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REVIEW ARTICLE

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Mechanisms of immune-deposit formation and the mediation of immune renal injury

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Abstract The passive trapping of preformed immune complexes is responsible for some forms of glomerulonephritis that are associated with mesangial or subendothelial deposits. The biochemical characteristics of circulating antigens play important roles in determining the biologic activity of immune complexes in these cases. Examples of circulating immune complex diseases include the classic acute and chronic serum sickness models in rabbits, and human lupus nephritis. Immune deposits also form "in situ". In situ immune deposit formation may occur at subepithelial, subendothelial, and mesangial sites. In situ immune-complex formation has been most frequently studied in the Heymann nephritis models of membranous nephropathy with subepithelial immune deposits. While the autoantigenic target in Heymann nephritis has been identified as megalin, the pathogenic antigenic target in human membranous nephropathy had been unknown until the recent identification of neutral endopeptidase as one target. It is likely that there is no universal antigen in human membranous nephropathy. Immune complexes can damage glomerular structures by attracting circulating inflammatory cells or activating resident glomerular cells to release vasoactive substances, cytokines, and activators of coagulation. However, the principal mediator of immune complex-mediated glomerular injury is the complement system, especially C5b-9 membrane attack complex formation. C5b-9 inserts in sublytic quantities into the membranes of glomerular cells, where it produces cell activation, converting normal cells into resident inflammatory effector cells that cause injury. Excessive activation of the complement system is normally prevented by a series of circulating and cell-bound complement regulatory proteins. Genetic deficiencies or mutations of these proteins can lead to the spontaneous development of glomerular disease. The identification of specific antigens in human disease may lead to the development of fundamental therapies. Particularly promising future therapeutic approaches include selective immunosuppression and interference in complement activation and C5b-9-mediated cell injury.

Key words Complement · Glomerulonephritis · Membranous nephropathy · C5b-9 · Complement inhibitor · Hemolytic uremic syndrome

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Introduction

In most forms of glomerulonephritis, injury occurs secondary to the formation of immune deposits, containing immunoglobulins, complement, and other proteins, at various intraglomerular locations. The type of injury that results is dependent on four factors: the mechanism of the deposit formation, the site of deposit formation, the composition of the deposits and the amount of the deposits. It must be understood that an immune complex reflects a dynamic interaction between antigen and antibody, rather than a permanent physical structure or unit. The composition of the immune complex can change, either in the circulation or within tissue. The purpose of this review is to summarize mechanisms of immune-deposit formation and their consequences in immune renal injury.

Circulating immune-complex trapping

The passive trapping of preformed immune complexes was thought to be responsible for many forms of human glomerulonephritis, because the kidney is a particularly susceptible target for immune deposit formation. Immune complexes in the circulation are delivered at a high rate because the kidney receives 25% of the cardiac output. Intraglomerular pressure is higher than in other capillary

