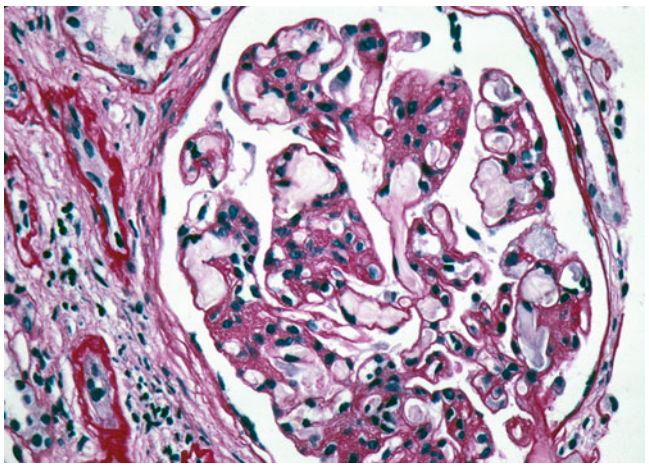

Fig. 19.2 Lipoprotein glomerulopathy (PAS stain). From Ponticelli and Glassock [1]; by permission of Oxford University Press

Lipoprotein Glomerulopathy

This is a rare disorder identified by thrombotic-like deposits of lipoproteins in glomeruli [1, 6–9]. In many cases a hereditary dispostion is noted, linked to the ApoE gene on Chromosome 19. A familial distribution is found in about one-third of a cases, but the specific mode of inheritance is not fully understood. The clinical findings at presentation are nonspecific and consist of proteinuria (often in the nephrotic range), hypertension, and impaired renal function. Almost any age or gender can be affected, but patients are usually between the age of 20 and 40 years at the time of discovery [1, 6–9]. Hyperlipidemia (hypertriglyceridemia—type II phenotype) is often present and elevated plasma apoE is almost always present [1, 6–9].

The diagnosis is made by renal biopsy. By light microscopy the glomerular capillaries are distended and filled with a hematoxylin and eosin pale-staining substance positive for lipid (Oil Red O stain) [6–9] (Fig. 19.2). These deposits contain apoE, apoB, and apoA by immunofluorescence microscopy, and Ig are absent. Electron microscopy shows deposits of various sizes forming lamellated structures. No fibrils are present [6–9].

The disease is likely caused by heterogenous mutations in the apoE gene (e.g., apoE Sendai, Tokyo, Maebashi) resulting in an instability of the molecule and its aggregation in glomerular capillaries [10, 11]. ApoE is also synthesized by glomerular cells and can regulate glomerular mesangial function in an autocrine fashion, including that of Fc receptors [10]. Dysfunction of the latter may participate in the accumulation of lipoprotein deposits in the mesangial and endothelial cells by altering uptake and clearance.

The disease is usually slowly progressive, but at highly variable rates. Treatment with steroids or immunosuppressive agents is ineffective. The best treatment is with fibric-acid derivatives (fibrates) which can both slow progression and cause the disappearance of the lipoprotein deposits in addition to improving the hyperlipidemia [1, 6–9, 12]. Treatment with LDL apheresis and nicotinic acid derivatives (niceritrol) can also be beneficial [13, 14]. The disease can recur in renal transplants attesting to its systemic nature [1].

C1q Nephropathy

This is an uncommon, but not rare, disorder that can only be diagnosed with certainty by immunofluorescence microscopy (like IgA nephropathy) [1, 15–19]. It accounts for <3 % of all forms of primary glomerular disease causing nephrotic syndrome in adults and children [16–19]. Although it is more common in African-Americans, it has been described in many ancestral groups. Rarely familial cases have been described. Males predominate (about 2:1) and both adults and children can be affected [16–19]. The clinical features are nonspecific. Proteinuria (often in the nephrotic range), hypertension, and hematuria are common. Acute renal failure (often in association with gross hematuria) and progressive renal failure can develop [20, 21]. Rapidly progressive glomerulonephritis (with superimposed crescents) has been observed [8]. Systemic features suggesting an autoimmune disease are uniformly absent. Serum complement component values are typically normal, but rarely an acute nephritic syndrome with hypocomplementemia can develop [22]. Antinuclear antibodies and anti-C1q autoantibodies are not found [1, 16–19].

The diagnosis is exclusively made by renal biopsy and immunofluorescence microscopy [1, 16–19, 23, 24]. By light microscopy a variety of findings are present, including minimal change disease (MCD), mesangial proliferative glomerulonephritis (MesPGN), and focal and segmental

glomerulosclerosis (FSGS). The latter is the most common lesion observed and can frequently be of the "collapsing variant." The association with polymorphisms in MYH9 gene may explain the frequent association of C1q nephropathy and collapsing FSGS in African-Americans [25]. The characteristic findings are revealed in immunofluorescence microscopy [16–19]. Intense mesangial deposition of C1q accompanied by C3 in an amorphous or granular pattern is characteristic. Polyclonal IgG and to a lesser extent IgM is also found in 85–95 % of cases. This pattern resembles that seen in lupus nephritis, but no clinical or laboratory features of SLE are present (by definition). Electron microscopy shows mesangial electron-dense deposits, but tubuloreticular inclusions are rarely found.

The pathogenesis of this disorder is obscure [1, 16–19]. Certain features suggest in situ or circulating deposition of immune complexes. The antigen is unknown.

The clinical manifestation of renal disease tends to persist, but spontaneous remissions have been reported [26], especially following a bout of acute renal failure and gross hematuria. Progressive renal disease is the rule and the 3 year renal survival is about 80 % [1, 16–19]. Collapsing FSGS and persistent marked proteinuria portend a poorer prognosis [16–19].

Treatment is quite uncertain. Partial or complete remission of nephrotic syndrome can follow steroid treatment in about 10–20 % of cases, which is probably higher than a spontaneous remission rate [1, 16–19, 21]. No randomized controlled trials of treatment have been conducted. Patients with MCD probably respond better to steroid therapy than those with FSGS. The effect of cytotoxic immunosuppressive drugs and calcineurin inhibitors is largely unknown. Preliminary anecdotes suggest a possible role for rituximab therapy [27]. Recurrent disease in renal transplants has not yet been observed, but C1q deposition can be observed in renal transplant biopsies, irrespective of the original disease [28].

Idiopathic Nodular Glomerulosclerosis

The development of "nodular" lesions in an intercapillary (mesangial) distribution is a common finding in advanced diabetic nephropathy (the Kimmelstiel–Wilson lesion) and in some forms of membranoproliferative glomerulonephritis and monoclonal light-chain deposition diseases as well [1, 29–32]. When diseases known to provoke this pattern of glomerular histology are absent, one can designate the disorder as "idiopathic" nodular glomerulosclerosis (ING) [29–32].

It is a rather uncommon lesion and tends to be observed in older adults (typically >65 years of age), especially women, and in the presence of a history of heavy smoking [29–32]. It has not been reported in children. All cases are sporadic. Blood sugar and hemoglobin A1c levels are normal (by definition), and no monoclonal gammopathies should be present (serum free light chain kappa/lambda ratios should be normal). Serum complement component levels are normal. Dyslipidemia, hypertension, proteinuria (including nephrotic syndrome), and atherosclerosis are common [1, 29–32].

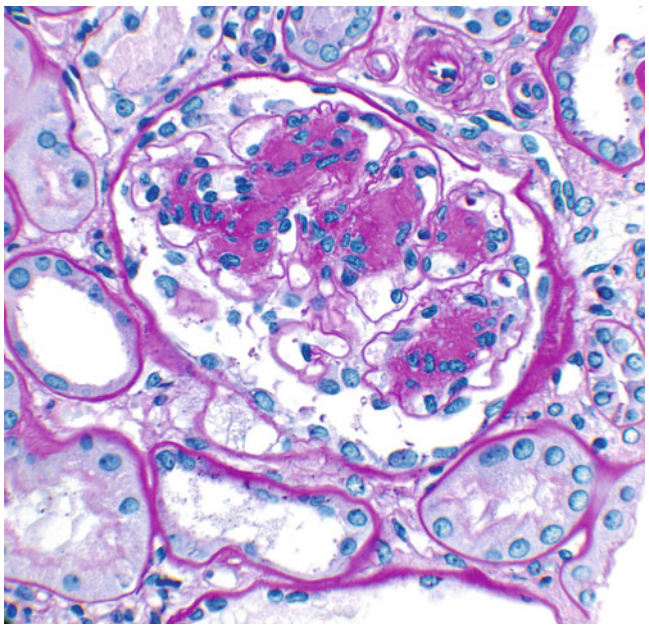

Fig. 19.3 Idiopathic nodular glomerulosclerosis (PAS stain). From Ponticelli and Glassock [1]; by permission of Oxford University Press

By light microscopy the lesion is characterized by diffuse and nodular mesangial sclerosis and thickening of the glomerular basement membrane (GBM) (Fig. 19.3) [29–32]. Severe arteriolar sclerosing lesions are also seen. Amyloid deposition is absent. Immunofluorescence microscopy may show diffuse linear albumin deposits along the GBM but Ig and C3 are absent. The electron microscopic features are nonspecific. No electron-dense or fibrillary deposits are present. The GBM is thickened.

The pathogenesis of ING is unknown, but many features suggest chronic endothelial injury and repair, perhaps connected to smoking and intermittent renal hypoxia [28–32].

Most, but not all, patients progress to ESRD. Due to late discovery the median time from diagnosis to ESRD is about 2 years [1, 29–32]. Other than stopping smoking and control of dyslipidemia and hypertension (preferably with angiotensin-II inhibition), there is no known effective therapy for this condition. A recurrence in a renal allograft has been recently reported [33].

Fibrillary and Immunotactoid Glomerulonephritis

These two clinical-pathologic entities are often discussed together, even if they may have different underlying mechanisms [1, 34–39]. They share in common the presence of

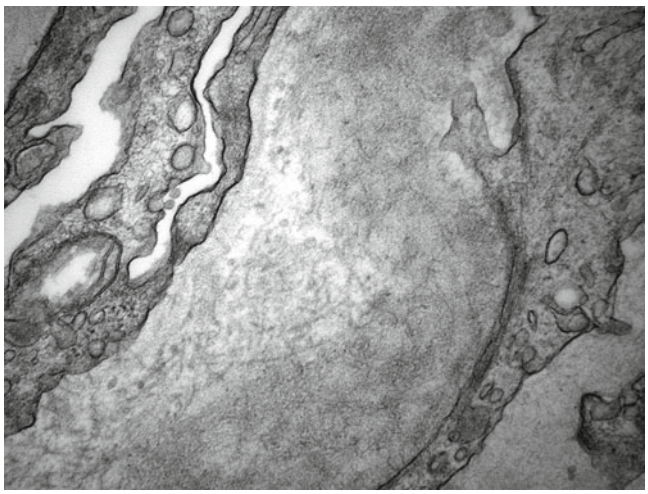

Fig. 19.4 Fibrillary glomerulonephritis: Electron microscopy showing randomly oriented fibrillary deposits within the thickened glomerular basement membrane. The fibrils measure ~19nm in thickness. 46,000X. Courtesy Dr. Sanjeev Sethi - Mayo Clinic

congo red negative (non-amyloid) deposits of immunoglobulins (Ig) that acquire a fibrillary (organized) substructure. They are often also called glomerulonephritis with organized monoclonal immunoglobulin deposits (GOMID) when the deposits of Ig are monoclonal in nature. The main distinctions between fibrillary (FGN) and immunotactoid (ITGN) glomerulonephritis are that the fibrils are smaller in FGN (12–24 nM in diameter) compared to ITGN (>30 nM in diameter and microtubular in appearance with parallel arrays) and that ITGN is much more commonly associated with a monoclonal gammopathy [34–39].

FGN is much more common than ITGN. Both disorders commonly evoke proteinuria, in the nephrotic range, hematuria, and hypertension. The serum complement component C3 level is usually normal, but rarely it may be reduced (37–43). Most patients are older adults (ages 50–65 years of age). Women are more commonly affected than men. Over 90 % of the reported cases are Caucasian. Both are relatively uncommon disorders, seen in about 0.6 % of renal biopsies for FGN and 0.06 % of renal biopsies for ITGN [38].

By light microscopy both commonly show a pattern of membranoproliferative glomerulonephritis (MPGN) with Ig and C3 deposition and subendothelial electron-dense deposits with a fibrillar substructure (Fig. 19.4). Less commonly, a pattern of diffuse proliferative glomerulonephritis can be seen. Membranous nephropathy is a distinctly uncommon lesion. Superimposed crescentic disease may be present in cases with a rapidly progressive course.

Immunofluorescence microscopy shows diffuse glomerular deposits of IgG in nearly all cases of FGN and ITGN. IgG4 is the most common subclass in FGN. The deposits of IgG in ITGN are more commonly monoclonal in ITGN [1, 34–39]. C3 and C1q deposits are also found in both FGN and ITGN. By electron microscopy the characteristic fibrils or tactoids (microtubules) are seen in FGN or ITGN respectively. In FGN the fibrils are haphazardly arranged, and in ITGN they tend to occur in parallel arrays [1, 34–39]. Typical fibrils of amyloid (8–11 nM in diameter, non-branching) are not seen.

The pathogenesis of FGN and ITGN is not well understood. Extrarenal deposition of fibrillar material is very uncommon. However, it is likely that the fibrillar deposits are derived from the circulation. Something leads to their deposition in glomeruli. ITGN has been associated with various lymphoproliferative disorders, chronic hepatitis C viral infection, and vasculitis with hypocomplementemia. There may be some overlap between ITGN and type II (mixed IgG/IgM). cryoglobulinemia [34–38, 40]. FGN has been associated with autoimmune diseases (such as SLE, Crohn's disease and Graves' disease) [40].

The clinical course of both FGN and ITGN is progressive with at least 50 % reaching ESRD within 2 years of diagnosis [1, 34–40]. Elevated serum creatinine at discovery and persistent nephrotic-range proteinuria are indicative of a poor prognosis [40]. Fatalities usually are the consequence of underlying lymphoproliferative disease in ITGN.

Treatment of both FGN and ITGN with steroids or immunosuppressive agents generally yields unsatisfactory results with 10–15 % of treated cases entering a partial remission [1, 8, 34–40]. Rarely, a complete remission of ITGN can develop after successful treatment of the underlying lymphoid malignancy. Preliminary anecdotal reports of the use of rituximab in FGN are promising [41]. Efforts to control blood pressure and to diminish proteinuria (with angiotensin-II inhibition) may slow progression to ESRD.

A recurrence of the disease in renal transplants is seen in about 35–40 % of cases [42].

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Monoclonal Immunoglobulin Deposition Disease

20

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Introduction

Monoclonal immunoglobulin deposition disease (MIDD) is a systemic disease characterized by the deposition of monoclonal immunoglobulin proteins as amorphous, non-congophilic material in body tissues. Light-chain deposition disease (LCDD) is the most common subtype and was first described as a systemic disease by Randall et al. in the 1970s [1]. Renal dysfunction is the most common initial manifestation, but immunoglobulins can deposit in many different visceral organs, resulting in a myriad of clinical presentations. MIDD also includes the less common subtypes of light- and heavy-chain deposition disease (LHCDD) and heavy-chain deposition disease (HCDD). MIDD can be viewed as part of the larger category of paraprotein-associated disorders, which also include myeloma cast nephropathy, light-chain proximal tubulopathy, cryoglobulinemic glomerulonephritis, light-chain amyloidosis (AL), and heavy-chain amyloidosis (AH).

The central pathogenetic factor in MIDD is the abnormal production of monoclonal immunoglobulins by plasma cells or other B cells, resulting in end-organ damage due to an excess of immunoglobulin peptides that are predisposed to tissue deposition in a non-fibrillar state. Some affected individuals have an underlying plasma cell dyscrasia, such as multiple myeloma or monoclonal gammopathy of uncertain significance (MGUS), but others have no demonstrable paraprotein on serum or urine protein electrophoresis [2]. In a necropsy study of 57 patients with multiple myeloma, LCDD was present in approximately 5 % of subjects [3]. A larger case series has shown a higher percentage of 18 % [4]. Kappa light chains are more commonly associated with LCDD than lambda light chains, in contrast to AL where lambda light chains are more common [2].

The clinical picture is most often dominated by the renal manifestations, with nephrotic syndrome and renal dysfunction being the most common presentations [2, 5–9]. The next most common organs involved are the heart, lungs, liver, and peripheral nerves [6, 10]. The most common glomerular pathology seen in the kidney on light microscopy is nodular glomerulosclerosis. However, immunofluorescence demonstrating the deposition of light chains along the tubular and glomerular basement membranes, as well as the presence of "powdery" electron-dense deposits on electron microscopy, is essential for diagnosis, as the light microscopy findings are nonspecific. In fact, the diagnosis is often unsuspected and found only after immunofluorescence is performed [7].

The natural history of MIDD and its prognosis are variable and are largely dependent on the severity of renal failure on diagnosis and the presence or absence of an underlying multiple myeloma [4]. Current treatment strategies are targeted at reducing the production of immunoglobulins and include means such as systemic chemotherapy and high-dose alkylating agents in conjunction with autologous stem-cell transplant. The role of renal transplantation is still controversial given the risk of recurrent disease and the uncertain impact on survival.

Pathology

As nephrotic syndrome and renal insufficiency are common presenting signs, the diagnosis of MIDD is often made by renal biopsy. MIDD has several distinct findings on light microscopy, immunofluorescence, and electron microscopy. The most commonly seen lesion on light microscopy is nodular glomerulosclerosis, characterized by periodic acid-Schiff-positive material and mesangial expansion, which can appear indistinguishable from diabetic glomerulosclerosis

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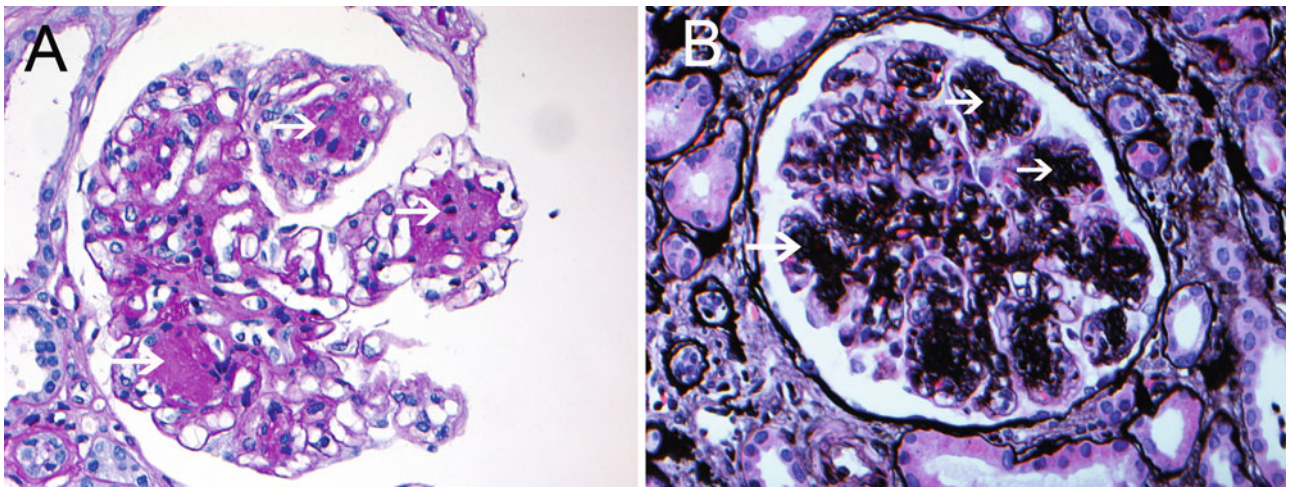

Fig. 20.1 Light chain deposition disease. Glomeruli showing (A) PAS and (B) silver positive mesangial nodules. Note the lobular accentuation of the glomerular tufts. Arrows point to mesangial nodules. (PAS- periodic acid Schiff stain, Silver- Jones methanamine silver stain, both 40x). Courtesy Dr. Sanjeev Sethi, Mayo Clinic

(Fig. 20.1). In several case series, the presence of nodular glomerulosclerosis is seen in approximately one-third to one-half of biopsies [2, 7, 11], but has been reported as high as 61 % in a more recent case series [9]. Tubular, and sometimes glomerular, basement membrane thickening is the most common finding, which may be present in up to 87 % of cases of LCDD [2, 12], though some authors have shown this finding to be variable [8, 9]. There may also be some tubular atrophy and interstitial infiltration with inflammatory cells present [12, 13]. Some biopsies may have features more consistent with a membranoproliferative glomerulonephritis, and crescents have been reported [9, 14–16].

Immunofluorescence is the most sensitive technique to diagnose MIDD on renal biopsy and is essential for diagnosis [2, 6, 9]. The light chains present in LCDD are more commonly kappa than lambda, with the occurrence of lambda light chains only accounting for 10–30 % of cases [2, 8, 13, 17]. A recent case series by Nasr et al. of 64 patients with MIDD showed that 84 % of LCDD patients and 67 % of LHCDD patients had a kappa light chain present on immunofluorescence [9]. In addition, kappa light chain nephropathy is more likely to be associated with nodular glomerulosclerosis than lambda light chain nephropathy [7]. The light chains show a predilection for the basement membranes in the tubules and the glomeruli [5] (Fig. 20.2). The most common site of basement membrane deposition is the tubule, followed by the glomerulus and the blood vessel [6]. Electron microscopy reveals granular, "powdery," electron-dense deposits along outer aspect of the tubular basement membrane and the inner side of the glomerular basement membrane, as well as in the mesangium (Fig. 20.3) [12].

The heavy chain in both LHCDD and HCDD is more likely to be of the γ[gamma] subtype, and immunofluorescence

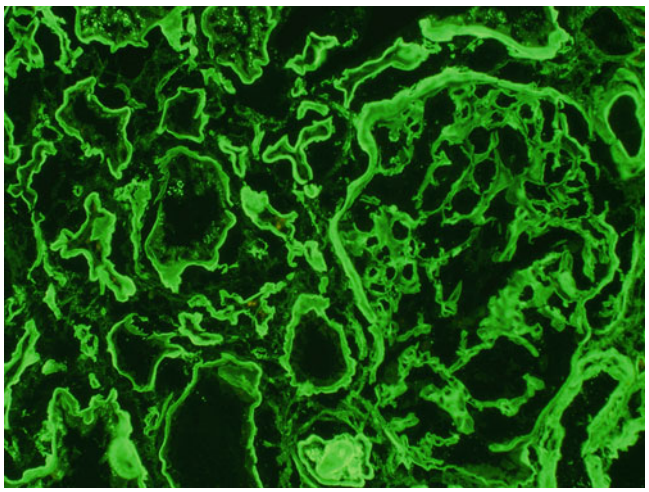

Fig. 20.2 By immunofluorescence, a stain shows bright linear glomerular and tubular basement membrane staining for kappa light chain; staining for lambda light chain and other immunoreactants was negative

reveals IgG staining that is monotypic on IgG subclass staining. The staining pattern and deposits on electron microscopy are otherwise indistinguishable from LCDD. All IgG subclasses (1–4) have been reported and there are rare cases of IgA-HCDD [18].

There have been uncommon cases of MIDD in which electron-dense deposits were demonstrated on electron microscopy despite negative immunofluorescence. This discrepancy is more common in patients with coincident myeloma cast nephropathy (MCN) [2, 11].

As immunoglobulins are capable of causing several different disease processes, it is not surprising that features

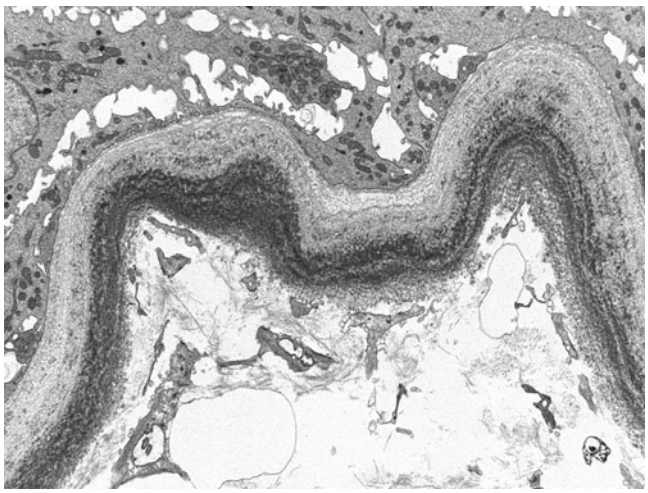

Fig. 20.3 Electron micrograph of a tubule shows finely granular electron-dense deposits along the tubular basement membrane. Similar deposits were seen along glomerular basement membranes and in the mesangium

of other paraprotein-associated diseases can frequently be seen on renal biopsy. LCDD may co-occur with AH, AL, and MCN. Pozzi et al. reported a case series of 63 patients in which 3 % had amyloidosis on renal biopsy [2]. In one small case series, all three patients had renal biopsies with features consistent with both LCDD and AL [19]. Other tissues that were sampled in two of these patients—including skin, liver, heart, and lung—also showed the Congo Red positivity diagnostic of AL, together with the discrete light-chain deposits along the basement membranes consistent with LCDD. The authors concluded that the same light chain was capable of forming either the fibrillar deposits of AL or the non-fibrillar deposits of LCDD, depending on environmental factors. There may, however, be no Congo Red positivity on renal biopsy, allowing the coexistence of AL to go unnoticed in the absence of other tissue samples [20].

In patients with multiple myeloma, MIDD may also occur in the presence of MCN. In a case series of 34 patients by Lin et al. on patients with MIDD, the biopsies with both MCN and LCDD were characterized by the significant tubular damage from the cast nephropathy, with less severe glomerular disease and less significant deposition of light chains along the basement membrane [8]. The clinical features and outcomes of these patients were more consistent with MCN patients than with pure MIDD patients. In another case series of 23 patients with LCDD and MCN, the glomeruli appeared normal in two-thirds of cases and nodular glomerulosclerosis was present in only three biopsies [11]. Again, the pathology was most notable for the tubular damage done by light chain casts, and the diagnosis of LCDD was made only by immunofluorescence. Of note, approximately one-third of cases did not demonstrate electron-dense deposits on electron microscopy. Lastly, in a recent series of 190 patients at the Mayo Clinic with multiple myeloma, 41 patients had MIDD and five had concurrent MCN and two had concurrent AL amyloidosis [21].

Lorenz et al. [22], in a recent case report, describe a patient who initially presented with hypertension and renal insufficiency and was found to have a monoclonal IgG kappa on serum immunofixation, and renal biopsy showed acute tubular necrosis, MCN, and focal arterial amyloidosis. She was treated with dexamethasone, thalidomide, and autologous stem-cell transplant and then had a recurrence 6 months later. Repeat biopsy revealed MCN, as well as Congo Red positive, positively birefringent deposits in the interstitium. Immunofluorescence demonstrated kappa light-chain positivity of the tubular basement membranes. Interestingly, no glomerular deposits were seen, and all three features—MCN, amyloidosis, and LCDD—were predominantly in the interstitium.

MIDD, though most often diagnosed by renal biopsy, is truly a systemic disease, with the possibility of immunoglobulin deposition in many tissues, including the heart, lung, and liver. Myocardial tissue demonstrates smooth, diffuse, and uniform staining in a perimuscular pattern [6, 10]. In lung tissue, there can be a more patchy deposition of eosinophilic material in alveolar walls, small airways, and vessels, with marked giant cell reaction. In MIDD, immunofluorescence shows either light or heavy chains within the basement membranes of the aforementioned structures, and electron microscopy demonstrates coarse extracellular granular deposits [23, 24]. Liver biopsy shows diffuse staining of the basement membranes, outlining the liver cell cords [6].

With treatment, there may be regression of the underlying nodular glomerulosclerosis and disappearance of the deposits [25, 26]. A case report describes a 53-year-old woman with LCDD who presented with nephrotic syndrome and nodular glomerulosclerosis on biopsy, received chemotherapy and an autologous stem-cell transplant, and achieved remission of her underlying disease. She then developed nephrotic syndrome 6 years later with a new IgG-lambda monoclonal gammopathy [27]. On repeat biopsy, she had IgG AH disease and no evidence of the prior nodular glomerulosclerosis. This case demonstrates the emergence of a second clone after autologous stem-cell transplant but also shows the reversibility of the glomerular lesion with effective treatment of the underlying disorder. Other authors, however, have reported persistence of light-chain deposits after chemotherapy and remission of underlying disease [28, 29].

Pathogenesis

The pathogenesis of MIDD is related to the primary structure of the protein itself, the amount of protein produced by a specific clone, the environment in which the immunoglobulin is presented, and the reaction of the cells in that environ-

ment. One thing that is clear from several large case series is that patients with multiple myeloma, and therefore those with the largest malignant clones, have a worse outcome than patients who simply have MGUS or no underlying plasma cell dyscrasia [2, 4, 6]. Though it is difficult to discern the exact cause for this discrepancy, one possibility is the amount of protein produced and presented to target organs dictates the severity of the disease. There are patients, however, with very aggressive and rapidly progressive MIDD in the absence of multiple myeloma, suggesting that it is more than just the absolute amount of immunoglobulin that dictates the severity of disease [30]. As LCDD is the most common subtype of MIDD, more is known about the pathogenesis of light chain, as opposed to heavy chain, deposition.

LCDD

In LCDD, the primary structure of the light chain likely has some importance in its ability to deposit in the body. Several proteins from patients with LCDD have been sequenced, and some conclusions can be drawn from analysis of the primary structure. The kappa light chains are more likely to be of the V-region subtype, of which Vκ[kappa]$_{IV}$ seems to be overrepresented, though κ[kappa]$_{I–IV}$ has been described [31–33]. There is no consistent structural motif or specific residue that has been shown to be responsible for the pathogenicity of these proteins; however, certain features have emerged. First, the amino acid substitutions appear to derive from somatic mutations, as opposed to germline mutations. Second, though the substitutions can occur throughout the protein, they are most common in the complementarity-containing region [31]. Third, the mutations described in both kappa and light chains are more likely to introduce hydrophobic residues, suggesting a disturbance in protein–protein interaction, destabilization of the protein, and subsequent deposition in tissues [31, 34, 35]. The predisposition to aggregation can be demonstrated in a murine model of LCDD, in which transfected mice with vectors containing kappa light chain sequence from a human subject with LCDD of the Vκ[kappa]$_{IV}$ subtype showed light-chain deposition in the kidney [36]. Lastly, posttranslational modification has also been implicated in creating pathologic light chains, as the isolate of one kappa light chain from a patient with LCDD had a mutation that resulted a new N-glycosylation site [37].

The mesangial cell is also an important factor in the pathogenesis of this disease. In a series of in vitro studies by Herrera et al., it has been demonstrated that mesangial cells are integral in the deposition of excess extracellular matrix (ECM) proteins that characterize the pathologic lesion of nodular glomerulosclerosis [38–40]. In one of the initial studies, cultured mesangial cells were incubated with light chains from patients with either amyloidosis, MCN, or LCDD [38]. The authors were able to show that mesangial cells cultured with amyloid light chains initially had an increase in ECM proteins, then a reduction to below control levels with a concurrent increase in collagenase activity. In contrast, mesangial cells cultured with LCDD light chains showed proliferation of mesangial cells followed by excess ECM formation, a reduction in collagenase activity, and mesangial nodules consistent with nodular glomerulosclerosis.

One important ECM protein that was present in these nodules was tenascin. Tenascin is a component of the mesangial ECM that is present in low levels in normal mesangium, but is present in much higher amounts in pathologic conditions of the kidney [41]. In addition, transforming growth factor β[beta] (TGF-β[beta]) is a known inducer of tenascin production and has been shown by the same authors to be present in the glomeruli of patients with LCDD [17]. Tenascin is degraded by the matrix metalloproteinase (MMP) 7. In the same in vitro model described above, the culture media from mesangial cells incubated with LCDD light chains showed a marked reduction in MMP-7 levels in comparison to those incubated with amyloid light chains [42] as well as an increase in TGF-β[beta] and platelet-derived growth factor β[beta] [40]. Interestingly, later studies have shown that MMP-7 is produced by mesangial cells, but the cells are for some reason unable to secrete it [40]. These experiments suggest that there is some intrinsic feature of the light chain itself that promotes or inhibits ECM production, alters regulation of ECM breakdown by MMPs and collagenase, and thereby produces different pathologic entities. One lingering question is how the same light chain is able to produce different types of deposits in the same individual or even in the same organ as described above (see Pathology).

HCDD

HCDD was first described as a distinct entity in 1992 by Tubbs et al. [43], where the proposed name was pseudo-γ[gamma] heavy-chain deposition disease. As mentioned above, the heavy chain in HCDD is more likely to be of the γ[gamma] class [8, 9], with some rarer cases of the α[alpha] subtype [16] and only one case of the μ[mu] subtype [44]. In most reported cases of γ[gamma]-HCDD, there is a deletion of the first constant (CH1) domain [18, 45–47], resulting in a truncated protein. One theory as to the mechanism of disease is that as these proteins lack the CHI portion, they cannot bind to the heavy-chain-binding protein (BiP) in the endoplasmic reticulum [48] and therefore are secreted into circulation rather than being degraded [49]. There may also be some amino acid substitutions in the complementarity-determining regions (CDRs) and framework regions that can change the property of the heavy chain, making it more likely to deposit in tissues [18, 50].

Further insight into the pathogenesis of HCDD can be gained by further subtyping the heavy chain. There have

been reports of γ[gamma]1–4 subtypes [18, 43, 45, 49]. All of the γ[gamma]3 subtypes and the majority of the γ[gamma]1 subtypes reported have had low serum complements and deposition of C1q and C3 on immunofluorescence [49]. However, those with γ[gamma]4-HCDD and the one case of γ[gamma]2-HCDD did not have low serum complement or deposition of C3 and C1q. This is consistent with what is known about the classical pathway of the complement system, where IgG3 binds C1q most avidly, followed by IgG1 and IgG2 [51]. IgG4 fixes complement very poorly and is unable to activate the classical pathway. In a review of all the cases reported of γ[gamma]-HCDD, 11/19 patients had hypocomplementemia, as opposed to the five reported cases of α[alpha]-HCDD, where no patients had hypocomplementemia [16]. Interestingly, in all cases of α[alpha]-HCDD, crescents were observed on light microscopy. As crescents are rare in MIDD, this feature highlights the subtle but real pathogenic differences between all the subtypes of MIDD based on the characteristics of the deposited immunoglobulin. More research into the properties of the various immunoglobulins in MIDD may help explain the varied pathologic and clinical features of these different subtypes.

Clinical Presentation

The clinical presentation of MIDD depends on the organ systems involved and therefore can have a wide variety of signs and symptoms (Table 20.1). In general, affected individuals are in the sixth decade of life, and men are affected more often than woman in a ratio of 2:1 [2, 8, 13]. Given the varying criteria used for the diagnosis of multiple myeloma versus MGUS in several studies, the incidence of multiple myeloma in patients with MIDD is somewhat unclear. In the case of LCDD, the percentage is likely approximately 25–40 % [5, 6, 8], but has been reported as high as 58–65 % [2, 7, 9]. Conversely, patients with multiple myeloma have a 5–22 % lifetime incidence of LCDD. The incidence of MM in patients with HCDD and LHCDD may be slightly lower, 29 % and 50 %, respectively, in a recent case series [9]. The remainder of affected individuals may have MGUS with fewer than 10 % plasma cells on bone marrow biopsy, rarely another lymphoproliferative disorder, or no discernible hematologic disorder by conventional criteria.

Table 20.1 Summary of clinical characteristics of light-chain deposition disease

Kidney	Renal insufficiency
	Nephrotic syndrome
	Proteinuria
	Microscopic hematuria
	Hypertension
Heart	Restrictive cardiomyopathy
	Congestive heart failure
	Cardiac arrhythmias
Lung	Interstitial lung disease
	Pulmonary nodules
	Cystic lung disease
Liver	Hepatomegaly
	Transaminase elevation
	Fulminant hepatic failure
Nervous system	Peripheral neuropathy
	Focal brain lesions
	Seizures
	Retinal vasculopathy

Serum protein electrophoresis (SPEP) is only positive in approximately 25 % of cases, but the majority of patients have an identifiable monoclonal protein by immunofixation in either the serum or urine, at a rate of 73–78 % and 79–90 %, respectively [2, 7, 13]. In one study of MIDD, the SPEP and urine protein electrophoresis (UPEP) were more likely to be positive in HCDD and LHCDD than LCDD, but in the patients with HCDD, the whole monoclonal Ig was detected by SPEP in only 4/7 patients and by UPEP in 1/7 [9]. The authors speculate that this may be due to its presence in very low titers or due to a rapid rate of deposition [52]. It is important to note that approximately 10 % of patients have no monoclonal protein detected by immunofixation, again emphasizing the importance of routine immunofluorescence on renal biopsy specimens. The diagnosis of MIDD by renal biopsy may precede the diagnosis of monoclonal gammopathy in up to 68 % of cases [8]. The newer free light-chain assay that quantifies the levels of light chains in the serum and gives a ratio of kappa:lambda has not only aided in the diagnosis of AL, LCDD, and other plasma cell dyscrasias but also correlates with disease activity and can be followed during the course of treatment [53, 54]. Table 20.2 demonstrates the clinicopathologic correlates of LCDD, LHCDD, and HCDD, respectively.

Renal

Though there is some variability in presentation, most patients come to medical attention due to their renal dysfunction, and the diagnosis of MIDD is made by renal biopsy the majority of the time [5]. Some degree of renal insufficiency is common and approximately 90 % of individuals have an elevation in serum creatinine [5, 7–9]. Approximately 50 % of those affected have nephrotic-range proteinuria [7, 13], though some degree of proteinuria is almost always present [5, 7]. Patients with multiple myeloma-associated MIDD present with acute renal failure much more commonly and are more likely to need dialysis at presentation [8]. Hypertension is often present, in contrast to AL where patients are usually hypotensive [5].

Table 20.2 Clinical and pathologic features of MIDD based on data from three large case series [2, 8, 9]

	LCDD	HCDD	LHCDD
Light microscopy			
Nodular glomerulosclerosis	+++/+	++++	+++/+
Global glomerulosclerosis	+	+	+
TBM thickening	V	V	V
GBM thickening	NS	NS	NS
Crescents	+	+	+
Tubular atrophy/interstitial fibrosis	V	V	V
Immunofluorescence			
Anti-light chain on TBM	++++		++++
Anti-heavy chain on TBM		++++	++++
Anti-light chain on GBM	++++		++++
Anti-heavy chain on GBM		++++	++++
Electron microscopy			
Granular deposits along TBM	++++	++++	++++
Granular deposits along GBM	++++	++++	++++
Mesangial deposits	+++	++++	++++
Clinical features			
Creatinine at diagnosis (mg/dl)	3.8–4.0	4.8–5.6	3.1–5.3
Nephrotic-range proteinuria (>3 g/day)	++	+++/+	+/+
Nephrotic syndrome[a]	+	+++	+/+
Hypertension	++++	++++	+++/+
Diagnosis of multiple myeloma[b]	+++	++/+	+/+
+SPEP and immunofixation	++/+	+++/+	++++
+UPEP and immunofixation	+++/+	+++/+	++++

+ = 1–25 %, ++ = 25–50 %, +++ = 50–75 %, ++++ = >75 %, values separated by / have range that span two quartiles
NS not significant, *V* variable with wide range
[a]Definition of nephrotic syndrome—nephrotic-range proteinuria with hypoalbuminemia and peripheral edema
[b]Definition of multiple myeloma in Nasr et al. [9], renal MIDD plus ≥10 % monoclonal plasma cells in BM and monoclonal protein identified in serum and/or urine; Lin et al. [8], renal MIDD plus at least one of the following: (1) positive BM biopsy, (2) presence of osteolytic lesions, (3) hypercalcemia with positive SPEP/UPEP, or (4) ≥10 % BM plasmacytosis with low quantitative serum immunoglobulins; and Pozzi et al. [2], according to major and minor diagnostic criteria of multiple myeloma and not otherwise specified

Cardiac

Extrarenal disease in MIDD is common and cardiac disease can be present in up to 8-21 % of cases of MIDD [2, 9]. The exact prevalence of cardiac involvement is hard to quantify, as patients often primarily present with renal involvement, and once the diagnosis is confirmed by renal biopsy, there may not be a reason to pursue additional tissue. In addition, many of the symptoms may be ascribed to the renal dysfunction, such as peripheral and pulmonary edema. In a review of patients with confirmed cardiac LCDD by biopsy or autopsy, the most common clinical manifestations were arrhythmia, congestive heart failure, and conduction disease [6]. Transthoracic echocardiography (TTE) in general will have evidence of diastolic dysfunction and decreased compliance, consistent with a restrictive cardiomyopathy [55]. In a series of five patients with cardiac LCDD, of whom only four had TTE available, all demonstrated increase in LV wall thickness and preserved ejection fraction (50–85 %), as well as low voltage [10]. AV conduction delays were common, as were arrhythmias, including atrial fibrillation and atrial flutter. One patient had ventricular tachycardia. These clinical and echocardiographic characteristics are very similar to cardiac amyloidosis and other infiltrative cardiomyopathies, though unlike amyloidosis, patients rarely present with primarily cardiac symptoms.

Pulmonary

The pulmonary manifestations of MIDD are somewhat heterogeneous, and in some cases the immunoglobulin deposits are confined to the lung with no systemic involvement [56]. The most commonly described symptoms are dyspnea and mild cough, but patients may have no symptoms at all. In cases of LCDD, patients can present with nodules composed of light chains that can be peripheral or endobronchial [6, 57]. However, they can also have diffuse interstitial disease, and these patients have a worse prognosis and are more likely to have an underlying plasma cell disorder [24]. A few patients with cystic lung disease that has the radiographic appearance of lymphangioleiomyomatosis have also been described [23].

Hepatic

Patients with MIDD often have hepatomegaly and modest enzyme elevations, but liver dysfunction is uncommon [6]. There are rare cases of fulminant hepatic failure and subsequent death due to LCDD [58, 59].

Neurologic

Peripheral neuropathy can be present in 20 % of cases [6]. As paraproteins cannot cross the blood-brain barrier, central nervous system disease is rare; however, a few cases of LCDD in the brain have been described, with seizures being the most common presentation [60–62]. In all cases, there were collections of lymphocytes, either reactive or malignant, in the brain parenchyma that were the likely source of light chains,

though one patient was subsequently found to have a monoclonal gammopathy in the serum. In one case, high levels of IgG were detected in the cerebrospinal fluid [62].

Natural History

The natural history of MIDD is variable and is largely dependent on the presence or absence of multiple myeloma. It is well established that patients with multiple myeloma and LCDD, the most common form of MIDD, have a more rapid renal deterioration and shorter survival [2, 7, 8]. In a retrospective study of 63 patients with LCDD by Pozzi et al. [2], 88 % of whom received some form of treatment, the median survival was 4 years and the death rate was 17.5 per 100 patient-years. Renal survival, which is defined as an increase of greater than 50 % from baseline serum creatinine, was 67 % at 6 months and 31 % at 8 years. The authors found that factors associated with worse overall survival, as well as renal survival, were presence of multiple myeloma and symptomatic extrarenal disease. Factors associated with worse renal outcomes alone were presence of cast nephropathy and acute renal failure or rapidly progressive disease. This is consistent with earlier studies that have shown that patients who present with MIDD as well as acute renal failure due to MCN and multiple myeloma have a survival of only 8 months [6].

In a larger retrospective review of 118 patients with multiple myeloma and renal disease, Montseny et al. [4] looked at the outcomes of patients with LCDD, AL, and MCN. In this series, 22 patients had LCDD, and the incidence of LCDD was distributed evenly amongst the different stages (1–3) of multiple myeloma, as opposed to AL which was present mostly in stage 1 multiple myeloma and MCN which was present in stage 3 multiple myeloma. The LCDD patients had a median survival of 36 months versus 24 months in AL and 12 months in MCN. Once HD was initiated, patients with LCDD had a survival of 48 months versus 22 months in AL and 6 months in MCN. The predictors of survival in this study were age less than 70, low serum calcium, and low serum creatinine.

In the recent review by Nasr et al. [9] of 64 patients with MIDD of whom 56 patients had available data on follow-up, 32 patients (57 %) were treated with chemotherapy and 11 (34 %) of those patients progressed to ESRD over a mean follow-up of 34 months. Sixteen patients underwent autologous stem-cell transplantation (ASCT), and six of those patients progressed to ESRD. Despite these interventions, 39 % progressed to ESRD and another 4 % had worsening renal function, with a mean renal survival of 64 months for all patients with MIDD. There was no statistical difference in renal survival for LCDD, LHCDD, and HCDD. The mean patient survival was 90 months, also with no statistical difference between the different MIDD subtypes. In those patients who died (32 %), the mean time from biopsy to death was 18 months. This study was encouraging in that there is an overall trend of better survival, likely due to more effective therapy, which will be discussed further below.

Notably, cardiac disease is an important source of mortality in this population. Cardiac insufficiency, arrhythmia, or sudden death can be the cause of death in approximately 20 % of cases [2, 4]. Other causes of death include severe renal failure, cachexia, GI bleeding, and infection [4, 5].

Treatment

The long-term outcomes associated with the treatment of MIDD are somewhat difficult to characterize, as it is a rare disease and there have been several different chemotherapeutic regimens tried over the years. Just as the treatment of multiple myeloma has undergone a large shift with the use of bortezomib and autologous stem-cell transplantation, there has been a parallel shift in the treatment of MIDD with some promising results.

Patients in early studies who received chemotherapy for MIDD often received alkylating agents, most often melphalan, and prednisone in varying amounts, and showed some modest improvements in survival with 70 % survival at 5 years, as well as some improvement in renal parameters including proteinuria and creatinine [13]. The main determinant of how well patients responded was how severe their renal dysfunction was at initiation of chemotherapy. Multidrug chemotherapy with regimens such as vincristine, doxorubicin, and dexamethasone (VAD) has also been tried with some improvement in survival and progression of renal disease [2, 4]. However, one study observed a worsening in renal function and development of extrarenal symptoms in some patients treated by conventional chemotherapy [7].

Transplantation in patients with MIDD has had somewhat discouraging results. The majority of patients have had recurrence of the MIDD posttransplantation, despite treatment with chemotherapy and reduction in paraprotein levels prior to transplant, according to a review of the literature by Short et al. [30]. In this group of patients, six of seven patients had a recurrence, and four of seven patients died after renal transplant, with disseminated myeloma and sepsis being the most common causes of death. Another case series by Leung et al. [63] looked at the outcomes of seven patients with LCDD who received kidney transplants, three of whom had received chemotherapy with an alkylating agent and prednisone prior to transplant. These patients also had poor outcomes—three patients had early acute cellular rejection, two required allograft nephrectomy, and five patients (71 %) had recurrent disease in the allograft at a median time of 33 months. Median survival was 12 years after diagnosis,

6 years after transplant, and 3.6 years after recurrence. Overall survival was worse than age-matched kidney transplant recipients without LCDD. As some patients seem to have early and somewhat aggressive recurrence of their disease, it raises the concern of the effect of immunosuppression on the natural history of MIDD. In addition, these studies would suggest that transplantation in patients with MIDD is inadvisable.

However, more encouraging results have emerged with the use of high-dose chemotherapy and ASCT. Several retrospective studies of patients with MIDD and multiple myeloma who have received high-dose chemotherapy and ASCT have shown promising results in terms of regression of disease, improvement in survival, and durability of renal allografts. In the first review by Royer et al. [28], 11 patients with LCDD or LHCDD were described who received variable amounts of chemotherapy prior to undergoing mobilization with G-CSF or cyclophosphamide, high-dose melphalan, and ASCT. There was a reduction in the level of monoclonal immunoglobulin in eight of ten patients, and six achieved complete response (CR). Extrarenal manifestations improved mainly in the patients with CR, and the renal response was somewhat variable—some stabilized, some improved, but the nephrotic syndrome did regress in the three patients who had it at presentation. It should be noted that aside from the expected side effects of neutropenic fever, nausea, and mucositis, two of the dialysis patients had severe encephalopathy after receiving high-dose melphalan. In terms of relapse, three patients had recurrence of their myeloma at 24–30 months, but none developed extrarenal manifestations. Only one patient underwent renal transplant and had no recurrent disease, though the length of follow-up is not stated.

Similar results were found in the study by Hassoun et al. [33] that looked at seven patients with MIDD and multiple myeloma, five with LCDD, one with LHCDD, and one with light-chain proximal tubulopathy. These patients also had chemotherapy prior to undergoing mobilization with either G-CSF and cyclophosphamide or G-CSF alone, followed by melphalan and ASCT. Of the seven patients, six achieved CR with normalization of the free light-chain ratios, and of the four patients not on RRT, two had improved renal function, one had stable and one worse renal function, but proteinuria was improved in all four patients. Of the three patients who were dialysis dependent, two underwent transplantation and had normal creatinine clearance at 14 and 45 months. Another retrospective review by Telio et al. had similar results in terms of hematologic CR, but seven of eight patients had a greater than 50 % improvement in serum creatinine from baseline [64]. A prospective study done at Boston University Medical Center [65] of patients with MIDD included six patients—five with LCDD and one with light-chain proximal tubulopathy, some of whom had already received chemotherapy—and treated them with high-dose melphalan and ASCT. The follow-up was much shorter, median of 12 months, but results were also encouraging with five of six patients achieving CR, a 75 % reduction in proteinuria, regression of septal thickness in the patient with cardiomyopathy, and only one patient with the severe side effect of encephalopathy.

The advent of bortezomib, the proteasome inhibitor used in the treatment of multiple myeloma, has recently been used in patients with LCDD. In a small study of four patients with LCDD and no evidence of multiple myeloma who received bortezomib and dexamethasone, all patients had a rapid normalization of free light chains, a decrease in proteinuria and an improvement in creatinine, as well as improvement in blood pressure control. The main side effect was peripheral neuropathy. Three of the patients went on to high-dose melphalan and ASCT and achieved CR. The authors note that bortezomib has been shown to inhibit the NFκ[kappa]B pathway [66]. As NFκ[kappa]B is known to attract inflammatory cells to tissue and increase TGF-β[beta]1 levels, bortezomib may be acting via an additional anti-inflammatory mechanism to improve renal function in patients with LCDD. Another case report demonstrated the efficacy of bortezomib in a patient with LCDD and multiple myeloma who presented with acute renal failure [67]. The use of bortezomib is an exciting new development in the treatment of LCDD and holds promise for the future.