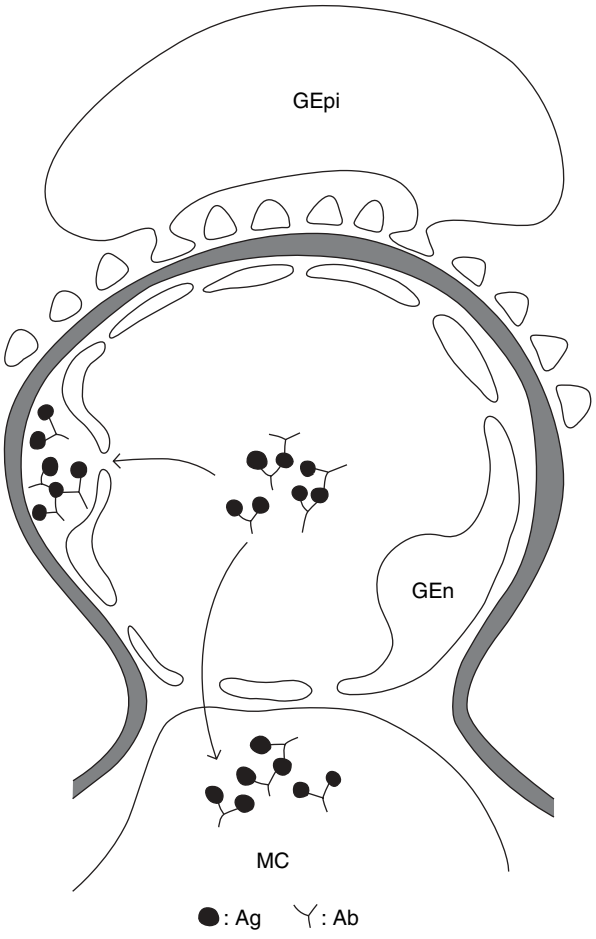

Fig. 1. Circulating immune formation. Circulating immune complexes deposit primarily in the mesangium or in the subendothelial space. The clinical and morphologic consequences of immune-complex deposition depend on the location and biochemical characteristics of the deposits. *GEn*, glomerular endothelial cell; *GEpi*, glomerular epithelial cell; *MC*, mesangial cell; *Ag*, antigen; *Ab*, antibody

beds, and more protein than usual may be forced across the glomerular capillary wall. The glomerular capillaries provide a large and highly permeable surface through which circulating immune complexes circulate. The capillary walls are also highly negatively charged structures that facilitate the localization of positively charged macromolecules. All of these properties facilitate the nonspecific trapping of circulating immune complexes in glomeruli.

Circulating-complex trapping occurs primarily in the mesangium and/or on the subendothelial, surface of the capillary wall (Fig. 1). Circulating preformed complexes are too large to cross the capillary wall intact to localize in the subepithelial space, although, in some circumstances, subendothelial immune complexes may dissociate to cross the glomerular basement membrane (GBM) and reform in situ in a subepithelial distribution.

The biochemical characteristics of the circulating antigen play important roles in determining the biologic activity of immune complexes. The size and blood clearance kinetics influence the properties of the macromolecular immune complexes.[1] When an antigen bears few epitopes (oligovalent) or when there is excess antigen, immune complexes tend to be small and soluble. Small immune complexes are inefficient at complement activation and attachment to Fc receptors; therefore, they tend to be relatively inert. As the antigen-to-antibody ratio nears equivalence, cross-linking is maximized and large insoluble immune complexes are formed. Large complexes have a high affinity for binding to Fc receptors and activate complement, which facilitates rapid removal from the circulation by phagocytosis. Medium-sized complexes that are less well-cleared but can activate complement and bind to Fc receptors tend to deposit in tissues and initiate glomerular injury.

The net charge and ability to bind to glomerular structures are important as well. Immune complexes containing cationic proteins tend to form glomerular deposits.[2,3] This is explained by the charge interactions between such soluble molecules and anionic sites in the basement membrane.[4,5] Furthermore, immune complexes containing cationic antigens exhibit a higher potential to activate complement compared to those of native or anionic antigens.[6,7] This property of cationic immune complexes may enhance their capacity to induce inflammatory reactions.

The best example of circulating immune complex disease is the classic acute serum sickness model in rabbits, induced by a single, large bolus injection of the foreign serum protein, bovine serum albumin (BSA). Binding of antibody to BSA results in circulating immune complexes, which accumulate in the glomerulus and other vessels and initiate acute glomerulonephritis and vasculitis. It is unclear whether there is an equivalent of acute serum sickness involving the kidney in humans, although post-infectious nephritis is felt by some to involve an analogous mechanism. Systemic lupus erythematosus (SLE) is an excellent example of an immune-complex disease in which the antigen is endogenous.[8,9] Circulating immune complexes are readily detected in patients with SLE, and contain a variety of specificities, including antibodies to double-stranded (ds) DNA, ribonucleoproteins, and others. Levels of circulating immune complexes as well as those of anti-ds DNA antibodies correlate with disease activity,[10] and anti-DNA and DNA immune complexes were found to be enriched in kidney eluates from lupus patients.[11] Thus, it has been speculated that immune deposits in the glomeruli in SLE reflect preformed immune-complex trapping. However, experimental evidence suggests the involvement of other pathogenic mechanisms in lupus nephritis as well. Immune complexes may also be formed in situ via charge interactions between anti-DNA/histone antibodies and DNA/histones already deposited in the glomerulus,[12–15] and some lupus autoantibodies also bind directly to intrinsic glomerular antigens, leading to in situ immune-complex formation, as described below.