Lastly, it should be noted that there were no deaths in any of the above studies associated with ASCT. However, in light of the small number of patients, the incidence of encephalopathy seems quite high. In addition, most of the patients in the first two studies had concurrent multiple myeloma, giving them a poor baseline prognosis relative to non-myeloma-associated MIDD, and therefore the benefits may be inflated. Nonetheless, the risk-benefit ratio seems strongly in favor of treatment with high-dose melphalan and ASCT to reduce the burden of immunoglobulins. The effect on renal function seems to be variable, but there is clear improvement in extrarenal manifestations. There is one case report of a patient with LCDD on dialysis who improved to the point of being able to come off dialysis after treatment with ASCT, but this is not the standard course [29]. The success of transplant remains to be seen, as there are still very few patients described, and the follow-up is brief. It does seem clear, however, that prior to committing a patient to renal transplantation, every attempt to induce a hematologic CR with normalization of the free light chains in the serum should be undertaken.

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Amyloidosis

21

Nelson Leung

Introduction

Amyloidosis represents a group of diseases characterized by the deposition of amyloid fibrils in various tissues [1]. The result is progressive organ failure that can lead to death. These fibrils are 7–12 nm in diameter and are randomly arranged. They have the ability to take up Congo red dye and give off an apple-green birefringence when viewed with polarized light. This is a pathognomonic feature which separates amyloid from other fibrils which cause kidney disease.

To date, over 24 types of protein are known to cause amyloidosis in human [2]. The fibrils form as a result of misfolding which causes the protein to take up a pathologic confirmation that allows for subsequent self-aggregation. Several amyloidogenic mechanisms have been identified [1, 2]. In some, there is a natural propensity in the native protein to misfold. With these proteins, amyloid is formed as a result of accumulation or overproduction. Examples of this include senile amyloidosis (wild type transthyretin) which accumulates as a result of aging, dialysis-related amyloidosis (Aβ[beta]$_2$m) where β[beta]2-microglobulin accumulates as a result of renal failure, and secondary amyloidosis (AA) where serum amyloid A is overproduced as a result of persistent inflammation or infection. Others are the result of a genetic mutation that produces an amyloidogenic protein (Table 21.1). Examples of this are (mutant) transthyretin (ATTR), fibrinogen α[alpha]-chain (AFib), lysozyme (ALys), apolipoprotein AI, and AII (AApoAI and AApoAII). Proteins can also become amyloidogenic after proteolytic modification. This is seen in Alzheimer's disease with β[beta]-amyloid precursor protein (APP) and immunoglobulin light chain (AL). Prion which causes transmissible spongiform encephalopathies such as Creutzfeldt–Jacob disease, Gerstmann–Sträussler–Scheinker syndrome, fatal familial insomnia, and kuru is also considered an amyloidogenic protein (APrP). In some, multiple mechanisms may be involved. The best example of this is AL amyloidosis in which the monoclonal light chain is overproduced by the plasma-cell clones. These light chains undergo a partial proteolytic digestion, and some have mutations which may enhance the amyloidogenic potential of the monoclonal light chain [1].

Different forms of amyloidosis have different organ tropism [2]. Some are quite specific such as the case of cystatin c (ACys) which causes the Icelandic form hereditary cerebral amyloid angiopathy resulting in cerebral hemorrhage, stroke, and dementia. Similarly, Aβ[beta] protein precursor (Aβ[beta]) is responsible for Alzheimer's disease and is localized to the central nervous system. In others, skin is a preferential organ of deposition. Lichenoid or macular amyloidosis is the result of keratin (AD) deposition possibly as a result of trauma. AIns (insulin) can be found in diabetic patients near their insulin injection site. Nodular localized cutaneous amyloidosis is a form of localized AL that is often produced by polyclonal plasma cells probably in response to a localized inflammatory reaction. Systemic amyloidosis occurs when visceral organs are involved. These forms are often fatal as the persistent deposition of amyloid results in progressive organ failure (Table 21.2).

Renal involvement is common in many forms of amyloidosis. Proteinuria frequently occurs and can be massive (>10 g/day) [3]. Renal insufficiency is variable and often depends on the severity of disease. Renal insufficiency with little proteinuria has also been described in a small subset of patients in which the amyloid preferentially deposits in walls of blood vessels instead of glomerular basement membranes or interstitium [4, 5]. Types of amyloid that involve the kidney include AL, AH (immunoglobulin heavy chain), AA, AFib, AApoAII, and ALECT2 (leukocyte cell-derived chemotaxin-2) [2, 6]. Renal involvement is uncommon in patients with ATTR which usually present with cardiomyopathy and neuropathy, but rare reports of renal involvement including end-stage renal disease have been reported. Finally, Aβ[beta]2m occurs only in dialysis-dependent

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Table 21.1 Mechanisms of amyloidogenesis

Age associated	Accumulation/ overproduction	Genetic/hereditary	Multifactorial	Infectious	Unknown
Senile	B2m	Transthyretin	AL	Prion	LECT2
Prolactin	SAA	Fibrinogen	AH		Beta
Calcitonin	Insulin	Lysozyme	ALH		IAPP
Amylin		Apolipoprotein AI	Calcitonin		
ANF		Apolipoprotein AII	Keratin		
		Gelsolin	Localized AL		
		Cystatin C			
		BRI protein			
		Lactoferrin			
		SAA (HPFS)			

B2m β[beta]-2-microglobulin, *SAA* serum amyloid A protein, *AL* immunoglobulin light chain, *AH* immunoglobulin heavy chain, *ALH* immunoglobulin light heavy chain, *LECT2* leukocyte chemotactic factor 2, *ANF* atrial natriuretic factor, *IAPP* islet amyloid polypeptide, *HPFS* hereditary periodic fever syndromes

Table 21.2 Distribution by amyloid type

Systemic	Localized	Central nervous system
AL	AL	Aβ[beta]
AH	ACal	ACys
AA	AIAPP	ABri
AFib	AIns	ADan
AApoAI	ALac (ocular)	APrp^{sc}
AApoAII	AD (pituitary)	
ALys	APro	
AGel	IAA/AANF	
AB2m		
ATTR		
Senile		

AL immunoglobulin light chain, *AH* immunoglobulin heavy chain, *AA* serum amyloid A protein, *AFib* fibrinogen α[alpha] chain, *AApoAI* apolipoprotein AI, *AApoAII* apolipoprotein AII, *ALys* lysozyme, *AGel* gelsolin, *AB2m* β[beta]-2-microglobulin, *ATTR* transthyretin (mutant), senile–transthyretin (native), *ACal* calcitonin, *AIAPP* amylin, *AIns* insulin, *ALac* lactoferrin, *AD* keratin, *APro* prolactin, *IAA* ANP (isolated atrial amyloid), *Aβ[beta]* beta protein precursor, *ACys* cystatin C, *ABri* BRI protein (British familial dementia), *ADan* Danish familial dementia, *APrp*^{sc} prion

patients due to a lack of clearance by dialysis [7]. Its main manifestations are soft tissue deposition (carpal tunnel) and arthropathy. However, autopsy studies have discovered systemic deposition usually in vascular beds including those of the kidneys. However, since these patients already had end-stage renal disease, the renal amyloidosis is never manifested.

Pathogenesis

The precursor amyloid proteins all share a common characteristic to misfold into β[beta]-sheet fibrillar protein [1]. This propensity can be natural or as a result of partial digestion as in the case of AL. The initial step involve formation of β[beta]-strands which allow the protein to be stacked on one another utilizing hydrogen bonds of alternating C=O and N–H groups. This self-assembly process eventually forms a sheet of polypeptides. The sheets are then mated together via a dry interface through a process known as the steric zipper [8]. In this cross-β[beta] model, sheets are mated together to form protofilaments. Four to six protofilaments are then braided together to form a fibril. Congo red dye is capable of intercalating the space formed between the protofilaments in a fibril which allows it to be used as a diagnostic tool. Ultrastructurally, fibrils from different precursor proteins are indistinguishable from one another despite the fact that precursor proteins come in different sizes and tertiary structures.

The pathogenic process of amyloid fibril is still not completely understood. Obviously, deposition of the amyloid fibrils certainly plays a big role. In cardiac amyloidosis, the heart is concentrically thickened resulting initially in diastolic dysfunction and later as disease progresses, systolic dysfunction. In the kidney, degree of proteinuria is associated with location of amyloid deposition while, glomerular filtration rate is determined by amount of deposition in the glomerulus. However, recent evidence suggests fibril deposition may not be necessary for cellular toxicity. Exposure to the precursor protein alone is sufficient for cytotoxicity to occur in cardiac myocytes [9]. The same phenomenon has also been noted in the kidney. Patients with massive proteinuria have been known to have very little amyloid deposits in their kidney. In fact, some of these patients were initially diagnosed as minimal-change disease [10]. Correct diagnosis was made when their biopsy was reviewed for failure to respond to steroids. Conversely, large amyloid deposits have been found in patients who responded to therapy and have normal proteinuria [11]. Electron microscopy of these patients suggests repair of the glomerular basement membrane can occur despite the presence of amyloid fibril [12].

Diagnosis

The diagnosis of amyloidosis requires the demonstration of the amyloid fibrils in the tissue (Fig. 21.1). The most commonly used method is Congo red staining [1, 3]. Congo red intercalates the fibrils and gives off an apple-green birefringence when viewed with polarized light. It is fairly sensitive but highly specific for amyloid fibrils. Thioflavin T is another stain that binds the β[beta]-sheet and gives off an enhanced fluorescence. However, it is considered to be less sensitive and specific than Congo red. Fibrils are also detected by electron microscopy. Characteristically, it is randomly arranged and has a diameter of 7–12 nm. These characteristics can be used to distinguish AL from other renal diseases with fibrillary deposits [13]. They include fibrillary glomerulonephritis, immunotactoid glomerulonephritis, cryoglobulinemia, hereditary nephropathies with fibronectin, and collagenofibrotic glomerulopathy. Fibrillary collagen can also be found in other glomerulopathies, most commonly in diabetes nephropathy, focal glomerulosclerosis, membranoproliferative glomerulonephritis, crescentic glomerulonephritis, and lupus. These diseases can be distinguished from amyloidosis by Congo red staining pattern and ultrastructural characteristics of the fibrils.

Various tissues have been used for the diagnostic evaluation of amyloidosis. In patients with renal manifestations, amyloid can be detected on the kidney biopsy in virtually all cases [14]. Historically, renal biopsy was felt to be risky because of the possibility of amyloid angiopathy. Patients with AL can also develop an acquired factor X deficiency further increasing their risk of bleeding. However, a recent study of 101 patients with amyloidosis found the rate of post-biopsy hemorrhage was no different than patients without amyloidosis if they were approved by the standard screening tests used for all patients [15]. Other high-yield tissue included the heart, although it is much more invasive. Fat aspirate is often used. It is positive in approximately 70 % of the patients [14]. Rectal biopsy had been popular in the past. This is usually performed via a flexible sigmoidoscopy and has a sensitivity of 70–80 %. However, it is probably best to biopsy the organ which is symptomatic in order to maximize the odds of obtaining amyloid for typing.

In the kidney, amyloid can be seen in all three compartments. Amyloid initially appears along the glomerular basement membranes [3]. In more advanced cases, extensive

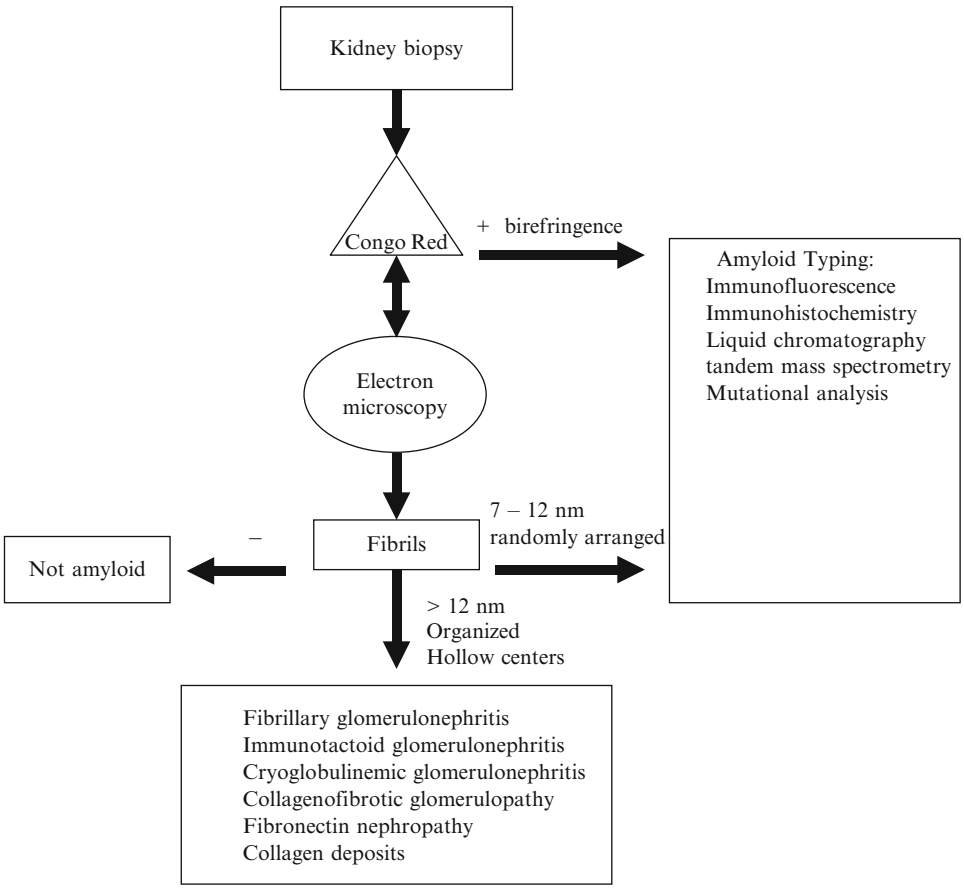

Fig. 21.1 Diagnostic approach for renal biopsies suspected of amyloidosis

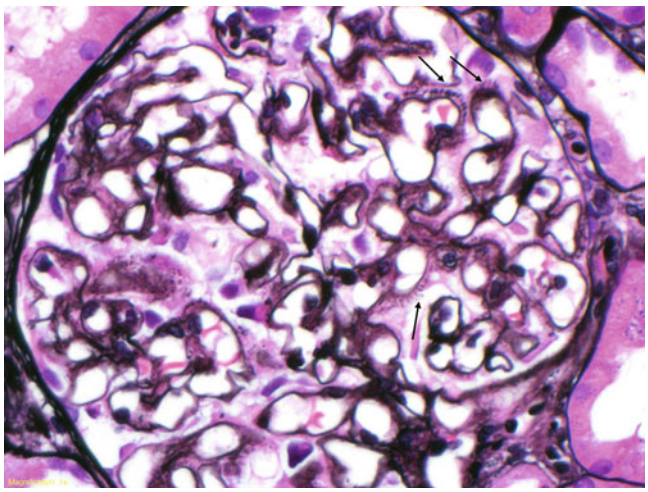

Fig. 21.2 Silver stain of a glomerulus. *Arrows* denote spicules along the glomerular basement membranes

deposition can be seen in the mesangium, becoming nodular in appearance in some cases. Vascular deposition is common, but in a small percentage of patients, it is the only place amyloid deposition occurs. On H&E stain, the deposits appear pink and amorphous. Amyloid deposits do not stain with periodic acid Schiff (PAS) or silver stain, but spicules can be seen along the glomerular basement membranes on silver stain (Fig. 21.2). In AL, immunofluorescence study should show a preferential staining for one of the light chains in areas where amyloid deposits have been identified. Light chain staining should be negative or equal and mild in intensity for all other types of amyloid.

Once amyloid is found in the tissue, typing of the amyloid protein is necessary. Treatment and prognosis differ for each subtype. Therefore, accurate typing is essential. Typing must be performed directly on the amyloid fibril. The use of surrogate markers such as circulating monoclonal protein or plasma-cell dyscrasia has led to misdiagnosis and treatment with cytotoxic agents [16]. It is paramount that AL or AH is confirmed before cytotoxic therapy is employed. Historically, potassium permanganate was used to distinguish AA from other forms of amyloid [3]. Applying potassium permanganate to the tissue prevents Congo red from binding to AA fibrils but not AL. However, immunohistochemical agents are now available. Antibodies to immunoglobulin light chains (κ[kappa] and λ[lamda]) and heavy chains, serum amyloid A protein, prealbumin (transthyretin), β[beta] 2-microglobulin, and fibrinogen are commercially available. Unfortunately, immunohistochemical identification is limited by the availability of the antibodies. Genetic testing has been used to identify many of the hereditary forms of amyloidosis. While this is helpful, caution is needed when interpreting the result. Differences in penetrance exist for different amyloidosis. The diagnosis is even more difficult when a monoclonal protein coexists or when dealing with senile amyloid where the amyloidogenic protein is wild type (non-mutated). Recently, the use of liquid chromatography–tandem mass spectrometry has made tremendous progress in the field of amyloid typing [17]. Tissues embedded on glass slides are dissected with a laser to capture amyloid-rich material. The tissue then undergoes tryptic digestion and is analyzed by the liquid chromatography–tandem mass spectrometry. The raw data are queried by multiple algorithms and the peptides are assigned a probability score. This technique allowed the identification of a new amyloid proteins [18]. The technique of SAP scintigraphy should be mentioned [19]. Serum amyloid P component is a molecule commonly found in all types of amyloid. Location and amount of amyloid deposits can be determined by injecting radiolabeled serum amyloid P component into the patient (Fig. 21.3). SAP scintigraphy is most useful in prognostication and assessment of response. And although a positive scan is virtually diagnostic for systemic amyloidosis, tissue biopsy is still required and should not be substituted [20].

AL

Previously referred to as primary amyloidosis, AL is the most common form of amyloidosis in industrialized countries [14]. The age adjusted incidence ranges from 5.1 to 12.8/million/year which appears to be stable. The median age at presentation is 64 years with a range of 32–90 years. It is almost always the result of a plasma-cell dyscrasia, but rarely it can occur with a lymphoma or lymphoplasmacytic lymphoma (Waldenström's macroglobulinemia). By immunofixation, a monoclonal protein is detectable in the serum and urine in 72 % and 73 % of patients, respectively. In the urine, the majority of the protein is made up of albumin with monoclonal protein representing only a small component. Eleven percent of the patients will have a negative monoclonal protein study by protein electrophoresis or immunofixation. These patients usually have a monoclonal light chain that exists in low levels. The sensitivity can be increased to 99 % by measuring serum-free light chain levels along with serum immunofixation [21]. Recent studies also showed serum-free light chain had a better correlation with outcomes of AL patients after treatment than the entire immunoglobulin [22]. Thus, measurement of serum-free light chain levels is essential in anyone suspected of having AL. A predilection toward lambda light chain exists in AL [14]. More than two-thirds of the patients with AL have a monoclonal lambda whereas the ratio is reversed in multiple myeloma. Approximately 18 % of the patients have >20 % plasma cells in the bone marrow, but coexistence with true multiple myeloma as defined by hypercalcemia, anemia, and lytic bone lesions is rare. AL is the most aggressive of the

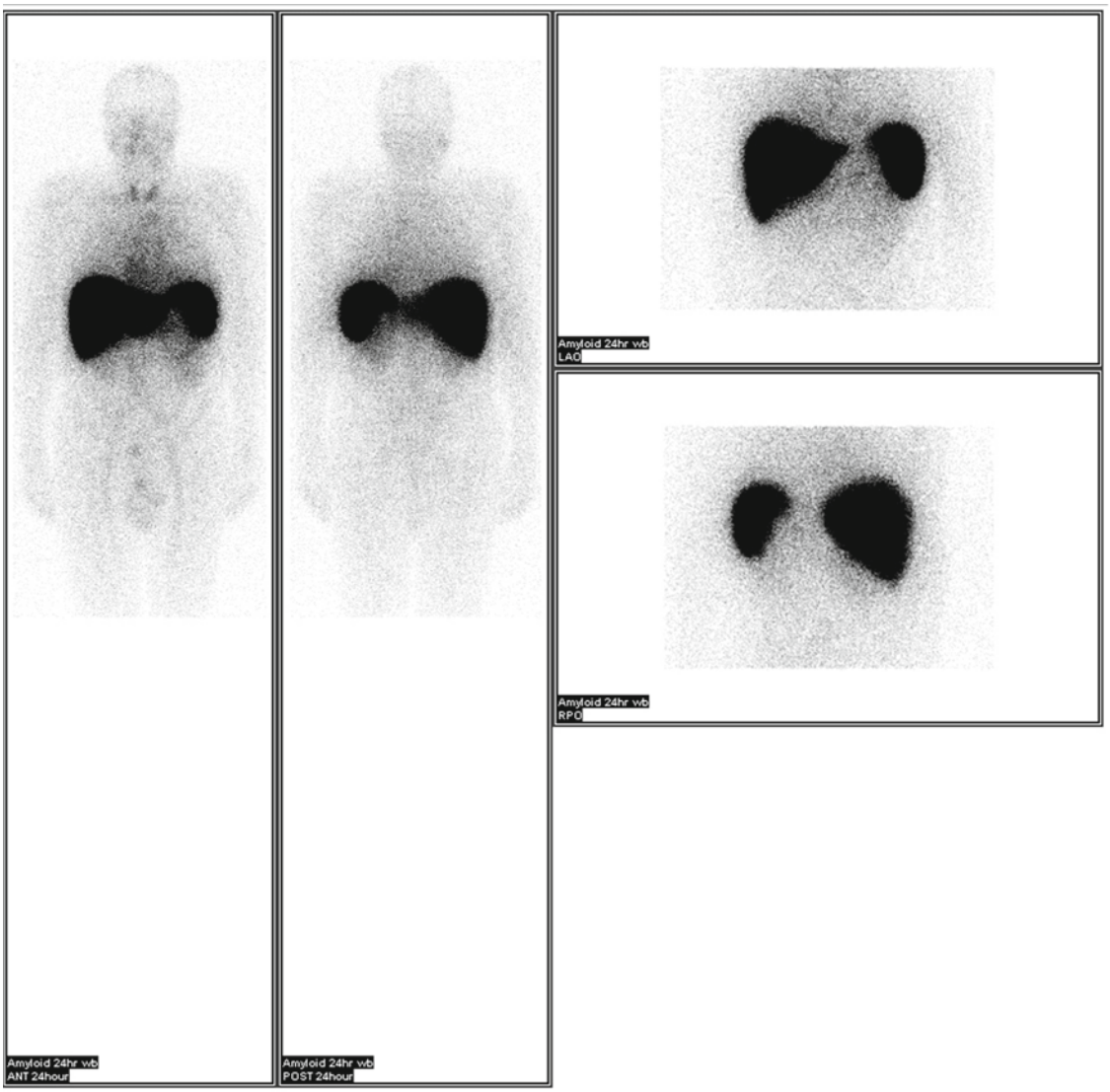

Fig. 21.3 SAP scintigraphy showing amyloid deposits in the liver and spleen. Images provided by Dr. Julian Gillmore

amyloidoses. Median survival is 13 months if left untreated with patients with advanced cardiac involvement or multiple myeloma faring the worst [23].

Kidney and heart are the most commonly affected visceral organs, but all visceral organs may be involved (liver, gut, spleen, and lung) as well as peripheral nerves, central nervous system, and soft tissues [14]. In a study of 474 patients, 73 % of patients presented with proteinuria and nearly half with renal insufficiency. The proteinuria was in the nephrotic range in approximately one-third of the patients. In a study of 145 patients, patients with lambda light chain were more likely to develop renal manifestations than ones with kappa [24]. The kappa to lambda ratio was 1:12 in patients with renal amyloidosis vs. 1:4 in those without ($p=0.02$). Patients with lambda light chain also appeared to have more severe proteinuria. Median proteinuria of lambda patients was 3.6 g/day vs. 0.7 g/day in kappa patients ($p=001$). When only patients with renal amyloidosis were analyzed, the disparity in proteinuria was maintained (7.2 g/day in lambda and 2.9 g/day in kappa). Elevated creatinine ($p=0.01$) and higher proteinuria ($p=0.03$) were risk factors for progression to end-stage renal disease (ESRD). ESRD eventually developed in 42 % of patients who presented with renal manifestations vs. 5 % of those without. This was similar to the rate (39 %) of ESRD reported in a recent Italian study of 198 biopsy-proven renal AL patients [25]. In the largest study to date with 923 patients from the UK, the rate of ESRD was slightly less at 23.9 % [26]. However, factors influencing progression to ESRD were similar (higher CKD stage and lower serum albumin). In this study, patients who had a hematologic response were less likely to require dialysis. Patients who progressed to ESRD had a significantly shorter survival [24].

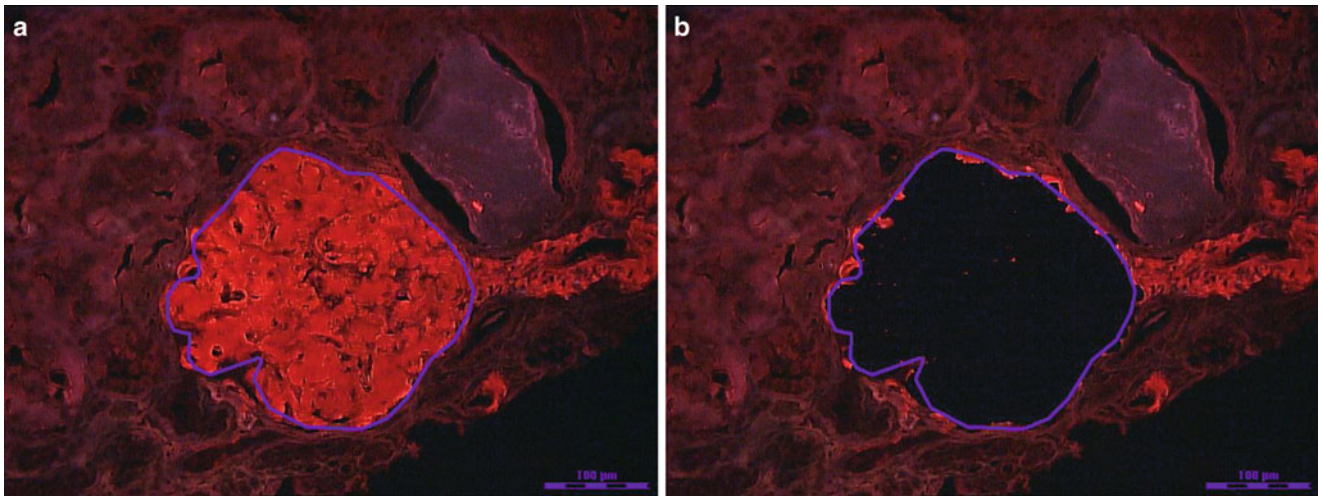

Fig. 21.4 (**a**) Photograph of a Congo red positive glomerulus traced for laser dissection and capture. (**b**) The glomerulus is dissected and captured for trypsin digestion and subsequent proteomic analysis by liquid chromatography and mass spectrometry. Photographs provided by Dr. Sanjeev Sethi

Data for the USA and Europe showed a median survival of 11 months for patients with ESRD. These data may represent bias since approximately 20 % of the patients died within the first month many of whom withdrew from dialysis. A more recent study with more effective treatment found a median survival of 39 months with many patients surviving long enough to receive kidney transplantation [26].

Histologically, AL deposits can be found in all three compartments of the kidney [3]. Within the glomerulus, deposits may range from minimal to massive. Deposits can also be found in the interstitium as well as in the wall of blood vessels. Patients who have vascular limited deposits on renal biopsy have less proteinuria (<1 g) and typically present with unexplained renal insufficiency [4, 5]. The deposits in AL should stain preferentially for just one of the immunoglobulin light chains [3]. Immunofluorescence staining with antibodies to immunoglobulin light chains is therefore essential for the diagnostic evaluation. In AL variants, the deposits can be composed of immunoglobulin heavy chain (AH) or both immunoglobulin light and heavy chain amyloidosis (ALH) [27]. The immunoglobulin subclass should be identified to insure the heavy chain is monotypic. Antibodies are available for IgG, IgA, and IgM subclasses. In ALH, both the immunoglobulin heavy chain and light chain should be monotypic. Deposits in the kidney must match the isotype of circulating monoclonal protein detected in the blood or urine. In cases where the light chains are not well stained with immunofluorescence, immunoperoxidase may be helpful. Liquid chromatography–mass spectrometry (LC–MS) has been found to be very accurate in typing amyloidosis and is extremely helpful in cases where immunohistochemistry stains are unrevealing or equivocal. It is now considered the gold standard for amyloid typing (Fig. 21.4) [17].

The treatment of AL had advanced considerably over the past two decades. The first effective treatment was melphalan and prednisone (MP). In two separate randomized controlled trials, MP extended median survival from 13 to 18 months [28–30]. Responders experienced significant reduction in proteinuria and alkaline phosphatase, but improvement in cardiac function was less common. Overall response rate was low in the 20–30 % range. Most of these patients achieved a hematologic partial response defined as >50 % reduction in the serum monoclonal protein, while hematologic complete response as defined by disappearance of the monoclonal protein was rare. In an attempt to reduce toxicity, high-dose dexamethasone was introduced. It was found to have some activity against AL. In a phase II trial, the median overall survival was 13.8 months [31]. The response was minor but was felt to be potentially beneficial if combined with other therapies. A median survival of 31 months was reported in patients treated with high-dose dexamethasone and undergone maintenance therapy with dexamethasone and alpha interferon [32].

The first therapy that significantly improved patient survival was high-dose melphalan followed by autologous stem cell transplantation (HDM–SCT) [33]. HDM–SCT was capable of achieving hematologic complete response at a high rate. In a study of 312 patients, a complete response was achieved in 40 % of the patients. In another large series of 270 patients, the partial response rate was 71 % with a complete response rate of 33 %. [34]. These high hematologic response rates helped extend the median overall survival to more than 4.6 years, and responders enjoy an even longer survival [33]. The factors associated with survival were the depth of the hematologic response and the severity of cardiac involvement at baseline. Improvement in proteinuria was

noted in patients particularly those who had hematologic complete response. Of the patients with renal involvement, 63 % had a renal response after achieving a hematologic complete response. In the patients without complete response, the renal response rate of 11 %. Overall, 31.6 % of the patients with renal involvement had a renal response.

Despite its advantages HDM–SCT does have one major drawback, treatment-related mortality (TRM). This is defined as mortality within 100 days of initiating treatment. TRM can be as high as 40 % in some centers, but it is ~10 % at major amyloidosis centers [35, 36]. Patients with severe cardiac involvement, multiorgan involvement, or advanced age are at the highest risk. Unfortunately, organ involvement may be hard to define, and cardiac assessment based on echocardiography tends to be operator dependent. A risk assessment scoring system using cardiac biomarkers troponin T (cTnT) and N-terminal pro-brain natriuretic peptide (NT-pro-BNP) developed by the Mayo Clinic has been found to accurately predict TRM [37]. This significantly simplified the risk assessment since these biomarkers are easy to obtain and measure. They also would not be influenced by the operator. The use of this scoring system has been credited with lowering the TRM down to 8–9 % in some centers [38].

In early 2000, melphalan and dexamethasone (MDex) was found to be an effective treatment for AL. In a small study of 46 patients not eligible for HDM–SCT, 66 % achieved a hematologic response with 33 % complete response [39]. Long-term follow-up of these patients revealed a median overall survival of 5.1 years [40]. Renal response was noted in 48.3 % of the patients with renal involvement. Median time to hematologic response was 4.5 months. This is a concern since disease progression can occur before the therapeutic effects take place. To evaluate its efficacy against HDM–SCT, a randomized trial was conducted on 100 patients. This study found the median survival of MDex-treated patients (56.9 months) was longer than that of HDM–SCT-treated patients (22.2 months, $p=0.04$) [41]. However, critics of the study pointed out that the HDM–SCT had extraordinary high early mortality. Ten patients died prior to HDM–SCT, and another 24 % of the 37 patients who underwent the procedure died within the first 100 days. Together 19 of the 50 patients randomized to HDM–SCT died within the first 130 days. Compare with the MDex group where seven patients died within the first 130 days with only two dying prior to therapy. The difference in mortality was most profound in patients considered high risk by the Mayo Clinic criteria ($p<0.0001$). Overall survival was similar in patients considered low risk regardless of treatment ($p=0.12$). The impact of the early mortality was demonstrated in the landmark analysis which found no differences in survival between the two groups after 6 months, $p=0.38$. These results highlight the importance of patient selection when considering aggressive treatment in these patients. In one study, the TRM was reduced with 50 % with patient selection alone [42]. Thus HDM–SCT may still be useful in the treatment of AL, but its use should be limited to low-risk individuals [38].

Recently, two new classes of drugs have demonstrated efficacy against multiple myeloma and have made their way to AL therapy. The first are the immunomodulatory drugs (IMiDs) represented by thalidomide and lenalidomide. The other is bortezomib, a proteosome inhibitor. These novel agents have shown remarkable activity against multiple myeloma especially when used in combination with other agents [43]. Lenalidomide and dexamethasone produced an overall hematologic response rate of 67 % with a complete response rate of 29 % in a small study of 34 patients [44]. Forty-one percent of the patients with renal involvement had a renal response. Because lenalidomide is renally cleared, dosage adjustment was necessary. Significant treatment-related toxicities were noted with the usual dose of 25 mg a day. Most of the toxicities improved when the dose was reduced to 15 mg a day. Worsening of renal function was also noted in 59 % of the patients. Majority of these patients had renal involvement from AL. Serum creatinine increased to >2.0 mg/dL in 38 % of the patients. Thalidomide had been studied in combination with cyclophosphamide and dexamethasone. In this study, patients with New York Heart Association class II or higher or patients with significant fluid retention were started on a lower dose of thalidomide (50 mg a day vs. 100 mg a day) and dexamethasone (20 mg a day for 4 days vs. 40 mg a day for 4 days) [45]. Hematologic response rates were similar between the dosing regimens, and a hematologic response by free light chain criteria was achieved in 72 % of the patients with 32 % complete response. Overall survival after starting treatment was 41 months but had not been reached when calculated from diagnosis since majority of the patients had had prior treatments. Toxicity was significant. In the study, 40 % experience fatigue or somnolence and 21 % had worsening fluid retention or congestive heart failure. The combination of bortezomib and dexamethasone had been reported in small study with 18 patients from a single center [46]. Of the 16 evaluable patients, 94 % achieved a hematologic response with 44 % complete response. Renal response was noted in 14 % of patients with renal involvement. The major toxicities were thrombocytopenia, fatigue, neurotoxicity, and orthostatic hypotension. Two multicenter retrospective studies had been published. In one study of 26 patients, overall hematologic response rate was 54 % with complete response rate of 31 %. Organ improvement was noted in 12 %, stabilization in 76 %, and progression in 12 %. Thrombocytopenia and hyponatremia were reported as grades 3 and 4 toxicity, and grade 2 neurotoxicity was reported in 42 %. In the other with 94 patients, the hematologic response rate was 71 % with 25 % in complete response. Organ response was noted in

29 % of patients with heart involvement and 19 % of patients with kidney involvement. Hematologic response and reduction in NT-pro-GNP were independently associated with better survival [47]. In all of the reports involving therapies with novel agents, the follow-ups were relatively short. Longer follow-ups are needed in order to fully evaluate the true effectiveness of these therapies.

While it is important to achieve hematologic response in AL, achievement of organ response is the ultimate goal. Much of this has to do with the differences between multiple myeloma and AL. First, the majority of patients with AL have only a low-grade plasma-cell dyscrasia. Few present or will ever progress to multiple myeloma [48]. Therefore, the tumor burden is not the problem but rather their byproduct, the monoclonal light chains. Second, patients with AL do not die from bone marrow failure as in multiple myeloma; they died of progressive organ failure which is the result of the amyloidogenic light chains. Obviously, eradicating the monoclonal light chains would be ideal but is not always possible. However, it is important to recognize that organ response can occur in the absence of complete hematologic response, while in some cases even a hematologic complete response does not guarantee organ response [49]. Therefore, it is important to measure the organ response along with hematologic response when making decision on further treatment. One of the easiest organ responses to measure is renal response defined as >50 % reduction (minimal of 0.5 g/day) in proteinuria with <25 % decline in renal function [20]. It has been reported in up to 60 % of the patients after HDM–SCT [49]. Patients who achieved renal response have a significant survival advantage over those who do not. In a study of 122 AL patients with renal involvement who had a minimum follow-up of 12 months after HDM–SCT, hematologic response was noted in 72.1 % and renal response in 43.4 % [50]. Hematologic response was noted in 96.2 % of the patients with renal response. The median survival had not been reached in patients with a both a hematologic and renal response. Patients who had either a renal or hematologic response had a median survival of 66 months vs. nonresponders who had a median survival of 4.7 months (Fig. 21.5). Renal response was noted after a median of 10–12 months, and complete resolution of proteinuria was seen at a median of 24 months. Independent features that predicted a renal response after HDM–SCT were lower proteinuria and lower cardiac troponin T.

Kidney transplantation had been successfully performed in patients with AL. Unfortunately, the early experience was discouraging due to high mortality rates [51]. Patients were often dying from cardiac complications, progression of disease, and infection. One study of patients from 1987 to 2008 found 21 patients who had undergone kidney transplantation. The status of their AL was not reported. Median follow-up was 50 months and the median estimated survival

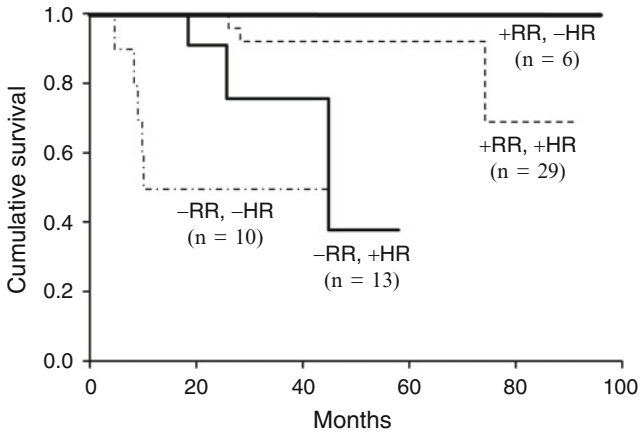

Fig. 21.5 Cumulative overall survival of patients with AL amyloidosis after autologous stem cell transplantation. Survival was calculated from day of stem cell transplantation. *HR* hematologic response defined by ≥50 % reduction in the M protein. *RR* renal response defined by ≥50 % reduction in proteinuria with <25 % reduction in renal function

was 89 months. Nine patients had died, with two from progression of their extrarenal amyloidosis, four from infection, one from gastrointestinal bleed, and two from unknown causes [26]. However, as treatment of AL improved, so did the outcomes after kidney transplantation. In one study, 15 patients with ESRD underwent HDM–SCT. The median survival for these patients was 25 months [52]. Three patients eventually received kidney transplantation with survival of 5.3–6.0 years. An alternative approach was to perform kidney transplantation first followed by HDM–SCT. The Mayo Clinic reported eight patients who underwent kidney transplantation prior to HDM–SCT. Two of the patient died prior to HDM–SCT, but the other six successfully underwent HDM–SCT. Median survival had not been reached after a median follow-up of 41 months. Recurrence occurred in one patient whose HDM–SCT was delayed for more than 3 years after kidney transplantation. This patient was able to achieve a hematologic complete response and the allograft remains in good function. Despite the immense immunosuppression of HDM–SCT, acute rejection can occur. One patient developed steroid-resistant cellular rejection immediate after leukocyte engraftment that required antithymocyte globulin. Patients can also receive kidney transplantation after achieving hematologic response without HDM–SCT. Neither patient nor allograft survival was different from patients who received HDM–SCT [53]. One of five patients developed recurrence in the allograft and was successful treated with additional AL therapy. With 30–40 % of the patients developing ESRD and the poor survival of patients on dialysis, kidney transplantation is an attractive alternative. It should be considered in patients who have minimal cardiac involvement who had achieved a hematologic complete response.

AA Amyloid

The fibrils in AA are derived from serum amyloid A (SAA) protein, an acute-phase reactant. It is synthesized by hepatocytes under the control of proinflammatory cytokines [54]. Elevated SAA levels can be seen in infectious, inflammatory, and malignant conditions; however, in order for amyloid formation to occur, the overproduction of SAA needs to be sustained over time. Common conditions that cause AA include chronic inflammatory arthritis such as rheumatoid arthritis, juvenile idiopathic arthritis, and ankylosing spondylitis. It can also be the result of chronic infections. Examples include bronchiectasis, osteomyelitis, infected pressure sores, or urinary tract infection in paraplegics. Malignant conditions such as lymphoma, mesothelioma, and Castleman's disease have also been associated with AA. Finally, a hereditary form is seen in patients with periodic fever syndromes [55].

Four types of periodic fever syndromes have been identified to cause AA [55]. The most common is the Familial Mediterranean Fever (FMF) which is an autosomal recessive disease resulting from the mutation in the MEFV gene on chromosome 16p. As its name implies, it affects people around the Mediterranean Sea, most commonly Armenians, Sephardic Jews, Greeks, Turks, and Arabs. It is characterized by periodic attacks of abdominal pain, serositis, arthritis, and fever. TNF-receptor-associated periodic fever syndrome (TRAPS) is the second most common and is the result of mutations in the TNFRSF1A gene which regulates the type 1 tumor necrosis factor (TNF) receptor. Over 40 mutations have been identified and the disease has an autosomal dominant pattern. It was first described in a boy with Scottish–Irish ancestry and was initially named Familial Hibernian Fever. Later studies reveal it is well distributed in many ethnicity and countries of origin. Its symptoms include but are not limited to recurrent abdominal pain, migratory myalgia, rash, periorbital edema, and testicular pain. Muckle–Wells syndrome is one of the cryopyrinopathies that includes familial cold autoinflammatory syndrome and multisystem inflammatory disease. They are characterized by urticaria like rash, arthralgia which is the result of mutations in the CIAS1 gene. Muckle–Wells syndrome is characterized by hearing loss and is the only cryopyrinopathy that is associated with amyloidosis. Most have an autosomal-dominant pattern of inheritance. Hyper IgD syndrome is an autosomal recessive disease involving the mutation of the mevalonate kinase (MVK) gene. Its distinguishing features are high levels of IgD and attacks triggered by vaccination. It is prominent in the Netherlands.

The most common presentation in AA is renal dysfunction [54]. In a study of 374 patients, 97 % presented with either proteinuria of >500 mg/day, serum creatinine >1.5 mg/dL, or both. Median proteinuria was 3.9 g/day and 12 % excretes >10 g/day. Median creatinine was 1.2 mg/day and 75 % had a serum creatinine of <3 mg/dL at diagnosis. ESRD was present in 11 % of the patients with another 33 % developing it during the course of their disease. Median time to ESRD was 256 months. Risk factors for ESRD include presence of Crohn's disease, chronic infection, hepatic amyloid deposits, and poor renal function at baseline. Other clinical features include hepatomegaly which was noted in 9 % of the patients. However, if hepatic involvement was assessed by SAP scintigraphy, then the number increased to 23 %. Cardiac involvement was only noted in 2 % of the patients by echocardiogram and only 1 % developed clinically significant cardiac failure. This may explain the long median survival of 133 months. Factors associated with poor survival include elevated SAA concentration, older age, and ESRD. In this study, no patient developed autonomic neuropathy. Adrenal amyloid deposits were found on SAP scintigraphy on 41 % of the patients, but only five required adrenocorticoid replacement.

Histologically, the amyloid deposition pattern in AA is indistinguishable from other types of renal amyloidosis [3]. One feature that is particular but not unique to AA is the formation of crescents [56]. This occurs most commonly in patients whose AA is secondary to rheumatoid arthritis. It is also more common in female than male. The coexistence of crescents on the kidney biopsy is often associated with rapidly progressive glomerulonephritis and rapid loss in renal function. Corticosteroids have been found to be effective at stabilizing and reversing the rapid loss in renal function [57].

Treatment of AA involves eliminating or controlling the underlying disease [54]. Complete remission of the renal amyloidosis has been reported after resection of the Castleman's disease, treatment of the periodic fever syndrome, or eradication of the infection [54, 58]. Steroids and TNF inhibitors are effective treatment for TRAPS [55]. Anakinra, an interleukin-1 receptor antagonist, is effective in Muckle–Wells and Hyper IgD syndromes [59]. Statins (HMG Co-A inhibitor) inhibit the production of mevalonate and have been found to be beneficial in Hyper IgD syndrome [60]. Colchicine is effective at preventing or reducing the symptoms of FMF in approximately 90 % of patients [59]. However, its benefits are limited once amyloidosis has developed. Eprodisate is a negatively charged, low molecular weight sulfonated molecule which can inhibit the interactions between amyloidogenic proteins in animal studies. A randomized placebo control trial was performed in patients with AA [61]. The primary endpoint of the study was a composite assessment of renal function or death. Secondary endpoints included slope of creatinine clearance, change in proteinuria, resolution of diarrhea, and change in amyloid content of abdominal fat. In this study, patients treated with eprodisate were less likely to have worsening renal function (27 %) than the placebo group (40 %, $p = 0.06$). There was no

significant difference in death rate between the two groups. Treatment with eprodisate also significantly decreased the slope of creatinine clearance (−10.9 ± 5.1 mg/min/1.73 m^2) vs. placebo (−15.6 ± 4.0 mL/min/1.73 m^2, $p = 0.02$). There were no significant differences in the change in proteinuria, diarrhea, and amyloid content in abdominal fat. A second randomized placebo-controlled trial is current underway to further clarify the results.

End-stage renal disease is a significant risk factor for mortality in AA [62]. Survival rates on dialysis are 82 %, 46 %, and 37 % at 1, 2, and 3 years. At autopsy, 10 of 13 patients were noted to have amyloid infiltration of the heart, much higher than suggested in the non ESRD AA patients [63]. Whether this represents advance-stage disease in the ESRD population or acceleration of amyloid deposition in the heart from ESRD remains undetermined. Kidney transplantation is an option for these patients. However, concerns had been raised about high mortality after kidney transplantation. Some studies suggested an increase in early mortality with 1-year patient survival of 69–79 % comparing with 97–100 % in the non-amyloidosis patients [64, 65]. Five-year survival was 52–69 % vs. 87–100 %. The causes of death were sepsis and cardiac related. Others reported no differences in the 1-year (93 % vs. 94 %) or 5-year (89 % vs. 90 %) survival rate between AA patients and non-amyloidosis patients, respectively [66]. This study is more recent and may reflect better management of immunosuppression. Recurrence of AA has been reported in the renal allograft. The true prevalence is unknown and may depend on the underlying disease. In FMF, colchicine has been found to be effective at preventing recurrence [67]. Patients who received 1.5 mg/day of colchicine had less proteinuria than patients receiving 0.5 mg/day suggesting a dose effect. Finally, recently anakinra was found to be effective in a patient who was resistant to high-dose colchicine [68].

Hereditary Renal Amyloidoses

Fibrinogen Alpha A[Alpha]-Chain Amyloidosis (AFib)

First sequenced in 1993, AFib is one the most common types of hereditary systemic amyloidosis involving the kidney in Northern Europe [69]. Families with AFib have also been identified in Peru, Mexico, and Africa. The disease is inherited via an autosomal-dominant pattern. Six mutations have been identified in the fibrinogen Aα-chain gene that are associated with amyloid formation [70]. Initially, AFib was thought to be one of the hereditary nonneuropathic systemic amyloidosis, but later descriptions discovered peripheral neuropathy was possible with certain mutations. Renal manifestations had been universal in this form of amyloidosis. In a study of 71 patients from multiple countries of origin, proteinuria or hypertension was documented in 72 % of the patients at presentation and renal insufficiency is present in 54 % [71]. Amyloid deposits had been found by radiolabeled SAP scintigraphy in the adrenal gland and spleen. Whether cardiac involvement is a feature in AFib is controversial. In one report, none of the 63 patients who underwent radiolabeled SAP scan demonstrated cardiac deposition or echocardiographic features of amyloid heart disease [71]. However, a smaller series of 22 patients found 52 % of the patients had echocardiographic evidence for amyloid infiltration [70]. In this study, three of the four patients who underwent endomyocardial biopsy were found to have a substantial amount of amyloid deposition. The deposits were also found on atheromatous plagues, but the significance of this was unclear. Median age of presentation was 58 years with a range of 33–83 years [71]. ESRD developed in 62 % and the median time to progression to ESRD of 4.6 years. Median survival from diagnosis was 10.9 years.

Recurrence is common in patients with AFib who received a kidney transplant alone. In a series of ten patients with 12 allografts, three grafts failed immediately due to technical problems [71]. In the nine remaining grafts, four had documented recurrence of which three subsequently failed at a median of 6.7 years. Only 1 graft remained functioning 12.2 years after transplantation despite having recurrence documented 7 years after transplant. Faster graft loss had been reported [72]. While fibrinogen synthesis has been identified in other cell types, in human, it appears to be exclusively produced in the liver [73]. As a result, orthotopic liver transplantation may completely eliminate the production of the mutant fibrinogen Aα-chain and prevent recurrence and disease progression. Although the data is limited, combined liver–kidney transplantation appeared to be effective at preventing recurrence in the kidney allograft. Results from the two largest series revealed none of the 12 patients who survived the perioperative period had a documented recurrence [70, 71]. On the other hand, regression of visceral amyloid deposits was noted on serial radiolabeled SAP imaging. In addition, patients had improvement of their symptoms from GI immobility improved and native kidney function. However, perioperative mortality was higher than non-amyloidosis patients especially those with ESRD and were on long-term dialysis prior to transplantation. Because of the higher morbidity and mortality, recommendation of preemptive liver transplantation in these patients remains controversial.

Lysozyme Amyloidosis (ALys)

First described in 1993, ALys is one of the nonneuropathic (Ostertag) forms of systemic amyloidosis [74]. Lysozyme is a ubiquitous bacteriolytic enzyme which is produced by the

macrophages, gastrointestinal cells, and hepatocytes. Currently, four mutations have been identified to be involved in amyloid formation. All of the mutations are located on exon 2. They are I56T, F57I, W64R, and D67H. In addition, a case of compound heterozygosity has been reported involving exon 2 (T70N) and exon 4 (W112R) [75]. Ethnic origins of families with ALys mutations include English, Italian, French, and Scandinavian descent. Diagnosis can be made by immunohistochemistry, but genetic confirmation is recommended.

The main feature of patients with ALys is GI manifestations. These include abdominal pain/discomfort to malabsorption syndrome [76]. Bleeding of the GI tract is also a common feature. One of the more serious complications is spontaneous rupture of the liver which has been reported in one family involving the mother and daughter [77]. Renal manifestations are the next most common. The presentation ranges from hypertension, proteinuria, to nephritic syndrome and ESRD. Time to ESRD is also quite variable ranging from 3 months to 18 years. Kidney transplantation has been successfully performed, but recurrence has also been reported. Unlike in AFib where liver is the exclusive source of the mutant protein, the mutant lysozyme is produced by cells in addition to hepatocytes. Thus, liver transplantation will not impede the production of the mutant protein and is not indicated in these patients.

AApoAI and AApoAII Amyloidoses

Apolipoproteins (Apo) are cofactor for lecithin cholesterol acyltransferase and are components of high-density lipoprotein (HDL). They are produced by cells of the liver and intestine and catabolized in the liver and kidney. AApoAI and AApoAII are part of the hereditary nonneuropathic systemic amyloidosis described by Ostertag in 1932.

To date, 13 mutations have been identified to cause amyloid formation in AApoAI and 4 in AApoAII [78, 79]. Renal dysfunction is the most common feature of both AApoAI and AApoAII, but other visceral organs can be involved including liver, heart, spleen, gonads, and skin [78–80]. Involvement of peripheral nerves is not a feature. Visual impairment has been reported in patients with severe hepatic involvement. The typical age of presentation for AApoAI is between 18 and 55 years of age, but this can vary depending on the mutation. Patients with Leu75Pro tend to present late in their seventh decade and can live into their 90s. Levels of HDL, ApoAI, and ApoAII are not elevated in these patients.

No treatment is currently available for either type of amyloidosis. Organ transplantation has been successfully performed. In one study, eight of ten patients with AApoAI who received a kidney transplantation remain alive 4–28 years after transplant [80]. Two patients died at 2 months (CMV) and 13 years. Recurrence was noted in five patients, but only one graft loss was reported 6.5 years after the recurrence was documented and 25 years after kidney transplantation. The other grafts remained functional at 5.3–8 years after recurrence was documented. Two patients underwent liver transplantation. Both patients experienced regression of their visceral amyloid deposits by SAP scintigraphy. Their overall well-being also improved and to date no sign of recurrence. This is interesting since liver is not the only source of ApoAI.

Latest Amyloidosis

LECT2 Amyloidosis (ALECT2)

ALECT2 is the newest member of amyloidosis family. The native protein is leukocyte chemotactic factor 2 (LECT2) [6]. Commercial immunohistochemistry testing is currently not available so diagnosis must be made by tandem mass spectrometry. Only a dozen cases have been described in the literature so far. In one kidney biopsy series where 285 of 21,598 biopsies were congophilic, 31 could not be typed by immunohistochemistry [81]. Of these, seven were later identified to be ALECT2. It was the third more common amyloid (2.5 %) in this series behind AL and AA. In the largest series to date, the mean age of presentation was 67 years [18]. Seven of ten patients were Mexican or Mexican-American. In the kidney, ALECT2 is highly congophilic and is deposited extensively in all compartments. Renal dysfunction appears to be the main clinical feature although ALECT2 has been identified in liver, spleen, colon, and adrenal gland. The significance of the extrarenal deposition is unknown. LECT2 level measured in limited number of patients has been either normal or undetectable suggesting systemic overproduction is not part of the pathophysiology. Systemic infection or inflammation is also not identified in these patients. Genetic analysis has not revealed a mutation, but so far, all patients tested are homozygous for the G allele of the LECT2 gene. Further genetic analysis however is needed before any conclusion can be made. No treatment is currently available for ALECT2.

Summary

Despite the similarity in the physical characteristics of the fibrils, amyloidosis is a tremendously heterogeneous group of diseases. Important differences are noted ranging from amyloidogenesis to organ tropism. These patterns reflect the origin of the amyloidogenic protein and provide insights into the pathogenesis. Tremendous advances have been made in the field of diagnostic and typing of amyloid fibrils. Advances have also being made in the treatment of amyloidosis which has resulted in significant increases in the survival of these patients.

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Glomerular Disease in Pregnancy

22

Andrew Smyth and Vesna D. Garovic

Introduction

Chronic renal disease affects approximately 4 % of women of childbearing age [1]. While diabetic nephropathy remains the most common etiology for glomerular disease in pregnant women, a variety of other primary renal diseases and those secondary to systemic diseases may occur.

In this chapter, we discuss the physiological changes of pregnancy that may affect glomerular disease presentation, activity, and diagnosis; specific glomerular diseases, both primary and secondary to systemic diseases, in the context of pregnancy; fetal and maternal complications, diagnosis, and differential diagnosis; and long-term effects. With respect to treatment, we will focus on medications that can be used to treat maternal disease, which are relatively safe with respect to fetal intrauterine exposure. The optimal approaches for the specific glomerular disease entities in nonpregnant, i.e., general population, patients will be addressed in the respective chapters. Also, some disease entities (such as Alport's syndrome and hereditary nephritides) are not discussed due to limited evidence, confined to case reports and small series. For these patients, optimal management should focus on the general principles of care of pregnant patients with glomerular disease, namely, preconception counseling with an emphasis on disease activity and baseline renal function, and close monitoring during pregnancy using a multidisciplinary team approach.

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Physiological Changes of Pregnancy in the Context of Glomerular Disease

Pregnancy is associated with important changes in renal physiology [2]. The combination of progesterone-induced ureteral smooth muscle relaxation and ureteral compression secondary to the enlarging fetus results in dilatation of the urinary collecting system, creating a physiological hydronephrosis, which is more prominent on the right side. The glomerular filtration rate (GFR) increases by up to 50 % above baseline levels, primarily due to elevations in cardiac output and renal blood flow [3]. Consequently, normal pregnancy is associated with a decrease in the serum creatinine concentration by an average of 0.4 mg/dL. Blood urea nitrogen levels follow the same downward trend, ultimately averaging at 8–10 mg/dL. Therefore, a serum creatinine of 0.9 mg/dL, which is within the normal range for a nonpregnant woman, may be indicative of underlying renal disease during pregnancy.

Pregnancy also results in a physiological increase in protein excretion due to a combination of an increased GFR and increased permeability of the glomerular basement membrane [4]. As a result, the normal urinary total protein excretion rate in pregnancy is elevated compared to the nonpregnant state, with an acceptable upper limit of normal of 300 mg/day [5]. This physiological increase in protein excretion during pregnancy is further exaggerated in patients with proteinuric renal disease, who commonly experience worsening of proteinuria toward the end of their pregnancies [6]. Worsening proteinuria coupled with either de novo or worsening of preexisting hypertension control may mimic either a flare of the underlying glomerular disease or preeclampsia. Several clinical and laboratory features may facilitate making the correct diagnosis and a renal biopsy should be considered when a noninvasive evaluation is nondiagnostic.

Another major adaptation that occurs during normal pregnancy is the modulation of both innate and adaptive immunity, with the goal of establishing maternal tolerance to a semi-allogeneic fetus expressing both maternal and paternal

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antigens. Several immunologic changes occur, most notably an increase in the number of CD4+/CD25+ regulatory cells (Tregs), which have a potent immunosuppressive effect, along with a shift from a Th1 cell-mediated to a Th2-antibody-mediated immune response, also referred to as Th2 polarization. The latter change, with its resulting relative suppression of Th1-mediated immunity, contributes to maternal tolerance of the fetus [7, 8]. A shift toward a Th2 response may contribute to a greater risk for flare in Th2-mediated diseases, such as systemic lupus erythematosus (SLE), during pregnancy [9]. Also, relative to normotensive pregnancies, the number of Tregs in patients with preeclampsia is decreased [8]. As SLE patients have fewer Tregs, which are also functionally defective, they may be at a greater overall risk for preeclampsia, which has been well documented for this patient population, as further reviewed in the discussion that follows.

Lupus Nephritis

SLE is a multisystem autoimmune connective tissue disorder that predominantly affects women of childbearing age. Fertility is preserved [10] in the absence of antiphospholipid syndrome, advanced renal insufficiency (i.e., creatinine ≥3.0 mg/dL), and/or previous treatment with cytotoxic alkylating agents. Although initial reports of pregnancy in these patients indicated a poor prognosis, more recent data show improved outcomes [11].

The presence of renal disease (lupus nephritis, LN) is one of the American College of Rheumatology (ACR) criteria [12] used to classify SLE, and up to 75 % of patients have clinically evident renal disease [13]. Pregnancy may have adverse short- and long-term effects on kidney function, including progression to end-stage renal disease (ESRD). These risks are determined, in part, by the severity of the underlying renal disease, with a particularly high risk with a serum creatinine ≥1.4 mg/dL.

Maternal and Fetal Complications

The presence of LN is associated with high risks for maternal and fetal complications, including spontaneous abortion, premature delivery, intrauterine growth retardation, and superimposed preeclampsia. A US study of patients with LN reported an increased risk of maternal mortality (odds ratio [OR] 17.8, 95 % confidence interval [CI] 7.2–44.0), caesarean section (OR 1.7, 95 % CI 1.6–1.9), preterm labor (OR 2.4, 95 % CI 2.1–2.6), and preeclampsia (OR 3.0, 95 % CI 2.7–3.3) [14]. Pregnancies in patients with stable renal disease are more likely to have good outcomes, emphasizing the importance of pregnancy planning, with conception occurring after a minimum of 6 months, but ideally, after 12–18 months of disease quiescence [15]. The rate of fetal loss is >50 % in the setting of active renal disease [16], and even higher if previous pregnancies were affected by complications [17].

Preeclampsia and HELLP syndrome (*h*emolysis, *e*levated *l*iver enzymes and *l*ow *p*latelet count) may occur in LN pregnancies, and due to their often similar presenting features, it may be difficult to distinguish them from a flare or new onset LN during pregnancy (Table 22.1). Preeclampsia is associated with negative or unchanged lupus serologies, the absence of extrarenal manifestations of SLE, proteinuria (in the absence of active urine sediment), and elevated serum uric acid levels. Decreased urinary output, elevations in serum creatinine, low serum complement levels, and increased titers of anti-DNA antibodies are typical of LN.

The ratio of complement activity (CH50) to complement split products (Ba) is preferred to measuring serum complement when differentiating a lupus flare from preeclampsia. In patients with a lupus flare, low CH50 levels are associated with elevations of complement split products, such as Ba, thus resulting in a lower ratio of CH50/Ba than in non-SLE patients with preeclampsia [18]. Newer biomarkers, including anti-angiogenic markers, such as soluble vascular endothelial receptor, 1 (sFlt-1) [19], may also be useful in making the

Table 22.1 Differential diagnosis of preeclampsia, HELLP syndrome and active lupus nephritis (either a flare or new onset)

	Preeclampsia	HELLP syndrome	Active lupus nephritis
Timing in pregnancy (weeks gestation)	After 20 weeks	After 20 weeks	Any gestational age
Complement (C3, C4)	Normal	Normal	May be decreased
Thrombocytopenia and neutropenia	Absent	Present	May be present
Active urine sediment or other organ involvement	Absent	Absent	Commonly present
Anti-dsDNA or Anti-C1q	Absent	Absent	May be present
Abnormal liver function tests	Absent	Present	Absent
Serum uric acid	Increased	Increased	Normal[a]
Hypertension (>140/90 mmHg)	Present	Absent in 10–15 %	Variable
Serum creatinine >1.2 mg/dL	Typically absent	<10 % of patients	Commonly present

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Anti-dsDNA anti-double-stranded DNA antibodies

[a]May be elevated with reduced GFR

Table 22.2 Laboratory testing of patients with lupus nephritis during pregnancy

Timing	Suggested laboratory investigations
Preconception counseling and/or first prenatal visit	• Urinalysis • Determination of proteinuria (optimally 24-h urine or protein/creatinine ratio) • Complete blood count • Serum creatinine • Antiphospholipid antibodies • Anti-SSA/Ro and Anti-SSB (La) antibodies: if positive, weekly fetal heart rate evaluation from weeks 16–24 and every other week from weeks 24–32 • Anti-double-stranded DNA antibodies • Complement studies • Liver function tests
Every month	• Urinalysis and measurement of proteinuria • Serum creatinine • If any tests are abnormal, obtain lupus serologies, complement studies, and consider renal biopsy before 32 weeks
Every trimester[a]	• Complete blood count • Anti-double-stranded DNA antibody • Complement studies • Liver function tests (for patients on azathioprine)

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[a]Additional testing may be needed depending on the disease activity and pregnancy course. For example, when initially negative, antiphospholipid antibodies should be retested in patients who develop deep venous thrombosis during pregnancy, while anti-SSA/Ro and anti-SSB/La antibodies should be rechecked, if fetal heart rate and/or echocardiogram abnormalities are present

distinction between a lupus flare and preeclampsia in the future. It is vital to distinguish LN (which will require immunosuppression) from preeclampsia, which is best managed with delivery.

If required, a renal biopsy can be performed safely in patients with optimal control of hypertension and stable coagulation parameters [20]. Of note, while early studies have reported high complication rates with renal biopsies in pregnancy, including perirenal hematoma (4.4 %) and gross hematuria (16.7 %) [21], more recent reports indicate complication rates that are similar to those in nonpregnant women [22, 23]. After 32 weeks of gestation, delivering the baby prior to contemplating a renal biopsy should be considered and discussed with obstetrical and neonatal providers.

LN Flare in Pregnancy

Studies to date have reported conflicting data [24–26] with respect to the likelihood of developing an LN flare during pregnancy. The most likely reason for these differing results is inconsistency in selecting appropriate controls, with some studies using nonpregnant controls and others using patients' own nonpregnant disease courses [27–29]. The studies based on the latter type of design consistently have reported an increased likelihood of LN flare during pregnancy, with the risk approaching 70 % [30]. A meta-analysis of 37 studies of SLE pregnancies reported a lupus flare in approximately one in four pregnancies [31]. As activation of LN may lead to adverse maternal and fetal outcomes, close monitoring, with monthly assessments of disease activity in pregnant LN patients, is indicated (Table 22.2, Fig. 22.1). This approach may facilitate early detection and timely treatment of an LN flare, if and when it occurs.

Maternal Mortality

Maternal deaths are relatively rare events and reporting bias makes the true rate difficult to determine. We recently reviewed published evidence of pregnancy-related maternal deaths in women with SLE and LN; all 17 maternal deaths occurred in those with active disease, with disease activity/complications and opportunistic infections being the two major causes [32]. These data further support current recommendations for the avoidance of pregnancy until SLE manifestations are quiescent, with a renal remission of ≥6 months. This may decrease the complication rates due to disease activity and reduce the use of aggressive immunosuppression and its related complications, including maternal death due to opportunistic infections. The presented evidence further supports the importance of a planned pregnancy, expert monitoring, and judicious use of immunosuppressive therapies.

Preconception Counseling and Antepartum Monitoring

Prior to attempting to conceive, women with SLE/LN should undergo a detailed evaluation of their disease activity, and renal function (Table 22.2), and their immunosuppressive medications should be changed to include those that are most appropriate for pregnancy (Table 22.3). The need to modify and/or

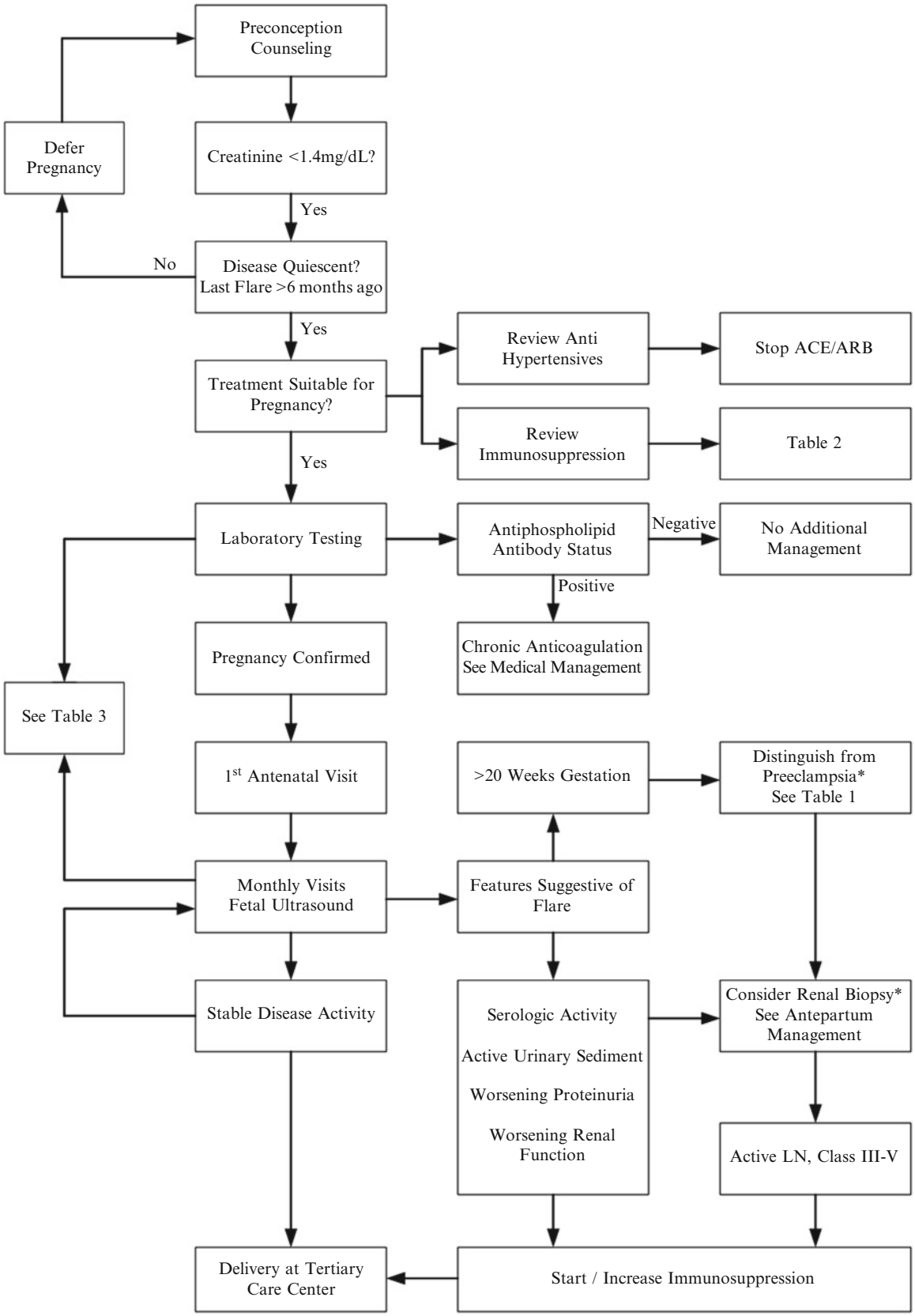

Fig. 22.1 Flow chart for management of active lupus nephritis during pregnancy. Reprinted with permission from Stanhope TJ, White WM, Moder KM, Smyth A, Garovic VD. Obstetric Nephrology: Lupus and Lupus Nephritis in Pregnancy. Clinical Journal of the American Society of Nephrology, 2012;7(12):2089–99

Table 22.3 Immunosuppressive medications and pregnancy

	Comments	FDA class[a]	Breast-feeding[b]
Anti-inflammatory drugs			
Nonsteroidal anti-inflammatory drugs (NSAID)	Avoidance after 28 weeks gestation because of the effects of NSAID-related prostaglandin inhibition on the fetal cardiovascular system (closure of ductus arteriosus)	C	Usually compatible
Immunosuppressive drugs			
Corticosteroids	Risks of use often outweighed by risk of underlying disease. Potential risks for orofacial clefts (3/1,000 births) and premature birth	C	Usually compatible
Hydroxychloroquine	Considered safe in pregnancy at 200–400 mg/day. Discontinuation during pregnancy associated with increased risk of lupus flare. May use for maintenance or mild flares	Not assigned	Usually compatible
Cyclosporine	Can be maintained in pregnancy at lowest effective dose. No significant increase in rate of congenital malformations	C	Not recommended
Tacrolimus	Can be maintained in pregnancy at lowest effective dose. Potential risks of neonatal hyperkalemia and renal dysfunction	C	Not recommended
Rituximab	Limited safety data. May alter fetal and neonatal B-cell development	C	Not recommended
IVIG (gamma globulin)	Data are lacking, but may be helpful for lupus nephritis flare refractory to medical therapy	C	Compatible
Azathioprine	May use for flare during pregnancy. Consider as alternative to mycophenolate. Avoid doses >1.5–2 mg/kg/day due to risk of suppressed neonatal hematopoiesis	D	Not recommended
Mycophenolate mofetil	Contraindicated during pregnancy due to teratogenicity	D	Not recommended
Cyclophosphamide	Useful when maternal disease is life threatening. High risk of fetal loss, but less pronounced in more recent studies	D	Not recommended
Methotrexate	High risk of miscarriage and congenital abnormality. Treatment should be withdrawn 3 months before pregnancy	X	Not recommended
Antihypertensive agents			
Centrally acting agents			
Methyldopa	Recommended for use, despite the risks of neuro-depression	B	Usually compatible
Clonidine	As efficacious as methyldopa, but has unproven safety	C	Possible milk effects
Beta-blockers			
Labetalol	Recommended for hypertensive urgency and may be associated with a risk of fetal bradycardia and neonatal hypoglycemia	C	Usually compatible
Atenolol	Contraindicated due to risk of intrauterine growth restriction	D	Not recommended
Calcium channel blockers			
Nifedipine	Recommended for use. With all calcium channel blockers, there is a risk of interactions with magnesium, resulting in profound hypotension	C	Usually compatible
Direct vasodilators			
Nitroprusside	Very effective in severe hypertension, but associated with cyanide and thiocyanate toxicity	C	Insufficient data; Possible infant risk
Hydralazine	Efficacious oral agent, but associated with maternal neuropathy, drug-induced lupus, and neonatal thrombocytopenia	C	Usually compatible
Diuretics			
Thiazides	Useful in chronic hypertension, heart failure, and renal disease and may cause volume contraction and electrolyte abnormalities	B	Possible milk effects
Spironolactone	Possible fetal antiandrogen effects	C	Usually compatible
Angiotensin-converting enzyme (ACE)-inhibitors and angiotensin II receptor blockers (ARB)	Contraindicated in pregnancy. Known fetal side effects include skull hypoplasia, anuria, renal failure, oligohydramnios, craniofacial deformation, and hypoplastic lung development	D	Possible infant risk

[a]According to the Food and Drug Administration pregnancy ratings: A—Controlled studies in women fail to demonstrate a risk to the fetus in the first trimester (and there is no evidence of a risk in later trimesters), and the possibility of fetal harm appears remote; B—Either animal-reproduction studies have not demonstrated a fetal risk, but there are no controlled studies in pregnant women, or animal-reproduction studies have shown an adverse effect (other than a decrease in fertility) that was not confirmed in controlled studies in women in the first trimester (and there is no evidence of a risk in later trimesters); C—Either studies in animals have revealed adverse effects on the fetus (teratogenic or embryocidal or other), and there are no controlled studies in women or studies in women, and animals are not available. Drugs should be given only if the potential benefit justifies the potential risk to the fetus; D—There is positive evidence of human fetal risk, but the benefits from use in pregnant women may be acceptable despite the risk (e.g., if the drug is needed in a life-threatening situation or for a serious disease for which safer drugs cannot be used or are ineffective); X—Studies in animals or human beings have demonstrated fetal abnormalities, or there is evidence of fetal risk based on human experience, or both, and the risk of the use of the drug in pregnant women clearly outweighs any possible benefit. The drug is contraindicated in women who are or may become pregnant

[b]According to either the World Health Organization and/or Thomson Lactation Ratings

discontinue immunosuppressive agents during pregnancy may contribute to an increased risk for LN flare.

Antihypertensive antihypertensive medications, angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) should be discontinued prior to pregnancy due to their known teratogenic effects [33] and replaced by a safe alternative(s) [34], such as methyldopa, labetalol, or nifedipine (Table 22.3). A baseline serology panel also should be obtained, which will facilitate the evaluation of disease activity during pregnancy.

Renal biopsy data should be reviewed during the counseling session, when available [35]. Patients with proliferative forms of LN (class III or IV) may need careful antenatal surveillance. An increased incidence of hypertension and preeclampsia has been reported in patients with class III or IV LN (37.1 %), compared with either class II or V (11.1 %) or age, parity, and duration of SLE-matched controls without renal involvement (11.6 %) [36]. During pregnancy, monthly prenatal visits, regular examinations (including serial fetal ultrasounds), and laboratory evaluations (Fig. 22.1, Table 22.2) are indicated.

Immunosuppression During Pregnancy

Immunosuppressive therapy may be required, for the first presentation of LN during the course of pregnancy, to maintain disease remission or to manage an LN flare during pregnancy. Both induction and maintenance regimens in pregnancy are based on corticosteroids, azathioprine, and calcineurin inhibitors (cyclosporine and tacrolimus) due to their acceptable safety profiles (Table 22.3), established mainly through their use in pregnant women with either inflammatory bowel disease or prior renal transplantation.

While previous studies have reported an association between corticosteroid use and orofacial clefts [37, 38], a more recent large-scale population study from Denmark did not confirm this association [39]. In addition, low-dose prednisone (5–10 mg/day) is unlikely to cause adrenal insufficiency and/or thymic hyperplasia in the fetus [40].

Azathioprine has been assigned to category "D" by the US Food and Drug Administration (FDA), mainly based on the data reporting increased risks for atrial and ventricular septal defects and preterm birth [41]. However, the results of a study of azathioprine use in pregnancies complicated by SLE reported favorable outcomes, supporting its use in pregnant patients with SLE or LN [42].

With respect to calcineurin inhibitors, cyclosporine is most commonly used due to its acceptable safety profile, which has been confirmed by both animal and human studies [43]. On the other hand, the use of tacrolimus over cyclosporine should be considered for induction, based on data suggesting that comparable rates of remission may be achieved with tacrolimus compared to cyclophosphamide [44]. While these data are insufficient to support the use of tacrolimus for induction in the general population, they may argue for its use in pregnant patients for whom mycophenolate mofetil and cyclophosphamide are contraindicated given their known teratogenic potentials [42, 43, 45, 46]. Optimally, mycophenolate mofetil should be discontinued 6 weeks prior to attempted conception [47].

In the postpartum period, caution is required, as a number of medications are secreted in breast milk and are not considered safe for infants (Table 22.3).

Antiphospholipid Antibodies/Syndrome

The presence of antiphospholipid antibodies, including lupus anticoagulant and anticardiolipin antibodies, in association with venous or arterial thromboses and/or pregnancy complications (such as recurrent miscarriages), constitute the diagnostic criteria of antiphospholipid syndrome (APS). Both the presence of antiphospholipid antibodies and APS may be seen as separate disease entities, or in association with SLE, with pregnancy complications including fetal loss, which generally occurs after 10 weeks of gestation, and an increased relative risk of preeclampsia [48, 49]. A meta-analysis reported the presence of antiphospholipid antibodies in about one quarter of SLE pregnancies [31]. Screening is recommended for the presence of these antibodies during the initial evaluation of pregnancies complicated by SLE or LN, as the risk of pregnancy loss has been correlated with the number of positive tests for the different antiphospholipid antibodies [50].

The mainstay of APS management is anticoagulation, while immunosuppression is reserved for those who, in addition to APS, have active SLE. Patients treated with warfarin before pregnancy should receive therapeutic anticoagulation, with either unfractionated heparin (UFH) or low molecular weight heparin (LMWH) while pregnant, as warfarin is contraindicated due to its teratogenic effects and the potential for life-threatening hemorrhage in the infant [51]. Pregnancy is a hypercoagulable state, and the risk for thrombotic events is further increased by the obstruction of venous return by the enlarged uterus. Therefore, for patients not treated before pregnancy, anticoagulation is indicated for all SLE patients positive for antiphospholipid antibodies and a history of thrombotic event(s), and for those without a previous history of thrombotic event(s), but meeting the obstetric criteria for APS, such as three or more pregnancy losses or a late pregnancy loss.

For women with antiphospholipid antibodies who do not meet the clinical criteria for APS, an acceptable approach is close clinical surveillance; other options include antepartum aspirin or prophylactic UFH or LMWH. Finally, even in the absence of antiphospholipid antibodies, prophylactic

anticoagulation should be considered for pregnant LN patients with nephrotic syndrome, as the hypercoagulable state inherent to nephrotic syndrome may worsen further with pregnancy, leading to an increased risk for thromboembolic complications [52].

In general, for patients requiring anticoagulation during pregnancy, heparin should be started immediately after confirmation of an intrauterine pregnancy and continued for at least 6 weeks postpartum. Specific therapeutic and prophylactic anticoagulation regimens, therapeutic goals, and monitoring strategies are available in the *American College of Chest Physicians Evidence-Based Clinical Practice Guidelines: Venous Thromboembolism, Thrombophilia, Antithrombotic therapy, and Pregnancy* [52].

Women with a history of antiphospholipid syndrome (APS) and arterial thrombotic events, stroke, in particular, should be advised against pregnancy due to the high risks for severe maternal complications, including death, which may sometimes occur despite chronic anticoagulation [53]. For these women, gestational carriers (surrogate mothers) can be considered for achieving parenting goals.

Systemic Sclerosis/Scleroderma

Scleroderma is more prevalent in women and has a mean age of onset of 40 years [54]. As a result, it is not commonly encountered in women of childbearing age. It is unclear if pregnancy increases the risk of scleroderma renal crisis, which presents with abrupt renin-mediated hypertension and renal impairment. Optimal treatment is with ACE inhibitors or ARBs [55], which poses a major challenge in pregnant patients, as these agents are generally considered to be contraindicated for use due to the risk of congenital malformations (Table 22.3). However, in the setting of life-threatening maternal disease, their use in pregnancy may be justified. Renal crisis affects 5 % of patients with systemic sclerosis and most commonly presents within the first 5 years of disease onset [56]. Therefore, women diagnosed with scleroderma should be counseled that delaying pregnancy may reduce the risk of renal crisis. Patients with CREST (calcinosis, Raynaud's, esophageal dysmotility, sclerodactyly, telangiectasia) syndrome, a limited form of scleroderma, typically do not have renal involvement and usually have good pregnancy outcomes.

IgA Nephropathy

Immunoglobulin A (IgA) nephropathy, the most prevalent primary glomerulonephritis, is most commonly diagnosed in the second and third decades and affects many women of childbearing age. It is uncertain if pregnancy adversely affects long-term renal outcomes, as most studies have included heterogenous populations and report inconsistent findings. A recent study reported that pregnancy does not affect long-term renal outcomes in patients with IgA nephropathy and near-normal kidney function [57]. Another reported that strategies resulting in the reduction of proteinuria prepregnancy were associated with preservation of postpartum renal function [58]. The risk of worsening renal disease during pregnancy is highest when the baseline GFR is <70/mL/min, and either the presence of uncontrolled hypertension or severe arteriolar and tubulointerstitial disease on renal biopsy [59].

The majority of agents used in the of management of IgA nephropathy are nonimmunosuppressive and include ACE inhibitors or ARBs, both of which are contraindicated during pregnancy (Table 22.3). Immunosuppressive agents are increasingly used for the treatment of IgA nephropathy, with clinical (hematuria, rising serum creatinine and rising proteinuria, despite anti-proteinuric therapy) and histologic evidence (necrotizing glomerular lesions) of active inflammatory changes. Most patients with mild, stable, or slowly progressive IgA nephropathy are not treated with immunosuppression. In general, ACE inhibitors should be discontinued prior to pregnancy and immunosuppressive agents should be reviewed and changed to pregnancy-safe alternatives (Table 22.3), ideally prior to conception or at the earliest indication of pregnancy in order to minimize fetal risks.

Diabetic Nephropathy

Diabetic nephropathy, a progressive disease characterized by proteinuria, hypertension, and reduced GFR, is present in approximately 6 % of pregnant women with type 1 diabetes [60]. As the majority of patients with type 2 diabetes are older when diagnosed, typically they have a lower prevalence of diabetic nephropathy during their childbearing years. The mainstay of management is strict glycemic control, which has resulted in improved perinatal survival to 95 % [61]. The risk of pregnancy complications is correlated with the degree of prepregnancy renal impairment [62].

Women with diabetic nephropathy are at an increased risk for preeclampsia, regardless of the severity of proteinuria in early pregnancy [63]. The risk for deterioration in renal function and/or progression to ESRD during or after pregnancy is highest with a baseline serum creatinine measurement of ≥1.4 mg/dL [61]. Ideally, these women should undergo preconception evaluation and counseling as to the potential risks associated with pregnancy.

ACE inhibitors or ARB should be changed to agents safer for use during pregnancy to maintain control of hypertension (Table 22.3). Studies have shown that prior treatment with an ACE inhibitor, in combination with intensive glycemic control for 3–6 months prior to conception, is associated with a renal protective effect during pregnancy [64].

Focal Segmental Glomerulosclerosis

Focal segmental glomerulosclerosis (FSGS) is one of the most common causes of nephrotic syndrome [65] and is the most common primary glomerular disorder that causes ESRD in the USA [66]. FSGS may present in association with IgA nephropathy, vasculitis, lupus nephritis, infection (e.g., HIV, parvovirus, cytomegalovirus, Epstein-Barr virus), or drugs/toxins (e.g., interferon, heroin, lithium, pamidronate). Primary FSGS, accounting for approximately 80 % of cases of FSGS [67], typically presents with acute or subacute onset of nephrotic syndrome (peripheral edema and hypoalbuminemia), in contrast to secondary FSGS, which usually presents with slowly progressive non-nephrotic range proteinuria. Renal insufficiency at presentation is more common in cases of secondary FSGS.

In pregnancy, it is important to distinguish FSGS from preeclampsia, as FSGS may require immunosuppression and preeclampsia is best treated with delivery. As previously mentioned, a renal biopsy can be performed safely during pregnancy. Renal biopsies from patients with preeclampsia may show features similar to FSGS [68], and biopsy findings should be correlated within the clinical context in order to make a definitive diagnosis.

Once the diagnosis of FSGS is made on renal biopsy, potential secondary causes that require specific therapy should be ruled out before presuming a diagnosis of primary FSGS. While ACE inhibitors or ARBs are contraindicated for use during pregnancy, the initiation of immunosuppressive therapy with glucocorticoids may be considered (Table 22.3). It may take up to 16 weeks [69] to achieve remission. Thereafter, the steroid dose can be slowly tapered over 3–6 months. Calcineurin inhibitors, which are reserved for second-line therapy [70], are relatively safe during pregnancy. Once remission is achieved, the therapy should be continued for a minimum of 12 months before tapering, in order to maintain remission. Both classes of agents are known to have direct effects on podocytes, independent of their immunosuppressive properties, and enhance podocyte survival [71, 72]. As these agents are only effective in approximately 50 % of cases [67], other agents have been tried in the nonpregnant state, including sirolimus. However, the use of sirolimus is contraindicated in pregnancy, with animal studies demonstrating embryotoxicity, fetotoxicity, impaired fetal growth and mortality. As there are no adequate and well-controlled human studies, for patients on sirolimus, its use should be discontinued at least 12 weeks before attempted conception [47]. Irrespective of pregnancy status, several studies have raised concerns with respect to the use of sirolimus in the treatment of FSGS and other primary glomerulopathies, as it may worsen renal function [73].

Blood pressure control, with agents suitable for use in pregnancy, is a key component of FSGS management, as is control of volume status with fluid restriction and a low-salt diet. Despite concerns that diuretics may increase the risk of fetal and maternal complications due to reduced plasma volume [74], these medications have been shown to be well tolerated in pregnancy and have not been associated with adverse perinatal effects [75].

Minimal Change Disease

Minimal change disease (MCD) is another common cause of nephrotic syndrome and is associated with 10–25 % of cases of nephrotic syndrome in adults [76]. MCD is often considered together with FSGS, as there is debate as to whether they are variants of the same disease or if they represent different diseases [77]. Circulating factors, such as soluble urokinase receptors (suPAR), are known to be normal in MCD, but elevated in primary FSGS [78]. Both diseases are characterized by diffuse podocyte foot process effacement and may respond to glucocorticoid therapy.

Similar to FSGS, management should focus on blood pressure control and treatment of volume overload with suitable antihypertensives, fluid restriction, and a low-salt diet. Again, ACE inhibitors and ARBs that are ideal for treatment in the nonpregnant state cannot be used safely during pregnancy. Glucocorticoid therapy, which is considered relatively safe in pregnancy, is the mainstay of management and leads to complete remission of proteinuria in the majority of cases. Adults generally achieve remission much slower than children with MCD [79]. When remission is achieved, slow tapering is required to maintain remission and to minimize the risk of adrenal suppression, which has been associated with an increased risk of relapse [80]. Other immunosuppressive agents are not recommended for first-line therapy and are generally only considered for frequent relapses of glucocorticoid-dependent disease. Relapses are common and affect approximately half of glucocorticoid-responsive adults [79]. Patients with relapsing MCD who wish to become pregnant can be safely treated with azathioprine or calcineurin inhibitors; in the nonpregnant state, cyclophosphamide or mycophenolate mofetil may also be considered, but are not used in pregnancy.

Membranous Nephropathy

Membranous nephropathy (MN) is a common cause of nephrotic syndrome and is characterized by diffuse thickening of the glomerular basement membrane and subepithelial electron-dense deposits. The majority of cases are idiopathic, and recently, the M-type phospholipase A2 receptor (PLA2R),

expressed on glomerular podocytes, has been identified as a major antigen in human idiopathic MN [81]. In women of childbearing age, MN usually occurs in association with SLE, drug exposure (such as penicillamine, NSAIDs, and biologic agents), hepatitis B, hepatitis C, malignancy, and syphilis.

Treatments employed in nonpregnant patients include ACE inhibitors and lipid-lowering therapy, anticoagulation, and immunosuppression (such as cyclophosphamide, glucocorticoids, calcineurin inhibitors, and rituximab). Pregnancy presents therapeutic challenges, as ACE inhibitors, lipid-lowering therapy, and warfarin are contraindicated. Immunosuppressive therapy that can safely be used during pregnancy is presented in Table 22.3.

Vasculitides

Takayasu's arteritis commonly affects women typically between the ages of 10 and 40 years [82], and, as such, it is the second most common vasculitis encountered during pregnancy, after SLE. It usually presents with renal involvement, hypertension, stroke, and heart failure and carries an increased risk for preeclampsia [83]. Other vasculitides are more commonly seen in men or in women after their childbearing years. As a result, there are few published studies reporting on these conditions during pregnancy.

ANCA vasculitides, including granulomatosis with polyangiitis (previously known as Wegener's granulomatosis), and microscopic polyangiitis rarely present during pregnancy or in the postpartum period. A 2008 review reported that only 36 pregnancies complicated with Wegener's granulomatosis have been reported in the literature since 1970 [84]. In eight patients treated with cyclophosphamide, only one pregnancy reached full term, an additional three ended in preterm deliveries, and four had abortions, either therapeutic ($n=2$) or spontaneous ($n=2$). These data suggest that pregnancies in patients with active disease may have poor outcomes, despite aggressive therapy, further supporting the current recommendation that a stable remission of 6 months duration be obtained prior to conception. Also, a transplacental transfer of MPO-ANCA from mother to fetus has been documented, which may or may not result in fetal/neonatal disease. In one case report, neonatal pulmonary hemorrhage and renal involvement were documented [85], while, in another, the newborn remained healthy despite the persistence of the transferred MPO-ANCA for several weeks [86].

Similar to other patients with glomerular disease, patients with quiescent vasculitis at conception may have better pregnancy outcomes. For those with active disease, cyclophosphamide may be considered while carefully weighing the benefits versus risks for congenital malformations and other related adverse effects. Of note, recent studies of pregnant patients with breast cancer reported no major fetal complications with concomitant cyclophosphamide use [87].

Preeclampsia

Preeclampsia is a pregnancy-specific disorder clinically characterized by hypertension (blood pressure ≥140/90 mmHg) and proteinuria (≥300 mg in a 24-h urine) [75]. It affects approximately 5 % of pregnancies and remains a leading cause of both maternal and fetal morbidity and mortality worldwide. The etiology and pathogenesis of this disorder remain elusive, resulting in a failure to develop specific preventive and treatment options.

Recent studies have provided evidence that preeclampsia is associated with elevated levels of the soluble receptor for vascular endothelial growth factor (VEGF) of placental origin [88]. This soluble receptor, commonly referred to as sFlt-1 (from fms-like tyrosine kinase receptor-1), may bind and neutralize VEGF and thus decrease free VEGF levels that are required for active fetal and placental angiogenesis in pregnancy. Low free VEGF levels may contribute to the pathogenesis of preeclampsia in a dual fashion by causing (1) endothelial dysfunction and (2) glomerular epithelial cell (podocyte) dysregulation, leading to the two main clinical findings of preeclampsia, hypertension, and proteinuria, respectively. A mouse model of podocyte-specific heterozygous VEGF-A expression supports the role of VEGF-A in glomerular development and maintenance [89]. These mice not only have decreased VEGF-A levels, but by 2½ weeks of age, develop proteinuria and severe endotheliosis, the pathologic hallmark of preeclampsia.

Renal Biopsy Findings

Glomerular endotheliosis, which is classic for, but not pathognomonic of, preeclampsia, is characterized by glomerular endothelial swelling with occlusion of the capillary lumens, leading to "bloodless" glomeruli (Fig. 22.2). In the most severe cases of preeclampsia, clinically commonly manifested as hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome, a renal biopsy may show thrombotic microangiopathy, with the characteristic finding of vessel thrombosis (Fig. 22.2). As the renal biopsy findings of thrombotic microangiopathy are indistinguishable from those encountered in thrombotic thrombocytopenic purpura (TTP) and pregnancy-associated atypical hemolytic-uremic syndrome (HUS), the clinical distinction among these conditions may be facilitated by certain clinical and laboratory features (Table 22.4).

In addition, a thrombotic microangiopathy (TMA) can occur in patients with antiphospholipid antibodies [90]. In these patients, it is possible that a hypercoagulable state,

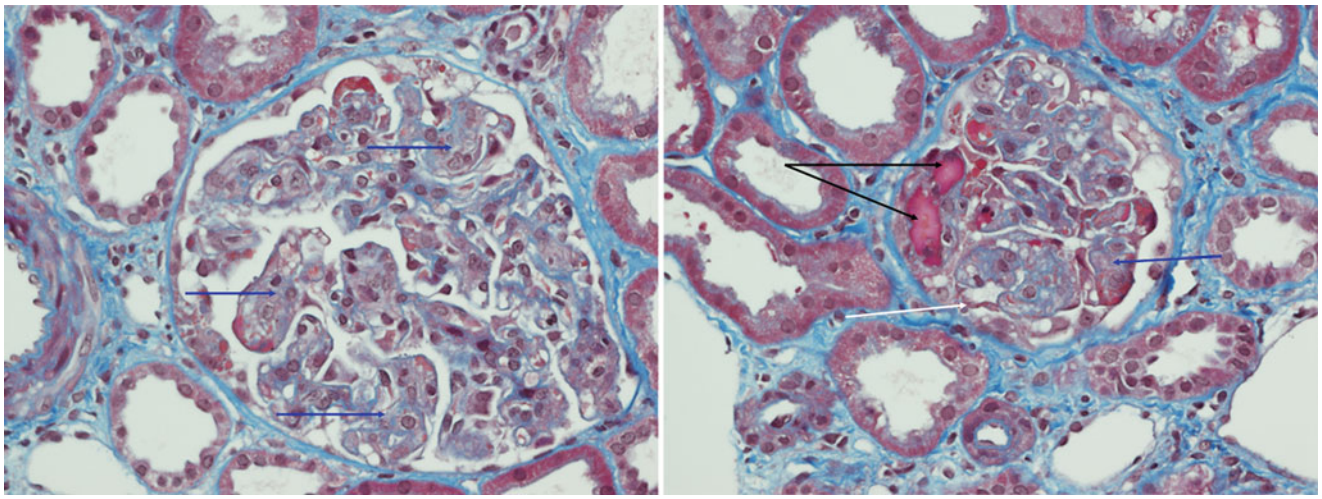

Fig. 22.2 Renal biopsy findings in preeclampsia. A 29-year-old pregnant woman presented at 21 weeks of gestation with hypertension (blood pressure of 160/100 mmHg) and acute-onset proteinuria of 22.6 g/24 h, with urinary sediment showing oval fat bodies and hyaline casts. Given the severity of her proteinuria, and after adequate blood pressure control with labetalol, a renal biopsy was performed to differentiate between early preeclampsia and renal parenchymal disease. The biopsy was uneventful and showed evidence of endotheliosis (*blue arrows*), mesangiolysis, and thrombotic microangiopathy (*black arrows*) with double contouring (*white arrow*). Unfortunately, her pregnancy ended with intrauterine fetal demise. Three months later, her blood pressure was adequately controlled on nifedipine, and the proteinuria had decreased to 260 mg/24 h

Table 22.4 Differential diagnosis of thrombotic microangiopathy in pregnancy: the role of clinical and laboratory findings

	HELLP syndrome	Atypical HUS	TTP
Time of onset	Late third trimester	Postpartum	Second and third trimester
Renal failure	Unusual	Common	Minimal or absent
Renal prognosis	Recovery	76 % ESRD [103]	Fair
Neurological findings	May be present	Minimal or absent	Dominant
Low platelet count	Present	Present	Present
Disseminated intravascular coagulation	May be present	Absent	Absent
Abnormal liver function tests	Present	Absent	Absent
Complement alternative pathway abnormalities	May be present [105]	Present	Absent
Low ADAMTS 13	Mild to moderate	Absent	Severe

which is characteristic of pregnancy, may contribute to the preexisting subclinical thrombotic state due to the presence of antiphospholipid antibodies, resulting in a clinically apparent syndrome of thrombotic microangiopathy during pregnancy. Finally, proteinuric renal diseases are known to increase the risk for preeclampsia. Consequently, renal biopsies during pregnancy or postpartum in preeclamptic patients may reveal the findings of glomerular disease that predated or occurred during pregnancy [91].

Mechanism of Renal Injury

The clinical signs of preeclampsia may be induced in pregnant rats by injecting the recombinant adenovirus encoding for the murine sFlt-1 gene [19]. These rats develop hypertension, proteinuria, and glomerular endotheliosis. In addition, studies in mice have shown that proteinuria may relate to the downregulation of nephrin [92], a structural component of the glomerular epithelial cell (podocyte) slit diaphragm, which is the main size-selective filtration barrier in the kidney. Studies in human pregnancies have confirmed that nephrin is downregulated in kidney sections of women who had severe preeclampsia, when compared to normal pregnancies [93]. In addition, the mechanism of proteinuria in preeclampsia may relate to podocyturia, i.e., urinary excretion of glomerular epithelial cells [94, 95]. Loss of a critical number of podocytes may impair the filtration barrier and, as such, predate proteinuria. Currently, the role of podocyturia in the prediction of preeclampsia is under investigation.

Long-Term Renal Prognosis

Early studies performed in the 1970s suggested that future renal function was not impaired in preeclamptic mothers [96] and that there was no future risk of hypertension [97]. More recent studies have shown that women with hypertensive pregnancy disorders, including preeclampsia, are at an increased risk for both cardiovascular and renal disease later in life [98, 99]. With respect to renal disease, a history of preeclampsia has been associated with a higher frequency [100], which is a risk factor for undergoing a kidney biopsy later in life [101] and, ultimately, developing ESRD [102]. Future research is needed to confirm these associations in a population-based study where both exposures and outcomes can be confirmed, while controlling for the copresence of other glomerular diseases and risk factors, such as diabetes and hypertension.

At present, women with a history of preeclampsia and proteinuria that persists 3–6 months postpartum need to be seen by a nephrologist for consideration of a renal biopsy. While the findings may be nonspecific in some cases, commonly representing residual focal segmental glomerular lesions [103], in other cases, a renal biopsy may reveal glomerular disease that either predated or occurred during pregnancy for which specific therapies are indicated.

Atypical Hemolytic-Uremic Syndrome (HUS)

Atypical hemolytic-uremic syndrome (HUS), characterized by hemolytic anemia, thrombocytopenia, and renal impairment, occurs due to uncontrolled complement activation. Classical HUS occurs due to infection with bacteria that produce Shiga-like toxin [104]. Pregnancy may trigger abnormal complement activation leading to atypical HUS. A recent retrospective cohort study of 100 patients with atypical HUS reported that 21 women developed atypical HUS in association with pregnancy, with complement abnormalities detected in 85.7 % ($n=18$) [105]. These patients were at an increased risk of fetal loss and preeclampsia, and 76 % had developed ESRD by last follow-up.

Recent work suggests that mutations in the genes encoding for complement regulatory proteins may increase the risk for preeclampsia in other disease entities, such as SLE and/or APL antibodies [106]. Also, complement abnormalities may be present in approximately 40 % of patients who develop HELLP syndrome during pregnancy [107]. Certain clinical and laboratory features may facilitate distinguishing thrombotic TTP, atypical HUS, and HELLP syndrome (Table 22.4). For now, the mainstay of treatment for atypical HUS in pregnant patients is plasma exchange, similar to the nonpregnant state. The safety of anti-complement-targeted agents in pregnancy, such as eculizumab, needs to be established. Whether complement dysregulation is involved in other pregnancy complications, such as preeclampsia, intrauterine growth retardation, and pregnancy loss, remains to be determined in future studies.

Conclusion

A number of glomerular diseases may occur in women of childbearing age. The occurrence of pregnancy in these women is not infrequent, particularly when their renal function is relatively preserved (i.e., creatinine <1.4 mg/dL). It is recommended that pregnancy in such patients be planned when the disease has been in remission for a minimum of 6 months in order to minimize maternal and fetal complications. Immunosuppressive agents should be optimized prior to conception to include those that are safe for pregnancy. Renal biopsy can be performed during pregnancy in patients with normal coagulation and adequate blood pressure control and may facilitate both the initiation of disease-specific treatment, and making the distinction between glomerular and pregnancy-specific diseases (such as preeclampsia). The complexity of medical management when caring for these patients calls for a multidisciplinary team approach, consisting of a nephrologist, rheumatologist, and obstetrician.

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Acute Interstitial Nephritis

23

Gerald B. Appel and Alice S. Appel

Definition

Acute interstitial nephritis (AIN) is a parenchymal renal disease with inflammatory infiltrates localized predominantly to the tubules and the interstitial area between the tubules, glomeruli, and blood vessels [1–10]. AIN may be primary (idiopathic) or secondary, resulting from medications, infectious agents, or autoimmune processes. Although acute bacterial pyelonephritis is technically a form of AIN, it is usually considered separately with other direct infections of the renal parenchyma. All forms of AIN are characterized by varying amounts of interstitial edema, tubular damage associated with tubulitis, and interstitial inflammation. The clinical manifestations are varied. Some patients present with symptoms and signs localized only to the kidney; others present with extrarenal findings as well. Typical renal findings include acute renal insufficiency and progressive renal failure or isolated evidence of tubular dysfunction. However, even AIN with severe clinical and histologic findings is a potentially reversible lesion and need not result in permanent renal dysfunction.