Despite the extensive literature on immune-complex disease, written over many decades, there is little direct evidence that the passive trapping of preformed immune complexes formed in the circulation leads to significant tissue injury at sites where the complexes become localized, including the glomerulus.[16]

In situ immune-complex formation

Immune deposits may form "in situ" when an antibody binds specifically either to antigens that are constituents of normal glomerular structures or to soluble antigens that become localized independently in the glomerulus, on the basis of nonspecific uptake by the mesangium or charge interactions with anionic sites in the glomerular capillary wall.

In the early 1980s, there was considerable debate regarding the relative importance of circulating immune complexes versus the in situ formation of immune deposits. The Heymann nephritis model of membranous nephropathy, which is described in more detail below, served an important role in clarifying this question. Initially it had been believed that immune-complex deposition in glomeruli was due to the glomerular trapping of circulating immune complexes formed by circulating tubular antigens and the corresponding antibodies. However, between 1978 and 1983, groups led by Hoedemaeker and Couser,[17–21] almost simultaneously reported studies that showed subepithelial immune complex formation following the direct perfusion of bloodless kidneys with pathogenic IgG antibody, thus establishing that subepithelial immune deposits in this classic model of membranous nephropathy developed through a novel mechanism, referred to as in situ immune-complex formation.[16]

The location of immune deposits that form in situ is determined by the site of expression of the relevant intrinsic glomerular antigen or the site where a nonimmunologic biochemical interaction leads to the trapping, or "planting" of an antigen. As a consequence, in situ immune-deposit formation may occur at subepithelial, subendothelial, or mesangial sites (Fig. 2). The clinical and morphologic consequences of this process depend on where the deposits form and which glomerular cells are targeted. However, in situ immune-complex formation has been most extensively studied in the Heymann models of membranous nephropathy involving subepithelial immune deposits, as discussed in the next section.

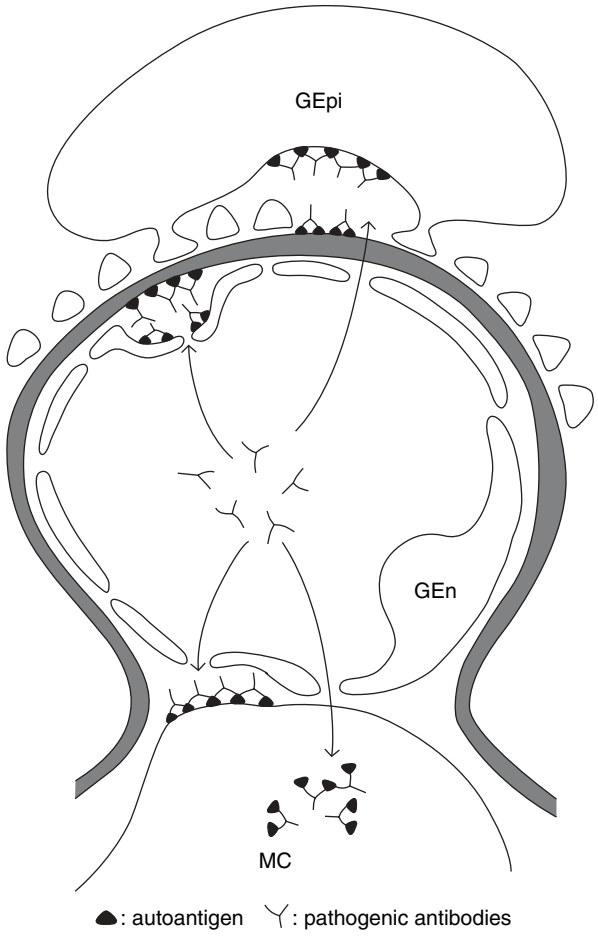

Fig. 2. In situ immune complex formation. In situ immune-deposit formation may occur at subepithelial, subendothelial, and mesangial sites. Immune complexes in mesangial or subendothelial sites lead to the recruitment of inflammatory cells, with subsequent inflammatory changes, i.e., nephritis. In contrast, circulating cells do not have access to subepithelial deposits, leading to resident glomerular cell injury mediated by noninflammatory mechanisms, i.e., nephropathy

Lessons about in situ immune-complex formation learned from membranous nephropathy

Subepithelial deposits are the characteristic features of membranous nephropathy, which is the most common cause of nephrotic syndrome in adults. Central to the pathogenesis of membranous nephropathy is the formation of immune complex deposits in the subepithelial space and the lamina rara externa of the GBM that cause a membrane-like thickening of the capillary wall.

The antibody constituent of immune complexes in membranous nephropathy consists of IgG (often IgG4) and so far largely unidentified antigens. Elution of pathogenic antibody has been largely unsuccessful in human membranous nephropathy, due to many problems, including the limited availability of suitable tissue and the need to employ relatively harsh elution procedures. Hepatitis B, hepatitis C, and *Helicobacter pylori* antigens; tumor antigens; and thyroglobulin have all been detected in the subepithelial deposits,[22–24] but there is no proof that these antigens are pathogenic, and autoimmune mechanisms may also be involved.

Our understanding of the molecular and kinetic concepts of subepithelial immune complex formation derives mainly from studies of Heymann nephritis in the rat,[25] which faithfully reproduces human membranous nephropathy. Following the discovery that immune deposits in Heymann nephritis form by antibody IgG binding to a native glomerular antigen, the autoantigenic target was identified as the podocyte-membrane protein, now called megalin.[26] Megalin, which was previously called glycoprotein (gp) 330, is a member of the low-density lipoprotein (LDL)-receptor family and is expressed both on the proximal tubular brush border and the soles of podocyte foot processes where immune complexes are initially formed.[19]

In rats, megalin seems to be expressed exclusively in glomeruli and the tubular border; it is not expressed on human podocytes or detected in subepithelial immune deposits in patients with membranous nephropathy.[27] Therefore, studies of this model should be interpreted, as a more general principle, to suggest that podocyte-membrane proteins are likely to serve as targets for immune-complex formation in situ and this results formation in pathogenic cascades downstream essentially similar to those defined experimentally.

Although the quest to identify the pathogenic antigens in human membranous nephropathy has been notoriously difficult and unproductive, despite many attempts, Ronco's group[28] recently provided the first breakthrough by identifying the neutral endopeptidase (NEP, or metallomembrane endopeptidase; MME) as a pathogenic antigenic target in one unusual form of human membranous nephropathy. Determination of the pathogenic mechanisms of immune-deposit formation was based on the finding that a genetically NEP-deficient mother developed antibodies to this enzyme during a first pregnancy; these antibodies were then transplacentally transferred into the fetal circulation of a second infant, who was not deficient in this enzyme. These antibodies localized in the glomerulus by binding to the normally expressed NEP antigen on the podocyte surface, causing the in situ formation of subepithelial immune deposits and neonatal membranous nephropathy.[28] Recent studies, by the same group, of three families, each with a newborn with neonatal membranous nephropathy, identified truncating mutations of the *MME* gene (coding for NEP) as the cause of alloimmunization during pregnancy.[29] However, NEP apparently serves as a target only in a very small number of patients; namely, the offspring of those rare mothers who lack a functional gene for this enzyme. It is likely that there is no universal antigen for membranous nephropathy in the human disease, but that other podocyte antigens are probably involved.