Councilman first described AIN in 1898 [11]. In examining the kidneys of children who had died of scarlet fever or diphtheria, he noted "an acute inflammation of the kidney characterized by cellular and fluid exudation in the interstitial tissue, accompanied by, but not dependent on, degeneration of the epithelium: the exudate is not purulent in character and the lesions may be both diffuse and focal." Councilman recognized that these kidneys were not infected by the organisms but were instead sterile and resulted from other inflammatory factors, "soluble" substances that were exerting a chemotactic role. Medication-related AIN was first noted with early antimicrobials and has become the most common etiology of AIN. Unique clinical presentations have been noted with several groups of medications such as nonsteroidal anti-inflammatory drugs. Over time several distinct clinical variants of idiopathic AIN have become well recognized and better defined. The optimal therapy for most forms of AIN remains to be determined [2].

Incidence and Etiology

The true incidence of AIN remains unknown [1, 2, 6, 7, 10]. Although clinical manifestations may be strongly suggestive, only a renal biopsy is diagnostic of AIN. Many cases do not come to biopsy. Milder cases may be overlooked or attributed to other renal disorders. In many patients the decision is made to alter potential offending medications and then, only if the lesion is not resolving, will a biopsy be considered. In other cases patients are too ill to undergo renal biopsy and the acute kidney injury may be attributed to acute tubular necrosis or to other forms of acute kidney injury.

Furthermore, the incidence of AIN will also vary in different populations [12–17]. In a Finnish study of military recruits, 174 of 314,000 (0.05 %) had a renal biopsy for persistent proteinuria or hematuria and only two (0.7 per 100,000) had interstitial nephritis [12]. In contrast, two autopsy series found AIN in 1 % of 25,000 autopsies and 7,980 autopsies, respectively. If large unselected series of all biopsied patients are examined, between 1 and 2 % will have AIN [13]. However, a more recent series examining over 1,000 renal biopsies from 1968 to 1997 found AIN, largely medication related, in 6.5 % of biopsies [15]. The incidence of AIN is higher than observed in earlier studies among patients biopsied for acute kidney injury (15–27 %) [16] and even higher in those with both normal-sized kidneys and acute kidney injury [17]. At Columbia University Medical Center with over 3,500 renal biopsies analyzed annually, the vast majority of non-transplant biopsies reveal glomerular disease, but among those with tubulointerstitial disease, AIN is the most common diagnosis.

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Table 23.1 Etiologies of acute interstitial nephritis

Medication-related AIN
AIN associated with infections
Direct invasion of the kidney
Remote infection
Idiopathic AIN—TINU syndrome
AIN associated with glomerular and systemic diseases
With anti-TBM antibodies
SLE and MCTD
Sarcoid
Sjogren's

Only a minority of patients with AIN have primary or idiopathic disease. Most patients have disease related to medications, infectious agents, or systemic or glomerular diseases (see Table 23.1). Excluding direct bacterial infection of the kidney (acute bacterial pyelonephritis), the most common etiology of AIN by far is medication associated [1, 3–10]. For example, 85 % of all AIN in one series was medication related with only 10 % related to remote infections and only 4 % classified as idiopathic [14].

Close to 100 different medications have been incriminated in producing AIN, with antibiotics of the beta-lactam class (penicillins and cephalosporins) frequently cited [18–21] (see Table 23.2). In particular, in over 100 patients methicillin has been reported to cause AIN. AIN has also been reported with numerous other penicillins and cephalosporins including both second and third generation cephalosporins. Despite wider usage of other antimicrobials and discontinuation of the use of methicillin, cases of beta-lactam-induced AIN continue to be reported. A recent study documented four cases of nafcillin-induced AIN within 1 year in a single institution [22]. Other antimicrobials including the sulfonamides, rifampin, and the quinolones have all clearly produced AIN [1, 3–10]. Vancomycin has also been identified as an inciting agent in AIN [23, 24]. While the antiviral agents, acyclovir and foscarnet, can also produce tubulointerstitial damage, this manifestation is more similar to acute tubular necrosis than to true interstitial nephritis [3, 25–28].

In addition to antimicrobials, a number of different structural classes of diuretics including thiazides, the loop diuretics, and triamterene may infrequently produce AIN [1, 3–10]. All classes of nonsteroidal anti-inflammatory drugs including Cox-2 inhibitors have also been associated with AIN [29, 30]. There are also increasing reports of AIN related to a variety of proton-pump inhibitors [31]. Of the many other pharmaceutical agents producing AIN, the only frequently offending agents are allopurinol, diphenylhydantoin, sulfinpyrazone, cimetidine and ranitidine, and 5-aminosalicylates such as azulfidine and mesalamine [32–34].

While some infectious agents produce parenchymal damage by direct invasion of the kidney (e.g., acute bacterial pyelonephritis), others may be associated with inflammatory

Table 23.2 Medications associated with AIN

Beta-lactam antibiotics
Methicillin[a]
Penicillin
Ampicillin
Apacillin
Flucloxacillin
Oxacillin
Mezlocillin
Nafcillin
Carbenicillin
Amoxicillin
Mezlocillin
Cefaclor
Cefotaxime
Cefoxitin
Cefuroxime
Cephalothin
Cephalexin
Cephradine
Cephaloridine
Other antimicrobials
Sulfonamides[a]
Trimethoprim-sulfamethoxazole[a]
Rifampin[a]
Polymyxin
Tetracyclines
Vancomycin
Isoniazid
Chloramphenicol
Ethambutol
Minocycline
Erythromycin
Para-aminosalicylic acid
Ciprofloxacin[a]
Norfloxacin
Piromidic acid
Spiramycin
Nonsteroidal anti-inflammatory agents
Ketoprofen[a]
Fenoprofen[a]
Indomethacin
Ibuprofen
Benoxaprofen
Phenazone
Mefenamic acid
Tolmetin
Diflunisol
Zomepirac
Piroxicam
Diclofenac
Suprofen
Oxyphenbutazone
Novominsulsone
Diuretics

(continued)

Table 23.2 (continued)

Beta-lactam antibiotics
Thiazides
Furosemide
Ethacrynic acid
Chlorthalidone
Triamterene
thiazide/amiloride
Other medications
Phenindione[a]
Glafenine[a]
Diphenylhydantoin[a]
Cimetidine[a]
Sulfinpyrazone[a]
Allopurinol[a]
Proton-pump inhibitors[a]
Carbamazepine
Clofibrate
Azathioprine
Aspirin
Phenylpropanolamine
Aldomet
Phenobarbital
Leukocyte interferon A
Haldol
Coumadin
Tofranil
Diazepam
Valproic Acid
Chlorprothixene
Captopril
Propranolol
Amphetamines
Doxepin
Quinine
Ranitidine and cimetidine[a]

[a]Indicates medication frequently associated with AIN

interstitial nephritis indirectly related to a systemic process. Such remote or systemic infections have been caused by bacteria, parasites, and viruses. Bacterial organisms associated with AIN include Streptococci, Brucella, Pseudomonas, Staphylococci, Legionella, Yersinia, *Mycobacterium leprae*, and Mycoplasma [1, 13–15, 35–41]. Leishmaniasitic parasitic infections including Kala-azar have also been associated with AIN as have cases of toxoplasmosis [42–44]. Epstein–Barr virus and measles infections have been associated with an interstitial renal inflammatory infiltrate [45–47]. One to two percent of patients with severe mononucleosis develop acute renal insufficiency which may be associated with biopsy documented AIN. Up to a third of biopsied patients with Kawasaki's disease have an inflammatory reaction typical of AIN [48]. While in HIV infection with or without glomerular lesions, prominent tubulointerstitial damage is common, its presence without glomerular pathology should prompt a search for invading infectious agents or medication-related toxicity [3, 49].

For many other infectious agents associated with renal dysfunction, it is unclear to what extent the interstitial inflammation relates to direct tissue invasion as opposed to remote effects of the infectious organisms. This is true for reports of AIN associated with leptospirosis, mycobacterial infections, rickettsial infections, and viral infections due to herpes, CMV, hantavirus, polyomavirus, and adenovirus. In addition, an acute interstitial infiltrate is a common finding in both sarcoidosis and Sjogren's syndrome [50–53].

Also AIN may be associated with a number of primary glomerulopathies [1, 3]. AIN with anti-TBM antibodies has occurred in a number of glomerulonephritides including Goodpasture's syndrome, membranous nephropathy, and familial nephritis. AIN may be associated with immune complex deposits along the TBM in systemic lupus and mixed connective tissue disease [1]. In SLE interstitial damage may occur along with the glomerular involvement or more rarely as an isolated occurrence [1, 54, 55]. TINU (tubulointerstitial nephritis and uveitis) syndrome is a unique form of idiopathic AIN [56, 57]. Recently, a new entity of hypocomplementemic tubulointerstitial nephritis has been reported [58, 59].

Pathogenesis and Pathophysiology

Despite much speculation, the pathogenesis of medication-related AIN is complex and remains to be defined [1–10]. There are no good experimental models. It is unclear why AIN results more frequently with some medications of a given class (e.g., methicillin) than with others (e.g., penicillin) [1–10]. Neither the medication concentration in the serum of many patients with medication-related AIN protein binding, route of excretion by renal tubular secretion, nor the type of underlying infection seems an adequate explanation for the wide discrepancy in incidence. Moreover, it is unknown what factors predispose individuals to develop AIN in response to medications previously taken without causing problems. For example patients developing AIN associated with NSAID use typically have taken the medication for prolonged periods of time before developing the adverse renal reaction [60, 61]. In one series several patients had more than one episode of AIN implying some form of predisposition [13, 62].

In many patients with medication-related AIN, there is clear evidence for an immunologic reaction [1–10]. The adverse renal reaction is uncommon, not dose related, and suggestive of an allergic immune response. In those with hypersensitivity features such as rash, fever, eosinophilia, and eosinophiluria all suggest an allergic immune response.

The pathology also supports an immunologic reaction by both the nature of the inflammatory cellular infiltrates and in some cases evidence of tubular basement membrane deposit (TBM) deposits. Prompt recurrence after rechallenge with the same or a similar drug also suggests an immune mechanism [62, 63]. A number of experimental models of tubulointerstitial nephritis unrelated to medications also suggest potential immunologic mechanisms of damage [1, 5].

The induction of AIN may relate to the kidney's excretory role for many drugs, with binding of drug hapten to kidney tubular or interstitial structural proteins [1–10, 63]. Although the small molecular weight of many medications makes them capable of eliciting only a weak immunologic response, when bound as haptens to other proteins, they may elicit a stronger response. Indeed, in some cases of penicillin AIN, the benzylpenicilloyl hapten and, for some cases of methicillin AIN, the dimethoxyphenyl penicilloyl hapten have been localized along the TBM's bound to kidney structural proteins [19, 20, 63–65]. However, these haptens have also been found bound to the interstitium of patients receiving these drugs, but who have not developed AIN [65]. It appears that under certain conditions in predisposed individuals, the drug-protein conjugate may initiate a response leading to AIN.

There is evidence for both humoral and cell-mediated immunologic reactions. Humoral mechanisms are supported in some patients by evidence of circulating anti-TBM antibodies [1, 59, 63, 65], while others have had circulating antibodies, which have bound to proximal tubule brush border antigens [66, 67]. In rare patients, immunofluorescence microscopy has confirmed the linear deposition of immunoglobulin or complement localized to the TBMs [19–21, 64, 68–71]. Rarely serum complement levels are depressed in medication-related AIN [18]. Increased serum IgE levels and interstitial inflammatory infiltrates with IgE-containing plasma cells are sometimes found, but despite these reported abnormalities, the majority of patients with medication-related AIN have no such findings [72–74].

A cell-mediated mechanism is supported by the prominent cellular interstitial infiltrates with lymphocytes and macrophages [1, 3]. A delayed hypersensitivity reaction to intradermal drug injection has also been occasionally documented [19, 64]. In NSAID-related AIN, the associated minimal change nephrotic syndrome has been cited as evidence of a cell-mediated lymphokine-directed reaction [27, 28, 60, 61]. The interstitial inflammatory infiltrates in medication-related AIN are composed predominantly of T lymphocytes rather than B lymphocytes, with a variable ratio of cytotoxic-suppressor to the helper-inducer T cell population [75–77]. There has been no difference in the individual T cell subtypes or T/B cell ratio between patients with beta-lactam-related AIN and those with NSAID-related AIN [77]. Drug-specific T cell clones may be involved in the pathogenesis of the lesions [78]. Although eosinophils are found in the biopsies of many patients with AIN, their role is also unclear [3]. They may be recruited by eosinophilic chemotactic factors and subsequently contribute to the interstitial damage through release of proteases, leukotrienes, superoxide radicals, and peroxidases [1–10].

The reduction in the glomerular filtration rate commonly seen probably relates directly to the inflammatory interstitial infiltration since the severity of the AIN correlates with the severity and diffuse nature of the inflammation. Interstitial edema may contribute to elevated intratubular pressures, and sloughed luminal cells and debris may lead to intratubular obstruction. The role of tubular back-leak across damaged epithelium, renal vasoconstriction, and tubuloglomerular feedback is unclear [72]. Structural damage from cellular infiltration and "tubulitis" or from release of mediators of inflammation may also play a role in the tubular defects seen in patients with medication-related AIN.

Pathology

The pathology of medication-related AIN is characterized by patchy to diffuse interstitial inflammatory infiltrates with variable amounts of edema and focal tubular damage [1–10, 13, 18, 19]. The inflammatory infiltrate is composed of lymphocytes, plasma cells, monocyte/macrophages, and sometimes significant numbers of eosinophils [3, 13] (Fig. 23.1). Eosinophils usually make up 2–10 % of the infiltrating cells [20, 73, 79]. Granuloma formation may be seen with palisading macrophages with an epithelioid character [3, 13, 69, 80–82]. Granulomatous interstitial nephritis may be seen in other forms of AIN such as sarcoid and TINU Syndrome [81]. "Tubulitis," defined as an invasion of the tubules by lymphocytes and other cells, is a characteristic feature of AIN and may be found in as many as 80 % of biopsies of AIN patients [3, 13, 67]. This more frequently involves the distal nephron [83]. In classic AIN the presence of tubulitis and only focal tubular necrosis contrasts with the more extensive tubular damage and sparser interstitial infiltrates found in acute tubular necrosis. Nevertheless, the distinction between these entities is not always clear-cut. The glomerular and vascular compartments are generally spared in medication-related AIN with the exception of disease related to NSAIDs.

Immunofluorescence microscopy is usually negative in medication-related AIN with neither complement nor immunoglobulins found along the tubules or within the interstitium [3, 18, 19, 68]. Rarely, there is linear or granular staining for IgG and C3 along TBMs [18, 59]. Likewise, by electron microscopy only rarely have TBM electron dense deposits been noted [68, 84].

The inflammatory infiltrates in most cases of NSAID-related AIN are characterized by lymphocytes and plasma

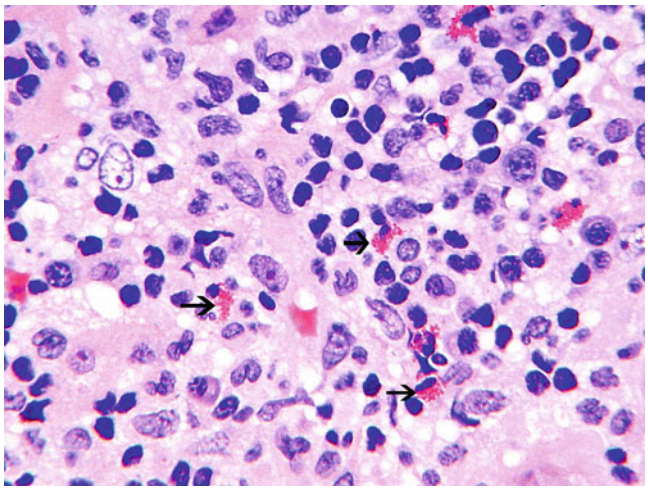

Fig. 23.1 Interstitial nephritis, showing a predominantly mononuclear infiltrate with few eosinophils (*black arrows*) (H&E ×40)

cells and less frequently by eosinophils [27, 28, 60, 61]. Most cases associated with the nephrotic syndrome have shown the lesion of minimal change disease, with normal glomeruli by light microscopy, negative immunofluorescence, and EM only effacement of the foot processes [27, 28, 60, 61]. Others cases have been associated with a membranous pattern of glomerular injury [85–87].

In AIN associated with infections in addition to edema and interstitial inflammation, neutrophils may be more prominent [35–40]. Although in AIN associated with systemic or glomerular disease the pathology of the interstitium varies, a patchy interstitial inflammatory infiltrate is common to all [1]. In immune complex diseases, such as lupus and cryoglobulinemia, there may be granular deposits of immunoglobulin and complement along the TBMs [54, 55]. In idiopathic hypocomplementemic interstitial nephritis, there are massive tubulointerstitial immune deposits [58]. The renal involvement in Sjogren's syndrome typically consists of a lymphocytic interstitial infiltration without immune deposits [52, 53]. In sarcoidosis a granulomatous interstitial nephritis is found in up to 20 % of patients [50, 51]. In TINU syndrome the renal pathology usually shows a variable inflammatory infiltrate of eosinophils, lymphocytes, and plasma cells occasionally with granuloma formation [56, 57].

Clinical Manifestations and Laboratory

The classic picture of medication-related AIN has best been described for the beta-lactam antibiotics, the penicillins and cephalosporins. Several hundred cases have been reported occurring in all decades of life and in both genders [18–21]. Although the duration of therapy often lasts for many weeks, the dose of the antibiotic has not been excessive [18]. The cardinal systemic features are the hypersensitivity triad of rash (30–50 %), fever (75–80 %), and peripheral blood eosinophilia (80 %) [18–21, 72]. However, at the time of onset of renal insufficiency, fewer than one-third of patients have the complete triad [18]. The rash is usually a classic drug eruption with maculopapular erythematous lesions on the trunk and upper extremities. The secondary fever spike often occurs 2–3 weeks into the course of the antibiotic therapy. Less common features are loin pain, arthralgias, lymphadenopathy, and other systemic organ involvement, e.g., hepatitis.

Urinary output may be maintained and nonoliguric acute kidney injury is more common than the oliguric form. Urinary findings include mild proteinuria, pyuria, and leukocyte casts. Hematuria is present in over 90 % of cases and gross hematuria is common in children. Large amounts of proteinuria and erythrocyte casts in the urine sediment are rare and can usually be explained by coincidental glomerular pathology [1–10, 88]. With the exception of NSAID-induced disease, only a few cases of AIN have been associated with glomerular pathology [32, 89, 90]. The finding of eosinophils in the urinary sediment, eosinophiluria, is both frequent and helpful in establishing a diagnosis [20, 21]. In a study of patients with acute kidney injury, all nine patients with methicillin AIN had eosinophiluria on Wright stain of the urinary sediment, while none of 43 other patients had this finding [20]. In a second study, 10 of 11 patients with AIN had eosinophiluria versus none of 30 patients with ATN, none of 10 with acute pyelonephritis, and only 1 of 15 with acute cystitis [91]. In this study the Hansel stain was superior to the Wright stain in confirming eosinophiluria. The precise number of urinary eosinophils diagnostic of AIN has been debated. However, one study suggested eosinophiluria of over 5 % of the total urinary leukocytes to be strongly suggestive of AIN [79]. However, urinary eosinophils are also commonly found in some cases of rapidly progressive glomerulonephritis, with renal atheroembolic disease and with acute prostatitis [79].

The kidneys in AIN typically are found to be normal sized or enlarged with increased cortical echogenicity by ultrasonography [1–10, 72]. Gallium scanning in drug-related AIN may show intense diffuse bilateral uptake of the gallium nuclide. Although this is rarely found in ATN, positive gallium scintigraphy has been noted in acute bacterial pyelonephritis and acute glomerulonephritis and even in several patients with minimal change nephrotic syndrome [92–94]. The urinary sodium and the fractional excretion of sodium have been elevated (>60 mEq/L and >1 % respectively) in only some studies [95]. Hypocomplementemia is rare [18].

Medications other than the beta-lactam antibiotics may be associated with AIN with similar or unique clinical features. In some patients there are no extrarenal manifestations accompanying the renal insufficiency [1–10]. Both sulfonamides and trimethoprim-sulfamethoxazole have been associated

with classic AIN with the full hypersensitivity triad [1–10, 18]. AIN associated with the use of the quinolone antibiotics has been reported frequently [96–98]. The picture often has both classic clinical and histopathologic features.

The pattern seen with rifampin is unique [99–101]. With either intermittent or discontinuous therapy or on rechallenge with rifampin, patients have experienced sudden onset of fever, flank pain, hematuria and acute kidney injury. Some cases are associated with myalgias, hemolysis, and thrombocytopenia. Renal biopsy reveals a spectrum of findings from classic AIN to typical acute tubular necrosis. In rare cases, continuous therapy with rifampin has produced AIN with the presence of nephrotic range proteinuria [89]. Other antimicrobials associated with AIN infrequently or in a less well-documented fashion are listed in Table 23.2.

Although diuretics typically produce renal insufficiency by causing intravascular volume depletion, a number of them including thiazides and chlorthalidone, furosemide, ethacrynic acid, and ticrynafen have rarely produced AIN [102, 103], which typically has occurred in patients with prior renal disease and often displays the classic hypersensitivity features of rash, fever, and eosinophilia.

While the NSAIDs are commonly associated with renal and electrolyte disturbances due to prostaglandin inhibition [29, 60, 61], when associated with AIN they present unique clinical features, including development in the older population receiving these medications and after a prolonged prior exposure to the drugs (months to years) [1, 10, 30, 60, 61]. The hypersensitivity triad of rash, fever, and eosinophilia is uncommon as are hematuria and eosinophiluria even when interstitial eosinophilia is found on renal biopsy [29]. Finally, AIN caused by the NSAIDs has often been associated with the nephrotic syndrome usually of the minimal change disease pattern [29, 30, 60, 61, 104]. The onset of the acute renal failure and the nephrotic syndrome are often concurrent, and both typically remit within weeks of discontinuing the NSAID. Membranous nephropathy and very rarely focal segmental glomerulosclerosis have also been noted in patients taking NSAIDs [85–87]. NSAID-associated AIN may be associated with more frequent permanent renal dysfunction perhaps as a result of its paucity of clinical findings and its insidious nature [1, 6, 13].

The analgesic glafenine and the anticoagulant phenindione, although not used in the USA, have been well associated with the induction of AIN. The anticonvulsants diphenylhydantoin, phenobarbital, and carbamazepine have all been associated with AIN, sometimes with clinical features of lymphadenopathy and evidence of associated glomerulonephritis or vasculitis. Allopurinol may also produce AIN with associated glomerular damage and vasculitis and occasionally with a granulomatous interstitial inflammation [33]. The uricosuric, antiplatelet agent sulfinpyrazone may also cause AIN. Both cimetidine and ranitidine have produced classic clinical AIN [34]. Recently proton-pump inhibitors (PPIs) have been reported to be a major source of medication-related AIN [31]. Medications used to treat inflammatory bowel disease such as mesalazine and azulfidine have also produced AIN [32].

Most patients with medication-related AIN present with nonoliguric renal failure [1–10, 18]. Severe renal failure requiring dialysis may occur in up to one quarter of the patients, but most patients recover function rapidly over days to weeks after discontinuation of the offending medication [13]. The number of patients left with residual renal dysfunction and to what extent is debated since only a minority have either repeat renal biopsies or the long-term follow-up necessary to resolve this question.

In some patients with medication-related AIN, tubular dysfunction (rather than acute kidney injury) dominates the clinical picture. Findings may include hyperkalemia, hyperchloremic metabolic non-anion gap acidosis, and rarely proximal tubular dysfunction marked by bicarbonaturia, hypophosphatemia, hypouricemia, glycosuria, and aminoaciduria [1–10, 105, 106]. Hypokalemia and hypomagnesemia due to renal tubular wasting have been reported [107].

In infection-related AIN, either oliguric or nonoliguric renal insufficiency is a common clinical manifestation which often remits with resolution of the infectious process. Idiopathic AIN may present as an isolated renal event or in association with other clinical features such as in the TINU syndrome. Both occur most frequently in young females [9, 56, 57, 108]. Patients may develop oliguric or nonoliguric renal insufficiency without any systemic features or with associated eosinophilia, fever, weight loss, and uveitis-iritis occurring before or during the episode of acute kidney injury. Some patients have elevated sedimentation rates and CRP levels, hypergammaglobulinemia, positive tests for rheumatoid factor, and tubular defects with glycosuria and aminoaciduria. Granulomatous bone marrow lesions have been found in some of these patients. In some young females the AIN may remit spontaneously while in older adults without treatment the disease is more likely to be progressive.

Prognosis and Treatment

Most patients with medication-related AIN recover from the acute kidney injury when the offending drug has been discontinued [1–10]. In one review of over 150 cases of beta-lactam-related AIN, only 17 % required dialysis and fewer than 3 % died as a result of the renal injury [18]. A second study found that almost 70 % of cases of renal insufficiency were completely reversible with another 12 % being partially reversible [13]. However, few studies have a repeat

biopsy to document full recovery or the long-term follow-up necessary to attest to this. Factors predictive of a poor renal recovery include longer persistence of the acute kidney injury, elderly age group, more diffuse interstitial damage and tubular atrophy on biopsy, the presence of granulomatous interstitial lesions, and especially interstitial fibrosis on biopsy [14, 18, 109]. It is likely that many of these patients have suffered an irreversible loss of functional renal tissue and/or renal "reserve." The long-term significance of this loss of functional tissue and GFR has not yet been determined in terms of morbidity or ultimate progression to end-stage renal disease.

In AIN associated with NSAID use, remissions of both the renal failure and nephrotic syndrome typically occur within weeks of stopping the drugs [1, 29]. However, in some cases the renal dysfunction may persist for months. In some series NSAIDs have been associated with less complete recovery of renal function [13]. Rarely such cases may progress to stage 5 chronic kidney disease.

The optimal treatment of medication-related AIN remains unclear due to the lack of controlled randomized studies and its infrequent occurrence [2]. There is little debate over discontinuing potential offending medications. In some patients it is necessary to withdraw or substitute several medications simultaneously when the offending medication is unclear. Cross-reacting medications of similar structure or class should be avoided since worsening of renal function has clearly been documented with rechallenge with different beta-lactam drugs in penicillin-related AIN [62, 64]. All NSAIDs regardless of differences in chemical structure should be avoided in patients felt to have NSAID-related AIN.

Supportive care in patients with AIN is similar to that in other patients with acute kidney injury and includes attention to management of electrolytes and acid–base balance, and volume status. Both the morbidity and mortality of drug-associated AIN is less than in many other forms of acute kidney injury. In part this is due to the nonoliguric nature of many cases of AIN and in part due to the less severe nature of underlying comorbidities. Dialysis is infrequently needed or required for only a short time interval. However, in some patients recovery is delayed for many months and some suffer irreversible renal damage [18, 109]. A recent investigation of biopsy-proven drug-induced AIN in 72 Chinese patients reported that only 18 (25 %) did not recover promptly upon withdrawal of the suspected drug. Older age, longer time to hospitalization from the onset of the disease process, and greater interstitial inflammatory cell infiltrates on renal biopsy were all associated with delayed recovery of kidney function in this group [110].

The value of corticosteroids or any other immunosuppressive drug in medication-related AIN has been widely debated [1–10]. Corticosteroids have been the most widely used immunosuppressives for the treatment of both medication-related and idiopathic AIN [1–10]. They have not been used in a large randomized trial. The goal of therapy is both to promote prompter recovery of the acute kidney injury and to prevent or ameliorate residual structural and functional renal damage. Several retrospective series support, but do not prove, the benefits of corticosteroids on improvement of GFR and of less residual damage on repeat renal biopsy. One nonrandomized study compared a 10-day course of 60 mg daily of prednisone to no therapy in 16 patients with methicillin-related AIN with severe acute kidney injury [20]. The steroid group had a lower final serum creatinine (1.4 vs. 1.9 mg/dL), a shorter duration of renal dysfunction, and a greater percentage of patients with renal function returning to baseline (75 % vs. 33 %). Another trial reviewed 27 patients with AIN, of whom 15 were drug related, and found that patients who did not improve following discontinuation of the offending medication benefited from corticosteroids [111]. An uncontrolled trial of seven patients with severe AIN, six of whom required dialysis, used several daily pulses of 500–1,000 mg of methylprednisolone leading to recovery and a fall in serum creatinine within 72 h in all patients [64]. Another retrospective study found that although high-dose daily prednisone (40–80 mg daily for 4–6 weeks) did not alter the recovery time from acute kidney injury in medication-related AIN, it did lead to a statistically lower serum creatinine at 8 weeks [11]. A number of studies, likewise, reported that patients with AIN treated with steroids have an improved GFR at long-term follow-up when compared to patients not receiving steroid therapy [1, 109]. Moreover, another retrospective analysis found no benefit of corticosteroid treatment in ultimate outcome in medication-related AIN [112].

A recent multicenter nonrandomized study analyzed the influence of steroids in 61 patients with biopsy-proven medication-related AIN [113]. AIN was attributed to antibiotics in 34 cases, NSAIDs in 23 cases, and other drugs in four patients. Fifty-two of the patients received steroids as opposed to nine who did not. The steroid-treated group had a significantly lower serum creatinine at final follow-up, and more patients in the untreated group remained in stage 5 chronic kidney disease. Of the steroid-treated patients, those treated more quickly after discontinuing the offending medication had a higher percent with complete recovery of renal function than did those treated later. Although neither randomized nor placebo-controlled, this study provides additional evidence both for a role of corticosteroids in recovery from AIN and for a role of early steroid treatment in leading to more complete recovery and prevention of chronic kidney disease [2].

Based on these limited data, some clinicians have recommended use of corticosteroids for most patients with AIN without clear contraindications [1, 2]. Therapy has included

either daily prednisone (60 mg daily) or alternate day prednisone (120 mg every other day) or several daily pulses of IV methylprednisolone (500–1,000 mg) followed by daily or every other day prednisone for up to 4–6 weeks. In NSAID-related AIN with and without the nephrotic syndrome corticosteroid therapy has been successful in treating the acute kidney injury and reversing the proteinuria. While some patients have experienced dramatic improvement of the acute renal dysfunction after steroid therapy, until recently it has been unclear whether all patients should be treated and how promptly therapy should be initiated.

Recent data support the short-term use of corticosteroids both to speed recovery of acute renal failure and to promote improved residual renal function in medication-related AIN. Of course, the risks of immunosuppressive treatment must be weighed against potential benefits. For example, in patients with AIN related to antimicrobials, steroid therapy may not be considered beneficial until infections are adequately controlled. Immunosuppressives such as oral or intravenous cyclophosphamide, cyclosporine, as well as plasmapheresis have rarely been used in patients with medication-related AIN [114, 115]. Although there is some animal data supporting their use, there is insufficient human data to recommend them [1, 2, 116]. Recently one study used mycophenolate mofetil (MMF) in a group of eight patients with steroid-dependent or steroid-intolerant AIN with good results [117]. Corticosteroids should still be considered the first-line therapy since MMF was used in a select group of patients and long-term follow-up and repeat biopsies were not performed.

AIN associated with systemic infections usually resolves with appropriate antimicrobial treatment of the underlying infection [1–10]. In the recent literature, this lesion has been reported less frequently than medication-related AIN perhaps due to the widespread use of antibiotics. The risks of a short course of corticosteroid therapy in this population with ongoing infections must be weighed carefully. Children with idiopathic AIN typically experience complete recovery of renal function either spontaneously or with steroid therapy. This is especially true of those with TINU syndrome. Repeat biopsies have often shown total resolution of the inflammatory infiltrates and only rarely is there significant residual fibrosis. It is unclear whether adults fare as well and whether corticosteroid therapy is necessary for good recovery of long-term function in this population [1, 56, 57]. Again, there is only limited data on the treatment of idiopathic AIN or TINU syndrome with immunosuppressive medications other than corticosteroids. AIN related to sarcoidosis and Sjogren's syndrome often responds to corticosteroid therapy. There are only anecdotal reports of the use of mycophenolate or rituximab in the treatment of Sjogren's-related interstitial nephritis.

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Chronic Interstitial Nephritis

24

Rowan G. Walker, Timothy D. Hewitson, and Gavin J. Becker

Introduction

The renal interstitium consists of the space between tubules, glomeruli and vessels. Chronic tubulointerstitial nephritis is characterised histologically by tubular cell damage with interstitial changes consisting of infiltration with mononuclear cells (macrophages, lymphocytes and fibroblasts) in a matrix which is expanded with increased amounts of collagen (particularly collagens I and II, which are involved in tissue fibrosis), proteoglycans and fluid. Chronic tubulointerstitial nephritis is a common final pathway towards chronic renal failure regardless of whether the primary insult is glomerular, as in glomerulonephritis or diabetes, or vascular, as in hypertension or renal ischaemia, or directly affects the tubulointerstitium. Histopathological studies have also repeatedly shown that tubulointerstitial pathology correlates better with the decline in renal function than any other pathology [1–3] and also predicts outcome [4].

The terms chronic interstitial nephritis and chronic tubulointerstitial nephritis (TIN) are effectively synonymous. In this chapter we will concentrate on diseases where the primary target of the insult is believed to be the tubular interstitium. Two important entities, reflux nephropathy and uric acid nephropathy, are addressed in separate chapters.

Chronic TIN has been relatively neglected in terms of diagnosis and therapy, in comparison with the more substantial advances that have been made in glomerular diseases and in the vasculopathies. It is important to recognise that addressing the mechanisms, which promote glomerular and vascular disease, will also benefit patients with chronic tubulointerstitial nephritis. In diseases that primarily affect the tubulointerstitium such as reflux nephropathy, focal and segmental glomerulosclerosis (FSGS) and vascular sclerosis frequently occur along with clinical progressive renal failure and hypertension.

In turn, this secondary glomerulosclerosis resulting from glomerular dropout, hyperfiltration and proteinuria will lead to further tubular damage and TIN, while vascular sclerosis will result in tubular ischaemia, hence aggravating the chronic progressive chronic TIN.

Accordingly the therapies, which address chronic progressive diseases of all types, should be employed in all patients with chronic tubulointerstitial nephritis. These include blood pressure control and steps to ameliorate glomerular hyperfiltration and reduce proteinuria mainly centred on blockade of the renin–angiotensin–aldosterone system.

Pathogenesis

The Normal Renal Interstitium

The renal interstitium is comprised essentially of a loose extracellular matrix (ECM) of collagens (especially types I and III), proteoglycans and fluid, in which both resident cells and nonresident cells are dispersed. These cellular elements include matrix-producing fibroblasts, macrophages and dendritic cells. As the interstitial matrix is situated between the tubules and vessels, it is clearly important for both the structural and the functional integrity of the kidney. Any expansion to the width or alteration to the nature of the matrix will have the potential to have profound or important effects on fluid and electrolyte balance and on kidney function because the matrix structure and integrity affect such functions as diffusion of oxygen from the peritubular capillaries to the tubules and the transfer of solute and water (Fig. 24.1).

Mechanisms of Tubulointerstitial Injury

The tubulointerstitium may be injured in a variety of ways. Examples include toxic insults (e.g. heavy metals), drugs

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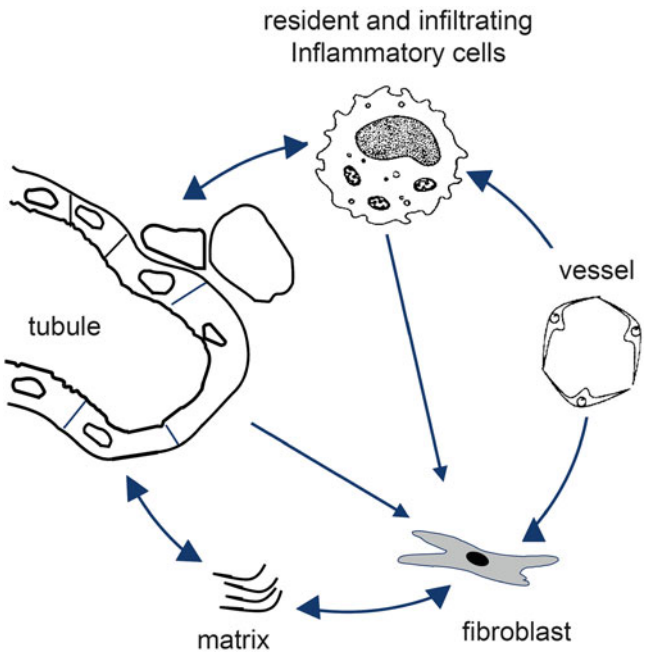

Fig. 24.1 Simplistic schematic representation of the general relationship between inflammatory cells (resident and infiltrating), vessels, fibroblasts, tubules and extracellular matrix in the normal kidney. The interrelation and interaction between these elements in response to a variety of insults may result in tubulointerstitial injury and chronic tubulointerstitial nephritis

(e.g. analgesics), crystals (e.g. calcium phosphate, uric acid), infections, obstruction, lipid deposition, immunologic mechanisms and acute elevations in capillary pressure and by ischemia [5–7]. Glomerular disease can also injure the tubulointerstitium through mechanisms that may involve glomerular cytokine release [8], direct effects of proteinuria on the tubules [9] or ischaemia [6], amongst others.

Experimental models of renal disease provide insights into possible processes responsible for these changes. Proteinuria has been found to be a major mechanism for initiating tubular injury; studies suggest that this may be due to cytokines and pro-oxidant molecules present in the proteinuric urine [9], as well as complement components that are then activated to form the complement membrane attack complex (C5b-9) which in turn can directly activate the tubular cells [10]. Ischaemia induced by intrarenal vasoconstriction and/or loss of peritubular capillaries also leads to tubular injury and fibrosis that is often most severe in the outer medulla and juxtamedullary regions where the kidney normally has borderline oxygen saturation [11].

Response to Trauma

While the type and size of injury are important, the kidney's response is surprisingly universal, resembling the generic wound healing process that occurs in all organs [12, 13].

The tubulointerstitial reaction to trauma begins, as elsewhere, with inflammation. In the case of physical trauma, platelet aggregation forms a hemostatic plug, acts as a provisional matrix and initiates an inflammatory response. Likewise, in immunological and toxic injuries, the immune response triggers the recruitment of inflammatory cells. Injuries to the tubules and peritubular capillaries are often primary events and result in the release of chemotactic substances (chemokines and lipid factors) and expression of leukocyte adhesion molecules (such as ICAM-1 and osteopontin). Tubular cells also express HLA antigens and secrete complement components and vasoactive mediators, which may further stimulate or attract inflammatory cells. Tubular cell proliferation and hypertrophy are part of an early attempt to regenerate and repair injured tissue.

Antibodies have been used to histochemically identify the infiltrating haematogenous cells in various forms of inflammation. Important cells involved in the process include haematogenous cells and connective tissue cells such as resident macrophages, lymphocytes and fibroblasts. In the early stages of acute inflammation, polymorphonuclear granulocytes (polymorphs) are the predominant infiltrating leucocytes. If the tissue is infected by pus-producing bacteria, there is sustained and enhanced polymorph infiltration. The intensity of this response subsides as polymorphs are lost though apoptosis. In chronic interstitial nephritis, the interstitial infiltrate is similar in nearly all forms of tubulointerstitial injury [14]. T lymphocytes form the majority of haematogenous cells present, with monocytes, B cells, natural killer cells and plasma cells making up the remainder. Within the T-lymphocyte population, there is variability in the relative proportion of T-cell subsets [14].

Monocyte infiltration into extravascular tissue is a key event. Following activation, macrophages also secrete a wide range of biologically active mediators which are involved in tissue destruction (proteases, oxygen-derived free radicals), chemotaxis (cytokines, chemokines), vascular haemodynamics (thromboxane A_2, prostaglandins) and production of matrix (growth factors, "remodelling" collagenases) [15]. There is, however, heterogeneity amongst macrophage populations, with different stimuli producing different activation states. Macrophage accumulation may persist with their numbers supplemented by local proliferation [16] and re-circulation [17].

What follows in chronic interstitial nephritis is an attempt at wound healing, a universal response to the inflammation that follows injury. It consists of a series of consecutive but overlapping events: these include cell proliferation, migration, extracellular matrix deposition (collectively known as fibrogenesis), resolution and remodelling.

Fibroblasts are the principal cells in fibrogenesis; selective deletion of fibroblasts by transfection with a so-called death gene is sufficient to prevent experimental renal interstitial fibrosis after injury [18]. The paucity of specific

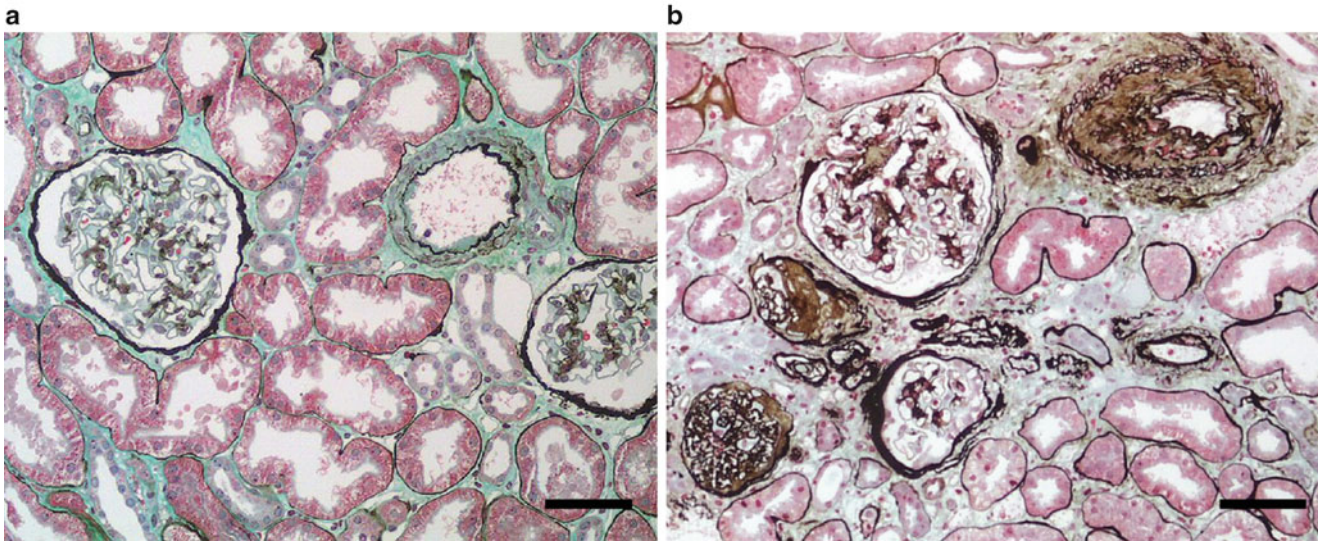

Fig. 24.2 (**a**) Normal kidney. (**b**) Tubulointerstitial disease with tubular atrophy and tubular basement membrane thickening, perivascular fibrosis, and glomerular sclerosis and interstitial fibrosis [Silver methenamine/Masson's trichrome stain; scale bar = 100 μm (microns)]

markers for fibroblasts, however, has meant that they have been difficult to identify. Activated fibroblasts (myofibroblasts), however, can be localised from their expression of alpha-smooth muscle actin, an actin isoform usually only found in vascular smooth muscle cells. The origin of (myo) fibroblasts in chronic tubulointerstitial nephritis is controversial. Rapid proliferation of resident fibroblasts seems to be an important early event in response to injury, mitogenesis exponentially increasing myofibroblasts at the site of renal injury within days [19]. Fibroblasts may also be derived from tubular epithelia in a process termed epithelial–mesenchymal transition [20], from circulating precursors [21], and after migration from adjacent perivascular areas. It remains to be seen if these processes are temporally distinct in fibrogenesis.

In each case, fibroblasts are the main source of the extracellular matrix proteins, mostly collagen and fibronectin, which provide structural integrity, while the myofibroblast provides force for wound contraction. Importantly, fibrogenesis continues as long as these cells persist in the wound, their removal or loss by apoptosis being part of the process that limits the extent of scarring.

The process of fibrogenesis is regulated by a plethora of cytokines and growth factors. Transforming growth factor-β (TGF-β) and platelet-derived growth factor (PDGF) have consistently been shown to be important in a variety of situations [5]. Conversely, a number of naturally occurring renoprotective factors act as a counterbalance and limit fibrogenesis. The most studied of these, hepatocyte growth factor (HGF) and bone morphogenic protein 7 (BMP7), downregulate profibrotic TGF-β signalling by interfering with signal transduction [22].

Finally, the matrix is subject to remodelling by collagenous and non-collagenous proteinases, including metalloproteinases and plasmin/plasminogen family. Although the functional significance of these proteases is poorly understood, it is widely thought that the balance between collagen synthesis and degradation is an important factor in determining the extent of matrix accumulation [23].

The inflammatory and wound healing response is therefore an attempt to repair injury and restore tissue function. However, the tubulointerstitium has a very limited capacity to regenerate after a prolonged insult. Whereas acute wounds go through this linear series of events, chronic non-healing wounds do not. Some areas of chronic wounds are in different phases at the same time, and progression to the next phase does not occur in the same co-ordinated manner. Loss of vascular perfusion in this area may result in hypoxia, further exacerbating the failure of repair. The end result of chronic interstitial nephritis is the failure of wound healing and accumulation of excess matrix, so-called pathological scarring (Fig. 24.2).

Epidemiology

Although chronic TIN characterises progressive renal disease of all aetiologies, idiopathic forms of chronic TIN account for relatively few patients reaching end-stage renal disease (ESRD). For instance, of the primary renal disease diagnoses in the incident cases ($n = 2{,}210$) recorded in 2005 in the Australia and New Zealand Dialysis and Transplant Registry, only 130 (6 %) had an "uncertain diagnosis". Of the remaining cases ($n = 1{,}880$), importantly only 57 cases (<3%) were recorded where the primary insult was attributed to the interstitium [24].

If one includes those particular conditions where the kidneys are macroscopically abnormal, such as reflux nephropathy

(3 %), analgesic nephropathy (3 %), obstructive uropathy (2 %) and cystic renal disease (7 %), and where the major histologic lesions are also "tubulointerstitial", this expands the proportion of TIN causes of ESRD towards 20 %. Reports from other areas of the world have also indicated quite a variable incidence of chronic tubulointerstitial disease in patients with ESRD, with a range from 42 % in Scotland to 3 % in the USA [25]. The marked variability in incidence may relate to differences in how diagnoses are made and in particular the use of renal biopsy, differences in aetiologies and toxin/drug exposure and different available measures to prevent or treat the various conditions.

Clinical Manifestations

Most patients with primary chronic TIN will exhibit proteinuria in the sub-nephrotic range. The urinary sediments will be relatively "inactive" in appearance. White blood cells and tubuloepithelial cells may be observed, and rarely white blood cell casts or tubuloepithelial cell casts may be present. If measured, a (partial) urinary concentrating defect is often apparent (perhaps with accompanying polyuria and nocturia), which may occasionally be severe enough to result in a nephrogenic diabetes insipidus. Other tubular defects occur, which may relate to the main site of injury, resulting in either proximal tubule defects (with aminoaciduria, glycosuria, phosphaturia, proximal renal tubular acidosis [RTA] or, rarely, Fanconi's syndrome) or distal tubular defects (especially type IV RTA). Although many diseases affecting the tubulointerstitium are also associated with the inability to conserve salt on a low-salt diet (resulting in salt-wasting syndromes), there is evidence that certain chronic tubulointerstitial diseases, particularly if they are associated with microvascular disease, may also be associated with a relative inability to excrete salt, especially when the patient has a relatively high-salt diet [26]. This apparent phenomenon probably explains why some tubulointerstitial diseases, such as lead nephropathy, gouty nephropathy, cyclosporine nephropathy and analgesic nephropathy, are frequently associated with salt-sensitive hypertension. Thus, in summary, primary or idiopathic and acquired tubulointerstitial diseases may as a consequence present problems of either salt excretion or salt conservation [26].

Pathology

Since progressive interstitial disease (particularly interstitial fibrosis) is part of a common final pathway for all causes of renal disease [1], the identification of any specific histologic characteristics in chronic TIN is problematic. In effect, all of the major histologic features of TIN are "nonspecific". The pathological diagnosis of primary TIN is often established rather by the absence of specific glomerular or vascular features, thus pointing to the tubulointerstitium as the likely area of primary insult in the pathologic process. Notably, as progressive tubulointerstitial injury occurs, the glomeruli may become secondarily involved, with hyalinosis and glomerular sclerosis occurring as a consequence of glomerular hypertension and so-called hyperfiltration injury.

Interstitial Fibrosis and Tubular Atrophy

Interstitial fibrosis is a fundamental histologic feature of chronic TIN and is characterised by an increased interstitial space relative to glomerular, vascular and tubular volume. This is due in large part to the accumulation of excessive connective tissue. The pattern may vary and be either focal or diffuse depending on the nature of the original insult.

Likewise, tubular atrophy is a nonspecific lesion. Tubular atrophy is observed frequently in areas of developing interstitial fibrosis. The tubular basement membrane (TBM) is often thickened; tubules may be dilated or atrophic and are often separated from each other by dense interstitial fibrosis. In some conditions (e.g. lithium-associated nephropathy), the degree of tubular atrophy can be correlated with a measurable renal functional abnormality such as impairment of urine-concentrating ability or impairment of urinary acidification.

Fibrosis on histology represents a disproportionate amount of ECM and may be due to either an increased deposition of ECM or the atrophy of renal parenchyma [27].

Glomerular Sclerosis

Glomerular sclerosis is accepted as occurring secondarily to the tubulointerstitial insult. The normal glomerular structure is replaced eventually by global fibrosis. In progressive renal disease with associated hyperfiltration, surviving glomeruli may be observed to be normal or show focal and segmental hyalinosis lesions, as might be the case in any renal disease under such conditions.

Occasionally glomeruli are structurally well preserved but are non-functional due to periglomerular fibrosis that effectively prevents outflow of the urine into the proximal tubules ("atubular glomeruli") [28]. Disruption of the tubular segments may be the result of tubulointerstitial injury and lead to nephron loss which can also occur, or tubulointerstitial injury may indirectly lead to periglomerular fibrosis that constricts the outflow of the urine from Bowman's space into the proximal tubule, again leading to the development of "atubular glomeruli".

The inflammatory cells of the renal interstitial infiltrate (macrophages and lymphocytes) may also release oxidants and vasoactive compounds such as angiotensin II that can lead to local vasoconstriction and modulate glomerular hemodynamics and impair sodium excretion. Indeed, there is evidence that they may contribute to the pathogenesis of some forms of salt-sensitive hypertension [29].

Cast Formation

The presence of casts within tubular lumina is also a common and nonspecific finding. The casts are usually strongly PAS positive and are largely composed of Tamm–Horsfall protein but may also contain desquamated tubuloepithelial cells embedded in the Tamm–Horsfall protein as well as other products such as immunoglobulins [30]. Occasionally the casts take on a homogenous waxy appearance in dilated tubules, suggesting a chronic obstructive element to the tubulointerstitial injury.

Interstitial Cell Infiltrate

Interstitial cell infiltrate is not a consistent finding. Cells in the infiltrate include lymphocytes and monocyte–macrophages. Depending on the aetiology of the tubulointerstitial disease, there may be other cell types such as neutrophils, eosinophils or plasma cells. Cells infiltrating into the interstitium are usually observed in patches (focal) but may be more diffuse.

Loss of Peritubular Capillaries

Again it has long been recognised that a loss of peritubular capillaries parallels progression. It is important to remember, however, that this reduction in capillaries may be both causative and a consequence of increased ECM accumulation.

Aetiologies

Analgesic Nephropathy

Definition and Epidemiology

Analgesic nephropathy was first recognised as a clinical entity as early as the 1950s in Swiss watch factory workers, who were habitually taking large amounts of over-the-counter analgesics, particularly combination analgesics containing phenacetin. Shortly thereafter analgesic nephropathy was recognised as an important cause of ESRD, especially in certain areas of Europe and Australia. In two Australian States, Queensland and New South Wales, as many as 25 % of patients with ESRD in the 1970s had analgesic nephropathy identified as the primary disease, and in the 1980s in Belgium the prevalence was nearly 20 % [31]. In contrast, analgesic nephropathy was substantially less frequently diagnosed in the USA, which presumably related to a lesser clinical awareness of the entity as well as the lower availability of compound analgesic mixtures, particularly those containing phenacetin.

The eventual recognition of compound analgesic mixtures particularly those containing phenacetin as a risk factor for renal disease importantly led to the prohibition of such compounds in a number of countries. Whereas the restriction of compound analgesic sales in Scandinavian countries Finland, Canada, and the UK led to variable effects on the incidence of ESRD, in Australia a very significant decline in the incidence of ESRD related to analgesic nephropathy was observed [32]. Following the introduction of the legislation, the number of new patients commencing dialysis fell significantly from 1980 (Fig. 24.3). Controversy has continued as

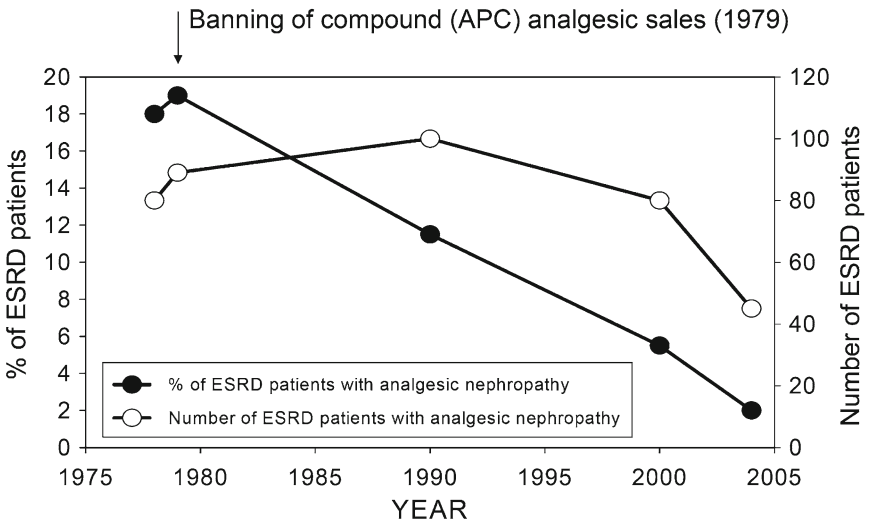

Fig. 24.3 Relationship between the incidences of ESRD attributed to analgesic nephropathy in Australia between 1978 and 2005. Graph compares the total number of patients with analgesic nephropathy as the diagnosis and the proportion of patients with ESRD for whom the diagnosis was analgesic nephropathy. Note that the sale of aspirin–phenacetin–codeine (APC) compounds was banned in 1979. Data from Australia and New Zealand Transplant Registry (ANZDATA) registry reports for 1979–2005, ANZDATA, Adelaide, Australia

to whether the reduction in incidence related to the banning of the compounds rather than the elimination of phenacetin from the mixtures [33].

The decline in patients requiring dialysis was most pronounced for those between the ages of 40 and 49 years. In the early 1990s, the percentage of hemodialysis patients with analgesic nephropathy as an incident disease was 9 % in Australia, 3 % in Europe and 0.8 % in the USA [31]. Analgesic nephropathy as a presenting cause of ESRD in Australia accounts currently for only 2.8 % of cases [24] (Fig. 24.3).

So in summary, the withdrawal of phenacetin was not associated with a complete eradication of analgesic nephropathy, but it was associated with a marked diminution in the incidence of ESRD attributed to analgesic nephropathy. Considerable evidence (experimental, pharmacologic and epidemiologic) [34, 35] evolved, suggesting that succeeding analgesic mixtures not containing phenacetin but containing compounds such as acetaminophen, pyrazolones and even aspirin [36] were capable of producing the typical kidney pathology lesions (see below).

Aetiologic Agents

The clinical syndrome of classical analgesic nephropathy resulted from the prolonged misuse or abuse of compound analgesics containing aspirin or antipyrine, combined with phenacetin, acetaminophen (paracetamol) or salicylamide, and caffeine or codeine. Lately, it has been suggested that a similar lesion can be induced with chronic nonsteroidal anti-inflammatory agent (NSAID) use. NSAIDs reported to induce analgesic nephropathy include alclofenac, antipyrine, benoxaprofen, fenoprofen, ibuprofen, indometacin (indomethacin), mefenamic acid and naproxen; there are also reports suggesting aspirin and acetaminophen may have contributory roles. While the role of non-phenacetin compounds in causing analgesic nephropathy remains controversial [31], there is some evidence that the chronic use of aspirin or acetaminophen may accelerate renal progression of any aetiology [37]. In this regard, it is important to recognise that agents that block intrarenal prostaglandin synthesis, such as NSAIDs, can be acutely nephrotoxic and can cause an acute but not always reversible decline in glomerular filtration rate (GFR) or acute tubular necrosis, especially in the setting of volume depletion, in the elderly and in diabetics [38–41]. Acute papillary necrosis may also occur particularly if large doses are ingested, and acute renal papillary necrosis induced by NSAIDS has certainly been demonstrated in animal studies [42]. NSAIDs may also be idiosyncratically associated with a minimal change-like lesion glomerular with acute presumably allergic TIN.

Establishing a link between NSAIDS and chronic kidney lesions is problematic. The data is scarce and epidemiological in nature. The studies have been criticised for poor study design, and the odds ratio for risk of end-stage renal disease varies from decreased risk (RR = 0.6) to markedly increased (RR = 10) depending on duration of exposure to NSAIDS [43, 44], age and gender and adjustment for other analgesics.

Because of the uncertainties surrounding toxicologic data and pathogenesis, it follows that it is difficult to establish the critical amounts and periods of intakes of the various analgesic agents required to produce analgesic nephropathy. Epidemiologic studies generally show a dose-dependent risk for developing analgesic nephropathy with compound analgesics, especially with those containing phenacetin, and it has been estimated that there is a 15- to 20-fold increased relative risk for the development of chronic renal disease when the total consumption of phenacetin exceeds 1,000 g [45]. For NSAIDS, an association was demonstrated between prolonged exposure (up to 26,000 doses) and the demonstration of renal papillary necrosis on imaging [46] but with only modest effects on kidney function.

Limitations of study design and of diagnosis have made the interpretation of case–control and other epidemiological studies [43, 47–49] highly problematic [50]. At the current time, it has not been possible either to establish a clear link between prolonged analgesic exposure and progression of underlying chronic kidney disease of any aetiology or to reach a clear understanding of the risk of end-stage kidney disease associated with analgesic use. Interestingly, despite the widespread perception that analgesic use may promote progressive CKD, a recent analysis from the Danish National Registry of over 6,600 ESRD patients reported that over one-third received NSAIDS for a median of 40 days in the 3 years before starting dialysis [51].

Pathogenesis and Pathology

The principal site of renal injury with chronic analgesic abuse is in the medulla and papillae, sites that are vulnerable because of the concentration of toxic metabolites built up by the countercurrent mechanism and because of the low oxygen tension present in these anatomical regions. The injury may relate to the net effects of several metabolites [52]. Phenacetin, for example, is converted to acetaminophen, which can deplete cells of glutathione and result in the generation of oxidative and alkylating metabolites. Aspirin [53, 54] and NSAIDs [55, 56] can result in the reduction of vasodilatory prostaglandins; caffeine may be metabolised to adenosine, with vasoconstrictive effects within the kidney. Collectively, these substances may lead to oxidant and ischemic injury to the medulla and papillae, an effect that is exacerbated in the setting of volume depletion [57]. Renal papillary necrosis with features of an ischemic infarct may develop, with atrophy of the overlying cortex leading to chronic interstitial changes.

The quintessential pathologic lesion of analgesic nephropathy is renal papillary necrosis [58], a coagulative necrosis

involving the medulla, including the loops of Henle, the vasa recta, the medullary interstitial cells and the collecting ducts. The cortical changes of chronic interstitial nephritis overlying the necrotic papilla are secondary and comprise tubular atrophy, interstitial fibrosis and a mononuclear cellular infiltrate. The presence of a golden-brown lipofuscin-like pigment in tubular cells, and the characteristic analgesic microangiopathy or capillary sclerosis, is highly indicative of an analgesic aetiology. Glomerular changes of focal glomerular sclerosis and hyalinosis associated with proteinuria are similar to those described in reflux-associated nephropathy and tend to be late features.

Clinical Features

The vast majority of patients are female [59] and have a history of chronic pain syndromes and other somatic complaints or symptoms suggestive of a broader analgesic syndrome [60]. An addictive and dependent personality trait characterised by introversion, psychoneurosis and an external locus of control has been reported, and an association with cigarette smoking has been noted. Peptic ulcer disease and gastrointestinal symptoms are also present in many patients. Denial of analgesic abuse is common.

The renal function abnormalities of analgesic nephropathy (other than a slowly progressive impairment of GFR) include impaired urine-concentrating ability (often an early defect possibly associated with polyuria), urinary acidification defects and impaired sodium conservation [54]. The urinalysis frequently shows pyuria with or without urinary tract infection, micro- or macrohaematuria may be present, and non-nephrotic range proteinuria. Proteinuria occurs in at least half of the patients [61] and increases progressively as GFR decreases with advanced disease. The proteinuria is of both tubular and glomerular origin, the latter as a consequence of secondary glomerular sclerosis that develops as renal function deteriorates. Hypertension is a very common clinical feature [54].

Although patients with any form of chronic kidney disease are at greatly increased risk of cardiovascular mortality, cardiovascular disease, fatal or nonfatal myocardial infarction, heart failure or stroke, patients with analgesic nephropathy may be at even greater risk for premature atherosclerosis [62]. Atheromatous renal artery stenosis is also more common in analgesic abusers. The increased atherogenic tendency probably relates to multiple factors, including hypertension, smoking and hyperlipidemia, and perhaps the formation of atherogenic oxidised low-density lipoproteins under the oxidative influence of phenacetin [63].

An important association of analgesic nephropathy is the increased risk of transitional cell carcinoma of the uroepithelium (renal pelvis, ureter, bladder and proximal urethra) [64]. This should be especially considered in patients with gross haematuria.

Diagnosis

The disease is suggested by a history of analgesic abuse coupled with urographic, sonographic and/or tomographic findings showing papillary calcifications and bilateral atrophic but often asymmetric kidneys with irregular contours ("bumpy"). The imaging findings are not always easily distinguishable from those of reflux-associated nephropathy. Papillary calcification is a very helpful diagnostic feature and may be best detected by a noncontrast CT scan [65]. The differential diagnosis of papillary necrosis, the key underlying pathology in analgesic nephropathy, includes diabetic nephropathy, sickle-cell nephropathy and, very rarely, obstructive uropathy and reflux-associated nephropathy. Papillary deposits of calcium can also be seen in medullary sponge kidney and all forms of nephrocalcinosis.

Treatment

Management consists of avoiding phenacetin-containing analgesic mixtures (which are largely not available now) and reducing or ideally completely stopping ingestion of other analgesic medications [66, 67] as no specific treatments are available. Management is essentially similar to that for all patients with chronic kidney disease, including careful monitoring of blood pressure and the use of renoprotective agents such as ACE inhibitors for those with impaired kidney function (stage 3 CKD with an eGFR <60 mL/min/1.73 m^2). Because of the increased incidence of uroepithelial tumours, long-term follow-up is necessary. A cause of flank pain may be obstruction due to detached (necrotic) papillae lodging in the ureter. In the presence of infection, this complication can be a life-threatening clinical scenario.

Lithium Nephropathy

In the mid-1970s, it was recognised that long-term administration of lithium salts to patients with severe unipolar and bipolar affective illness may be associated with the development of chronic kidney disease. Several different forms of renal injury were identified [68]. It should be emphasised, however, that because the therapeutic index of the lithium ion is narrow, the most important complication of short- or long-term lithium administration remains the development of acute lithium intoxication. The kidneys are effectively exclusively responsible for the excretion of lithium and, therefore, are pivotal to the development of this important complication.

Lithium-Associated Polyuria, Polydipsia and Nephrogenic Diabetes Insipidus

Lithium ingestion is associated with the development of resistance to vasopressin (antidiuretic hormone), resulting in polyuria (usually defined as a urine volume exceeding

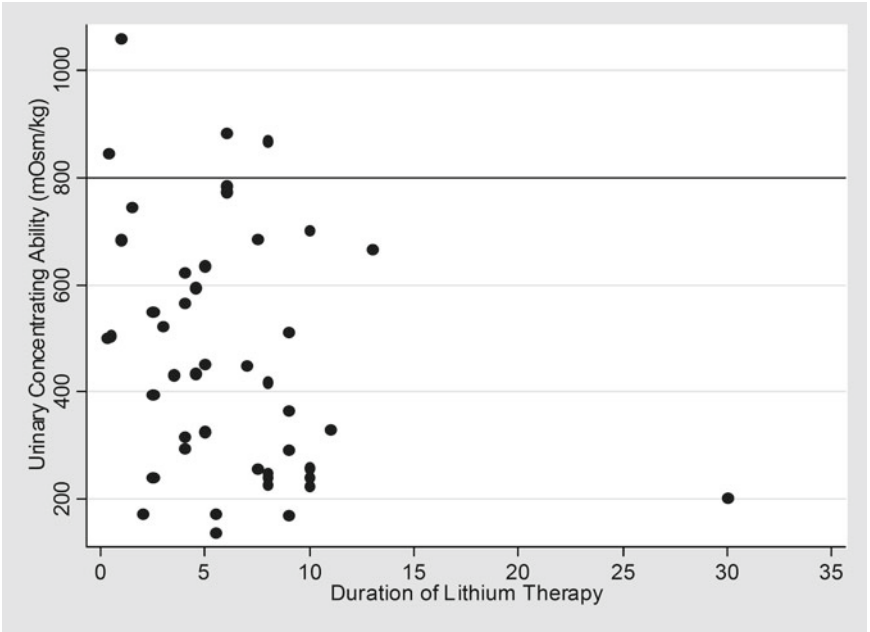

Fig. 24.4 Relationship between urinary concentrating ability (measured after overnight fluid restriction in mOsm/kg) to duration of lithium therapy in 46 of 50 patients on long-term maintenance lithium for unipolar and bipolar affective disorders. Note that only very few patients with short durations of lithium therapy achieved urinary concentrations above 800 mOsm/kg. Correlation coefficient; $r = -0.5200$ (p-value < 0.001). Adapted from Walker [68], with permission

3,000 mL/24 h) and polydipsia in up to 40 % of patients. Lithium is the most common cause of iatrogenic diabetes insipidus [69–74]. Lithium accumulates in the collecting tubule cells after entering these cells through sodium channels in the luminal membrane. It then interferes with the ability of vasopressin to increase water reabsorption [75] by inhibiting adenylate cyclase and hence cyclic AMP (cAMP) production and also by decreasing the apical membrane expression of aquaporin 2 [76], the collecting tubule water channel. Although this common side effect is widely regarded as reversible, lithium-induced impairment of urine-concentrating ability was shown in some patients to persist many months after cessation of lithium therapy. Interestingly, lithium is also less commonly a cause of hypercalcemia. This complication is a consequence of an increased release of parathyroid hormone (PTH) and is associated with local (parathyroid) cAMP production. Persistent hypercalcemia could potentially exaggerate the tubular concentrating defect and contribute to the development of chronic interstitial nephritis in lithium-treated patients (see section "Hypercalcemic Nephropathy").

A lithium-induced impairment in distal urinary acidification [type 1 (distal) RTA] may be seen in parallel with nephrogenic diabetes insipidus with lithium therapy. A lithium-induced impairment in distal urinary acidification [type 1 (distal) RTA] is a partial functional defect that is rarely of clinical importance. A lithium-induced decrease in the activity of the H+ ATPase pump in the distal nephron (collecting ducts) may be the main cause of this defect.

Acute Lithium Toxicity

Acute impairment of GFR and tubular injury (acute tubular necrosis) associated with episodes of acute lithium intoxication have for many years been recognised potential complications of lithium therapy. The risk is greatly increased in the presence of volume depletion [77].

Histologically, the most distinctive change associated with lithium occurs in the distal convoluted tubules where there is ballooning, swelling and vacuolation of the cell cytoplasm, accompanied by strands and granules of periodic acid-Schiff (PAS)-positive staining material (glycogen). This lesion is not only acute but also quite reversible [78, 79].

Chronic Lithium Nephropathy

A new observation in the 1970s was the recognition that, in lithium-treated patients, the polyuria (and polydipsia) was not always reversible [80–86].

A large number of studies subsequently demonstrated a correlation between the duration of lithium therapy and persistent impairment of urine-concentrating ability [68] (Fig. 24.4). Biopsies in these patients subsequently revealed primarily a focal chronic interstitial nephropathy with interstitial fibrosis and tubular atrophy, and more recently the degree of associated glomerular sclerosis has been recognised [87].

Another characteristic finding is the presence of microcystic changes in the distal tubule. More recently, these cystic changes have been well demonstrated using MR imaging [88]. Patients at particular risk seemed to be those with severely impaired urine-concentrating defects and those with a history of repeated episodes of acute lithium intoxication.

Interestingly, most patients with chronic lithium nephropathy have a relatively well-preserved GFR [73, 89–92] in relation to the distal tubular defect(s). Although a small number of patients have chronic kidney disease with renal impairment, in most studies there is no correlation between GFR

and the duration of lithium therapy (unlike with the concentrating defect). At least in one study, the slow rate of deterioration of renal function and final creatinine clearance was related to duration of therapy [93]. In patients taking lithium who do not have repeated episodes of acute lithium intoxication, the role of lithium in chronic interstitial disease remains controversial. Nonetheless, the number of reported cases of either serious renal insufficiency or chronic kidney disease continues to cause concern as there are clearly cases of elevated serum creatinine (>177 μmol/L or >2.0 mg/dL) with no obvious cause other than the long-term ingestion of lithium. In Lepkifker et al.'s review [94], it was estimated that 20 % of patients on long-term lithium develop renal insufficiency [70, 94–96]. Controversy also persists because although differences in urinary concentrating ability between patients on lithium and patients not on lithium are apparent, patients with affective disorders do not concentrate urine normally [97] (Fig. 24.5). Furthermore, other similar degrees of functional change [97, 98] and chronic interstitial change, including interstitial fibrosis, have been noted in patients with affective disorders not being treated with lithium [99], although the characteristic microcystic dilatation of the distal tubules is generally absent. The role of other psychotropic medication in this setting is unknown. If chronic TIN does occur in association with long-term stable maintenance therapy with lithium, it probably requires many years of therapy [93, 100, 101] and the consequent degree of renal insufficiency, as indicated above, is likely to be relatively mild. Perhaps the most important factor in preservation of renal function and histology is prevention of episodes of acute intoxication.

Proteinuria is not a particular feature of lithium-associated nephropathy, but proteinuria has certainly been reported in association with lithium-induced minimal lesion nephrotic syndrome and focal glomerular sclerosis [102, 103].

Management of Lithium-Associated Renal Disease

After eliminating other possible causes of polyuria and polydipsia [104], especially psychogenic polydipsia (or potomania), one should first consider reducing the dose of lithium, aiming for therapeutic concentrations (12-h serum lithium concentrations) of 0.4–0.6 mmol/L (0.28–0.42 mg/dL) or converting to a single daily dose [105] aiming for a lower trough serum lithium concentrations. For prophylaxis, levels of 0.4–0.6 mmol/L (12 h standard trough serum lithium concentrations) may be sub-therapeutic in a proportion of patients [106], compared to recommended levels of at least 0.5–0.8 mmol/L (identical in patients with bipolar and unipolar illness) [107], but also likely to be associated with fewer side effects. Values of 0.8–1.0 mmol/L are likely to have marked efficacy but many side effects. The potassium-sparing diuretic amiloride can also reduce the urine output by up to 50 % and has the added advantage of blocking lithium uptake through the sodium channels in the collecting duct [108]. Amiloride is most effective when the concentrating defect is mild and potentially reversible and has generally been disappointing in patients with severe defects in urine-concentrating ability. Although thiazide diuretics are effective in treating lithium-induced polyuria, they should be avoided as they pose risks for the induction of acute lithium intoxication because the resultant volume contraction causes an increase in sodium and lithium reabsorption in the proximal tubule. Avoidance of NSAIDs if possible is also recommended.

A practical approach to the patient on long-term lithium treatment should include a regular (at least yearly) estimation of renal function measured as serum creatinine (or estimated GFR), estimation of spot urine albumin/creatinine ratio and measurement of 24-h urine volume [95, 107, 109, 110]. Because progressive renal injury with a reduced GFR (or raised serum creatinine) in a patient without prior acute lithium intoxication is relatively unusual, a raised serum creatinine should probably in the first instance be treated with dose reduction. For persistently elevated serum creatinine estimations, a renal biopsy could be considered, although the result would not often lead to a recommendation to cease lithium entirely. At all times, the risk of discontinuation of lithium in a patient with a severe unipolar or bipolar affective disorder needs to be measured against the minimal risk of progressive renal injury. As for analgesic nephropathy, the management of lithium-associated nephropathy with chronic kidney disease is similar to that for all patients with chronic kidney disease and includes careful monitoring of blood pressure and the use of renoprotective agents such as ACE inhibitors for those with impaired kidney function (stage 3 CKD with an eGFR <60 mL/min/1.73 m^2). Care is required when introducing any new agent to a patient taking lithium to check serum lithium estimations because of the potential of some agents to acutely alter GFR and/or renal clearance of lithium.

Also, a range of other therapies exist for bipolar and unipolar affective disorders including anticonvulsants such as carbamazepine, valproate and lamotrigine, which may be alternative medications in cases of severe lithium-induced renal symptoms or renal insufficiency.

Heavy Metals

Lead Nephropathy

Acute lead intoxication is rare but may present with abdominal pain, encephalopathy, haemolytic anaemia, peripheral neuropathy [111, 112] and proximal tubular dysfunction (as manifested by either a proximal RTA or Fanconi's syndrome) [113]. In contrast, chronic lead intoxication, which may develop insidiously and even follow decades after acute lead

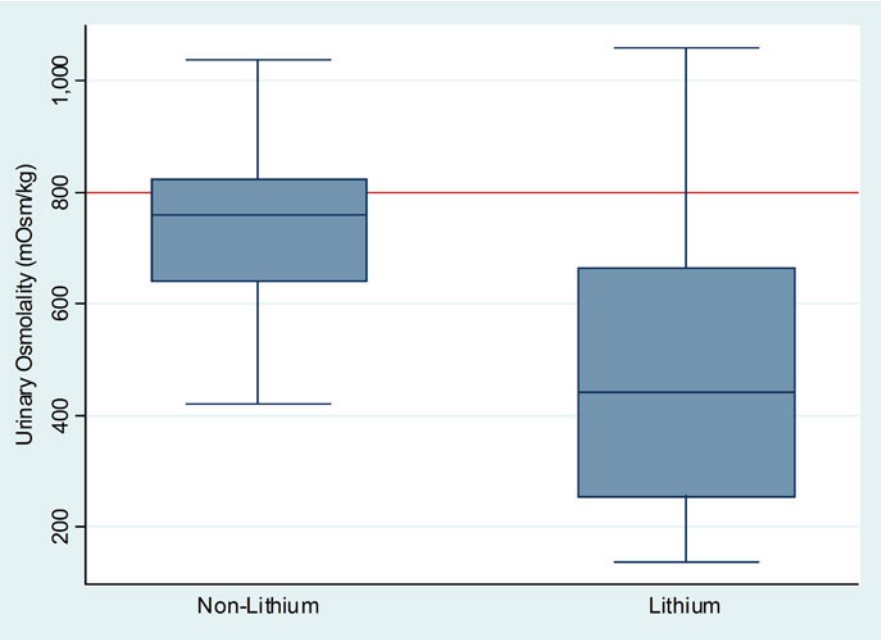

Fig. 24.5 Box plot quartiles of urinary concentrating ability (measured after overnight fluid restriction in mOsm/kg) in 46 (of 50) patients (Lithium) on long-term maintenance lithium for unipolar and bipolar affective disorders compared to 27 (of 32) patients with affective disorders not on lithium (non-lithium). Note the number of non-lithium patients with urinary concentrations below 800 mOsm/kg (adapted from Walker RG, Doctoral Thesis "*Lithium Nephrotoxicity*", 1986, University of Melbourne)

poisoning [114], is much more subtle with impaired renal function, episodic gout ("saturnine") and hypertension being the main manifestations [115]. Occasionally, patients may exhibit other signs of chronic lead intoxication including peripheral motor neuropathies, anaemia with "basophilic" stippling and perivascular cerebellar calcifications [116]. Individuals known to be at risk from lead intoxication include children exposed to lead-based paints, adults drinking "moonshine" and residents living close to lead-smelting factories and other industries expelling lead into the environment [117, 118].

Tubular functional disturbances are usually the earliest manifestations of chronic lead nephropathy leading to hyperuricaemia (resulting from diminished urate secretion), aminoaciduria or renal glycosuria. Chronic exposure can ultimately lead to a chronic interstitial nephritis. The pathogenesis of the renal disease may be related to the proximal tubule reabsorption of filtered lead, with subsequent accumulation in the proximal tubular cells. Histologically, proximal tubular injury, with intranuclear inclusion bodies composed of a lead–protein complex, may be observed initially but with prolonged lead exposure the major histologic features of chronic interstitial nephropathy: progressive tubular atrophy and interstitial fibrosis associated with chronic renal insufficiency occur in the presence of a relatively normal urinalysis, but associated with hypertension, and gout [119]. Populations with high rates of diabetes, hypertension and/or chronic kidney disease appear to be at greater risk of developing adverse renal effects from lead [118]. Ideally, all patients with significant hyperuricaemia and renal insufficiency should have a history of occupational lead exposure excluded [120], as confusion may occur with chronic urate nephropathy in which urate deposits (tophi) may form in the renal interstitium.

Several epidemiologic studies in the general population have also suggested that low-level lead exposure may be associated with chronic kidney disease with renal impairment and/or hypertension. In these studies, an increase in lead burden has been shown, but no causal association has been established [121–123], leading to some investigators to question whether chronic lead exposure truly promotes renal failure in recent years [124].

Diagnosis of chronic lead intoxication can be made by the calcium disodium edetate ($CaNa_2$ EDTA) lead chelation test or K X-ray fluorescence [125, 126].

Treatment

In the industrial and occupational settings including foundry workers and individuals working with lead-based paints and glazes, preventative measures to minimise exposure and low-level absorption are essential. In established cases, removal of the source of lead is obviously important. Otherwise, treatment involves the use of infusions of $CaNa_2$ EDTA. The likelihood of a satisfactory response to $CaNa_2$ EDTA chelation will be influenced by the degree of already established interstitial fibrosis.

Cadmium

Cadmium is a metal used in a variety of industries, especially in the manufacturing of alloys and electrical equipment. Historically, a major outbreak of cadmium toxicity including nephrotoxicity occurred in Japan as a result of industrial

contamination of the Jinzu River in the Toyama prefecture, leading to contamination of rice crops. The disease, named *itai-itai* [127, 128] or "ouch-ouch", primarily affected older women and was characterised by proximal tubular dysfunction [129], anaemia, severe osteomalacia and, rarely, progressive chronic interstitial disease [130]. Functional changes accompanying cadmium nephropathy include proteinuria, enzymuria, aminoaciduria, glycosuria, polyuria, hypercalciuria and increased urinary uric acid and of course urinary cadmium [131]. Cadmium workers also have a higher incidence of hypertension and renal calculi (the latter caused by hypercalciuria). Cadmium accumulates in renal tubular lining cells [132] bound to a small (30 % cystine) protein *metallothionein*, which actually protects against nephrotoxicity by binding cadmium in a nontoxic form. Chronic interstitial fibrosis and renal functional abnormalities have been reported to occur when cadmium levels in the renal cortex exceed a concentration of ~200 μg/g [133].

Prevention is the only known effective treatment. Interestingly, exposure to cadmium industrially has been associated with an increased relative risk of developing lung cancer [134] and also renal cell carcinoma [135].

Arsenic

Arsenic, present in insecticides, weed killers, wallpaper and paints, rarely causes renal disease. Chronic arsenic toxicity manifests most commonly as sensory and motor neuropathies, distal extremity hyperkeratosis, palmar desquamation, diarrhoea and nausea, Aldrich–Mees lines (linear white bands on the nails) and anaemia. However, both proximal tubular dysfunction [renal tubular acidosis (RTA)] and eventually chronic interstitial fibrosis may occur [136]. The diagnosis is established by demonstrating elevated urinary arsenic levels.

Radiation Nephritis

In "*acute radiation nephritis*", oedema, hypertension (including occasionally accelerated hypertension), dyspnea, headache and nocturia are possible manifestations. Proteinuria may be marked, whereas the urine sediment is relatively unchanged. Anaemia (normochromic, normocytic or microangiopathic) is likely to be present as is mild-to-moderate impairment of kidney function. Progression to a "chronic" form of radiation nephritis may be a consequence of acute radiation injury, or "*chronic radiation nephritis*" may present as a more indolent process, with proteinuria and progressive chronic kidney disease and even ESRD. The incidence of new cases appears to be fortunately low. Considering all cases in the Australian and New Zealand Dialysis and Transplant Registry (ANZDATA Registry) in the last 10 years, there have been less than two per annum [24].

The precise pathogenetic mechanism of radiation nephritis remains obscure. Histologic features are similar between human and laboratory animals [137], and degenerative, inflammatory and thrombotic changes that appear early advance ultimately to severe glomerular sclerosis, tubular atrophy with associated thickening of the TBM and interstitial fibrosis. Interstitial disease may occur in the absence of glomerular lesions, which vary from milder mesangial cell changes (increased cells, increased matrix and mesangiolysis) to frank glomerular capillary and arteriolar necrosis [138], which may include evidence of thrombosis [139]. Capillary walls are thickened and may typically show "splitting". Vessels may show patchy intimal proliferation and thickening, intimal fibrosis and fibrinoid necrosis [140]. Severe disease is characterised by progressive interstitial fibrosis [141] and the presence of interstitial inflammatory cells.

The similarities in certain pathologic characteristics of radiation nephritis with those of haemolytic uremic syndrome and thrombotic thrombocytopenic purpura (HUS/TTP), and the prevalence of the lesions of HUS and associated deterioration in renal function in bone marrow transplant recipients treated with combined radiotherapy and chemotherapy lead to speculation that endothelial cell injury, possibly leading to local intravascular coagulation, is one possible pathogenetic mechanism. Radiation nephritis has also been seen in bone marrow transplant recipients following total body irradiation and particularly in patients receiving cyclosporine [142]. Whether cyclosporine A enhances the development of radiation nephropathy is not established.

Prevention is the major therapeutic approach to radiation nephritis. The risk of developing this condition may possibly be minimised by proper shielding of the kidneys and/or by fractionating the total body irradiation into several small doses over several days. Minimising the total dose [143] of irradiation to the kidney to less than 20–30 Gy (1 Gy = 100 rad) is recommended, but no specific treatment is available for established radiation nephritis.

Balkan Nephropathy

Aetiology and Pathogenesis

Balkan (endemic) nephropathy is a slowly progressive form of chronic TIN found almost exclusively in some well-defined areas in Croatia, Serbia, Bosnia, Bulgaria and Romania [144]. Balkan nephropathy is further geographically localised to a few areas along the Danube's tributaries in topographical regions characterised by plains and low hills that have generally a high humidity and high rainfall. Although described originally, more than 50 years ago [145], the precise aetiology of Balkan nephropathy has never been established. A variety of factors have been either considered or implicated, including genetic factors, environmental

agents (such as trace elements [146] and fungal and plant toxins) and immune disturbances.

It has been argued that the inheritance of Balkan nephropathy, the familial nature of which has also been recognised for many years, is more likely to be polygenic and that the environment might markedly influence the manifestations of the genotype. In the endemic areas, likely affected individuals are villagers, but several members of one family (or household) across one or more generations may be affected with many unaffected households being present in the same area. An early Bulgarian genetic study favoured an autosomal dominant form of inheritance [147]. Later chromosomal breakage and spontaneous aberration studies demonstrated linkage to the 3q25 band on chromosome 3 [148–150], in both affected individuals and in healthy relatives of individuals with Balkan nephropathy considered to be at high risk.

More indirectly, genetic variations in metabolic susceptibility such as the ability to metabolise the drug debrisoquine, a partial deficiency of lecithin–cholesterol acyltransferase (LCAT) (a rare cause of progressive chronic kidney disease) demonstrated in the healthy relatives of patients with Balkan nephropathy, and low delta-aminolevulinate dehydratase activity in Balkan nephropathy sufferers and their healthy relatives add support to the possible genetic nature of this condition or to the genetic predisposition to the condition in the presence of an environmental toxin. Any possible associations of these abnormalities and Balkan nephropathy remain unconfirmed [151–153].

A search for potential viral aetiologies for Balkan nephropathy has also not been fruitful. Equally, the disease also appears not to be mediated by any primary immune-mediated disease process. Despite significant parenchymal damage, interstitial inflammatory cells are not prominent and immune reactants (such as C3, IgG and IgM) are only focally present in the mesangium, along the TBM, and in the walls of blood vessels.

Various trace elements either in excess (lead, cadmium, manganese, copper and silica) or in deficiency (selenium) [154–158] also do not appear likely in the pathogenesis.

Environmental toxins, geochemical [159, 160], fungal and plant, have also been investigated as possible contributors to the pathogenic pathway. Pliocene-age coals, prevalent in endemic Balkan nephropathy areas [161] and which potentially produce soluble carcinogenic polycyclic aromatic hydrocarbons and aromatic amines, provide a theoretical link between a toxic nephropathy and uroepithelial tumours frequently observed in the full Balkan nephropathy syndrome. Ochratoxin A, a mycotoxin, is a product of the fungus Penicillium and has in particular been implicated [162, 163] because of its combined nephrotoxic and oncogenic properties, but proof remains still less than convincing that mycotoxin-induced apoptosis in animals has led to the postulate that apoptosis may be a cause of the tubular atrophy and subsequent chronic interstitial injury in Balkan nephropathy [164–166].

Perhaps the most exciting association has been with contamination of wheat flour with aristolochic acids, which were derived from seeds of Aristolochia clematis. Aristolochic acids present in Chinese herbal preparations have been linked to chronic tubulointerstitial disease in Belgium (from Aristolochia fangchi) [167], and Australia [168], and to a Fanconi's syndrome in Japan (from Aristolochia manshuriensis) [169]. The clinical and histologic features from the interstitial disease associated with the Belgium cases (termed Chinese herbal nephropathy) are rather similar to Balkan nephropathy (see below) [170–174].

Clinical Presentation and Diagnosis

Apart from the epidemiologic features described above, the diagnosis of Balkan nephropathy is based on clinical, functional and histologic characteristics. Balkan nephropathy is a tubulointerstitial kidney disease progressing slowly to ESRD, usually in the fifth or sixth decade of life. Normochromic normocytic anaemia disproportionate to the degree of renal impairment is an early feature, whereas salt retention and/or hypertension occurs in the late stages.

Proteinuria is usually mild but like other progressive nephropathies may increase in advanced stages of the disease [175]. The urinary sediment is usually unremarkable in Balkan nephropathy, with occasional mild increases in urinary leukocytes and erythrocytes. Macroscopic haematuria suggests the presence of a uroepithelial carcinoma (bladder, ureter or renal pelvis) [176, 177]. The increased incidence of tumours of this type is similar to that observed in both analgesic nephropathy and Chinese herbs nephropathy.

Abnormal renal tubular function such as impaired acidification, decreased ammonia and uric acid excretion, and urine-concentrating defects with renal salt wasting may precede the reduction in GFR. A symmetric, smooth reduction of the kidney size is characteristic which is an interesting contrast to the irregular outlines of the kidneys in analgesic nephropathy, another condition characterised by a high incidence of uroepithelial tumours but where the key pathology is papillary necrosis.

Proximal tubular functional abnormalities with normal GFRs accompanying tubular lesions on renal biopsy are characteristic. Renal histology in the early stage of disease shows predominantly focal tubular atrophy, interstitial oedema and sclerosis, and sometimes mononuclear cell infiltrates. Progression of disease is associated with marked tubular atrophy and interstitial fibrosis. Major changes in the interstitial vasculature (capillaries), such as endothelial cell oedema with consequent narrowing of the capillary lumen, have been found in the post-glomerular vascular network. These changes correlate with the development of interstitial oedema and fibrosis. Early glomerular changes

are mild and focal, with moderately increased numbers of mesangial and endothelial cells. In the advanced stages of the disease, most glomeruli become more globally hyalinised and/or sclerotic.

Treatment

The treatment of Balkan nephropathy is primarily supportive as the unknown aetiology means there is no effective preventive measure. For advancing disease with proteinuria and/or hypertension or reduced GFR, ACE inhibitors are recommended.

Chinese Herbs Nephropathy (Aristolochic Acid Nephropathy)

Regular intake of Chinese medicinal herbs containing aristolochic acids is now recognised as a cause of tubulointerstitial fibrosis [178] and a risk factor for urothelial cancer [179].

In Belgium, in 1992, an outbreak of a rapidly progressive renal failure associated with progressive tubulointerstitial fibrosis occurred. It was found to be associated with Chinese herbs that were used as a part of a "slimming" regimen and that one of the herbs (Stephania) had been inadvertently replaced with Aristolochia fangchi [172]. Affected patients were found to be predominantly young and middle-aged women [171] with at least a 6-month history of ingesting the herbs. The renal failure was often discovered incidentally and was characterised by progressive loss of renal function over several months. The histologic lesion is strikingly similar to that observed in Balkan nephropathy, and like Balkan nephropathy, the condition is also associated with a marked increased incidence of uroepithelial tumours.

There is excellent evidence that the aristolochic acids are responsible for the tubulointerstitial injury [180].

First, there is a direct relationship between the dose of Aristolochic herbs ingested and the degree of renal failure observed in these patients [173, 174]. Second, the administration of aristolochic acid to experimental animals has also resulted in tubulointerstitial disease and uroepithelial atypia [181].

Although most patients are managed conservatively, one pilot study suggests that progression of renal failure could be slowed by prednisone therapy (1 mg/kg for 1 month followed by a slow tapering of the dosage) [182].

Hypokalaemic Nephropathy

For several decades, it has been known that impaired urine-concentrating ability characterised symptomatically by nocturia, polyuria, and polydipsia may be a feature of chronic hypokalaemia [183] usually when the plasma potassium concentration is consistently <3.0 mmol/L. The renal defect, which develops gradually over several weeks, is associated with decreased collecting tubule responsiveness to vasopressin, possibly occurring in part through decreased expression of aquaporin 2, the water channel that fuses with the luminal membrane under the influence of vasopressin. Hypokalaemia also increases the tubular production of ammonia and ammonium ions [184] and enhances bicarbonate reabsorption. Mild-to-moderate hypokalaemia can impair the ability to excrete a sodium load. An opposite effect may be seen with severe hypokalaemia (plasma potassium concentration usually 2 mmol/L). In this setting, maximum sodium chloride reabsorption may be impaired; the exact mechanism is unclear.

Histologic Abnormalities

Chronic potassium depletion may ultimately result in chronic interstitial nephropathy. However initially, characteristic histologic lesions are vacuoles in the cytoplasm of epithelial cells of the proximal tubule and occasionally the distal tubule. It is generally considered that this abnormality generally requires at least 1 month to develop but is essentially reversible with potassium repletion. Prolonged hypokalaemia may lead to more severe changes, predominantly in the renal medulla, including interstitial fibrosis, tubular atrophy and cyst formation. Experimental evidence suggests that this may relate to intrarenal vasoconstriction induced by hypokalaemia; indeed, both angiotensin receptor antagonists and endothelin receptor antagonists can partially ameliorate the injury [185]. The genesis of cysts in patients with hypokalaemic nephropathy may relate to ammonia production stimulated by hypokalaemia, and the associated intracellular acidosis stimulates cell proliferation [186]. Local ammonia production stimulated by hypokalaemia can also lead to intrarenal complement activation that may contribute to the chronic renal injury. Although correction of hypokalaemia may be associated with a decrease in the number and size of cysts, the tubulointerstitial lesions may not be irreversible. At least experimentally, there is evidence that the chronic lesions may lead to the development of salt sensitivity [187]. Patients abusing diuretics [188] or laxatives or binge eaters and excessive dieters and those with primary hyperaldosteronism are at increased risk of developing the chronic irreversible interstitial lesions [189].

Hypercalcemic Nephropathy

There are many causes of hypercalcemia such as primary hyperparathyroidism [190], sarcoidosis (see below), myeloma and other adenocarcinomata [191], tuberculosis [192] and drugs [193], with potential to have renal manifestations. The commonest functional manifestation of

hypercalcemia is impaired urine-concentrating ability with associated polyuria and polydipsia. Although incompletely understood the impairment of urinary concentrating ability relates both to reduction in medullary solute content and to disruption of the cellular (distal convoluted tubule and collecting duct) response to vasopressin. Hypercalcemia can also cause acute renal insufficiency including acute interstitial nephritis, although this is an uncommon event and is usually reversible with correction of the hypercalcemia. Tubular obstruction with calcium phosphate crystalline casts, most commonly seen in relation to malignant disease where severe hyperphosphatemia is present, can contribute to or result in acute oliguric renal insufficiency. If irreversible renal failure occurs as a rare outcome of long-standing hypercalcemia, it will almost invariably be associated with evidence of crystal deposition in the renal interstitium.

Indeed, the most distinctive histologic feature of long-standing hypercalcemia is the occurrence of calcific deposits in the interstitium ("*nephrocalcinosis*"). Depending on the cause of the metabolic defect, these deposits can be microscopic (detected on renal biopsy) or macroscopic (detected by renal imaging (ultrasound or plain X-ray) and are most often evident in the renal medulla. The other features of chronic interstitial nephritis such as interstitial fibrosis, tubular atrophy and secondary glomerular sclerosis will be present on renal biopsy as well.

Renal Interstitial Disease in Sarcoidosis

One of the more common presentations of sarcoidosis with renal involvement results from hypercalcemia (see above) [194–196] that such patients develop as a consequence of enhanced 1,25–dihydroxyvitamin D3 production by activated macrophages in the lymph nodes and lungs. All the renal manifestations of hypercalcemia described above may occur. More rare is the development of a usually steroid responsive acute interstitial nephritis with mononuclear cells and noncaseating granulomas. This lesion may progress to chronic interstitial disease with typically tubular atrophy and interstitial fibrosis. Glomerular lesions including membranous nephropathy, a proliferative/crescentic glomerulonephritis, or focal glomerulosclerosis may also rarely occur and may be associated with microhaematuria, macrohaematuria and/or proteinuria [197].

Renal Interstitial Disease in Systemic Lupus Erythematosus

Tubulointerstitial disease may very occasionally be the only manifestation of lupus nephritis [198, 199]. It has been suggested that in some unusual cases, the tubulointerstitial nephritis may be due to peritubular capillaritis secondary to the immune complex depositions in the capillary wall of the interstitium [200]. Normally, however, the presence of an interstitial infiltrate and associated tubular injury with or without immune deposits along the TBM is a frequent finding in lupus nephritis associated with concurrent glomerular disease. The severity of the tubulointerstitial involvement is predictive of a poorer prognosis and progressive disease, correlating positively with the presence of hypertension and an elevated plasma creatinine. This possibility of interstitial involvement without glomerular disease may be suggested progressive decline in glomerular filtration and a urinalysis that shows relatively benign or indeed normal urine sediment. Tubular dysfunction such as type I or type IV RTA, hyperkalemia (from impaired distal tubule secretion) or hypokalaemia (secondary to salt wasting and hyperaldosteronism) may also suggest interstitial disease.

Corticosteroid therapy is quite likely to be effective in preventing or slowing progression [199].

Sjögren's Syndrome

In patients with Sjögren's syndrome [201], abnormalities in tubular function, including Fanconi's syndrome, type 1 (distal) RTA (25 % of patients), hypokalaemia and nephrogenic diabetes insipidus (impaired tubular responsiveness to vasopressin), may occur. Acidosis is usually mild, but some patients present with quite a low plasma bicarbonate concentration (<10 mmol/L) and a potentially symptomatic low plasma potassium concentration (<1.5–2.0 mmol/L) resulting from concurrent urinary potassium wasting [202].

The mechanism responsible for type I RTA may be related to loss of the H+ ATPase pump in the intercalated cells of the collecting tubules [203]. Hypokalaemia may occur in the absence of RTA [203]. The primary defect is thought to be sodium wasting; thus, hypokalaemia may occur because sodium delivery to the potassium secretory site in the collecting tubules is increased and/or because of associated volume depletion which increases the secretion of aldosterone [201].

In patients with Sjögren's syndrome, associated chronic interstitial disease, the histologic lesions are characterised by a lymphocytic and plasmacytic interstitial cell infiltrate, tubular cell injury and, rarely, granuloma formation. This progresses over time to tubular atrophy and interstitial fibrosis. Treatment with corticosteroids at the stage of cellular infiltration may be beneficial [204] but will be ineffective if irreversible tubulointerstitial injury has occurred. Regardless, progression to ESRD is uncommon. Glomerular manifestations (membranoproliferative glomerulonephritis and membranous nephropathy) are rare but have been reported.

Epstein–Barr Virus-Associated Chronic Interstitial Nephritis

The relationship between acute interstitial nephritis and EBV viral infections has been recognised and reported on a number of occasions. In a number of cases, acute renal failure or renal insufficiency has been a feature [205–207].

Relatively recent evidence exists, suggesting that some cases of idiopathic chronic interstitial nephritis with progressive loss of renal function may be associated with chronic intrarenal infection with Epstein–Barr virus (EBV). In a number of cases of interstitial nephritis of uncertain aetiology, EBV DNA was localised to proximal tubular cells by in situ hybridisation; the proximal tubular cells were also shown to express CD21, which is the receptor for EBV on B lymphocytes [208].

Concluding Remarks

More than 30 years ago, several groups [1–3] documented the relationship between tubulointerstitial injury, which manifests primarily as interstitial fibrosis, and glomerular filtration rate (serum creatinine) and the potential for risk of progressive loss of function over time.

Almost universally, therapy directed at the chronic interstitial nephritides will be given with the aim of preventing progressive renal injury and the development of interstitial fibrosis. In conditions where the tubulointerstitium is the target of acute inflammation or nephritis and particularly where the renal lesion is secondary to a systemic illness (e.g. systemic lupus or sarcoidosis), there is a role for corticosteroids (see also Chap. 23).

Careful monitoring for the development of uroepithelial tumours is also necessary in those diseases in which these tumours occur at increased frequency, most notably analgesic nephropathy, Balkan nephropathy and the interstitial disease induced with Chinese herbs (Aristolochia sp.) and probably cadmium toxicity.

Acceptable therapies exist but are as yet not fully evidence based with appropriately designed randomised clinical trials, but in chronic progressive impairment, these therapies have at least some theoretical potential to influence the development of interstitial fibrosis directly [13]. For instance, control of hypertension and hyperlipidemia has the potential to reduce vascular injury and, therefore, reduce ischemia in the tubulointerstitium. In addition, reducing glomerular hyperfiltration (e.g. with a low-protein diet) theoretically would reduce the workload on tubular epithelial cells, leading to less ammoniogenesis, reduced complement activation and a lower production of various oxygen radicals. All of these changes have the potential to stimulate interstitial inflammation and injury.

Most clinicians would favour the use of ACE inhibitors, which reduce glomerular and systemic pressures, decrease proteinuria and increase renal blood flow. These drugs have shown the ability to slow progression of chronic kidney disease in all experimental and human renal disease settings where they have been tested. Other agents, such as drugs aimed at blocking other vasoactive mediators (such as endothelin) or cytokines (PDGF and TGF-β1 antagonists), are on the horizon but are still not available clinically.

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Reflux Nephropathy and Vesicoureteral Reflux

25

Marc Cendron

Introduction

Vesicoureteral reflux (VUR) is a clinically silent condition which is usually diagnosed either prenatally in association with hydronephrosis or postnatally in the face of (1) a urinary tract infection, (2) a family history of VUR, or (3) some other urologic abnormality such as duplication anomaly of the upper urinary tract. With VUR, urine regurgitates back up the ureter in a retrograde matter from the bladder. Primary reflux refers to reflux that occurs in isolation of any other condition. It is usually due to some anatomic defect at the level of the ureterovesical junction. Secondary reflux refers to reflux that is seen in association with some other pathology, such as ectopic insertion of the ureter into the bladder neck or the urethra. This chapter will solely be devoted to issues related to primary vesicoureteral reflux and its associated renal condition, reflux nephropathy.