Mechanisms of immune-complex-mediated tissue injury

When immune complexes form *in situ* or passively deposit in glomeruli from the circulation, they can attract and activate infiltrating leukocytes or intrinsic glomerular cells to release many local mediators of inflammation, including complement, growth factors, vasoactive substances, cytokines, and activators of coagulation.

Receptors for the Fc region of immunoglobulins are expressed on many cells of the immune system and kidney. Fc receptors initiate a number of responses, including Fc receptor-mediated phagocytosis and antibody-dependent cell-mediated cytotoxicity. However, antibodies specific for a given antigen make up only a very small proportion of the total amount of immunoglobulin in plasma. Therefore, the Fc receptors distinguish antibody molecules bound to something from the vast majority of free antibody molecules. This condition is met by two changes that occur when antibodies bind to antigen: the aggregation of antibodies and conformational changes in the Fc portion of the molecule. The cross-linking of Fc receptors by immunoglobulins in immune complexes initiates inflammation, which is amplified by cytokines and activated complement components.

The Fcγ receptor, which binds the Fc part of the constant region of IgG, is one of the most important classes of immune complex-binding receptors. Two general types of Fcγ receptor are recognized – the activator receptors, characterized by the presence of a cytoplasmic immunotyrosine activator motif responsible for cell signaling, and the inhibitor receptors, characterized by the presence of a cytoplasmic immunotyrosine inhibitory motif. In NZB/NZW F1 mice, a model of lupus nephritis, the animals lacking activator Fcγ receptor on circulating leukocytes were protected from immune complex-induced glomerular injury.[30] Protection of the kidney by activator Fcγ receptor deficiency was also observed in a model of anti-GBM disease.[31,32] Furthermore, mice lacking inhibitor Fcγ receptor on a C57BL/6 background developed a spontaneous lupus-like disease with glomerular sclerosis.[33] Recent studies by Alpers' group (Muhlfeld et al.[34]) showed exacerbation of cryoglobulin-associated membranoproliferative glomerulonephritis in a mouse model without inhibitor Fcγ receptor. Despite the presence of Fcγ receptors on intrinsic renal cells, experimental studies show that glomerular damage is mediated entirely through Fcγ receptors on circulating bone marrow-derived cells.[35,36] All of these studies emphasize a crucial role for Fcγ receptors in immune-complex diseases and potentially demonstrate that immunoglobulin and complement deposition could be uncoupled from inflammatory consequences in some models.

However, another group has reported the successful induction of crescentic nephrotoxic nephritis in antibody-deficient, μ-chain-deficient mice.[37] It is, therefore, likely that both Fcγ receptor-dependent and Fcγ receptor-independent mechanisms involving T cells may be operative in different phases of various diseases.

Complement activation associated with immune-complex formation

The principal mediator of immunoglobulin-induced glomerular injury is the complement system. When immune deposits form, local complement activation occurs, leading to subsequent tissue injury.

Subendothelial deposits are readily accessible to circulating inflammatory cells, such as neutrophils and platelets, and cause severe damage, with endothelial cell injury, the activation of pro-coagulant cascades, fibrin deposition, and exudative lesions, as seen in type I mesangioproliferative glomerulonephritis and post-infectious glomerulonephritis.

Mesangial deposits injure and activate resident mesangial cells, which then de-differentiate to express α-smooth muscle actin; the cells then proliferate and overproduce growth factors, cytokines, and extracellular matrix, as seen in IgA nephropathy and other diseases.

Subepithelial deposits, as observed in membranous nephropathy, are characterized by complement-mediated podocyte injury and the lack of any apparent inflammatory reaction. This is because subepithelial immune complexes are separated from the circulation by the GBM, and the mediators released by activated podocytes are carried by filtration forces into the urine, rather than toward the circulation.