This chapter will define reflux nephropathy, outline the pathophysiology of vesicoureteral reflux, discuss its association with urinary tract infection, and then briefly review the evaluation and management of vesicoureteral reflux.

Reflux Nephropathy

Reflux nephropathy refers to lesions found in the renal parenchyma seen in association with vesicoureteral reflux. The term reflux nephropathy was coined by Bailey, in the early 1970s, to describe renal abnormalities which were noted in kidneys in patients diagnosed with vesicoureteral reflux [1]. Reflux nephropathy is a pathologic entity that should require histological confirmation by biopsy but has come to represent lesions seen on imaging studies of the kidney such as intravenous urogram (IVP) (Fig. 25.1), ultrasound, computed tomography (CT) scan (Fig. 25.2), or renal scintigraphy (DMSA renal scan). Few if any studies, however, have correlated histologic observations with radiologic findings except in nephrectomy specimens [2]. On IVP, reflux nephropathy is identified by areas of decreased uptake of the contrast material in the renal parenchyma. The renal parenchyma may also appear thinned out [3–5]. On ultrasound, areas of parenchymal thinning with reduced renal size and an increase in the parenchymal echogenicity can be seen. Reflux nephropathy on DMSA is seen as areas of decreased uptake of the radionuclide [6]. The true definition of reflux nephropathy is clouded by the fact that, in the face of VUR, renal lesions may be caused by several factors that will be discussed further.

Historically, reflux nephropathy was initially understood to be renal parenchymal lesions found in the kidneys of patients who experienced a febrile urinary tract infection or pyelonephritis in the face of VUR [7, 8]. A classic cascade of events due to bacterial infection of the kidneys was well described by Roberts [9]. Schematically the process of renal scarring starts with the colonization of the urinary tract by a bacterial organism such as *Escherichia coli* which is capable of adhering to the urothelium. Colonization occurs because of many factors including ineffective bladder emptying, decreased immune response, and urologic anomalies such as vesicoureteral reflux. With ascent of urine up into the kidney, the bacteria can enter the renal parenchyma and cause pyelonephritis as demonstrated by Ransley and Risdon who referred to the event as the "big bang" effect [10, 11]. Development of renal scarring is dependent on the inflammatory response in the renal medulla initiated by invasion of the renal tissue by bacteria. A local inflammatory response will develop and may result, ultimately in local renal tissue damage. Roberts has postulated that bacteria adhering to the renotubular cells may elicit an immune response which causes release of superoxide which will, in turn, damage both bacteria and tubular cells. The damage subsequently causes an interstitial inflammatory response which leads to

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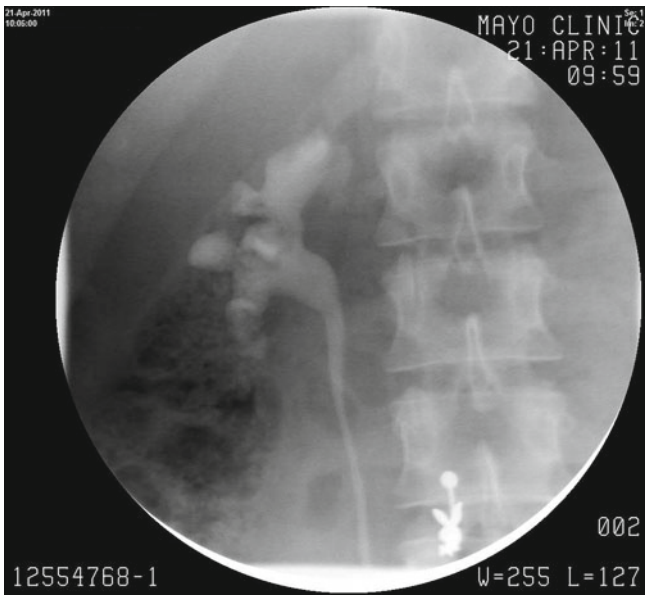

Fig 25.1 Voiding cystourethrogram revealed right vesicoureteral reflux to blunted calices (not shown). Images provided by Dr. Akira Kawashima, Department of Radiology, Mayo Clinic, Rochester, MN

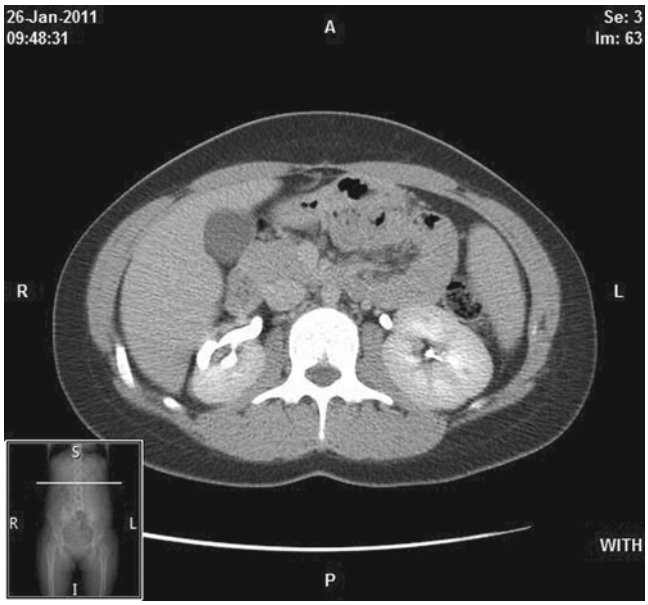

Fig 25.2 Delayed enhanced CT scan obtained at the level of the right mid-kidney demonstrates a dilated calyx with associated cortical scanning laterally, characteristic of chronic atrophic pyelonephritis. Images provided by Dr. Akira Kawashima, Department of Radiology, Mayo Clinic, Rochester, MN

deposition of collagen and destruction of the normal tubular arrangement.

A schematic representation of cascade events causing renal damage in the face of bacterial invasion is described on Fig. 25.3. The final outcome is seen as areas of the kidneys which are replaced by areas of scar tissue and damaged nephrons [12, 13]. The pathological features of

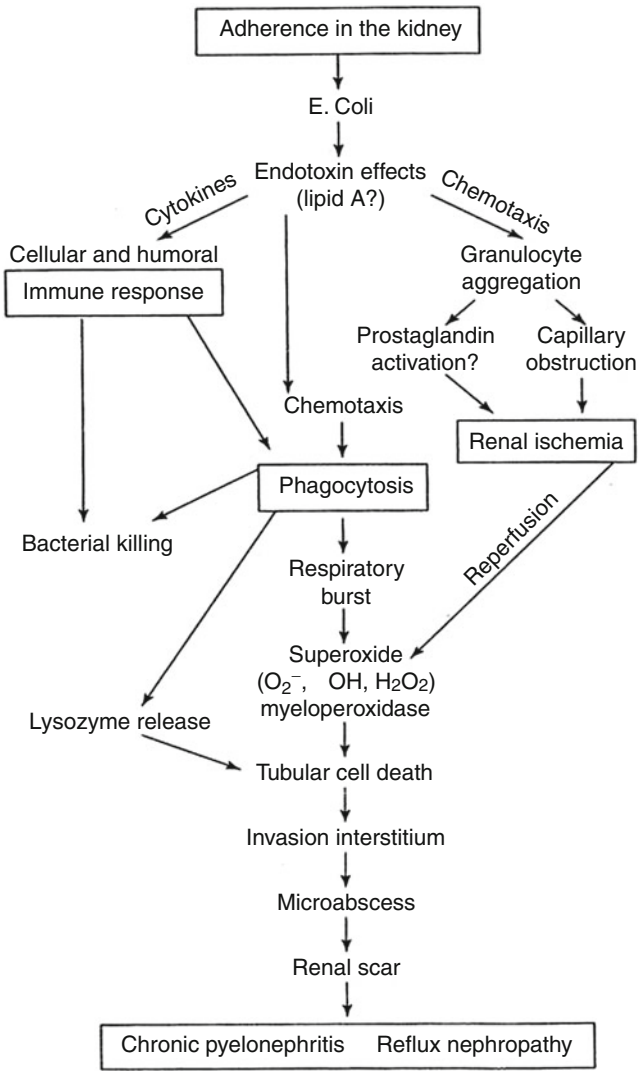

Fig. 25.3 Schematic representation of the pathologic events in the formation of pyelonephritic scar. From: Roberts JA. Vesicoureteral reflux and pyelonephritis in the monkey: a review. J Urol 1992;148(5 Pt 2):1721–5. With permission

chronic pyelonephritis can be identified microscopically as areas of fibrosis and cortical thinning overlying dilated and distorted calyces (Figs. 25.4 and 25.5). The "scarred" kidney will usually be seen to be smaller than normal with variability in the areas of scarring. The scarred area shows dilated and atrophic tubules with a preservation of the large blood vessels. Normal parenchyma is usually seen adjacent to areas of scar. Periglomerular fibrosis and varying degrees of glomerular sclerosis can also be observed [13]. The changes are not exclusive of the area of scarring but can also be seen in normal areas of the kidneys which may indicate that the autoimmune process of the renal damage may be at work diffusely in patients who have had a pyelonephritis. Anomalies may also be noted and include periarterial fibrosis and changes consistent with medullary fibrosis.

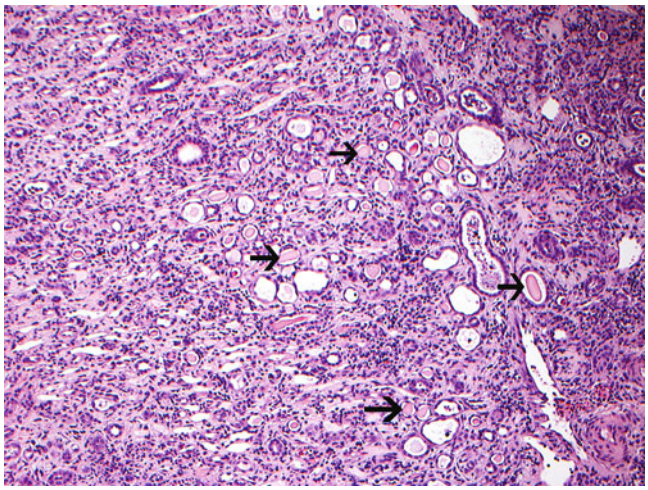

Fig 25.4 Reflux nephropathy showing features of chronic pyelonephritis. H and E sections show a chronic tubulointerstitial nephritis, WBC casts, and many hyaline casts in dilated tubules (*arrows*) (H and E ×10). Courtesy Dr. Sanjeev Sethi, Department of Radiology, Mayo Clinic, Rochester, MN

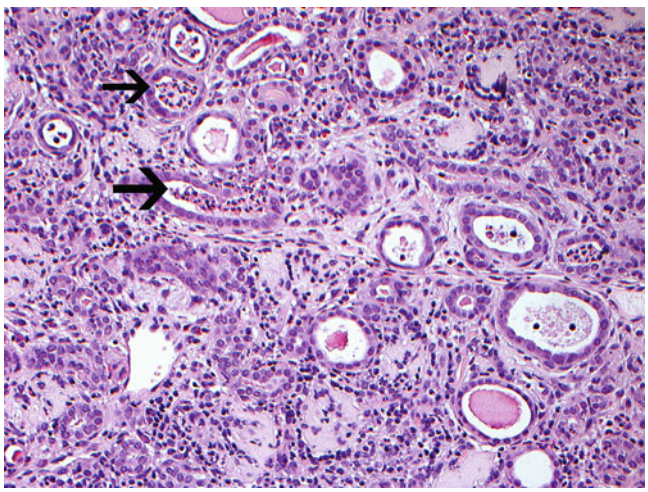

Fig 25.5 Chronic pyelonephritis with WBC casts (*arrows*). Note ruptured tubule with WBC cast (H and E ×20). Courtesy Dr. Sanjeev Sethi, Department of Radiology, Mayo Clinic, Rochester, MN

Recently, the etiologic implication of infection as the sole cause of renal damage in reflux nephropathy has been challenged because some patients with VUR have no documentation of infection present with evidence of renal damage as documented by either ultrasound or (more specifically) by DMSA scan. These renal lesions may, in fact, be different than those associated with urinary tract infection and are referred to renal dysplasia [14]. Renal dysplasia is a pathological diagnosis in which evidence of primitive renal tissue is found in association with significant medullary fibrosis and areas of abnormal tissue such as cartilage. Renal dysplasia is found commonly in other conditions such as posterior urethral valves, upper urinary tract obstruction, and duplicated collecting systems with upper pole pathology and can be classified as either cystic or solid [15]. Mackie and Stephens theorized that renal dysplasia may actually be the result of altered kidney development associated with abnormalities in the embryology of the mesonephric duct and metanephric blastema [16]. It has become clear over the last few years that a number of signaling molecules and transcription factors play an important role in the developmental process of the ureteral bud and of the kidney. Abnormalities in ureteral bud development and improper induction of the metanephric blastema may occur when expression of these molecules is altered leading to a field defect resulting in both kidney and the urinary tract abnormalities. Several animal studies using mouse models point towards a complex system of receptors and transcription factors which influence the growth and elongation of the distal ureter (GDNF/RET-signaling pathway) [17]. The theory of Mackie and Stephens regarding abnormal renal development in the face of abnormal ureteral bud insertion is being confirmed on a molecular basis in these animal models [18]. Current understanding is that the mesenchyme adjacent to abnormally positioned ureteral bud may not be competent to respond to inductive signals and this leads to abnormal renal development more specifically renal dysplasia.

Obstruction to the normal flow of urine from the kidneys has also been postulated as a possible etiologic factor for renal dysplasia [19].

It is therefore felt that reflux nephropathy associated with VUR may be a spectrum of disease and close attention should be paid to the etiologic factors of the renal abnormalities observed. In general, renal dysplasia is seen in patients diagnosed prenatally or early in life who have not had any evidence of urinary tract infection [20]. The features of renal dysplasia on DMSA scan usually are that of a diffuse, reduced uptake of the radionuclide; whereas reflux nephropathy associated with recurrent urinary tract infection is seen focally, the defects of DMSA scan appear to be localized (segmental) in the upper and lower poles and associated with relatively high grade reflux [21, 22]. As will be discussed later, these distinctions are important in managing children with reflux nephropathy.

Pathophysiology of Vesicoureteral Reflux

Vesicoureteral reflux is, in all likelihood, the result of maldevelopment or immaturity of the ureterovesical junction (UVJ). Histologically, the distal ureter is seen to enter the outer muscle layer of the bladder and then course underneath the mucosa of the bladder. This arrangement has been postulated to act as a flap-valve mechanism whereabouts the submucosal roof of the submucosal portion of the ureter will be compressed as the bladder fills. Urine will still be allowed

to flow down the ureter as the peristaltic activity of the ureteral muscle will propel the urine away from the kidney. The length of intramural tunnel is felt to be important. If the length of ureter underneath the mucosa of the bladder, or if the diameter of the distal ureter is large, the flap-valve mechanism will be ineffective and ascent of urine up the ureter will occur. Other factors may, in fact, be involved such as the anchoring of the ureter to the muscle of the bladder. Paquin, in 1959, showed that the length of intramural tunnel is essential to preventing the reflux of urine. He postulated that a normal, non-refluxing ureter had a tunnel length to ureteral diameter ratio of 5 to 1, whereas in refluxing ureters that ratio was much smaller [23]. The appearance of the ureteral orifice may not be important but its location within the bladder is certainly crucial as more laterally displaced ureteral orifices will indeed be associated with reflux. The histologic events associated with the development of the ureterovesical junction have been well described by F. Douglas Stephens and may explain the development of a faulty ureterovesical valve [24].

Reflux of urine from the bladder back up into the ureter and renal collecting system has been recognized since the time of Galen [25]. VUR became identified as an etiologic factor for pyelonephritis from the classic studies by Hutch who, in 1952, studied a group of paraplegic patients diagnosed with neurogenic dysfunction of the bladder and vesicoureteral reflux. Reflux of infected urine into the upper urinary tract was hypothesized to be the cause of chronic pyelonephritis and subsequent renal damage [26]. Subsequently Hodson in 1959 observed that reflux seemed to be more common in children with urinary tract infection and that there was a correlation between reflux and chronic pyelonephritis as documented by VCUG (voiding cystourethrogram) and IVU (intravenous urogram) [27]. The increasing use of imaging studies of the urinary tract such as the VCUG, led to the recognition that reflux is associated with upper urinary tract infections and renal parenchymal lesions. The presence of intrarenal reflux (reflux of contrast into the medulla of the kidney) which can be occasionally demonstrated on VCUG has also been associated with renal scarring [28].

When the association between vesicoureteral reflux and urinary tract infection became more established, additional information became available and a relationship between renal abnormalities and reflux was also observed. In the early 1970s, Rolleston reported that severe reflux in infants seemed to have a higher likelihood of associated renal damage [8]. This led to the belief that renal damage associated with vesicoureteral reflux may be acquired and was caused by recurrent, ascending urinary tract infection. However, this notion was disputed by Stecker and associates who reported the presence of renal parenchymal lesions which were found in a small series of patients with reflux but who had never had any evidence of a urinary tract infection [29].

Presentation of Vesicoureteral Reflux

Primary vesicoureteral reflux can be classified by its mode of presentation. Until the advent of prenatal screening by ultrasound, vesicoureteral reflux was usually identified during a work-up of a febrile urinary tract infection. However, over the last 20 years it has become apparent that reflux can be detected prior to the advent of a urinary tract infection. Prenatal ultrasound has revealed a presence of variable degrees of hydronephrosis in a significant number of fetuses. Postnatal evaluation with VCUGs has revealed the presence of vesicoureteral reflux in a significant number of cases (approx. 20 %) [30]. Although vesicoureteral reflux cannot be diagnosed in utero, its presence can be inferred from findings on conventional ultrasound during prenatal screening. Radiologic findings include variation in the degree of dilatation of the upper urinary tract during prolonged observation of the fetus with increasing size of the upper urinary tract during emptying of the bladder [31]. Such findings should lead to postnatal evaluation with a VCUG [32].

Prenatal hydronephrosis is mostly reported in males, can be variable in its degree and grade, and is not associated with urinary tract infection. Renal abnormalities have also been observed in patients found to have VUR diagnosed in the perinatal period. The extent of the renal abnormalities can vary with up to 10 % of patients showing a poorly or non-functioning kidney on the side of the reflux [2].

The classic presentation of vesicoureteral reflux after birth is in the setting of a febrile urinary tract infection (UTI) occurring in an infant or an older child. The features of this type of vesicoureteral reflux are that it usually affects females, is usually diagnosed later in life (especially during toilet training time), and is usually associated with lower grades of vesicoureteral reflux [33]. In addition, 25–50 % of patients who present with acute pyelonephritis are found to have VUR. It should be emphasized, however, that the relationship of VUR and UTI is not cause and effect. One key concept is that, in the face of VUR, bacteria that have entered the bladder have easy access to the upper urinary tract [34]. Most children who have lower grades of VUR and few UTIs have, in general, as they grow older, a benign outcome [35, 36]. Nevertheless, a concern exists in that renal lesions can be observed in up to 13.5 % of patients who have had recurrent urinary tract infections involving the upper urinary tract or pyelonephritis [37]. But the causal relationship between UTIs and renal scarring is difficult to define despite a fairly large body of literature based on mostly retrospective studies [38, 39].

Evidence suggests that several factors may contribute to renal damage and that a genetic predisposition may exist [40, 41]. The age at which a child with VUR has an episode of pyelonephritis and the number of episodes of infection appear to be related to the severity of renal damage.

Pylkkanen et al. reported that infants younger than 1 year of age carry the highest risk of developing renal damage and the highest incidence of congenital urinary tract anomalies, whereas after puberty new renal damage does not appear to occur [41]. In addition, the occurrence of renal lesions is directly related to the frequency of the episodes of upper urinary tract infection; the more infections, the more renal damage is seen [42]. Genetic predisposition for renal damage in association with VUR has also been suggested [43, 44]. Association with ACE gene polymorphism with urinary tract infections and renal lesions in young children has been suggested [45]. Clearly the molecular aspects and the genetics of renal maldevelopment and injury seen in association with VUR will require further studies.

Approximately 30 % of siblings of patients with reflux will also be noted to have reflux [46, 47]. This category of patients usually does not have a history of urinary tract infection and, overall, seems to have a fairly good prognosis with few patients exhibiting any renal lesions [48].

The prevalence of vesicoureteral reflux has been hard to estimate as most patients present either prenatally, with a urinary tract infection or on family screening. A number of patients are diagnosed after being screened for reflux when familial reflux is reported [49]. Obviously a large portion of the population has not been screened for reflux but the incidence of vesicoureteral reflux has been estimated to be 1–2 % of live births [50]. Reflux is not distributed equally amongst all races. It appears that children of West African ancestry have very low incidence of VUR [51–54].

Diagnostic Evaluation of Vesicoureteral Reflux

Diagnosis of reflux is achieved by demonstrating retrograde flow of urine up into the kidney. This is best carried out by either a voiding cystourethrogram or a radionuclide cystogram. Each of these modalities will be described separately. As discussed earlier prenatal diagnosis of vesicoureteral reflux cannot be made but can be inferred by features of a well carried out prenatal ultrasound. In the early 1990s, with improvement in ultrasound technology, several articles described what is now referred to as prenatally diagnosed reflux [31, 55–57]. Ultrasound diagnosis prenatally of the possibility of vesicoureteral reflux is usually inferred when hydronephrosis is noted. A recent meta-analysis on the postnatal outcome of antenatally diagnosed hydronephrosis indicates that the overall risk of vesicoureteral reflux in the population of fetuses diagnosed with antenatal hydronephrosis is approximately 8.6 % [58]. An important feature of vesicoureteral reflux associated with antenatal hydronephrosis is that the degree of prenatal hydronephrosis does not correlate with the presence or degree of vesicoureteral reflux. In fact, the rate of vesicoureteral reflux in patients screened postnatally with VCUG seems to be pretty much constant in the various grades (mild, moderate, severe hydronephrosis ranging from 4.4 % presence of vesicoureteral reflux to 14 % in the moderate degrees and 8.5 % in the severe degree of hydronephrosis). It is therefore felt that hydronephrosis is an indicator of urologic pathology but is not necessarily a predictor of vesicoureteral reflux when diagnosed prenatally. If there is a suspicion for vesicoureteral reflux then postnatal evaluation is warranted. The optimal timing of the postnatal follow-up remains debatable, but current recommendations are for an ultrasound 2–3 days after birth and a VCUG or RNC (radionuclide cystogram) within a month after birth. In general, a VCUG is also recommended in females who have not had a urinary tract infection and have a family history of vesicoureteral reflux or hydronephrosis diagnosed prenatally. In males, a VCUG is favored since it will provide better anatomic resolution and will help rule out any lower urinary tract abnormalities such as posterior urethral valves.

The voiding cystourethrogram is the principle method of assessing the lower urinary tract in children. The first images of the bladder and urethra were reported back in 1905, but it was not until the early 1930s that instillation of contrast material into the bladder was evaluated fluoroscopically during voiding [59]. VCUG is carried out by inserting a catheter in the bladder. The bladder is filled until the child voids. Sequential images of the voiding phase are obtained. Filling and emptying the bladder (cycling) has been shown to increase the yield for the diagnosis of vesicoureteral reflux [60]. VCUG will allow quantification of the amount of reflux and will outline both the bladder and the upper urinary tract anatomy as well as the anatomy of the urethra [61]. As noted the current accepted classification of the severity of reflux is based on VCUG findings (Fig. 25.5) [62]. Other pathological conditions of the bladder can be noted on VCUG such as bladder diverticula which are associated with reflux as well as posterior urethral valves, neurogenic bladder dysfunction, urethral strictures, and ureteroceles as well as reflux into one or both poles of a duplex kidney.

Bladder conditions associated with vesicoureteral reflux include:

- Prune-Belly syndrome
- Duplication of the upper urinary tract
- Posterior urethral valves
- Neurogenic bladder
- Bladder diverticulum
- Ureterocele

The VCUG is recognized to be a fairly invasive test, which may cause a significant amount of psychological stress to the child if not performed correctly by those skilled in the imaging of children. In addition, use of ionizing radiation near the growing child's gonads from VCUG is concerning. Recent improvement in fluoroscopic techniques using digital techniques will significantly reduce the exposure to radiation [63].

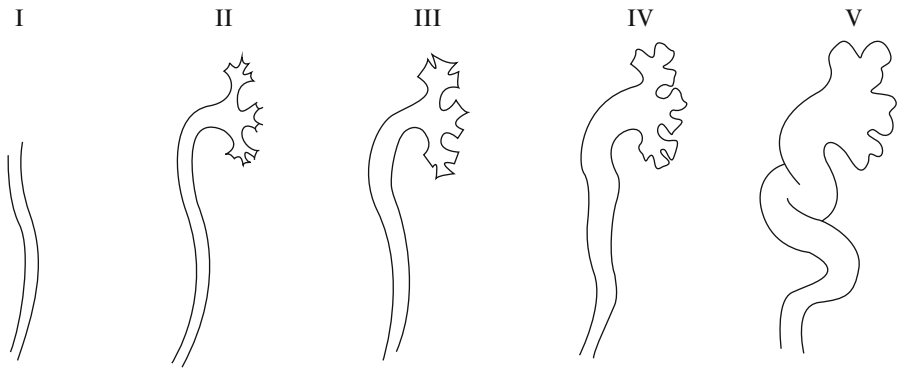

Fig. 25.6 International Reflux Study classification [60]

The radionuclide cystogram (RNC) has also become widely accepted for the evaluation of vesicoureteral reflux. Its first practical application was described in the early 1960s and has become a popular way to evaluate for vesicoureteral reflux in children who are followed for reflux [3]. Urethral catheterization is required and a radionuclide solution is instilled into the bladder. The advantage of this technique is that the amount of radiation associated with the radionuclide is less than that with a VCUG [64, 65]. In addition, the nuclear cystogram may have a greater sensitivity as it provides continuous monitoring of the bladder as it fills and empties. However, no anatomic determination of the lower or upper urinary tract can be achieved using the nuclear cystogram. The classification of the reflux is also different as it can only show mild, moderate, or severe degrees (grades I, II, III). It is also sometimes hard to see grade I vesicoureteral reflux (international reflux classification) (Fig. 25.6) on a radionuclide cystogram because of the activity in the bladder. The limitations of the radionuclide cystogram have led some authors to favor the VCUG as the initial study to diagnose reflux. The radionuclide cystogram is then used for subsequent follow-up studies [66, 67]. Cycling the bladder will increase the sensitivities as described by Fettich and Kenda [68]. To avoid catheterization of the bladder, several alternate techniques have been attempted to diagnose vesicoureteral reflux but none have been shown to have the diagnostic accuracy of either the VCUG or RNC. For example, the indirect radionuclide cystogram (IRC) described by Merrick et al. has been associated with a high rate of false-negative and false-positive studies [69, 70].

In order to fully evaluate the urinary tract in the face of vesicoureteral reflux, current recommendations are to carry out an ultrasound study which will give anatomic details of the kidneys as well as indications as to the anatomy of the upper collecting system. The ultrasound will also help to evaluate the lower urinary tract and show whether or not the bladder empties properly. However, ultrasound is not a very helpful test in monitoring kidneys except to evaluate for their growth. A large study by Blane and colleagues showed that a significant number of kidneys in patients with vesicoureteral reflux were normal by ultrasound (74 %) without evidence of ureteral or renal pelvic dilatation [71]. It was, therefore, felt that conventional renal ultrasonography is not a helpful test to diagnose reflux but can serve as a screening test to look for anomalies of either the bladder or kidney and acquired conditions.

With regard to renal abnormalities associated with vesicoureteral reflux, recent studies have suggested that the dimercaptosuccinic acid (DMSA) renal scan may be the most important study in the evaluation of patients who present with a urinary tract infection and vesicoureteral reflux. DMSA scan had been shown to be a useful tool in assessing both for acute and permanent renal damages in children with urinary tract infection [6, 72]. Evaluating a patient after a febrile urinary tract infection and diagnosed with vesicoureteral reflux may, in fact, help identify those at risk for long-term sequelae of vesicoureteral reflux nephropathy. DMSA uses an agent that labels tubular cells tubular which enables good cortical imaging of the kidneys and can be used to evaluate children with both urinary tract infections (UTI) and vesicoureteral reflux. DMSA scans can demonstrate evidence of pyelonephritis in the acute setting as well as evidence of permanent renal lesions if performed several months after resolution of the infection. There are, however, no published guidelines for the use of the DMSA in children with reflux. Clearly from the urologic and nephrologic standpoints, the presence of renal lesions is an important piece of information. A recent study of 303 children under age 2 who were diagnosed with UTI and investigated with a DMSA scan as well as a VCUG (top down approach to reflux) within 3 months after UTI demonstrated that 50 % of the patients showed renal lesions on DMSA scan. Only 26 % of those patients, however, had vesicoureteral reflux. Of note is that the grade of vesicoureteral reflux correlated significantly with the presence of renal lesions [73].

DMSA scanning has been shown to be more accurate than intravenous urography (IVU) in evaluating for the presence of renal lesions subsequent to urinary tract infection in the

face of reflux [4, 5]. Rushton and colleagues followed patients with acute pyelonephritis with serial DMSA scan at the time of the acute episode and then several months later. They observed that the areas identified as foci of acute pyelonephritis later appeared as areas of renal damage and therefore confirmed that acute pyelonephritis can lead to renal lesions [22]. Technical improvements have not led to ultrasonography replacing DMSA renal scanning in the ability to detect parenchymal lesions. In fact, small focal lesions less than 1 cm are not picked up by renal ultrasound [71]. Investigators have proposed that DMSA scan may be the study of choice in children with febrile urinary tract infection [74]. Mingin and colleagues suggested that abnormalities seen on DMSA scan correlated with the presence of grades III–V reflux in children with febrile urinary tract infection, and these children had a greater chance of having a breakthrough infection (60 %) than those without renal lesion [75]. DMSA may detect those patients who may be at risk for renal damage in the face of VUR and urinary tract infection. Therefore, it has been suggested that the DMSA scan be the first study in patients with a febrile UTI with evidence of upper urinary tract dilatation seen on ultrasound and that VCUG or RNC be carried out only in those patients with evidence of renal lesions on DMSA scan [73].

Magnetic resonance urography (MR urography) has been studied as potentially new diagnostic modality for renal and urinary tract evaluations. While MR urography may provide improved spatial and contrast resolution than DMSA scan, it remains an experimental tool as no definite criteria or categories of renal lesions have been published. It has been suggested, however, that MR urography may distinguish renal dysplasia from post-pyelonephritic renal lesions but further studies will be needed to validate this possibility [76]. It should be kept in mind that MR urography is more expensive and more time-consuming and requires anesthesia or sedation in the pediatric population.

The Natural History of Vesicoureteral Reflux

Vesicoureteral reflux is known to resolve spontaneously in a number of infants and children. Several studies, both prospective and retrospective, have tried to assess the rate of resolution [77–82]. Elder et al. following a thorough review of the literature, provided resolution curves that showed that reflux resolution was more likely to occur in younger children and that at 5 years, 92 % of patients with grade I and 81 % of patients with grade II reflux showed resolution of the reflux irrespective of the age at presentation and whether the reflux was unilateral or bilateral (Fig. 25.7) [38]. Unfortunately the data suffers from several problems including heterogenicity of definitions of the outcomes. Reflux grading has not been consistent in all studies and may not be comparable. Resolution rate has been looked at both in terms of ureters as well as patients. This makes the data hard to interpret. Several means of evaluating for reflux have also been reported upon and these include both voiding cystourethrograms as well as radionuclide cystograms. Also patient selection is not always consistent in some of these studies. Reflux has been shown in some series to be intermittent and reports of resolutions have then been modified when reappearance has been noted at a later study [50]. Finally, in all studies, a significant number of patients were noted to be lost to follow-up. More recently, a nomogram used to predict reflux resolution in primary vesicoureteral reflux showed that resolution was dependant on several factors which

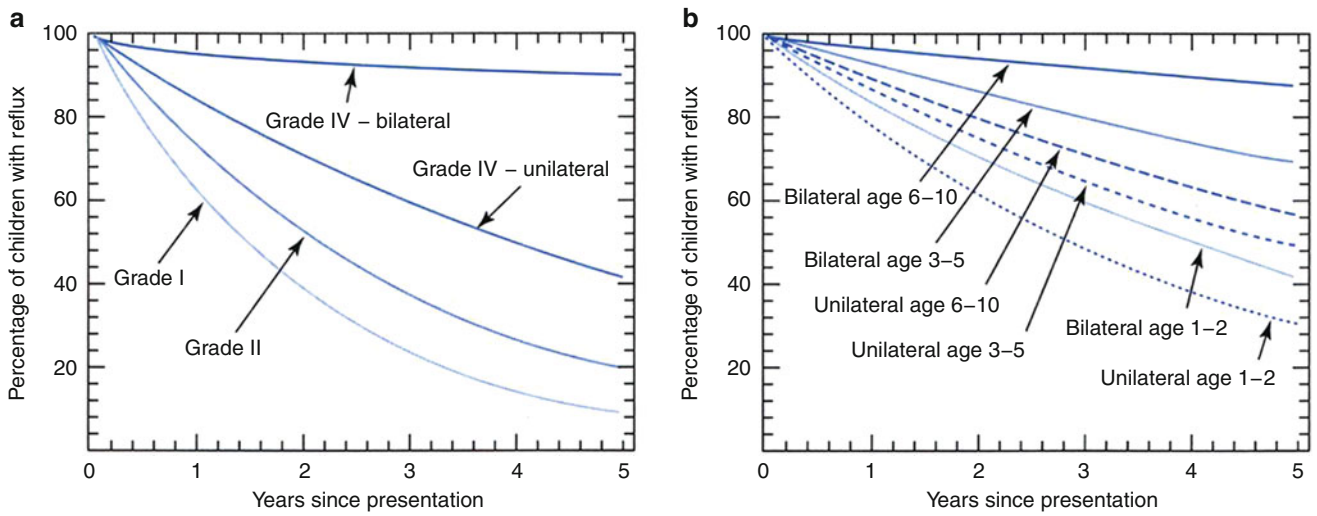

Fig. 25.7 Curves showing the likelihood of spontaneous reflux resolution in percentages of patients with VUR followed up to 5 years: (**a**) for VUR grades I, II, and IV and (**b**) for VUR grade III by patient age at presentation [38]

Table 25.1 Rates of VUR resolution in children according to grade [38]

Series	No. of patients	Grade I (%)	Grade II (%)	Grade III (%)	Grade IV (%)
Bellinger and Duckett [10]	269[a]	87	63	53	33
Goldraich and Goldraich [2]	202	80(I/II)		50(III/IV)	
Huang et al. [11]	214	92	76	62	32
Greenfield et al. [12]	601	69	56	49	
Smellie et al. [6]	149			73[b]	44[b]
Schwab et al. [13]	214	83	77	68	36
Estrada et al. 2007 (personal communication)		86(I/II)		72(III)	54(IV/V)

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[a]Renal units, not patients
[b]10-year data: results disparate for unilateral vs. bilateral refluxes

included age at presentation, gender, grade, laterality, mode of presentation, and ureteral anatomy [82]. Nevertheless, certain conclusions can be gleaned from the data. First of all, reflux resolution seems to occur more likely in younger children and children with lower grades of reflux (reflux grades I and II). Resolution of grade III reflux seemed to vary depending on laterality. If the reflux was unilateral and children were under age two, reflux disappeared in 70 % of cases. If the patient has bilateral grade III vesicoureteral reflux and was older than five, the resolution rate for reflux was much lower (12.5 %). For unilateral grade IV reflux, resolution rate was in the order of 58 % after 5 years, but if the patient had bilateral grade IV reflux, only 10 % demonstrated resolution (Table 25.1). Grade V reflux resolved in less than 5 % of patients [38, 82–85].

Resolution of reflux over time has been the proposed basis for medical management of vesicoureteral reflux. In addition to the age of the patient, the grade of reflux, and laterality, the status of bladder function has come to be considered as an important factor in reflux resolution. Koff, in 1992, recognized the relationship between abnormal voiding patterns and vesicoureteral reflux [86]. Subsequently, Snodgrass and Koff independently showed that children with vesicoureteral reflux and urinary tract infection showed symptoms of voiding dysfunction. Symptoms of voiding dysfunction include infrequent voiding, frequency-urgency, incomplete bladder emptying, and day and night incontinence [87, 88]. Patients with voiding dysfunction appear to have a higher rate of constipation and seem to be predisposed to urinary tract infection. Farhat and colleagues felt that a proper evaluation for children with vesicoureteral reflux and urinary tract infection should include investigation for voiding dysfunction. This group recommended the use of a validated dysfunctional scoring system using test questions geared at evaluating urinary and bowel habits to screen for patients who may have dysfunction elimination syndromes [89]. Using this questionnaire the Pediatric Urology Group from Toronto studied a large group of patients with vesicoureteral reflux. Children with high symptom score who were managed with behavior modification consisting in timed voiding with coordinated relaxation of the external sphincter and complete bladder emptying seemed to demonstrate a higher rate of reflux resolution which correlated to reduction in the symptom score. Children who did not have much reduction in the symptom score tended to demonstrate persistence of the reflux [90].

In addition to behavior modification, anticholinergic therapy has also been used to improve bladder function. The goal of anticholinergic therapy is to reduce intravesical pressures as high pressures in the bladder may favor reflux. A combination of anticholinergic therapy and behavior modification using timed voiding has been shown to be potentially beneficial [91]. Thirty to forty percent of patients show improvement and resolution of reflux in this study. The drawbacks of using an anticholinergic are side effects (dry mouth, facial flushing, reduced sweating, blurred vision, and, most importantly, constipation). The side effects are dose related and can be lessened using certain medications such as hyoscyamine or trospium which appear to have a lower rate of side effects. Treatment of constipation may also reduce the risk for urinary tract infection.

Sequellae of Reflux Nephropathy

As discussed earlier renal lesions seen in association with vesicoureteral reflux may be either due to abnormal development of the kidney (dysplasia) or due to the sequelae of infection (chronic pyelonephritis). Clearly a higher prevalence of renal lesions has been reported in children with reflux whatever the cause of reflux might be [92]. Renal lesions are very important clinically for the nephrologist and pediatric urologist as they may affect children into adulthood. These potential sequelae include hypertension, loss of renal function and even end-stage renal failure, effects on somatic growth, risk for further infection, and potential effects on pregnancy.

In the 1970s and 1980s, studies used IVP to determine the presence of renal lesions but it is now recognized that IVP is an insensitive technique to screen for renal lesions. Beetz et al. retrospectively studied 189 patients who underwent treatment for vesicoureteral reflux and were followed for a several years. At almost 11 years of follow-up, 61 patients had evidence of renal lesions on IVP and 11.5 % were noted to be hypertensive [93]. Patients who did not have any evidence of renal lesions had a much lower incidence of hypertension (2.3 %). Smellie, in a long-term large cohort study of 226 patients who presented in childhood with urinary tract infection and vesicoureteral reflux at an average age of 5 years in the pre-ultrasound era, reported that after 18–35 years, 7.5 % of patients had hypertension [94]. Of the 17 patients in that cohort with hypertension, 15 had documented renal scarring on IVP at the time of initial presentation. Only one of these 226 patients was found to have end-stage renal failure in adulthood.

Reflux nephropathy has been felt to be one of the most common disorders causing hypertension in childhood and then in adulthood. Hypertension is felt to be, in this situation, mediated by activation of the renin-angiotensin system with higher levels of renin reported in several studies that evaluated patients with renal lesions [95, 96]. In a recent long-term study, Goonasekera et al. followed prospectively a cohort of patients found to have reflux nephropathy over a span of 15 years. Eighteen percent of the patients were noted to have hypertension after 15 years with most of these patients becoming hypertensive between ages 15 and 30 years [97]. Wolfish et al., following 129 patients with primary uncomplicated vesicoureteral reflux for over 10 years, found that patients with no renal lesions had no evidence of hypertension [98]. Unfortunately, a review of the literature reveals that almost all the studies reporting on hypertension in children with vesicoureteral reflux and reflux nephropathy are flawed by their retrospective nature, varied patient selection criteria, and little or no follow-up into adulthood. In addition, methodological problems inherent to these studies include poor reporting of the degree of reflux, uneven documentation of infection status, and variable methods to define both renal lesions and hypertension.

Hypertension may vary with age, time of presentation, degree of parenchymal damage, unilaterality or bilaterality of the damage, and length of follow-up. Retrospective studies that have followed children with vesicoureteral reflux and renal scarring demonstrate a variable rate of hypertension between 15 and 20 %. Long-term studies in adulthood show a 30–40 % incidence of hypertension in patients with long-standing reflux nephropathy. This, in all likelihood, represents the natural history of renal damage in patients with reflux. The incidence of hypertension goes up as patient age as documented by a large epidemiological study that demonstrated a 28 % prevalence of hypertension in adults ages 35–74 [99]. In a large series of 294 patients, Zhang and Bailey demonstrated an incidence of 38 % of hypertension in patients found to have reflux nephropathy who enrolled in the study as teenagers. In this study, the risk of hypertension seemed to increase with age and was more common in patients with severe, bilateral parenchymal lesions [100]. In a more recent study, Kohler et al., found that 58 % of patients with bilateral renal scarring had hypertension, while those with unilateral scarring had rate of hypertension of 33 %. Interestingly, 33 patients with a history of reflux but no renal scarring were also found to have hypertension but to a milder degree. The concern is that deterioration of renal function as documented by serum creatinine determination was only seen in patients with bilateral scarring or scarring in solitary kidneys and was associated with a 92 % incidence of hypertension [101].

Given the clear association between the developments of hypertension and renal lesions in patients with vesicoureteral reflux, it would seem reasonable to recommend long-term blood pressure screening in those patients. Currently no clear-cut guidelines based on long-term, prospective studies have been published. However, recommendations should include a blood pressure measurement at least once a year throughout lifetime for patients with renal lesions and who have had vesicoureteral reflux. Parents of patients with vesicoureteral reflux should also be instructed about the potential long-term complications of hypertension.

Reflux nephropathy is also associated with chronic renal insufficiency although the exact rate of renal failure in patients with reflux nephropathy is somewhat unclear. Progressive deterioration of renal function is not felt to be necessarily related to either recurrent urinary tract infection or the persistence of reflux. Patients who have been free of urinary tract infection may still progress to renal insufficiency. Approximately 20 years ago, reflux nephropathy was felt to be responsible for 22 % of pediatric cases of end-stage renal disease in Great Britain [102]. Currently in the USA reflux nephropathy is felt to be the third most common etiology for chronic renal failure in children (8 %) [103]. Recently, the Italian Pediatric Registry of Renal Failure reported an incidence of 25 % of patients with end-stage renal failure having a history of vesicoureteral reflux [104]. The true incidence of end-stage renal failure due to vesicoureteral reflux and renal scarring may be difficult to ascertain. There is no clear change in the incidence of end-stage renal disease secondary to reflux nephropathy over the last 40 years despite increased awareness and early management [105]. This would suggest that a significant number of patients with reflux indeed have intrinsic renal disease (dysplasia) at baseline and that it will neither improve with time nor will it be amenable to treatment. Correlation between the degree of parenchymal damage and decreased renal function has been well documented, but the disease process is still

incompletely understood. Proteinuria has been found to be a hallmark of progression in renal insufficiency [106] and thus should be used to monitor patients with renal lesions.

A number of patients with reflux nephropathy and end-stage renal disease have a focal segmental glomerulosclerosis pattern of injury on renal biopsy. Four mechanisms have been proposed to explain the development of renal damage: immunologic injury, macromolecular trapping and mesenchymal dysfunction, vascular alteration, and hypertension with adaptive hemodynamic alterations that may lead to glomerular hyperfiltration [107]. A subset of the population diagnosed with end-stage nephropathy have no history of urinary tract infection, are found to have high-grade vesicoureteral reflux, and are males [108]. In light of these findings, it has been suggested that patients who progress to end-stage renal disease are those patients who have congenital renal dysplasia in the face of high-grade reflux [109]. The North American Pediatric Renal Transplant Cooperative Study reviewed all patients who underwent renal transplantation between 1987 and 1995 and found that reflux nephropathy was the etiology of ESRD in 5.7 % of pediatric patients [110].

Renal damage seen in association with VUR may cause variable degrees of renal insufficiency that may affect the growth and development of the child. Patients with significant renal lesions, especially bilaterally, should be monitored closely. The Birmingham Cooperative Study found no difference in the overall growth between surgically and medically managed patients with vesicoureteral reflux, but Polito et al. found a decrease in both height and weight in a group of patients with bilateral renal lesions when compared to age-matched controls [111, 112]. When these patients were followed after puberty, catch-up growth to normal heights and weights was noted [113]. Close monitoring of those patients with renal lesions should therefore include height and weight charting, blood pressure measurement, as well as annual evaluation of renal function from serum studies and urinalysis.

Management of Vesicoureteral Reflux

The natural history of VUR has been hard to map out as reflux may be a heterogeneous condition with variable presentation and unpredictable outcome. Traditionally, once the diagnosis of reflux has been made in a child, the tendency has been to treat these patients either medically or surgically in order to prevent ascending urinary tract infection and pyelonephritis [114]. Unfortunately very few long-term studies monitoring patients from the time of diagnosis into adulthood are available [94]. And there is no consensus as to the best approach to vesicoureteral reflux since there are no clearly defined outcome measures for this condition.

The management of vesicoureteral reflux can be divided into three general categories: observational, medical, and surgical. Observational management does not entail any therapeutic intervention. The patient is watched for UTIs and reflux is monitored on a yearly basis. Medical treatment of vesicoureteral reflux involves prevention of urinary tract infection and bladder management as well as prevention of constipation. Surgical management includes either endoscopic treatment which entails injection of a bulking agent under the intravesical portion of the ureter or open surgical repositioning of the ureter in order to create a flap-valve mechanism. None of these approaches to VUR have been studied in a prospective, randomized fashion. There are, therefore, few evidence-based guidelines for the management of VUR. The outcomes measures for evaluating success of therapy have not been clearly defined. Most would agree that prevention of renal damage and its progression once it has been observed would be the ultimate goals. In patients with congenital renal damage, however, this goal may not be attainable. Prevention of ascending urinary tract infection may prevent pyelonephritis. This is the stated goal of surgical intervention which seeks to eradicate retrograde flow of urine up into the ureter by recreating the antireflux valve mechanism. In that situation, the outcome measure is disappearance of reflux.

Observational treatment involves monitoring the patient for urinary tract infection and ensuring that the patient adheres to good voiding habits. It assumes that sterile reflux is of no consequence on the kidneys and that VUR will, over time, resolve. The approach was studied in the 1960s and 1970s and a concern that emerged was that a significant number (up to 21 %) of patients developed new renal lesions in the face of urinary tract infections [38, 115, 116]. More recently, however, cessation of antibiotic coverage has been found to be safe in older patients with persistent low-grade VUR [35, 117].

The concern that recurrent urinary tract infections in the face of VUR could cause further renal damage led to the use of low-dose, once daily antibiotic prophylaxis. Several studies indeed showed a reduced rate of renal scarring in children on continuous antibiotics followed for several years [78, 94, 118]. New scar formation was not completely prevented, however, and was still seen in a small number of patients (3 %) who experienced symptomatic breakthrough infections while on antibiotic prophylaxis [77]. The two major drawbacks of antibiotic prophylaxis are compliance and breakthrough urinary tract infections. Compliance with antibiotic therapy seems to be quite variable and is associated with failure to follow-up [119]. Noncompliance rates have been reported to be as high as 88 % [50]. Breakthrough infections are due to colonization of the urinary tract by organism resistant to the antibiotic being used for prophylaxis. Breakthrough infection rates have been reported to occur in up to a third of

patients enrolled in the International Reflux Study and up to 40 % in a meta-analysis [120] by Wheeler et al. and occur more frequently in girls and in children with voiding dysfunction [117]. While there is general consensus that antibiotic prophylaxis prevents UTIs in children with reflux, this approach has not been evaluated in a prospective, randomized manner. A recent meta-analysis of randomized controlled trials evaluating antibiotic prophylaxis and surgical treatment of reflux revealed that combined treatments yielded a 60 % reduction in febrile UTIs. However, there appeared to be no significant reduction in the rate of either new renal scarring or progression of renal damage [120].

Surgical treatment is aimed at restoring the one-way valve mechanism of the ureterovesical junction in order to prevent regurgitation of infected urine into the upper urinary tract. This is usually accomplished by either injecting a bulking agent under the distal, intramural ureter (endoscopic procedure) or lengthening the submucosal tunnel of the distal ureter. Endoscopic treatment was popularized by Puri and O'Donnell using a paste of polytetrafluoroethylene particles [121]. The procedure is considered minimally invasive and is carried out under general anesthesia on an outpatient basis. Several compounds have been used but currently the only one to have received FDA approval is a suspension of dextranomer microspheres in a carrier gel of stabilized sodium hyaluronate [122]. Complications of the procedure are very low and results show a reflux resolution rate of 70–80 % after up to three injection procedures [123]. To date, no study has demonstrated a reduction in the rate of renal scarring after endoscopic treatment.

Since Hutch's report on successful surgical correction of VUR several procedures have been used to create an antireflux mechanism [26]. Open surgical treatment of VUR appears to have a 95.6 % success rate (in all grades of VUR) in eradicating reflux according to an exhaustive review of the literature [38]. Higher grades of reflux (grade V), however, have been found to associate with a much lower rate of success, with persistent VUR noted in up to 19 % of cases following surgery. Complications of open surgical repair include distal ureteral obstruction (1 % of cases) and persistence of the reflux [124]. The International Reflux Study showed that there was no difference in renal scarring between patients randomized to antibiotic prophylaxis or to surgery after 5 years [116].

Treatment of VUR is tailored to the individual and the clinical situation. In general, indications for surgical management of VUR include (1) breakthrough infections despite consistent antibiotic prophylaxis, (2) noncompliance with antibiotic prophylaxis, (3) severe grades of VUR (IV or V), (4) failure of renal growth, (5) appearance of new renal lesions, (6) persistent reflux with little or no resolution after 3 or 4 years, and (7) reflux associated with congenital anomalies at the level of the bladder or ureter (i.e., bladder diverticulum, ectopic ureter).

At the present time, no strict guidelines for the management of VUR have been agreed upon based on the grades of reflux. However, most urologists would agree that grade I VUR can be managed by observation since it is not associated with high rates of pyelonephritis or renal damage. Grade II or III VUR is initially managed with antibiotic prophylaxis or observation. If the reflux fails to resolve after 2 or 3 years, endoscopic or open surgical management can be proposed. Grade IV reflux may resolve quickly in boys and, thus, antibiotic prophylaxis with radiologic monitoring can be carried out for 1–2 years. If no resolution is noted or if a child is older than 3 years, surgical therapy is a reasonable option. Grade V VUR is, in general, approached surgically [125].

In conclusion, reflux nephropathy appears to occur in two distinct forms: congenital abnormality of the kidney (dysplasia) or post-infection, acquired scarring (post-pyelonephritis). Clinical presentation of each of these entities may be different but the ultimate outcome for reflux nephropathy should be the maintenance and preservation of functional renal parenchyma by early diagnosis and careful nephro-urologic monitoring, prevention of infections, and long-term follow-up into adulthood. Infants and children with unexplained fevers should be screened for the possibility of a urinary tract infection by getting a urinalysis and a urine culture if the urinalysis is positive for the presence of leukocytes. Those patients diagnosed with pyelonephritis should be treated promptly and evaluated with a VCUG (or RNC) and DMSA scan. Should scarring be found, then long-term monitoring must be carried out into adulthood.

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Uric Acid and the Kidney

26

Duk-Hee Kang

Introduction

Uric acid is produced during the metabolism of purines by the degradation of xanthine by xanthine oxidase or its isoform, xanthine dehydrogenase (reviewed in [1] and Fig. 26.1). In most mammals, uric acid is further oxidized to water-soluble allantoin by the hepatic enzyme, urate oxidase (uricase), and serum uric acid level is maintained at relatively low levels (1–3 mg/dL, 60–180 μ[mu]M). During early hominoid evolution (12–20 million years ago), however, a series of mutations occurred, first affecting the promoter region and then the actual gene, eventually rendering uricase nonfunctional in humans and higher primates [2, 3]. As a consequence, serum uric acid levels in humans are higher (3–15 mg/dL, 180–900 μ[mu]M) and less regulated than in most mammals.

The best known consequence of an elevated uric acid in humans is gout. Because of the limited solubility of uric acid, gout is characterized by the deposition of urate crystals in synovial joints, occasionally with tophi formation. However, there are also a number of well-known renal and cardiovascular manifestations associated with hyperuricemia. There are three major different types of uric acid-induced renal disease: acute uric acid nephropathy, chronic uric acid nephropathy, and uric acid nephrolithiasis. In the following chapter, the major associations of uric acid with renal disease will be reviewed. Newer data will be presented regarding the role of uric acid in the progression of other renal diseases and in mediating intrarenal vascular disease and hypertension. Clinical implications and treatment strategies of asymptomatic hyperuricemia will be also discussed.

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Uric Acid Metabolism and Homeostasis

Production of Uric Acid

Uric acid is a weak acid trioxypurine (M.W. 168) that is composed of a pyrimidine and imidazole substructure with oxygen molecules, which is produced from metabolic conversion of either dietary or endogenous purines, primarily in the liver, muscle, and intestine [1]. The immediate precursor of uric acid is xanthine, which is degraded into uric acid by xanthine oxidoreductase. Xanthine oxidoreductase may attain two interconvertible forms, namely, xanthine dehydrogenase or xanthine oxidase [4]. Most xanthine oxidoreductase is in the xanthine dehydrogenase form in vivo [4, 5], which is transformed into xanthine oxidase by irreversible proteolytic cleavage or reversible oxidation in specific environment [6] including hypoxia. Xanthine oxidase uses molecular oxygen as electron acceptor and generates superoxide anion and other reactive oxygen species as by-products in the process of uric acid degradation, whereas xanthine dehydrogenase generates the reduced form of nicotinamide adenine dinucleotide (Fig. 26.1). Both exogenous (present in fatty meat, organ meats, and seafood) and endogenous purines are major sources of uric acid in humans. Approximately two-thirds of total body urate is produced endogenously, while the remaining one-third is accounted for by dietary purines. Purine-rich foods include beer and other alcoholic beverages, anchovies, sardines in oil, fish roes, herring, organ meat (liver, kidneys, sweetbreads), legumes (dried beans, peas), meat extracts, consomme, gravies, mushrooms, spinach, asparagus, and cauliflower [7].

Excretion of Uric Acid in Humans

The primary site of excretion of uric acid is the kidney. The normal urinary urate excretion is in the range of 250–750 mg/day, approximately 70 % of the daily urate production [8]. Although urate (the form of uric acid at blood pH of 7.4) is

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freely filtered in the glomerulus, there is evidence that both reabsorption and secretion occur in the proximal tubule and as a consequence the fractional urate excretion is only 8–10 % in the normal adult. Some adaptation occurs with renal disease, in which the fractional excretion of urate will increase to the 10–20 %. The remainder of uric acid excretion occurs through the gut, where uric acid is degraded by uricolytic bacteria. The gastrointestinal tract may eliminate up to one-third of the daily uric acid load in the setting of renal failure [9, 10].

The classic paradigm of uric acid excretion consists of a four-step model with glomerular filtration, reabsorption, secretion, and postsecretory reabsorption; the latter three processes occur in the proximal convoluted tubule [11, 12]. Ideas of the handling of uric acid by the kidney have changed greatly over the past few decades, with the identification, characterization, and isolation of transporters and channels mainly or exclusively restricted to urate transport [13–15]. Membrane vesicle studies have suggested the existence of two major mechanisms modulating urate reabsorption and secretion, consisting of a voltage-sensitive pathway and a urate/organic anion exchanger. Recently several of these transporters/channels have been identified (Fig. 26.2).

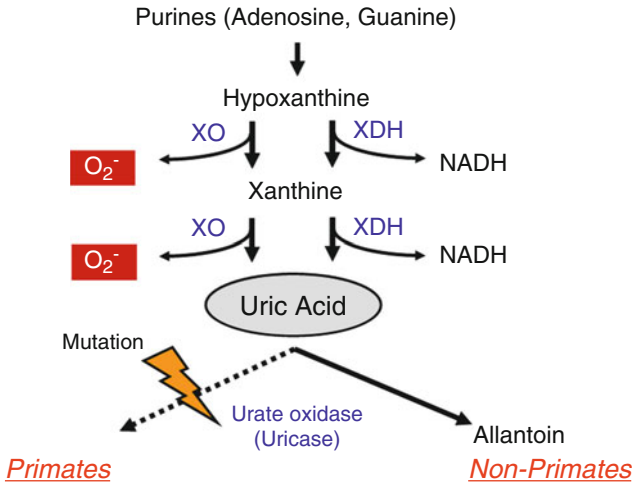

Fig. 26.1 Production and metabolism of uric acid. *XO* xanthine oxidase, *XDH* xanthine dehydrogenase, *NAD* nicotinamide adenine dinucleotide

Uric acid transporter-1 (URAT-1), a member of the organic anion transporter (OAT) family, is identified as the major organic anion exchanger for uric acid on the apical (luminal brush border) side of the proximal tubular cell [13]. In the human kidney, urate is transported via URAT-1 across the apical membrane of proximal tubular cells, in exchange for anions being transported back into the tubular lumen to maintain electrical balance. Urate then moves across the basolateral membrane into the blood by other OATs, most likely OAT-1 and OAT-3 [16, 17]. URAT-1 has a high affinity for urate in exchange for lactate, ketones, α-ketoglutarate, and related compounds. Pyrazinamide, probenecid, losartan, and benzbromarone all inhibit urate uptake in exchange for chloride at the luminal side of the cell by competition with the urate exchanger. In humans, urate secretion is relatively minimal and URAT-1 is thought to be the major mechanism for regulating blood level of uric acid.

OAT4 is one of the apical urate transporters in the proximal tubule which has recently been identified as an

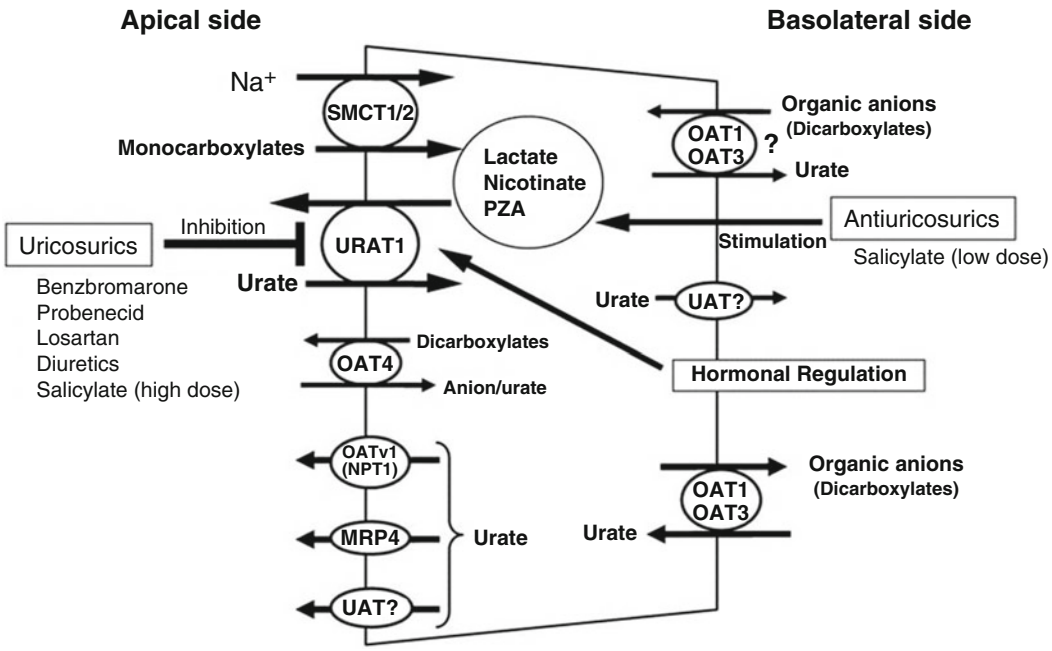

Fig. 26.2 Models of transcellular urate transport in the proximal tubule. From Anzai et al. [15]; used with permission

organic anion–decarboxylate exchanger. OAT4 may be also a target of the uricosuric agent probenecid [18, 19].

Urate secretion may be mediated principally by a voltage-sensitive transporter, which is expressed ubiquitously and localizes to the apical side of the proximal tubule in the kidney. This may be the role of the urate transporter (UAT), which is in the galectin family [14]. UAT/galectin 9 is thought to be a housekeeping urate channel that serves in the efflux of urate produced by intracellular purine metabolism [20]. The role of UAT/galectin 9 as a urate transporter/channel in the apical membrane of proximal tubules remains to be elucidated.

Recently, a novel human renal apical organic anion efflux transporter, called MRP4, has been identified [21]. MRP4 is a member of the ATP-binding cassette transporter family. It may mediate the secretion of urate and other organic anions such as cAMP, cGMP, and methotrexate across the apical membrane of human renal proximal tubular cells. Human MRP4 is an ATP-dependent unidirectional efflux pump for urate with multiple allosteric substrate-binding sites [22].

Another protein involved in renal transport of urate is Tamm–Horsfall protein (THP), also known as uromodulin. THP is exclusively expressed and secreted by epithelial cells of the thick ascending limb and co-localizes with the Na, K, 2Cl transporter in lipid rafts in the apical cell membrane, suggesting a functional interaction [23]. Mutations in the human uromodulin gene have been identified in subjects with medullary cystic kidney disease type 2 and in patients with familial juvenile hyperuricemic nephropathy (FJHN) (see Section "Familial Juvenile Hyperuricemic Nephropathy") [24, 25]. It is not yet known how the THP mutation leads to hyperuricemia, as most evidence suggests that uric acid handling is restricted to the proximal tubule. There is some evidence, however, that some urate secretion in the rat can occur distal to the proximal tubule [26]. Furthermore, there is also an evidence that the THP mutation may lead to sodium and water wasting and possibly stimulate urate reabsorption proximally [27].

Another novel insight regarding renal urate transport is a potential link between sodium and urate reabsorption as in a cotransport process [28]. A model of indirect coupling of sodium transport via SMCT1/2 and urate transport via URAT-1 with transcellular urate transport in the proximal tubule is shown in Fig. 26.2.

Uric Acid-Induced Nephropathy

Chronic Uric Acid Nephropathy

In patients who have had gout for many years, renal disease is common. Natural history studies prior to the availability of uric acid-lowering drugs reported that up to 25 % of gouty subjects developed proteinuria, 50 % developed renal insufficiency, and 10–25 % developed end-stage renal disease [29, 30]. Renal histologic changes are particularly common and have been observed in autopsy studies of 75–99 % of patients with gout. The classic definition of chronic uric acid nephropathy (also known as chronic urate nephropathy or gout nephropathy) is a form of chronic kidney disease (CKD) induced by the deposition of monosodium urate crystals in the distal collecting duct and the medullary interstitium with a secondary inflammatory reaction. The other primary histologic findings consist of arteriolosclerosis, glomerulosclerosis, and tubulointerstitial fibrosis. The exact pathophysiological mechanism for renal tophi formation is not clear since hyperuricemia and simple oversaturation of the urine is not a prerequisite [31].

This characteristic pathologic pattern has historically been termed "gouty nephropathy" and was thought to be a major etiology of chronic renal disease in the past [32]. Studies performed primarily in the 1970s and 1980s challenged the concept that gouty nephropathy was a true entity. Uric acid crystal deposition is usually focal and this does not adequately explain the diffuse pattern of the disease. Moreover, uric crystal deposition could be found in some autopsies where no renal disease was present [33]. One should also consider that most of the gouty subjects who had or developed renal disease had concomitant hypertension or were elderly as both conditions are associated with the development of microvascular disease, glomerulosclerosis, and tubulointerstitial fibrosis [34–36]. Yü and Berger [34–36] argued that, although gout was often associated with renal disease, mechanisms directly related to uric acid seem not to be responsible for renal pathology. For example, when they excluded gouty subjects with hypertension or who were older, they found little evidence that gout could cause renal disease.

Importantly, the effect of uric acid-lowering therapy on renal function in gout remains debatable. Although some studies suggested that lowering uric acid could improve the renal disease in gout [37, 38], other studies could not demonstrate any significant improvement [39]. Even in studies in which an improvement in renal disease was observed, the question remains whether better uric acid control consequently resulted in less use of nonsteroidal agents, which are considered to be nephrotoxic [40]. Finally, it became evident that some subjects with high uric acid and renal disease had other concomitant disorders such as lead nephropathy [41] or hereditary familial hyperuricemic nephropathy [42]. Although some investigations suggested that markedly elevated uric acid levels might cause chronic renal disease (usually at levels >13 mg/dL in men or >10 mg/dL in women) [43], most authorities concluded that uric acid was unlikely to cause a chronic nephropathy directly. Therefore, many authorities considered the term "gouty nephropathy" a misnomer [31, 44].

New Insights into Chronic Uric Acid Nephropathy

Despite skepticism about the clinical implication of chronic uric acid nephropathy, renewed interest in the role of gout and asymptomatic hyperuricemia has emerged in the last several years. First, it was recognized that there were questionable inherent assumptions in previous investigations [45]. For example, one implicit assumption was that primary pathogenesis of chronic uric acid nephropathy had to be uric acid crystal deposition with an exclusion of the possibility of other effects of hyperuricemia as a mediator of renal disease. It may also be inappropriate to use the presence of hypertension to explain every case of renal insufficiency in the gouty patient, since most subjects with essential hypertension have relatively preserved renal function. Furthermore, the analysis also assumed that the presence of hypertension was a separate cause of renal disease and that it had to be independent of the uric acid. These ideas led to a proposal to reinvestigate the role of uric acid in chronic renal disease.

Subsequently, a number of studies using multivariable regression modeling have examined the relationship between serum uric acid levels and the subsequent risk for developing chronic renal disease. Interestingly, several studies of the general population found that hyperuricemia was a strong independent risk factor for developing renal insufficiency. A serum uric acid of >7 mg/dL in men and >6 mg/dL in women confers a 10.8-fold increased risk in women and a 3.8-fold increased risk in men compared to those with normal uric acid levels [46]. This increased relative risk was independent of age, body mass index, systolic blood pressure, total cholesterol, serum albumin, glucose, smoking, alcohol use, exercise habits, proteinuria, and presence of hematuria. Indeed, an elevated uric acid was more predictive for the development of renal insufficiency than proteinuria. An elevated uric acid was also independently associated with a markedly increased risk of renal failure in another study of more than 49,000 male railroad workers [47]. Additional evidence that hyperuricemia may have a role in kidney disease was provided by a recent randomized, controlled clinical trial in which allopurinol therapy resulted in a slowing of renal disease progression in subjects with hyperuricemia and renal insufficiency associated with a significant decrease in uric acid level [48].

Another major breakthrough in understanding the role of uric acid on the progression of kidney disease came from experimental studies in which chronic mild hyperuricemia was induced in rats [49, 50]. Because rats have functional uricase, mild hyperuricemia can be induced by administering low concentrations of the uricase inhibitor, oxonic acid, to the diet [49, 50]. This resulted in serum uric acid levels that were only 1.5- to 3.0-fold greater than normal, and importantly this did not result in intratubular or interstitial urate crystal deposition. Over time, however, rats developed hypertension and progressive renal disease. Early in the course, the rats developed arteriolar thickening and rarely hyalinosis of the preglomerular arterioles, often accompanied by glomerular hypertrophy [50, 51]. Over time proteinuria developed and was followed by worsening vascular disease, glomerulosclerosis, and interstitial fibrosis [50]. The lesion was identical to that observed with nephrosclerosis of hypertension, with aging-associated glomerulosclerosis, and with gouty nephropathy, except for the absence of crystal deposition that had been observed in the latter condition. These findings raised the possibility that chronic hyperuricemia may cause renal disease and hypertension via a crystal-independent pathway.

Clinical Manifestations and Diagnosis of Chronic Uric Acid Nephropathy

Although histologic evidence of renal disease is almost invariably present, most subjects with long-standing gout will have either normal plasma creatinine or only mild renal insufficiency [34–36]. Renal blood flow is usually disproportionately low for the degree of renal insufficiency [34–36]. Fractional excretion of uric acid is usually less than 10 %. Proteinuria occurs in the minority of cases and, when present, is usually in the non-nephrotic range. The urinary sediment is also usually benign. However, hypertension is frequent, occurring in 50–60 % of subjects and increasing in prevalence as renal function worsens.

The diagnosis of chronic uric acid nephropathy is classically defined by histology, demonstrating chronic glomerulosclerosis, tubulointerstitial fibrosis, renal microvascular disease, and focal medullary crystal deposition. Chronic hyperuricemia can lead to precipitation of uric acid crystals (tophus formation) mainly in the distal collecting ducts and the interstitium. The crystal evokes a foreign body reaction with central accumulation of crystalloid material (monosodium urate) surrounded by a rim of inflammatory cells (Fig. 26.3). Recent experimental studies, however, suggest that crystal deposition is not necessary. Therefore, the diagnosis may become problematic because the other histologic findings are indistinguishable from benign nephrosclerosis (renal injury from essential hypertension) or with aging-associated renal disease. A disproportionately elevated serum uric acid in relation to the degree of renal insufficiency (such as a uric acid level of >9 mg/dL for a serum creatinine of <1.5 mg/dL, a uric acid of 10 mg/dL for a serum creatinine of 1.5–2.0 mg/dL, and a serum uric acid of >12 mg/dL when serum creatinine is >2.0 mg/dL) should make one consider the diagnosis of chronic uric acid nephropathy.

Management of Chronic Uric Acid (Gouty) Nephropathy

Although no specific studies have addressed management of chronic uric acid nephropathy, it is reasonable to treat gouty nephropathy similar to chronic renal disease of other etiologies.

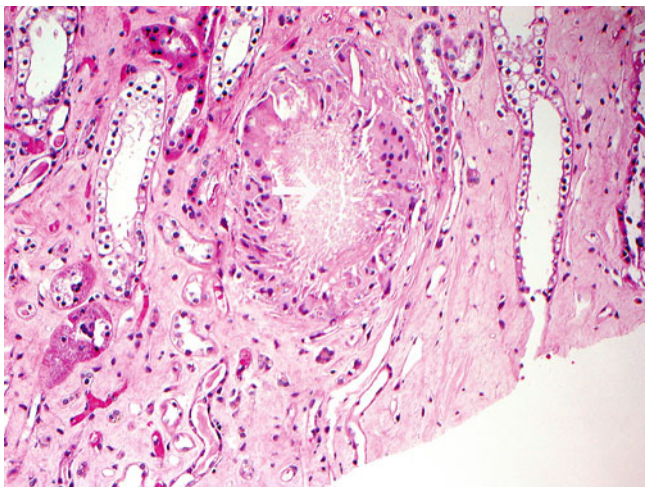

Fig. 26.3 Uric acid crystals (*white arrow*) surrounded by macrophages including giant cells (H and E ×40)

Therefore, one should achieve good blood pressure control (targeting a blood pressure of <140/90 mm Hg in nonproteinuric patients and 125/75 mm Hg in proteinuric patients). Given the known benefit of blocking the renin–angiotensin system on other renal diseases, and the observation that renin–angiotensin system blockade also prevents the renal injury induced with experimental hyperuricemia [49–51], we would recommend the use of an angiotensin receptor blocker and/or an angiotensin-converting enzyme inhibitor as part of the clinical regimen. Furthermore, losartan may be a particularly good choice as it is also uricosuric and will lower uric acid levels by affecting the urate–anion exchange in the proximal tubule through a mechanism that is not shared with other angiotensin receptor blockers [52]. The use of thiazide or loop diuretics is discouraged, as these drugs have as a side effect of raising the uric acid, and recent studies suggest they provide less renal protection than other antihypertensive agents available [45]. If a diuretic is required, the use of amiloride or spironolactone could be attempted with a careful monitoring for the development of hyperkalemia. Some subjects will nevertheless require a thiazide to control blood pressure.

The role of hypouricemic therapy in chronic uric acid nephropathy still remains controversial, and no definitive studies have been performed to resolve this important issue. Based on the experimental studies, it does seem reasonable to lower serum uric acid in subjects with hyperuricemia, particularly in individuals with markedly elevated levels (>8 mg/dL). In the setting of renal insufficiency, most uricosuric agents are relatively ineffective, although some success with benziodarone has been reported [53]. Allopurinol is the most commonly used hypouricemic agent because of its ability to lower serum uric acid regardless of the cause of hyperuricemia and the convenient once daily dosing.

Table 26.1 Guideline for allopurinol dose according to renal function [5]

Creatinine clearance (mL/min)	Maintenance dose allopurinol
0	100 mg every 3 days
10	100 mg every 2 days
20	100 mg/day
40	150 mg/day
60	200 mg/day
80	250 mg/day
100	350 mg/day
120	350 mg/day
140	400 mg/day

Allopurinol reduces the conversion of xanthine to uric acid through inhibition of xanthine oxidase, which is the rate-limiting step. Approximately 20 % of patients experience side effects with allopurinol and up to 5 % discontinuing therapy [54]. The most serious side effect is a rare hypersensitivity syndrome, which results in fever, rash, eosinophilia, hepatitis, renal failure, and in some cases death. In addition, oxypurinol, the functional metabolite of allopurinol, builds up in the setting of renal insufficiency. Dosing guidelines for allopurinol according to creatinine clearance have been published in an attempt to reduce the risk of the hypersensitivity syndrome (Table 26.1) [55]. A promising drug that is currently undergoing clinical trials is febuxostat, a nonpurine xanthine oxidase inhibitor that appears to have minimal side effects. Febuxostat may be more effective than allopurinol and for which no dose adjustment in renal disease is needed [56]. In addition to pharmacologic therapies, patients should be instructed to reduce ingestion of foods that can raise uric acid, such as foods with high-purine content, ethanol, and fructose. Management of hyperuricemia in CKD of other etiologies will be discussed in detail in the following sections.

Acute Uric Acid Nephropathy

Acute uric acid nephropathy is characterized by acute oliguric renal failure with a rapid rise in serum uric acid level, which is almost always associated with uric acid precipitation within the tubules. This is most often due to overproduction and overexcretion of uric acid in patients with hematologic malignancy [57]. Tumor cell necrosis ("tumor lysis syndrome") leads to a brisk increase of purine metabolism, hyperuricemia, and characteristic hyperuricosuria. Uncommon causes of acute uric acid nephropathy include tissue catabolism due to seizures or treatment of solid tumors, primary overproduction of uric acid due to the rare syndrome of hypoxanthine–guanine phosphoribosyltransferase deficiency, or hyperuricosuria due to decreased uric acid reabsorption in the proximal tubule, as seen in the patients with a Fanconi-like syndrome.

Serum uric acid levels may increase to greater than 15 mg/dL, resulting in a marked increase in urinary urate excretion that exceeds its solubility. Due to sudden oversaturation of uric acid in the urine, uric acid precipitates as crystals or sludge in tubules and collecting ducts. These precipitates cause tubular obstruction and acute renal failure. Interstitial fibrosis or tophus formation is generally not encountered.

Clinical Manifestation of Acute Uric Acid Nephropathy

Acute uric acid nephropathy is typically asymptomatic, although flank pain can occur if there is significant renal pelvic or ureteral obstruction. The diagnosis should be suspected when acute renal failure develops in any of the above clinical settings in association with marked hyperuricemia (plasma uric acid concentration generally above 15 mg/dL). This is in contrast to most other forms of acute renal failure in which the plasma uric acid concentration is less than 12 mg/dL, with the exception of prerenal azotemia where there is an increase in proximal sodium and urate reabsorption. Diagnosis is facilitated by the characteristic clinical syndrome and with a urinary uric acid/urinary creatinine ratio of >1 mg/mg (or >0.66 mM/mM) [29] and by the presence of urate crystals in the urinary sediment.

Treatment of Acute Uric Acid Nephropathy

Prevention is the best therapy for acute uric acid nephropathy. Patients about to receive chemotherapy or radiation for a malignancy with rapid cell turnover should be pretreated with allopurinol plus fluid loading (with saline and possibly mannitol) to maintain a high urine output (over 2.5 L/day) [58, 59]. Historically, treatment consisted of forced alkaline diuresis (to facilitate solubilizing the urate) and large doses of xanthine oxidase inhibitors (typically allopurinol 300–600 mg/day). Recently, recombinant uricase (rasburicase) has become available, which can be administered intravenously and which effectively lowers serum uric acid levels and corrects acute kidney injury more rapidly than allopurinol [60]. Dialysis can also be used to acutely lower the serum uric acid levels. The natural course is one similar to that for acute renal failure of any etiology with a period of oliguria, followed by partial or complete clinical recovery. However, some degree of residual renal injury/damage is common.

Familial Juvenile Hyperuricemic Nephropathy

FJHN is an autosomal dominant disorder associated with juvenile onset of gout and progressive renal disease. Histologically, the kidney usually shows patchy areas of tubular atrophy and fibrosis, focal interstitial infiltration of lymphocytes and histiocytes, and globally or segmentally sclerosed glomeruli. Associated gross thickening and sometimes reduplication of the basement membrane in distal tubules and collecting ducts have been observed [61–63], but urate crystal deposition is rare. Gout may or may not occur in the individual. Urate production is normal in all cases as judged by urinary excretion on a purine-free diet. A decreased fractional urate excretion (FE_{ur}) for age and sex precedes any decrease in the glomerular filtration rate in otherwise apparently healthy patients and suggests a primary defect in urate handling with secondary (or late associated) renal damage. Subjects often are normotensive initially, but hypertension is common as the renal disease progresses. Hemodynamic studies have shown that there is severe renal vasoconstriction, with marked depression in renal plasma flow relative to the glomerular filtration rate.

Recently the disease was shown to be due to a mutation in uromodulin, also known as the gene encoding the THP [25]. The TH protein is produced only by the thick ascending limb tubular epithelial cells in the kidney, raising questions of how this mutation could result in hyperuricemia and renal failure. Interestingly, mutations in uromodulin have also been shown to be the cause of type 2 autosomal dominant medullary cystic kidney disease [25]. Indeed, a recent study also suggests that this latter entity is commonly associated with severe hyperuricemia and clinically mimics the phenotype of familial hyperuricemic nephropathy [25]. However, it is clear that FJHN is a syndrome, not a result of defect in a single gene [64].

The pathogenesis of the renal injury remains to be fully elucidated. Interestingly, medullary cystic kidney disease is known to be associated with salt wasting, but it remains unclear if patients with FJHN also have a salt- or water-wasting defect. Preliminary results in FJHN patients, however, suggest that they have a defect in urine concentration, and this correlated inversely with the serum uric acid levels [15]. Furthermore, mice with the uromodulin mutation also show a mild water- and sodium-wasting phenotype and have evidence for upregulation of sodium transporters in their proximal tubules, as well as a relative defect in urinary urate excretion when adjusted for the sodium excretion [65]. This provides the interesting possibility that the hyperuricemia in FJHN is due to increased proximal sodium and urate reabsorption secondary to renal salt loss.

Diagnosis and Treatment of FJHN

Diagnosis is suggested by a positive family history; the early onset of renal insufficiency in the setting of elevated uric acid levels (often >9 mg/dL) disproportionate to age, sex, or degree of renal dysfunction; and by FE_{ur} of <5 %. The grossly decreased mean fractional excretion of uric acid found in affected children is even more striking when compared with the normally high FE_{ur} of their healthy counterparts (range from 12 % to 30 %). However, the very low FE_{ur} in FJHN increases if renal failure progresses, and

consequently in early uremia, the FE_{ur} may be normal for a while [61] and thus obscure the diagnosis. Confirmation of FJHN can now be obtained through leukocyte DNA analysis for the mutation [62].

Treatment is largely supportive. Aggressive control of high blood pressure is generally thought to be crucial for a successful outcome. The role of allopurinol (and hence a decrease of hyperuricemia) in ameliorating the progression of the renal disease has been stressed by some researchers [63, 66, 67] but is disputed by others [68, 69]. The efficacy of allopurinol clearly relates to the degree of renal damage at the time of treatment initiation, and patient compliance is also important.

Hyperuricemia in Patients with Chronic Kidney Disease

We have discussed the role of hyperuricemia as a cause of kidney disease; however, hyperuricemia may be also seen in patients with established renal diseases, mainly in the setting of reduced renal clearance of uric acid associated with progressive loss of GFR [70]. As discussed earlier, there is some retention of uric acid with renal insufficiency of various causes, but this is usually mild due to compensatory increases in the fractional excretion of uric acid and an increase in enteric excretion in the gut [9, 10]. Indeed, gouty arthritis is uncommon in CKD. This may relate to the fact that uric acid level above 10 mg/dL is unusual in advanced renal failure and that uremia is known to inhibit neutrophil chemotaxis and function [71], and hence the inflammatory response may be partially subdued.

There has been a long-history controversy whether it is beneficial to lower serum uric acid levels in CKD patients and if so, what target level of uric acid needs to be achieved. Asymptomatic hyperuricemia in CKD patients was regarded biologically inert and was not an indication of aggressive hypouricemic therapy without a history of gouty arthritis or biopsy-proven chronic uric acid nephropathy or excessively high serum uric acid level. However, many recent studies provide new evidence that uric acid has contributory role in hypertension and renal and cardiovascular disease, which may change the therapeutic guideline of hyperuricemia in different population of subjects.

There is compelling evidence that hyperuricemia is an independent risk factor for CKD in the general population [46]. In one study, risk for CKD in a subject with hyperuricemia was greater than for that associated with proteinuria [72]. Hyperuricemia has also been reported to be an independent risk factor for renal disease progression in patients with glomerular diseases [73–75] and contrast-induced nephropathy [76] and also may confer increased risk in subjects with essential hypertension [77]. Additional evidence that hyperuricemia may have a role in kidney disease was provided by a prospective clinical trial in 54 hyperuricemia CKD patients with random assignment of allopurinol or placebo for 1 year. Allopurinol treatment resulted in a decrease in serum uric acid associated with a significant slowing of renal disease progression. [48]. Another study reported that diabetic patients with elevated serum uric acid levels were at highest risk for developing progressive nephropathy [78]. In this study, a multiple logistic regression model revealed a significant relationship between uric acid and prevalence of hypertension [odds ratio (OR) = 1.8; 95 % confidence interval (CI) 1.6–2.0] after adjustment for age, sex, and duration of diabetes. The prevalence of macroalbuminuria was significantly higher in patients with hyperuricemia than in those with hypo- and normouricemia. Uric acid was also independently associated with macroalbuminuria, after adjustment for age, sex, HbA_{1c} levels, creatinine clearance, duration of diabetes, and blood pressure levels (OR = 1.5; 95 % CI 1.1–2.0). There have been several recent epidemiologic studies demonstrating the role of uric acid in development of new kidney disease and/or worsening renal function in subjects with different clinical background and comorbidities [79–83].

Besides the effect of hyperuricemia on renal disease progression, an elevated uric acid is also regarded as a cardiovascular risk factor. Given the high prevalence of cardiovascular morbidity in CKD patients, clinical implication of hyperuricemia on cardiovascular complication in CKD subjects may need to be readdressed. A recent prospective study in 294 newly diagnosed patients with CKD stage 5 followed for an average of 6 years revealed that subjects with markedly increased serum uric acid levels (≥9.0 mg/dL) had a twofold increased risk for mortality after adjusting for numerous comorbidities [84]. Although high uric acid levels were associated with lipid levels, calcium/phosphate metabolism, and levels of inflammation markers, an elevated uric acid level itself may represent a true risk factor for cardiovascular disease and mortality in CKD patients [85, 86]. In a randomized, prospective study in 113 patients with estimated GFR (eGFR) <60 ml/min, Goicoechea et al. demonstrated that allopurinol (100 mg/day) was able to slow the progression of renal disease after a mean time of 23.4 ± 7.8 months [87]. No changes in blood pressure or in albuminuria induced by allopurinol have been observed. Interestingly, allopurinol treatment also reduced cardiovascular and hospitalization risk in these subjects.

Potential Mechanisms of Uric Acid-Induced Promotion of Chronic Kidney Disease and Cardiovascular Disease

According to the recent experimental data, uric acid may induce preglomerular arterial disease, renal inflammation, and hypertension via an activation of RAS and COX-2 [51, 88].

There is still limited information, however, about the pathogenic mechanism to verify the causative role of uric acid in renal and cardiovascular diseases. Uric acid is also a mitogen for vascular smooth muscle cells. Rat aortic vascular smooth muscle cells showed de novo expression of COX-2 mRNA after incubation with uric acid [88]. Incubation of the smooth muscle cells with either a COX-2 inhibitor or with a TX-A2 receptor inhibitor prevented a proliferation response to uric acid. COX-2 was also shown to be expressed de novo in the preglomerular vessels of hyperuricemia RK rats, and its expression correlated both with the uric acid levels and with the degree of smooth muscle cell proliferation. These findings suggest a critical role for uric acid-mediated COX-2 generated thromboxane in vascular smooth muscle cell proliferation in animal models of chronic progressive renal disease. In addition to COX-2, it is likely that angiotensin II is also contributing to uric acid-induced vasculopathy. Preglomerular vasculopathy in rats with oxonic acid-induced hyperuricemia can be largely prevented by blocking the RAS, and scientists have also reported that uric acid-mediated vascular smooth muscle cell proliferation can be partially inhibited by blocking the angiotensin II type 1 receptor [51]. Therefore, both angiotensin II and COX-2 are involved in the vascular proliferation and inflammation observed in in vitro and in vivo animal studies.

Once thickening of the afferent arterioles and macrophage infiltration in vessel wall were induced, preglomerular vasculopathy may potentiate renal injury by causing ischemia to the postglomerular circulation. The reduction in lumen could also provide a stimulus for the increase in renin expression we observed and might also contribute to the development of the marked hypertension in these rats [49, 51, 89]. Furthermore, there is evidence that the arteriolopathy also leads to ineffective autoregulation and increased transmission of systemic pressures to the glomerulus [90], which can also potentiate renal damage.

Uric acid also induced the proinflammatory cytokine, monocyte chemoattractant protein-1 (MCP-1), and de novo expression of C-reactive protein (CRP) in vascular smooth muscle cells, which was further shown to be due to direct entry of uric acid into VSMC with activation of mitogen-activated protein (MAP) kinase and nuclear transcription factor (NF-kB) [91, 92].

In addition, uric acid can become pro-oxidative under certain circumstances [93]. Consistent with this possibility, there is evidence that increased serum uric acid promotes oxidation of low-density lipoprotein cholesterol to then facilitate lipid peroxidation. On the other hand, some investigators have reported that uric acid may function as an antioxidant, particularly as a scavenger of peroxynitrite [94]. These investigators propose that an increase in uric acid may be beneficial and represent a host response to combat the inflammatory processes associated with atherosclerosis and hypertensive vascular damage. However, these benefits may be overwhelmed by the detrimental effect of uric acid.

Treatment Guidelines of Hyperuricemia in Chronic Kidney Disease

Asymptomatic hyperuricemia in patients with CKD generally remains untreated because of concerns over the potential toxicity with urate-lowering therapies, in particular the potentially fatal allopurinol hypersensitivity syndrome [55]. However, recent evidence showing the association of hyperuricemia with obesity, insulin resistance, hypertension, metabolic syndrome, and progression of kidney disease has led to the suggestion that hyperuricemia may be an independent risk factor for the development of cardiovascular and renal disease and thus warrant treatment in its own right. Especially given the high prevalence of cardiovascular and metabolic comorbidity in CKD patients, nephrologists should carefully consider whether to treat hyperuricemia or not and which option is the most appropriate in individual patients if they decide to prescribe antihyperuricemic medicine. However, it should be emphasized that aggressive treatment of hyperuricemia in CKD patients is not considered as standard practice yet until more robust evidence is available.

Management of Hyperuricemia

Lifestyle modification with low-purine diet is the first option for treating hyperuricemia in CKD patients. Careful monitoring of nutritional status is necessary to avoid malnutrition. Dietary purines make a substantial contribution to serum uric acid levels, and low-purine diet can reduce serum uric acid levels [95]. The association between alcohol and gout has recently been confirmed in a 12-year study [96] revealing the relative risk of gout was increased from 1.32 for men who consumed 10.0–14.9 g alcohol/day to 2.5 for men who consumed ≥50 g alcohol/day compared with nondrinkers. Although consumption of seafood and meat was associated with hyperuricemia and increased risk of gout, moderate intake of purine-rich vegetables or protein was not associated with an increased risk of gout. From a practical perspective, restriction of dietary purines from animal rather than from vegetable sources may be appropriate. Traditional dietary advice for hyperuricemia has centered around a low-purine and low-protein diet. Reduction of serum uric acid by approximately 1–2 mg/dL may occur with such a diet [95].

There are medications that can raise plasma uric acid levels, which include loop and thiazide diuretics, cyclosporin, aspirin (low dose), pyrazinamide, ethambutol, or nicotinic

acid. Therefore, clinicians need to be alert to discontinue or reduce doses of these drugs. In contrast, the angiotensin II receptor antagonist losartan has been reported to have a uricosuric effect [97], and the calcium channel blocker amlodipine increases uric acid clearance and reduces uric acid concentrations in comparison with perindopril [98]. In patients with primary hyperlipidemia, atorvastatin, but not simvastatin, has been shown to reduce uric acid concentrations [99]. Fenofibrate, but not other fibrates, has been shown to enhance renal uric acid clearance and reduce uric acid concentrations [100]. However, it should be noted that the fibrates have also been associated with an increase in serum urea and creatinine [101]. Sevelamer, a phosphate binder, can also lower serum uric acid levels [102]. In summary, clinicians should carefully consider the therapeutic options for conditions associated with hyperuricemia such as hypertension and hyperlipidemia. In patients with difficult-to-control hyperuricemia, use of an agent that assists in reducing serum uric acid (or at least that does not increase serum uric acid) may be beneficial.

Antihyperuricemic Treatment : Old and New Drugs

Currently available options for reducing SUA include either reducing uric acid production by the use of xanthine oxidase inhibitors (allopurinol) or increasing renal the excretion of uric acid through the use of uricosuric agents (probenecid, benzbromarone).

Allopurinol is the most commonly used hypouricemic agent because of its ability to lower SUA regardless of the cause of hyperuricemia and the convenience of once daily dosing. It reduces the production of uric acid at its rate-limiting step through inhibition of xanthine oxidase. Allopurinol is rapidly metabolized to oxypurinol, which is responsible for most of the xanthine oxidase inhibition. The half-life of allopurinol is 1–2 h and that of oxypurinol is 18–30 h in those with normal renal function, but its action is prolonged in CKD extending to a week in those with a creatinine clearance (CrCL) of <3 mL/min [103]. Approximately 20 % of patients experience side effects with allopurinol, with up to 5 % ultimately discontinuing therapy [54]. The most serious side effect is a rare hypersensitivity syndrome, which results in fever, rash, eosinophilia, hepatitis, renal failure, and in some cases death. The exact mechanism of the allopurinol hypersensitivity syndrome is unclear, but it has been postulated to be related to elevated serum oxypurinol levels as well as to immunological processes [104].

Careful dosing of allopurinol is necessary in patients with renal impairment (Table 26.1) [5]. However, such dosing regimens are often ineffective in controlling hyperuricemia and gout [104]. Furthermore, the ability of such dosing regimens to reduce allopurinol hypersensitivity syndrome remains unclear [105]. The net result is often undertreatment of a potentially curable disorder. Although there are multiple reports of clinicians using higher than recommended doses of allopurinol [106], there is a lack of evidence as to the benefit of such therapy in controlling hyperuricemia [105].

Uricosuric agents, such as probenecid and benzbromarone, lower serum uric acid by increasing renal uric acid excretion. One potential complication is the deposition of uric acid crystals within the kidney, which can result in urate nephropathy and/or the formation of uric acid stones. The risk of these complications can be reduced by gradual increases in drug dose, ensuring urine volume is ≥1,500 mL/day and maintaining an alkaline urine (pH 6.4–6.8). Probenecid was the first uricosuric drug available. Its use is limited as efficacy of probenecid declines as renal function declines, and it is ineffective when CrCL is <60 mL/min. Probenecid is usually well tolerated at the recommended doses of 1–3 g/day. Of note, the uricosuric effect of probenecid is blocked by the simultaneous administration of aspirin. Benzbromarone is a potent uricosuric agent, which lowers SUA by inhibiting postsecretory tubular resorption of uric acid [107]. Low-dose benzbromarone (50–100 mg/day) has been reported to be more potent than 300 mg/day of allopurinol [53] and equipotent to 1–1.5 g/day of probenecid [108]. Benzbromarone appears to have only slightly impaired efficacy in patients with impaired renal function [109], and in renal transplant recipients, it has been reported to be beneficial in patients with a CrCL >25 mL/min. Benzbromarone therapy has also been associated with a faster tophus reduction than has allopurinol therapy [109]. Hepatic toxicity, rarely leading to death, has been reported in patients taking high doses of benzbromarone [110]. Benzbromarone is unavailable in the USA because of concerns over the potential for hepatotoxicity. However, in the largest series published, there was no significant liver toxicity in 200 patients treated for a mean of 5 years with 75–125 mg/day of benzbromarone [111]. Benzbromarone remains a therapeutic option particularly for patients with significant renal impairment or with intolerance to allopurinol, or in transplant recipients who are taking azathioprine.

In the last few years, several new hypouricemic agents have emerged. One of them is febuxostat, a non-purine-selective inhibitor of xanthine oxidase. Unlike allopurinol, febuxostat does not resemble purines or pyrimidines structurally. In comparison to allopurinol, which only weakly inhibits the oxidized form of xanthine oxidase, febuxostat inhibits both the oxidized and reduced forms of xanthine oxidase [112]. Febuxostat has been shown to be safe and effective in lowering uric acid concentrations in randomized double-blind studies. Importantly, there is no need for dose

Table 26.2 General guideline for management of hyperuricemia in CKD patients

1. First, we need to decide to treat hyperuricemia or not according to the patients' characteristics
1.1. Try to find the aggravating factors of hyperuricemia and correct them
1.2. Lifestyle modification with diet
1.3. Consider several drugs with uric acid-lowering effects such as losartan, statin, sevelamer, or AST120
2. If FE_{UA} is normal or high, prescribe xanthine oxidase inhibitor, but with adequate dose, careful monitoring, and consideration of drug reaction
3. If patient is not tolerable to xanthine oxidase inhibitor, uricosuric agents can be tried according to patients' residual renal function, comorbidity, and concurrent medication
3.1. Benzbromarone is more effective in patients with > stage III CKD than other uricosuric agents
3.2. Careful monitoring of side effects is necessary
4. High-flux dialysis is helpful for controlling hyperuricemia in HD patients

adjustment in renal disease. In patients with normal (CrCL 80 mL/min/1.73 m^2), mild (CrCL 50–80 mL/min/1.73 m^2), moderate (CrCL 30–59 mL/min/1.73 m^2), or severe (CrCL 10–20 mL/min/1.73 m^2) impairment in renal function, 80 mg febuxostat daily for 7 days has been reported to be safe without requirement for dose adjustment [113]. Further data will be required to address the safety, efficacy, and effect on renal function of febuxostat in CKD patients. General recommendations for the management of hyperuricemia in CKD patients are summarized in Table 26.2.

Significance and Treatment of Asymptomatic Hyperuricemia

Asymptomatic hyperuricemia is defined as hyperuricemia without the sign of urate deposition in joints or other organs, which is found in 5–8 % of the general population and corresponding to two-thirds or more patients with hyperuricemia. Almost 10 % of adults are documented to have hyperuricemia at least once in their lifetime [114]. Most do not need further workup or treatment. However, those at substantially higher risk for complications of hyperuricemia—gout, urolithiasis, or acute uric acid nephropathy—merit an evaluation to determine the cause and the appropriate treatment. Hyperuricemia is also occasionally a sign of underlying disease or environmental exposure including specific drug or toxin. Table 26.3 shows major causes of hyperuricemia, which has been arbitrarily defined as >7.0 mg/dL in men and >6.5 mg/dL in women. Clinical significance of asymptomatic hyperuricemia is uncertain at this point of time although elevated uric acid level seems to be more than simply an epiphenomenon of reduced renal function in animal models. However, in many epidemiologic studies, hyperuricemia per se is not clearly shown to be an independent risk factor for cardiovascular and renal disease after controlling other risk factors such as aging, high blood pressure, and insulin resistance. Furthermore, it is not established yet whether to screen serum uric acid levels as a risk factor of cardiovascular disease in general population.

Table 26.3 Causes of hyperuricemia

Genetic causes
Familial hyperuricemic nephropathy (mutation of uromodulin)
Lesch-Nyhan syndrome (HGPRT mutation)
Phosphoribosyl pyrophosphate synthetase mutation (PRPPS)
Dietary causes
Diet high in purines (organ meats, shellfish, fatty meats)
Diet high in fructose (high fructose corn syrup, table sugar, honey)
Ethanol
Low-salt diet
Drugs
Thiazides
Loop diuretics
Calcineurin inhibitors (cyclosporine > tacrolimus)
Pyrazinamide
Low-dose aspirin
Volume depletion
Hypoxia (systemic or tissue)
Increased cell turnover (myeloproliferative disorders, polycythemia vera)
Conditions associated with higher uric acid levels
Renal failure
Obesity metabolic syndrome
Untreated hypertension
African-American race
Preeclampsia
Vigorous exercise

Recent investigations suggest that higher levels of serum uric acid may confer risk for kidney injury both acutely and chronically. For example, a single-center study of 190 patients undergoing cardiac surgery reported a graded increased risk for postoperative acute kidney injury, which was directly associated with higher levels of uric acid [115]. In a prospective study of over 1,000 participants with type 2 diabetes followed over 3.5 years, higher baseline serum uric acid was independently associated with greater reductions in eGFR as well as incident coronary heart disease [116].

Evaluation of Asymptomatic Hyperuricemia

Decisions regarding pharmacologic treatment of asymptomatic hyperuricemia should be based upon an estimate of the risk in each individual for the development of gouty arthritis, tophi, uric acid stones, chronic renal insufficiency, or acute uric acid nephropathy. This estimated risk should be weighed against the potential benefits and risks of drug treatment.

If asymptomatic hyperuricemia is identified in an individual, he or she should be screened for the causes and the degree of hyperuricemia. Underlying disorder or environmental exposure causing hyperuricemia and requiring specific treatment should be sought vigilantly, and hyperuricemia-inducing drugs or toxins should be removed or substituted with the goal of improving the hyperuricemic state. A thorough medical history, physical examination, and laboratory evaluation should be performed and directed at discovering potentially treatable causes of hyperuricemia such as lymphoproliferative and myeloproliferative disorders, psoriasis, vitamin B12 deficiency, preeclampsia, and lead nephropathy or lead exposure (Table 26.2). If the above evaluation is normal, a 24-h urine specimen should be collected while the individual is receiving a standard diet, excluding alcohol and drugs known to affect uric acid metabolism. Measurement of uric acid and creatinine in this specimen will usually distinguish between uric acid overproduction (with hyperuricosuria defined as urinary uric acid excretion greater than 800 mg/day or 12 mg/kg/day) and reduced urinary uric acid clearance (with normal urinary uric acid excretion) [113].

The distinction between overproduction and underexcretion can guide further investigation of the underlying cause of hyperuricemia and may be useful in directing the choice of antihyperuricemic medication. The vast majority of hyperuricemic individuals (80–90 %) demonstrate excess dietary purine consumption, urate underexcretion, or both. The remainder exhibit overproduction of urate from endogenous sources. In each patient, FE_{ur} needs to be determined in the 24-h urine specimen. Underexcretion is defined as a ratio of less than 6 %. If hyperuricosuria is present, the urine collection should be repeated after 5 days on a low-purine diet. If urate excretion is greater than 670 mg/day on a low-purine diet, inherited causes of overproduction such as various enzyme defects, disordered ATP metabolism, or disorders resulting in increased rates of cell turnover should be considered before making a diagnosis of primary hyperuricemia. If uric acid excretion and serum urate levels decline to normal levels on a low-purine diet, excess dietary purine consumption can be suspected.

Most patients with underexcretion of urate and a normal GFR have no other demonstrable abnormality in renal function. The defect in renal urate transport (which could be manifested functionally as either reduced secretion or enhanced reabsorption) may be acquired or intrinsic (possibly as an inherited trait) or secondary to reduced renal perfusion by volume depletion.