The complement system, which is an important mediator of inflammation and tissue injury, is a family of more than 20 serum and cell-surface proteins that function as a cascade. Immune complexes formed by IgG and nephritogenic antigens bind to complement factor C1q and activate the C1 complex, leading to the formation of C3 convertase and the enzymatic cleavage of the central complement component C3. Activation of C3, the most abundant of the serum complement proteins, results in the release of the chemotactic factor C3a and covalent attachment of the C3b fragment to host cells, which is an important step for amplification through the alternative pathway and for continued activation of the terminal membrane attack complex, C5b-9.

Local complement activation by the classical, lectin, or alternative pathways also generates chemotactic factors such as C3a and C5a. C3a and C5a function in a synergistic manner with Fc-receptor crosslinking to activate mast cells and stimulate inflammatory cells, thereby leading to tissue injury.

However, the most nephritogenic effect of complement activation appears to be the generation of the membrane attack complex, or C5b-9. C5b-9 inserts in sublytic quantities into the membranes of glomerular cells, where it produces cell activation, converting normal cells into resident effector cells to cause injury. In 1980, Salant and colleagues[38] were the first to show, using generalized complement depletion with cobra venom factor, that podocyte injury in the Heymann nephritis model was complement-dependent. Later studies showed that the same effect could be achieved with C6 depletion alone, thus implicating the C5b-9 membrane attack complex in this process.[39–42]

C5b-9 formation damages podocytes via various mechanisms. Sublethal C5b-9 attack in glomerular epithelial cells triggers metabolic changes and the induction of the de novo synthesis of prostaglandins and proteases.[43,44] Complement activation induces a massive increase in the biosynthesis of nicotinamide adenine dinucleotide phosphate, reduced (NADPH)-oxido-reductase in podocytes, which, in turn, causes local production of oxygen radicals.[45,46] These products facilitate the degradation of the GBM.

C5b-9 activates phospholipase A2 and induces phospholipid hydrolysis in podocytes, resulting in the impairment of endoplasmic reticulum (ER) membrane integrity and subsequent ER stress.[47] Sublytic C5b-9 attack induces DNA damage in podocytes both in vitro and in vivo.[48] Furthermore, complement activation leads to the reversible disruption of actin microfilaments in cultured podocytes, and the dissociation of nephrin from the actin cytoskeleton in experimental membranous nephropathy.[49,50] Thus, proteinuria in membranous nephropathy is a consequence of GBM damage caused by oxidants and proteases derived from podocytes, as well as podocyte cytoskeletal changes induced by C5b-9. For more details of the podocyte response to complement attack which leads to proteinuria, readers are referred to our recent review of membranous nephropathy.[51]

C5b-9 formation is crucial in the injury of other glomerular cell types as well. Complement attack activates mesangial cells and induces the production of various disease mediators.[52,53] Furthermore, complement attack directly stimulates mesangial cell proliferation.[54] Cell proliferation and mesangial expansion in experimental glomerulonephritis models resembling IgA nephropathy also reflect C5b-9-induced cell activation, with the overproduction of platelet-derived growth factor (PDGF) and transforming growth factor (TGF)-β, which are representative mediators of mesangial injury.[55,56] In a mesangioproliferative glomerulonephritis model of anti-Thy1 nephritis in rats, C6 deficiency resulted in lack of initial mesangial damage and subsequent renal-disease phenotypes.[57]

The relevance of complement activation in human mesangioproliferative glomerulonephritis was demonstrated in IgA nephropathy. Zwirner and colleagues[58] showed that the presence of complement activation was associated with more severe renal disease and suggested that activated C3 could serve as a clinical predictor of the disease course. In IgA nephropathy, it is speculated that IgA activates the complement cascade via the lectin pathway.[59–63]

An essential role of C5b-9 formation in glomerular endothelial injury has also been demonstrated in a variety of animal models, including a thrombotic microangiopathy model induced by immune-complex formation on glomerular endothelial cells.[64,65] Endothelial injury in lupus occurs when C5b-9 causes the upregulation of leukocyte adhesion molecules, as well as cell lysis, detachment, and apoptosis.[66]