Treatment of Asymptomatic Hyperuricemia

There is still no consensus on the treatment of individuals with asymptomatic hyperuricemia. Treatment of asymptomatic hyperuricemia is not necessary in most patients, unless perhaps they have very high levels of uric acid or are otherwise at risk of complications, such as those with a personal or strong family history of gout, urolithiasis, or uric acid nephropathy [114]. Although current clinical data do not justify antihyperuricemic treatment of most asymptomatic patients by risk/benefit analysis, some specific circumstances warrant at least consideration for the institution of treatment despite an absence of symptom. First is persistent hyperuricemia in the infrequent patients with sustained serum urate concentrations greater than 13 mg/dL in men and 10 mg/dL in women since these high values may carry some nephrotoxic risk, perhaps related to the likelihood of some component of uric acid overproduction. Second, excretion of urinary uric acid in excess of 1,100 mg/day is associated with a 50 % risk of uric acid calculi [117, 118]. Management of these individuals should begin with dietary purine restriction. Finally patients about to receive radiotherapy or chemotherapy that is likely to result in extensive tumor cytolysis should be treated to prevent acute uric acid nephropathy [58] as detailed in the section on acute uric acid nephropathy.

Summary

Alteration of uric acid metabolism and excretion is associated with a spectrum of renal disease. Emerging evidence suggests that hyperuricemia may not only cause acute renal failure as a consequence of crystal formation within the tubular lumina but that chronic hyperuricemia may also promote progressive renal disease via crystal-independent pathways. Indeed, uric acid may contribute to chronic renal disease from gouty nephropathy as well as play a role in lead nephropathy, primary renal diseases, diabetic nephropathy, and cyclosporine nephropathy. Recent evidence suggests that uric acid may be a previously unrecognized contributor to cardiovascular risk. Despite a strong evidence of a possible causal role for uric acid, however, much controversy still remains. In order to determine the complex role of uric acid in the pathogenesis of chronic disease processes, well-designed clinical intervention studies looking at the effect of lowering uric acid on cardiovascular, metabolic, and renal disease outcomes are needed.

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Plasmapheresis

27

François Madore and Catherine Girardin

Introduction

Plasmapheresis is an extracorporeal blood purification method for the treatment of conditions in which "circulating factors" are believed to contribute to disease pathophysiology. The basic premise of plasmapheresis is that removal of these pathogenic circulating factors will reduce further damage and will permit reversal of the pathologic process. Plasmapheresis has been applied over the several last decades as primary or adjunctive treatment for a wide variety of systemic conditions with or without renal involvement including hematologic, metabolic, and immunologic disorders. Plasmapheresis has also been used for a number of primary renal diseases. The past and current use of this complex and expensive treatment has not always been based on good quality randomized controlled trials. However, in recent years, indications for plasmapheresis have been reexamined according to the quality of evidence for safety and efficacy from published clinical trials and experience [1–3].

Apheresis Technology and Procedural Issues

The basic components of any plasmapheresis treatment are (a) withdrawal of venous blood, (b) separation of plasma from blood cells, (c) reinfusion of cells with or without infusion of a replacement solution, and (d) disposal or processing of the separated plasma. The procedure usually requires blood flow of 50–150 mL/min with administration of appropriate anticoagulant and lasts approximately 2–3 h.

Separation of Plasma from Blood Cells

Extracorporeal plasma separation can be achieved either by centrifugation or membrane filtration. In the USA and Canada, centrifugal separators are the dominant technology. Membrane filters are in use, for the most part, in Europe and Asia.

Centrifugation Technique

The use of centrifugal force causes the whole blood to separate into various components according to their specific gravity. Blood drawn from the patient is directed to a rapidly spinning centrifuge where the plasma is separated from the cellular components. The plasma is diverted to a collection container to be discarded, and the rest of the blood is returned to the patient with or without replacement fluid. Such centrifugation machines process blood using either a discontinuous or a continuous method. With discontinuous (or intermittent) apheresis, a discrete volume of blood is drawn into the centrifuge, separated, collected, and then returned (with or without replacement fluid) before a subsequent volume of blood is drawn and processed. Continuous-flow methods draw blood without interruption into the extracorporeal circuit while it is being separated, collected, and reinfused. Advantages of intermittent centrifugation include relative simplicity of operation, portability of the machines, and adequacy of a single-needle peripheral venipuncture. The disadvantages are slowness (typically >4 h) and the relatively large extracorporeal blood volume required (>225 mL). Continuous-flow centrifugation is faster and most operations (anticoagulation, collection procedures, and fluid replacement) are automated. Disadvantages include higher cost, relative immobility of the equipment, and the requirement for either two venipunctures or insertion of a dual lumen catheter.

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Membrane Filtration Technique

Extracorporeal plasma separation may also be achieved by membrane filtration (Fig. 27.1), with the membrane configured as either a hollow fiber or a flat plate. The pore size of such filters, 0.2–0.6 μ[mu]m in diameter, excludes erythrocytes, leukocytes, and platelets (which have mean sizes of 7, 10–13, and 3 μ[mu]m, respectively), allowing separation of the patient's plasma from the other blood components. Membrane plasma separation can be performed with standard dialysis equipment (either conventional or continuous). Membrane filtration has been shown to be as safe and efficient as centrifugal plasmapheresis [4–6].

There are a number of potential advantages of membrane filtration over centrifugal apheresis. The first advantage is its simplicity. Since the procedure can be performed using standard conventional or continuous hemodialysis machines, hemodialysis nurses may be comfortable using such a system. Second, the plasma separator membranes do not decrease the platelet count, whereas significant reduction in platelet count (up to 50 % reduction) may be observed with centrifugation. Third, no leukocyte loss is observed with membrane filtration, whereas in centrifugation, plasma may be contaminated with leukocytes. There are, however, a number of potential disadvantages to membrane filtration. The first disadvantage is that membrane filtration devices can only be used to isolate plasma. Centrifugation machines, on the other hand, can usually be adapted for either plasmapheresis or cytapheresis procedures, such as leukocytopheresis for leukemia, erythrocytopheresis for sickle cell disease, or thrombocytopheresis for symptomatic thrombocytosis. A second disadvantage of membrane filtration is the possible activation of complement and leukocytes on the artificial membrane. In addition, membranes may trigger anaphylactoid reactions; for instance, anaphylactoid reactions have been reported with membrane apheresis in patients taking angiotensin-converting enzyme inhibitors [7]. Finally, membrane filtration usually requires a large vein (central) catheter to obtain adequate blood flow rates, whereas peripheral venipunctures may suffice for centrifugal apheresis.

Processing of Separated Plasma

The plasma separated from the cells can be either discarded or reinfused to the patient after specific processing procedures. These specialized processing methods have been developed over the years with the goals of more selectively removing the pathogenic circulating factors and limiting the loss of plasma components. With these techniques, the plasma separated from the cells is further processed to eliminate only the purported pathogen(s). It is then returned to the patient without the need for replacement fluid. These approaches include adsorption techniques and selective filtration techniques (Fig. 27.1).

For adsorption techniques, the separated plasma is passed through a device (an adsorption column or equivalent) which has the capacity to remove specific components from the plasma by selectively binding them to the active component of the device. Adsorption columns have been designed to remove (1) IgG and immune complexes (using immobilized *Staphylococcus aureus* protein A which binds specifically to the Fc segment of IgG), (2) nonspecific immunoglobulins (using immobilized antihuman immunoglobulin antibodies), (3) anti-DNA and anticardiolipin antibodies (using immobilized dextran sulfate), (4) low-density lipoprotein (LDL) (using immobilized anti-LDL antibodies), and (5) endotoxin and inflammatory cytokines (using immobilized polymyxin B and others adsorbers) [8–12].

For selective filtration techniques, the separated plasma is passed through a membrane or another device which uses specific physicochemical properties to remove selected components from the plasma (Fig. 27.1). A variety of devices have been designed to remove selected components including (1) membrane filters with different pore diameters and filtration/adsorption characteristics (double filtration or cascade filtration) and (2) thermoregulation units to either warm the plasma (thermofiltration) or cool the plasma (cryofiltration) to enhance the removal of specific components [13–15].

The major advantage of selective plasmapheresis techniques over standard plasmapheresis is the possibility to reinfuse the treated plasma to the patient, therefore obviating the need to use either albumin or fresh frozen plasma. Hence, the side effects related to the use of replacement solutions (e.g., anaphylactoid reactions to fresh frozen plasma, coagulopathies induced by inadequate replacement of clotting factors, transmission of viral hepatitis and other infections) are eliminated. There are however disadvantages to selective plasmapheresis techniques. Additional equipment or devices are required which may add to the cost and the complexity of the therapies. These additional devices (i.e., membrane filters or ligands used in adsorption columns) may trigger allergic reactions or may cause adverse reactions in some patients. Finally, most of the selective procedures are considered investigational as only a limited number of studies have compared selective procedures to standard plasmapheresis [16, 17]. Therefore, clinical trials are needed to better appraise the place of these procedures among the various modalities of treatment available.

Anticoagulation

To prevent activation of the clotting mechanisms within the extracorporeal circuit, an anticoagulant is usually added to the patient's blood as it is withdrawn. The most frequently used anticoagulant for centrifugation procedures is citrate which chelates ionized calcium. A continuous infusion of acid citrate dextrose (ACD) is given intravenously during the

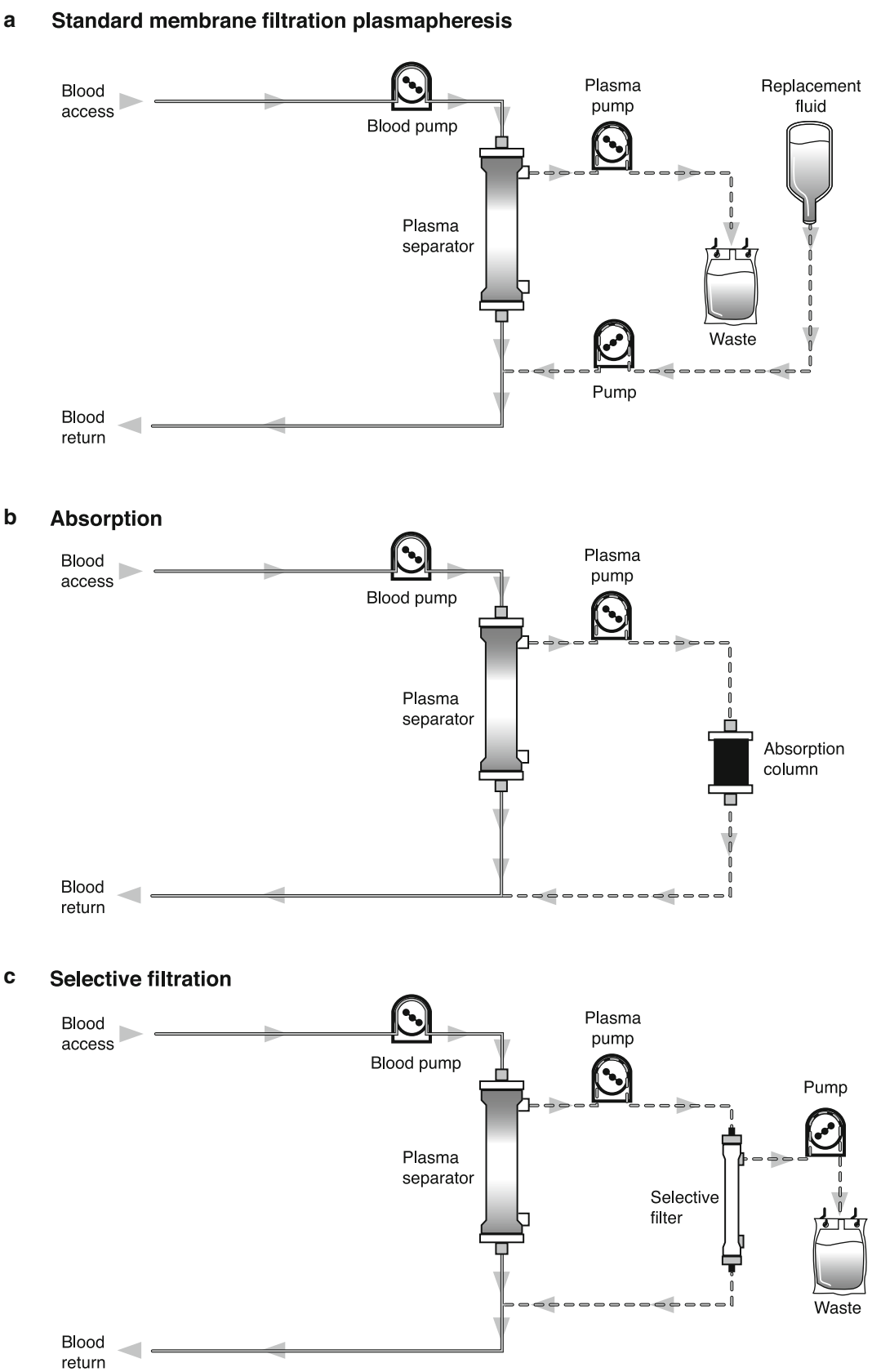

Fig. 27.1 Standard membrane filtration (**a**), adsorption (**b**), and selective filtration (**c**) systems. (**a**) Blood is pumped into a biocompatible membrane that allows the filtration of plasma while retaining cellular elements. Red cells, leukocytes, and platelets are reinfused to the patient along with replacement fluid. Plasma is pumped into a collection bag and discarded. (**b**) Following the separation of plasma and cellular elements, the separated plasma is passed through an adsorption column (or equivalent) which has the capacity to remove specific factors from the plasma by selectively binding to the active component of the device. The processed plasma is returned to the patient without the need for replacement fluid. (**c**) Following the separation of plasma and cellular elements, the separated plasma is passed through a membrane (or another device) which uses specific physicochemical properties to remove selected components from the plasma. The processed plasma is returned to the patient without the need for replacement fluid

procedure. The amount of citrate delivered to the patients is proportional to the volume of blood treated, the duration of the procedure, and the use of plasma products (which are collected and stored with citrate). Symptoms and signs of citrate toxicity include a metallic taste in the mouth, a decrease in blood pressure, paresthesia, muscle twitching, tetany, and prolonged Q-T interval. Symptomatic hypocalcemia resulting from citrate infusion complicates 1.5–9 % of treatments [18, 19]. Mild signs and symptoms usually resolve by simply interrupting the administration of citrate for a few minutes to allow time for metabolism of the citrate. In more symptomatic patients, infusion of calcium chloride or calcium gluconate should be given.

Standard unfractioned heparin is the most frequently used anticoagulant for membrane plasmapheresis. The required dose of heparin is approximately twice that needed for hemodialysis, because a substantial amount of the infused heparin is removed along with the plasma. If heparin is used in patients with a high risk of bleeding, the activated clotting time (ACT) or the partial thromboplastin time (PTT) should be monitored closely. Heparin-induced bleeding may be reversed with protamine.

Replacement Fluids

The typical replacement fluids are fresh frozen plasma or other plasma derivatives (e.g., cryosupernatant), 5 % albumin, colloidal starches, and crystalloids (e.g., 0.9 % saline, Ringer's lactate). The choice of fluid has implications for the efficacy of the procedure, oncotic pressure, coagulation, and spectrum of side effects. Fresh frozen plasma (FFP) replaces the normal proteins that have been removed. As a result, there is no depletion of coagulation factors or immunoglobulins. However, albumin or a combination of albumin and saline is usually preferred to FFP because of the risk of hypersensitivity reactions, citrate-induced symptoms, and transmission of viral infections with the latter. Albumin (5 %) is generally combined with 0.9 % saline on a 50 %:50 % (vol/vol) basis. Other alternatives to plasma include colloidal starches and 0.9 % normal saline. Colloidal starches (e.g., hetastarch, pentastarch) can be safely used as a partial replacement fluid in combination with 0.9 % saline or human albumin. Normal saline (0.9 %) can transiently satisfy volume replacement needs during plasmapheresis, but quickly equilibrates with the extravascular compartment, thereby limiting its use.

The exact composition of replacement fluids must tailored to the needs of the patient. For example, plasma is the replacement fluid of choice in patients with thrombotic thrombocytopenic purpura (TTP) because the infusion of normal plasma may contribute to the replacement of a deficient plasma factor. Plasma may also be preferable in patients at risk of bleeding (e.g., those with liver disease or disseminated intravascular coagulation) or requiring intensive therapy (e.g., daily exchanges for several weeks). When replacement solutions other than FFP are used, a post-pheresis depletion coagulopathy is expected in most patients. With a typical 1.0–1.5 plasma volume exchange, the immediate post-exchange levels of most coagulation factors are expected to be approximately 25 % of their pre-procedure values. The exceptions are factors VIII and IX, which are reduced to lesser degrees [20]. The importance of this coagulopathy depends on the clinical situation. Most patients with no underlying hemostatic risk tolerate these changes well and require no supplementation with coagulation factors. In patients with underlying increased bleeding risk, it is reasonable to include 25 % FFP replacement to ensure adequate post-pheresis hemostasis.

Vascular Access

Adequate vascular access is required to allow the high blood flow rates (i.e., 50–150 mL/min) necessary to remove the typical amount of plasma (4–4.5 L) over the course of a few hours during standard plasmapheresis procedures. For discontinuous (or intermittent) procedures, a single large vein or a single-lumen catheter is adequate. For continuous-flow techniques, two venous access sites are required, one for blood drawing and the other for blood return. Two large veins or a double-lumen catheter are thus required. For patients with large veins and for whom large number of procedures are not expected, antecubital and forearm veins may be adequate. For all other patients, a double-lumen catheter which is rigid enough to withstand significant flow and pressures is preferable. For urgent procedures and short-term therapy, a femoral line may be the best option. For patients with non-urgent indications who are likely to require long-term therapy, placement of a tunneled catheter is preferable.

Exchange Volume

In most instances, 4–4.5 L of plasma are removed or processed during a typical plasmapheresis procedure, which corresponds to 1.0–1.5 times the plasma volume. A single plasma volume exchange will remove approximately 63 % of the IgM and IgG found in the intravascular compartment, and an exchange equal to 1.5 times the plasma volume will remove roughly 78 % of the intravascular immunoglobulins [21]. The following formula can be used to estimate the plasma volume in adults:

$$\text{Plasma volume} = \left(0.65 \times \text{Weight}[\text{kg}]\right) \times \left(100 - \text{Hematocrit}[\%]\right)$$

For example, the plasma volume of a 70 kg man with a hematocrit of 40 % is estimated at 2.7 L. Therefore, a typical exchange volume of 1.5 times the plasma volume for this patient will be around 4 L (1.5 × 2.7 L).

Apheresis Schedule

The American Association of Blood Banks general recommendation for conditions requiring plasmapheresis is that one exchange be performed every second or third day, each exchange consisting of 1–1.5 plasma volumes, for a total of 3–5 procedures [22]. In some conditions, such as thrombotic thrombocytopenic purpura, plasma exchanges should be performed daily until the platelet count is normal for 2–3 consecutive days (see below). Another exception is anti-glomerular basement membrane mediated disease; in this disorder, plasma exchanges should be performed on a daily basis for at least 2 weeks (see below).

Apheresis Physiological Principles

Many of the disorders for which plasmapheresis is advocated are thought to have an immunological basis, and plasmapheresis is often thought to work by removing immunoglobulins or immune complexes [23]. Plasmapheresis has also been proposed for removing abnormal circulating proteins in disorders such as in multiple myeloma and other dysproteinemias [24]. The removal of thrombotic factors has been suggested to explain the effect of plasmapheresis in TTP [25]. Infusion of normal plasma may itself have beneficial effects. Indeed, there is evidence that replacement of a deficient plasma component may be the principal mechanism of action of plasmapheresis in TTP [26]. Other theoretical beneficial effects on immune function include depletion of complement products, fibrinogen, and some cytokines, alterations in idiotypic/anti-idiotypic antibody balance, and improvement in reticuloendothelial system function [27–29].

Efficacy of Plasmapheresis in Specific Renal Diseases

In the following section, the therapeutic use of plasmapheresis in specific acute renal conditions is reviewed. For each condition, the clinical studies that evaluated its efficacy are summarized with special emphasis on the results of randomized controlled trials, when available. Consensus plasma exchange regimens are presented for diseases in which there are evidences to support its use. Table 27.1 provides the list of renal conditions for which plasmapheresis may be attempted along with the American Society for Apheresis (ASFA) categories assignment and recommendation grade [2]. The ASFA categories are Category I—Therapeutic apheresis is acceptable, as first-line therapy, either as primary stand-alone treatment or in conjunction with other modes of treatment; Category II—Therapeutic apheresis is acceptable, as second-line therapy, either as a stand-alone treatment or in conjunction with other modes of treatment; Category III—Disorders for which the optimum role of apheresis therapy is not established; and Category IV—Disorders for which published evidence demonstrates or suggests apheresis to be ineffective or harmful. Clinical applications should be undertaken only under an approved research protocol. The above guidelines are not intended to mandate plasmapheresis for conditions in which it is clearly efficacious nor are they intended to deny or exclude patients from receiving plasmapheresis when some benefit, although small, may be realized. Given the complexity, expense, and risks of the procedure, however, the guidelines provide a framework for clinical decisions regarding the use of plasmapheresis.

Rapidly Progressive Glomerulonephritis

Causes of rapidly progressive glomerulonephritis (RPGN) can be divided into three main groups based upon the immunofluorescence pattern on renal biopsy. Anti-glomerular basement membrane (anti-GBM) antibody disease accounts for 15 % of cases, pauci-immune crescentic glomerulonephritis for 60 % of cases and immune complex crescentic glomerulonephritis for 25 % of cases.

Anti-Glomerular Basement Membrane Antibody Disease

Anti-GBM antibody disease typically presents as RPGN without or with pulmonary hemorrhage (Goodpasture's syndrome). Circulating anti-GBM antibodies are detected in greater than 90 % of patients and, in general, disease activity correlates with the titer of circulating antibodies. The rationale for plasmapheresis is the rapid removal of toxic anti-GBM antibodies.

The specific role of plasmapheresis in therapeutic regimens for anti-GBM disease has never been properly assessed by prospective randomized controlled trials. Only two controlled studies have evaluated the efficacy of plasmapheresis as an adjunct to conventional immunosuppressive therapy in this disease [30, 31]. Although small (17 and 20 patients), both studies suggested a benefit as evidenced by faster decline in anti-GBM antibody titers, lower serum creatinine after therapy, and fewer patients progressing to renal failure. However, the authors were cautious about accepting that plasmapheresis had been responsible for the improved outcome, as the groups receiving plasmapheresis had milder disease than the control groups.

Table 27.1 Indications for plasmapheresis in renal conditions

Conditions	ASFA category	Recommendation grade
Rapidly progressive glomerulonephritis:		
Anti-GBM disease	I	1a–b
Pauci-immune RPGN	I[a]	1a
Immune complex RPGN	III[b]	2b
Multiple myeloma:		
Myeloma cast nephropathy	II	2b
Hyperviscosity	I	1b
HUS—TTP:		
Primary—idiopathic (adult)	I	1a
Diarrhea associated (pediatric)	IV[c]	1c
Renal transplantation:		
ABO and HLA desensitization	II	1b
Antibody-mediated rejection	I	1b
Recurrent disease (FSGS)	I	1c

ASFA category: Category I—Therapeutic apheresis is acceptable, as first-line therapy, either as primary stand-alone treatment or in conjunction with other modes of treatment; Category II—Therapeutic apheresis is acceptable, as second-line therapy, either as a stand-alone treatment or in conjunction with other modes of treatment; Category III— Disorders for which the optimum role of apheresis therapy is not established; Category IV— Disorders for which published evidence demonstrates or suggests apheresis to be ineffective or harmful

Recommendation grade: Grade 1: Strong recommendation: a, high-quality evidence; b, moderate-quality evidence; c, low to very low-quality evidence; Grade 2: Weak recommendation: a, high-quality evidence; b, moderate-quality evidence; c, low to very low-quality evidence

ASFA, American Society for Apheresis, *RPGN* rapidly progressive glomerulonephritis, *GBM*, glomerular basement membrane, *HUS–TTP*, hemolytic uremic syndrome–thrombotic thrombocytopenic purpura, *FSGS*, focal segmental glomerulosclerosis

[a]Dialysis dependent or serum creatinine >500 μ[mu]mol/L—5.8 mg/dL

[b]Except for the following specific disorders: lupus nephritis (ASFA category IV) and cryoglobulinemia (ASFA category I)

[c]Except for children presenting with atypical HUS in the absence of diarrhea in whom plasmapheresis may be attempted

The results of more than 20 uncontrolled studies and case reports including close to 450 patients, published over the past 20 years support the results of the above controlled studies. In aggregate, these reports suggest that survival rates of greater than 80 % and renal preservation rates of greater than 45 % may be obtained with therapeutic regimens combining plasmapheresis with immunosuppressive drugs (reviewed in references [32, 33]). These results compare favorably with historical data suggesting patient survival of 45 % and progression to ESRD in 85 %.

Thus, there is evidence, although largely based on uncontrolled or retrospective studies with historical comparison, that plasmapheresis is a useful adjunct to immunosuppressive drugs. Plasmapheresis can accelerate disappearance of anti-GBM antibodies and improve renal function if instituted promptly. Several series have demonstrated that patients with severe disease (oliguria, dialysis, or serum creatinine >600 μ[mu]mol/L—6.8 mg/dL) are unlikely to regain renal function despite the use of plasmapheresis. Hence, in such patients, plasmapheresis should probably be reserved for treatment of pulmonary hemorrhage. The recommended regimen for anti-GBM disease is the following: daily plasmapheresis for 14 days, with 4 L exchanges and albumin solution as replacement fluid. Response to therapy should be monitored by repeated assessments of urine output, serum creatinine, and plasma anti-GBM levels.

Pauci-immune Rapidly Progressive Glomerulonephritis

Pauci-immune RPGN is characterized by minimal immune deposits in the glomerulus and the presence of antineutrophil cytoplasmic antibodies (either C-ANCA or P-ANCA) which may contribute to the pathophysiology of RPGN. In the majority of these patients, RPGN is caused by granulomatosis with polyangiitis (GPA, formally Wegener's granulomatosis) and microscopic polyangiitis (MPA) or "renal-limited" pauci-immune GN.

Seven randomized controlled trials have evaluated the efficacy of plasmapheresis as an adjunct to immunosuppressive therapy in patients with pauci-immune RPGN [34–39]. Three studies randomly assigned patients to receive immunosuppressive agents with or without plasmapheresis and found no statistically significant difference between the two groups as judged by serum creatinine or dialysis dependency [34, 35, 39]. Three other studies provided evidence of a benefit in subgroups of patients with severe disease [36–38].

Specifically, patients on dialysis or with a serum creatinine >800 μ[mu]mol/L—9 mg/dL were more likely to respond to plasmapheresis than patients with milder disease. Similarly, in a recent randomized controlled trial, the role of plasma exchange in patients with severe renal disease was studied in the methylprednisolone versus plasma exchange (MEPEX) trial [40]. This study enrolled 137 patients with a new diagnosis of pauci-immune RPGN (GPA or MPA) and a serum creatinine concentration above 5.8 mg/dL—500 μ[mu]mol/L. As compared with intravenous methylprednisolone, plasmapheresis was associated with a reduction in risk of progression to ESRD of 24 %, from 43 % to 19 %, at 12 months. However, none of the above randomized controlled trials reported improvements in patient survival with plasmapheresis.

In aggregate, these results suggest a benefit of plasmapheresis when used as an adjunct to immunosuppressive therapy in patients who have advanced renal dysfunction at presentation, as defined by a serum creatinine level above 500 μ[mu]mol/L—5.8 mg/dL and/or dialysis dependence. In addition, limited data suggest that patients with pulmonary hemorrhage may also benefit from prompt initiation of apheresis coupled with aggressive immunosuppressive therapy [41, 42]. Given the paucity of convincing data, however, it is impossible to provide firm recommendations regarding the specifics of therapy for patients with pauci-immune RPGN. It would seem prudent to reserve plasmapheresis for severe cases and to perform at least four plasmapheresis sessions during the first week of immunosuppressive therapy, using 4 L exchanges and albumin solution as replacement fluid. Response to therapy should be monitored with repeated assessments of urine output, serum creatinine, and possibly ANCA titers.

Immune Complex Crescentic Glomerulonephritis

Immune complex RPGN is characterized by granular immune deposits on immunofluorescence microscopy and is caused by a number of immune disorders including lupus nephritis, cryoglobulinemia, IgA nephropathy, Henoch–Schönlein purpura, post-streptococcal GN, and membranoproliferative GN. Plasmapheresis has been advocated mainly in the treatment of lupus, cryoglobulinemia, and IgA nephropathy/Henoch–Schönlein purpura.

Lupus Nephritis

Overt nephritis complicates 38–90 % of cases of systemic lupus erythematosus. Plasmapheresis has been advocated as a means of rapidly removing circulating autoantibodies and immune complexes that appear to play a key role in the pathophysiology of lupus nephritis.

Although initial case reports and uncontrolled case series suggested a benefit of plasmapheresis in severe lupus nephritis, a large multicenter prospective randomized controlled trial provided strong evidence against its use [43]. The Lupus Nephritis Collaborative Study Group assessed the value of plasmapheresis as an adjunct to prednisone and cyclophosphamide in 86 patients with severe lupus nephritis (serum creatinine value >2.0 mg/dL or 180 μ[mu]mol/L). Plasmapheresis caused a rapid reduction of serum anti-dsDNA antibodies and cryoglobulins. However, the percentage of patients progressing to renal failure (25 versus 17 %) and going into clinical remission (30 versus 28 %) was the same in both groups. Importantly, patients receiving plasmapheresis tended to have a worse outcome. Four other randomized controlled trials of plasmapheresis reported similar findings [44–47]. Plasmapheresis produced significant reduction in circulating immune complexes and anti-DNA antibodies, but the frequency and degree of partial or complete remission was the same in both plasmapheresis and control groups [44–47]. In addition, intensified treatment protocols that combine plasmapheresis with subsequent pulse cyclophosphamide have also been showed to provide no additional benefit when compared with pulse cyclophosphamide [48].

In summary, the results of multiple prospective randomized controlled trials do not support a role for plasmapheresis in the treatment of lupus nephritis. There is experimental and clinical evidence that plasmapheresis induces rapid removal of circulating immune complexes and anti-DNA antibodies, but plasmapheresis does not appear to influence renal function, remission rate, or mortality. However, plasmapheresis may have a role in lupus associated with antiphospholipid antibodies. Selected patients with acute renal failure and the antiphospholipid syndrome may respond to plasmapheresis, which presumably acts by removing the pathogenic antibody. In one uncontrolled case series of 12 cases, for example, renal recovery occurred in patients treated with plasma exchange [49]. Similarly, plasmapheresis may have a role in lupus associated with thrombotic thrombocytopenic purpura (see below).

Cryoglobulinemia

Cryoglobulins comprise immunoglobulins that reversibly precipitate at low temperature. The aggregates of cryoglobulins can deposit within the glomerular capillary lumen and cause damage by activating complement and recruiting leukocytes. Plasmapheresis has been reported to remove cryoglobulins efficiently. Case series and case reports suggest 55–87 % improvement in renal function and significant improvement in overall survival (from 55 % to 25 % mortality) with plasmapheresis [50–56]. Unfortunately, the specific role of plasmapheresis in therapeutic regimens for cryoglobulinemia has never been assessed by prospective randomized controlled trials. In addition, with the recognition that hepatitis C virus (HCV) is the etiologic agent for approximately 80 % cases of mixed essential cryoglobulinemia, a major theoretical concern with plasmapheresis is a possible

enhancement of HCV replication. Hence, it would appear prudent to limit plasmapheresis to patients with acute fulminant disease.

A reasonable plasmapheresis prescription is to exchange one plasma volume three times weekly for 2–3 weeks. The replacement fluid can be 5 % albumin, which must be warmed to prevent precipitation of circulating cryoglobulins [57]. The optimal method for assessing the efficacy of plasmapheresis is uncertain. Changes in the percent cryocrit after plasmapheresis do not correlate closely with clinical activity. Thus, response to therapy should be monitored with changes in clinical manifestations.

IgA Nephropathy and Henoch–Schönlein Purpura

IgA nephropathy and Henoch–Schönlein purpura which are characterized by IgA immune complexes deposits present with acute rapidly progressive glomerulonephritis in approximately 10 % of cases. Plasmapheresis has been advocated to rapidly remove circulating IgA immunoglobulins, although circulating IgA levels do not correlate with severity or activity of disease.

Uncontrolled reports in patients with crescentic, rapidly progressive glomerulonephritis suggest possible benefit from plasmapheresis and immunosuppressive therapy [58–61]. One report evaluated the efficacy of aggressive combination therapy (including pulse methylprednisolone, oral cyclophosphamide, and plasmapheresis) in six patients with crescentic glomerulonephritis due to IgA nephropathy [61]. All patients improved in the short term but subsequent deterioration in renal function was observed in more than half of these patients. No randomized controlled trials have evaluated the efficacy of plasmapheresis in therapeutic regimens for rapidly progressive IgA nephropathy and Henoch–Schönlein purpura.

In summary, no firm conclusions can be drawn regarding the efficacy of plasmapheresis in therapeutic regimens for rapidly progressive IgA nephropathy and Henoch–Schönlein purpura because of lack of evidence. Hence, its use should be limited to fulminant cases in the context of research protocols.

Hemolytic Uremic Syndrome and Thrombotic Thrombocytopenic Purpura

The presenting features of hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) are essentially the same in most patients, beginning with thrombocytopenia and microangiopathic hemolytic anemia without apparent cause; in addition, most patients have neurologic and/or renal abnormalities. Several factors may contribute to the pathogenesis of HUS–TTP including genetic factors, drugs (e.g., quinine, cyclosporine, antiplatelet agents, chemotherapeutic agents), infections (e.g., Shiga toxin-producing *Escherichia coli*), autoimmune disorders (e.g., antiphospholipid syndrome or systemic lupus erythematosus), and either congenital or acquired (e.g., autoantibodies) ADAMTS-13 deficiency. Therapeutic plasma exchange has been proposed to benefit patients with HUS–TTP by (1) replacing a deficient plasma factor and/or (2) removing circulating toxins that cause endothelial injury and/or platelet aggregation and promote formation of microthrombi.

There has been no controlled, prospective, randomized, appropriately blinded study comparing plasma exchange with placebo or drug therapy in the treatment of HUS-TTP. Most of the evidence in favor of the role of plasmapheresis originates from uncontrolled or retrospective studies and from comparison with historical data. Prior to the introduction of plasma infusion and plasmapheresis, the disease typically progressed rapidly and was almost uniformly fatal (93 % fatality rate; 79 % within 90 days) [62]. With plasmapheresis using fresh frozen plasma, remission rates of greater than 75 % and survival rates greater than 85 % are now consistently reported. It is unclear whether this benefit is due to plasma infusion alone and replacement of a deficient plasma factor or to plasma exchange and removal of a circulating toxic. Two randomized controlled trials compared plasma exchange to plasma infusion [63, 64]. Rock et al. randomized patients with TTP to either plasma exchange or plasma infusion with fresh frozen plasma and observed that patients receiving plasma exchange had a better response rate and superior survival [63]. Patients treated with plasma exchange received approximately three times as much plasma as those treated with plasma infusion alone (in whom the degree of plasma administration was limited by the risk of volume overload). Thus, the apparent enhanced benefit from plasma exchange may have been due to infusion of more plasma rather than to the removal of some toxic substance. Indeed, a smaller controlled trial did not observe a difference in outcome when patients were randomized to receive either daily infusions of 15 mL/kg of fresh frozen plasma or plasma exchange with a mixture of 15 mL/kg of fresh frozen plasma and 45 mL/kg of 5 % albumin as replacement fluid [64]. Thus, the exact roles of plasma removal versus plasma infusion in the beneficial effect of plasma exchange remain controversial. In practice, this question is rather semantical since it is often necessary to perform plasma exchange in order to administer the required large amount of plasma, given that these patients are often oligo-anuric and at risk for hypervolemia and pulmonary edema.

In summary, there is evidence, largely based on studies using historical controls, that plasma exchange improves renal outcome and mortality in adult patients with HUS-TTP. The most widely recognized plasma exchange protocol involves daily plasma exchange for 7–14 days, using 4 L exchanges and fresh frozen plasma (FFP) as replacement fluid. Response to therapy is monitored with repeated assessments of platelet

counts, serum lactate dehydrogenase (LDH) concentration, urine output, and serum creatinine values. Plasma exchange is initially performed daily until the platelet count has normalized and hemolysis largely ceased, as evidenced by a normal LDH concentration. When a normal platelet count has been achieved, plasma exchange is usually discontinued or gradually tapered by increasing the interval between treatments.

Since large volumes of FFP (approximately four units per liter of plasma removed) from many different donors are required during plasma exchange for HUS-TTP, a number of alternative plasma products have been suggested to increase efficacy and reduce complications (e.g., infections). The cryosupernatant fraction of plasma was initially suggested to be more effective than whole plasma as large von Willebrand multimers are largely removed in the cryoprecipitate [65, 66]. However, two small randomized, controlled trials found no difference in efficacy between whole plasma and cryoprecipitate-poor plasma [67, 68]. In an attempt to reduce infective complications, a number of methodologies for viral inactivation of FFP have been developed. Methylene blue and solvent detergent viral inactivation have been reported effective in inactivating viruses with lipid envelopes (e.g., hepatitis B and C viruses); however, neither method is effective against non-lipid enveloped viruses (e.g., hepatitis A virus, parvovirus), and neither method is capable of inactivating prions. Although some studies have suggested that these virally inactivated FFPs are as effective as standard FFPs for the treatment of HUS–TTP [69, 70], others have suggested that they may be less effective [71, 72]. In summary, plasma exchange should be performed in all patients with TTP using plasma as replacement fluid. Although there may be exceptions, most plasma products appear equally effective for the treatment of HUS-TTP.

Renal Failure Associated with Multiple Myeloma

Renal failure complicates 3–9 % of cases of multiple myeloma and portends a poor prognosis. Plasmapheresis has been suggested to prevent the renal toxicity associated with myeloma proteins by rapidly reducing their plasma concentration.

Three randomized controlled trials of plasmapheresis in multiple myeloma have been reported [73–75]. In all three trials, patients were randomized after correction of precipitating factors such as hypovolemia or metabolic acidosis. Zucchelli et al. randomly allocated 15 patients to plasmapheresis, chemotherapy, and hemodialysis and 14 patients to chemotherapy and preemptive intermittent peritoneal dialysis [74]. Renal outcome and patient survival were better among participants treated with plasmapheresis. By contrast, Johnson et al. randomized 21 patients to either chemotherapy plus forced diuresis or to the same regimen with plasmapheresis [73]. They reported no significant difference in renal recovery or patient survival despite more rapid lowering of plasma myeloma protein levels with plasmapheresis. The third randomized trial of plasmapheresis in myeloma cast nephropathy was published in 2005 by Clark and colleagues [75]. All study participants were treated with conventional chemotherapy (melphalan–prednisone or vincristine, doxorubicin and dexamethasone). Patients were randomized to either conventional chemotherapy plus plasmapheresis or to conventional chemotherapy alone. The study did not demonstrate improvement in renal outcome or patient survival with plasmapheresis.

In aggregate, the results of these three clinical trials argue against a role for plasmapheresis in the treatment of myeloma cast nephropathy. Whereas Zucchelli et al. showed improved renal and patient outcome with plasmapheresis, Johnson and co-workers and Clark et al. found no conclusive evidence that plasmapheresis provides any substantial benefit.

Nevertheless, there are clinical conditions for which plasmapheresis is clearly warranted. For example, plasmapheresis should be used in patients with hyperviscosity syndrome presenting with neurologic impairment. In addition, it is possible that plasmapheresis might provide additional benefit in specific subgroups of patients as even the largest clinical trial to date was relatively underpowered to detect small benefits of plasmapheresis in patient subgroups. In a small retrospective study, Leung et al. analyzed the efficacy of plasmapheresis in patients in whom the diagnosis of cast nephropathy was confirmed by biopsy and in whom serum levels of free light chains were used to guide therapy [76]. Renal recovery was highly correlated with significant reduction of free light chain levels [76]. This observation certainly warrants further study in specific research protocols.

Thus, the role of plasmapheresis in myeloma patients remains controversial. There is clearly no role for plasmapheresis in the treatment of undifferentiated renal failure. However, removal of light chains by plasmapheresis may be beneficial in specific cases (e.g., patients with hyperviscosity syndrome, patients unresponsive to standard therapies) or as part of research protocols.

Renal Transplantation

Over the past decades, plasmapheresis has been used in renal transplantation for (1) pretransplant desensitization in patients with preformed antibodies against donor human leukocyte antigen (HLA) and blood group ABO antigens, (2) treatment of acute and chronic humoral rejection, and (3) treatment of recurrent glomerular disease.

Pretransplant Desensitization

The increased risk of hyperacute antibody-mediated rejection (AMR) and subsequent allograft loss when transplanting against either preformed HLA or ABO antibodies has long engendered a general avoidance of this practice. However, reports of successful transplantation across HLA and/or ABO barriers using various desensitization protocols designed to reduce the amount of preexisting antibody have stimulated increased interest in this practice.

Anti-HLA Antibodies

Approximately 20 % of patients waiting for cadaveric transplantation have high titers of preformed cytotoxic antibodies that render them at high risk for AMR. Two types of protocols are principally used in an attempt to desensitize potential recipients with preformed HLA antibodies. One method uses high-dose intravenous immunoglobulin (IVIG) and the other uses low-dose IVIG and plasmapheresis. In the latter, alternate day plasmapheresis is used pretransplantation with 100 mg/kg of IVIG administered after each session. The number of pretransplant plasmapheresis treatments is estimated based on the baseline anti-HLA antibody titer. Once the antibody is no longer detectable and the pretransplant crossmatch is negative, transplantation proceeds. Plasmapheresis and IVIG are usually continued on the day of transplantation and postoperatively on an alternate day regimen. Albumin is generally used for replacement in plasmapheresis in most desensitization protocols to avoid the potential for HLA sensitization when using fresh frozen plasma. Most desensitization protocols also include adjunct immunosuppressive agents (e.g., tacrolimus, mycophenolate mofetil, prednisone, or rituximab).

In uncontrolled studies, plasmapheresis significantly reduced the titers of antibodies and provided acceptable rates of AMR [77–79]. However, only one study has directly compared high-dose IVIG with plasmapheresis/low-dose IVIG protocols [80]. In this retrospective comparison of three different desensitization protocols, the plasmapheresis-based desensitization protocols provided superior outcomes and lower AMR rates as compared to the high-dose IVIG protocol. Unfortunately, the efficacy of plasmapheresis has never been properly assessed by prospective randomized controlled trials. Hence, no firm conclusions can be drawn concerning the efficacy of this practice, although the use plasmapheresis is generally accepted.

Anti-ABO Antibodies

Approximately one-third of potential living donors are eliminated from consideration based on ABO incompatibility. Desensitization protocols which combine plasmapheresis to IVIG, or other preventive interventions (e.g., splenectomy, immunosuppressive therapy), have been suggested to allow successful engraftment despite ABO incompatibility. In controlled studies, short-term and long-term graft and patient survival rates in ABO-incompatible transplantation were similar to that of ABO-compatible transplantation [81, 82]. Unfortunately, the efficacy of plasmapheresis-based desensitization protocols over other protocols in this setting has never been properly assessed by prospective randomized controlled trials. Hence, no firm conclusions can be drawn concerning the efficacy of this practice, although the use plasmapheresis is generally accepted.

Acute and Chronic Rejection

Antibody-mediated rejection (AMR) resulting in graft dysfunction is estimated to occur in 3–10 % of all renal transplants and may be a component of 20–30 % of episodes of acute rejection. Plasmapheresis has been advocated as a means of rapidly removing preformed antibodies that are instrumental in the pathophysiology of the rejection process. A number of studies showed an improved response with plasmapheresis compared to historical controls [83–85]. In a representative study by Rocha et al., the one-year graft survival in renal allograft recipients with acute rejection with a component of antibody-mediated rejection was compared to those with acute cellular rejection alone [84]. Using a protocol of plasmapheresis and intravenous immune globulin (IVIG) for antibody-mediated rejection, the one-year graft survival for antibody-mediated rejection was 81 %, compared to 84 % with acute cellular rejection using standard treatment protocols. However, because of the nonrandomized nature of most studies published to date, the favorable results reported may reflect earlier diagnosis and treatment and not simply more effective therapy. In addition, many of the patients in the above mentioned protocols using plasmapheresis also received IVIG, steroids, and anti-lymphocyte therapy. Thus, the specific role of plasmapheresis in combination with other antirejection therapies is unclear. Further clinical trials are required to better define the relative role of plasmapheresis for this indication.

The literature on therapeutic plasmapheresis in chronic rejection is limited to a few uncontrolled series, and the results in general have been disappointing with improvement in graft function being, at best, modest and usually transient [86].

Recurrent Glomerular Disease

A few uncontrolled studies have suggested a role for plasmapheresis in the prevention and treatment of recurrent glomerular disease, particularly primary focal segmental glomerulosclerosis, after transplantation. Removal of a circulating factor by plasmapheresis or a protein adsorption column can reduce proteinuria and, in some cases, induce complete remission [87–92]. However, initial studies noted only a transient benefit, as the proteinuria in almost all patients

returned to pretreatment levels within several weeks [88]. However, better results were obtained in subsequent reports in which patients with recurrent disease were treated with plasma exchange for longer periods [87, 91, 92]. With these protocols, some remissions were maintained for up to 27 months. In addition, better results were obtained when plasmapheresis was started early (i.e., within 14 days of onset of proteinuria) [91, 92]. Prolonged beneficial results have also been reported in children treated with plasmapheresis and either cyclophosphamide or cyclosporine [93–95]. Thus, given the absence of conclusive data demonstrating a benefit and given the cost and potential side effects, plasmapheresis is not recommended as routine treatment for recurrent glomerular disease. However, given the encouraging preliminary results, the role of plasmapheresis deserves to be assessed further in randomized controlled trials.

Other Indications

Drug Overdose and Poisoning

Plasmapheresis is reported as a useful extracorporeal blood purification technique in the treatment of various intoxications and poisonings. The basic premise of plasmapheresis use in poisoning and drug overdose is that removal of circulating toxin/drug will reduce toxic-induced damage and minimize related complications. Protein bound toxins for which plasmapheresis has been reported to be useful include organophosphate herbicides (e.g., methyl parathion), Amanita phalloides (mushroom), and sodium chlorate [96–98]. Plasmapheresis in the treatment of drug overdose has been reported to be useful for the following agents: L-thyroxine, vincristine, cisplatin, and theophylline [99–102]. However, most reports evaluating treatment with plasmapheresis are case reports or case series in which many of the patients were treated concurrently with dialysis and/or specific antidotes. No randomized controlled trials have been conducted comparing plasmapheresis with other treatment modalities in the treatment of drug overdose and poisoning. Thus, because of the uncontrolled nature of all of the studies reported to date, it is impossible to provide firm recommendations. The use of plasmapheresis should be limited to research protocols or to exceptional cases presenting with life-threatening complications unresponsive to conventional therapy.

Sepsis and Multiple Organ Failure

Plasmapheresis has been proposed as a means of rapidly reducing circulating levels of bacterial toxins and endogenous inflammatory mediators released during sepsis and multiple organ failure. Several case reports and small uncontrolled studies have reported favorable outcome with the use of plasmapheresis in patients with sepsis [103–107]. Plasmapheresis has been reported to improve hemodynamic parameters and/or patient survival in this setting. However, in a recent randomized controlled trial, the theoretical benefits of extracorporeal adsorbent-based strategies or plasmapheresis were not demonstrated in sepsis patients [108]. One hundred forty-five patients with a clinical diagnosis of severe sepsis or septic shock were randomized to receive either standard therapy alone for sepsis ($n=76$) or standard therapy plus extracorporeal endotoxin adsorption ($n=67$) daily for the first 4 days following study entry. The proportion of responders (defined as a decrease in APACHE II score by $\geq$4 points from study entry to day 4) and survival were similar in both treatment groups. Despite this, there continues to be interest in removing endotoxin, cytokines, or other toxic substances. Multiple human trials are in progress and should allow more definitive recommendations to be made. At the present time, however, it is difficult to draw conclusions regarding the efficacy of plasmapheresis in sepsis patients because of limited data.

Complication of Plasmapheresis

The prevailing belief that "plasmapheresis is a benign procedure" undoubtedly contributed to its widespread use for unproven indications. The overall incidence of adverse reactions reported in the literature ranges from 1.6 % to 25 %, with severe reactions occurring in 0.5–3.1 % and death occurring in 0.03–0.05 % [18, 19, 108–110]. Table 27.2 summarizes the possible complications associated with plasmapheresis. The most frequent problems are fever, chills, and urticaria (which typically follow the administration of FFP and other plasma-containing replacement fluids) and paresthesias (due to binding of free calcium to citrate used as anticoagulant) [109]. More serious complications are related to anaphylaxis, vascular access, bleeding due to heparin or to coagulopathies induced by inadequate replacement of clotting factors, and transmission of viral hepatitis. Repeated plasmapheresis has been postulated to be immunosuppressive and to increase the risk of infections in patients receiving immunosuppressive agents. Indeed, repeated apheresis treatments with albumin replacement will predictably deplete the patient's reserve of immunoglobulins for several weeks. However, infection rates reported from randomized trials in the treatment of lupus nephritis and pauci-immune RPGN were the same in patients treated with plasmapheresis and those treated conventionally [34–36, 111].

Conclusion

In summary, use of plasmapheresis has changed in recent years reflecting the availability of evidence largely obtained from controlled prospective studies. However, the clinical efficacy of plasmapheresis for many renal conditions is

Table 27.2 Complications of plasmapheresis

Mechanisms	Complications
Anticoagulation:	
Citrate	Hypocalcemia Metabolic alkalosis
Heparin	Bleeding
Replacement fluid:	
FFP	Anaphylactoid reactions Infection (viral transmission)
Albumin	Coagulopathies[a] Infection (viral transmission)
Saline 0.9%	Coagulopathies[a]
Vascular access	Pneumothorax Hemothorax Catheter sepsis
Volume related	Hypotension Fluid overload
Medication	Anaphylactoid reactions with ACEI Drug removal
Immunosuppression	Infections

ACEI angiotensin-converting enzyme inhibitors, *FFP* fresh frozen plasma
[a]Induced by inadequate replacement of clotting factors

still controversial. Plasmapheresis appears to be a useful adjunct to conventional therapy in the treatment of anti-GBM nephritis, severe dialysis-dependent forms of pauci-immune RPGN, and HUS-TTP. Reported data also suggest a possible benefit of plasmapheresis in renal transplantation patients, but the case for plasmapheresis in this setting has not yet been fully supported by randomized clinical trials. In contrast, data from controlled trials do not support a role for plasmapheresis in lupus nephritis for instance. The more widespread application of prospective, randomized, controlled clinical trials should help to better define the value of plasmapheresis for treatment of renal diseases.

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