It should be noted that the balance between the harmful effects of complement activation and the beneficial effects of immune-complex processing and apoptotic debris disposal is important. While the complement system is considered to be a major mediator of tissue injury in SLE, a genetic deficiency of the early complement components is also associated with SLE. This is called the "lupus paradox",[67] because, on the one hand, complement is pathogenic but, on the other, it is protective. This is a good example to illustrate the necessity for a fine balance of the complement system in vivo. In the MRL/lpr mouse model of lupus nephritis, complement inhibition by soluble Crry,[68,69] or deficiency of factor B,[70] protected the animals from renal disease. In contrast, C3 –/– MRL/lpr mice were not protected from renal disease, and this lack of protection was associated with evidence of elevated immune-complex deposition in the glomerulus.[71]

Complement regulatory proteins as a protective mechanism

Complement activation is normally closely regulated by a series of circulating and cell-bound complement regulatory proteins that protect cells from injury.[72–74]

Our group[75,76] demonstrated that the overexpression of complement regulatory proteins in cultured glomerular cells served to protect against complement attack. Furthermore, the blockade of complement regulatory proteins by neutralizing antibodies resulted in aggravation of various renal disease models.[77–79] Quigg and colleagues[80,81] showed that the neutralization of a complement regulatory protein by pathogenic antibodies, which bound to podocyte surfaces and formed immune deposits, was essential to induce proteinuria in a Heymann nephritis model in rats. A protective role for complement regulatory proteins in renal disease has also been demonstrated, utilizing a gene-targeting approach, by our group and others.[82–86]

One may also ask whether a lack of complement regulatory proteins might result in renal disease. The answer is yes. Recent molecular biological analysis has demonstrated that genetic abnormalities of factor H, a soluble complement regulatory protein at the hit point of C3b, are associated with atypical hemolytic uremic syndrome (HUS).[87–89] It is estimated that 15% to 30% of atypical HUS can be ascribed to factor H mutations.[90,91] Recent studies employing surface plasmon resonance analysis have revealed a reduction of the binding affinity of mutated factor H to C3, demonstrating the functional importance of the mutations.[92] Furthermore, another membrane-bound complement regulatory protein, which works at the same step as factor H, has also been associated with atypical HUS.[93,94] All these findings suggest that inability to control the intravascular activation of the complement cascade can lead to endothelial injury, the formation of thrombus, and eventual HUS, emphasizing the substantial biological importance of complement regulatory proteins, especially in renal disease.

One question remains to be answered. While patients with heterozygous and some homozygous factor-H deficiencies develop atypical HUS, other homozygous factor H-deficient subjects suffer from membranoproliferative glomerulonephritis.[95] Both factor H-knockout mice and pigs with congenital deficiency of factor H develop membranoproliferative glomerulonephritis but not HUS.[96,97] This finding may be partly explained by the relative amounts of factor H, but it is still unknown why complete factor H deficiency in humans sometimes leads to atypical HUS, while, at other times, it results in membranoproliferative glomerulonephritis.

Future therapeutic approaches

The identification of specific antigens in human disease can lead to the development of new and specific therapies. In the field of neurology, Campylobacter has recently been identified to be responsible for the development of Guillain Barre syndrome.[98,99] Identification of NEP as a target in membranous nephropathy is a break-through in nephrology. Recent studies by Yoshizawa and colleagues[100,101] have shown that nephritis-associated plasmin receptor is the likely pathogenic antigen in acute post-streptococcal glomerulonephritis. This is a rapidly advancing area and one of the most promising frontiers in immune-complex disease research, because it offers the possibility of selectively targeting disease-specific elements of the immune response, or restoring tolerance to self antigens, without inducing generalized immunosuppression.

Other essential approaches to be attempted include the design of ways to interfere with complement activation and C5b-9-mediated cell injury. The utilization of complement regulatory proteins in various ways has considerable potential as a therapeutic tool in both glomerular and interstitial disease.

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