Renal Transplant Rejection

Philippa Dodd, Candice Roufosse, and Mark Harber

Contents

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Learning Objectives

1. To appreciate the risk factors associated with acute and chronic rejection
2. To explore the pathophysiology and diagnosis of acute and chronic rejection
3. Evaluation of treatment options and outcomes for rejection

Definitions

Renal allograft rejection manifests clinically as graft dysfunction evidenced by a rise in serum creatinine and an increase in urinary protein loss. From a clinical point of view, the time frame between transplantation and the onset of rejection defines either its acute or its chronic nature. For the pathologist however, activity and chronicity are graded in the renal allograft biopsy according to the scores for inflammation and scarring respectively, using the Banff Classification of renal allograft rejection [1], without reference to time posttransplantation. Subclinical rejection is defined as histological evidence of rejection in the absence of graft dysfunction, a pathologic entity identified by so-called protocol biopsy which is not performed in all transplant centres. In the absence of clinical graft dysfunction as well as robust data proving that treatment of subclinical lesions improves outcomes, how to manage this problem in the clinical practice of transplantation remains controversial.

Hyperacute rejection – occurs almost immediately upon reperfusion of the transplanted donor kidney on the operating table following cross-clamp release. This phenomenon is reflective of the interaction of recipient derived, usually high titre, preformed donor-specific anti-HLA antibody (DSA) with donor allo-antigens and associated complement activation. Or in the context of blood group incompatible (ABOi) transplantation.

Acute rejection—occurs within days (early or accelerated) or weeks (late) posttransplantation and may be antibody mediated or T-cell mediated.

- **Acute *antibody*-mediated rejection (AMR)** – occurs most often as a consequence of immunologic memory sustained through previously sensitizing events such blood transfusions, pregnancy, or previous transplant episodes. It may also occur in the face of high numbers of HLA donor and recipient mismatches and subsequent generation of de novo DSA.
- **Acute *T-cell* mediated rejection (TCMR)**—also known as cellular rejection, is the most common form of acute rejection and results either from direct interaction between recipient T cells and donor antigen expressed on donor cells or recipient T-cell interaction with donor alloantigen presented by recipient antigen-presenting cells (APCs).

Sub-acute rejection—usually occurs between three and six months posttransplantation, although it can occur at any point in the posttransplant period. It too may be antibody- or T-cell mediated. Where antibody-mediated rejection is concerned, graft dysfunction is most often reflective of the production of de novo DSA against donor antigen.

Chronic *T-cell* mediated rejection (cTCMR) – has relatively recently been recognized as a pathologic entity by the Banff working group and is characterized by marked tubulointerstitial inflammation in a scarred renal cortex with tubulitis and arterial intimal thickening and inflammation.

Chronic *antibody* mediated rejection (CAMR)—occurs months or years posttransplantation. Repeated or persistent immunologic injury has been strongly implicated in its development but the precise relationship between acute and chronic antibody-mediated rejection remains unclear. Its definition as a clinical-pathological entity has changed over time but the majority agree that the presence of glomerular double contouring, peri-tubular capillary basement membrane multi-lamination, interstitial fibrosis, tubular atrophy and vascular intimal hyperplasia are diagnostic histological features [2].

92.1 Introduction

Once the technical aspects of vascular surgery required for kidney transplantation were overcome, the immune response to the transplanted organ became the principal barrier to transplantation. And once Medawar, Billingham, and Brent demonstrated the immunological nature of rejection the next barrier was finding immunosuppression powerful enough to prevent graft destruction without killing the patient. In the early 1960s patients were subjected to enormous doses of steroids with predictable side effects and very poor graft and patient survival. Further understanding of the impact of sensitization and anti-HLA antibodies, led by Terasaki [3] in this era provided an explanation for and subsequent avoidance of hyperacute rejection and immediate allograft loss. Graft survival rates improved with the addition of 6-mercaptopurine and azathioprine [4] but it was only with the introduction of calcineurin inhibitors that acute rejection rates fell significantly [5]. Today, induction agents are in common-place use (anti-CD25mAb or depleting antibodies) combined with a

maintenance regimen consisting of tacrolimus and mycophenolate mofetil often in combination with corticosteroids.

92.2 Pathogenesis of Rejection

The normal response of the host to foreign antigens is the presentation of foreign peptides by host antigen-presenting cells (APCs) via major histocompatibility complex (MHC) molecules to host T cells via the T-cell receptor. In transplantation this normal immunological response is known as *indirect* presentation. However, unique to transplantation is the addition of donor APCs which can also present peptides to recipient T cells (known as *direct* presentation). Donor APCs can present (a) donor peptides (b) recipient peptides but via foreign MHC, therefore overcoming acquired tolerance and (c) the MHC of donor APCs can activate recipient T cells without peptide. This may all seem rather abstract but the MHC is extraordinarily polymorphic and MHC is highly expressed; the combination of high density, polymorphic allo-antigens and *direct* plus *indirect* presentation is thought to explain the extremely high frequency of recipient T cells that can recognize and react to donor antigens compared to conventional antigens. The corollary of this is that the alloimmune response, left unchecked is exceptionally vigorous.

The second, real-world element to rejection is sensitization to donor antigens following prior exposure to foreign tissue types. This most frequently results following previous transplantation, pregnancy, blood transfusion, or more rarely following infection. This can result in a very powerful amnestic response from T cells, natural killer cells, macrophages and B cells with the generation of donor-specific antibodies which may be complement fixing. An amnestic response is particularly problematic as donor-specific IgG antibodies are likely to be high avidity and previous T- and B-cell clonal expansion engenders a rapid and vigorous immunological response. With this in mind, the nature and timing of rejection fits into clinical paradigms.

92.2.1 Hyperacute Rejection

Hyperacute rejection is the catastrophic fixation of preformed, high titre and complement fixing antibodies to the renal vascular endothelium. Fibrin thrombi form in small vessels resulting in occlusion of the blood supply. This either results from a blood group incompatible transplant or secondary to blood group incompatible or very high levels of donor-specific antibodies. It declares itself to the unhappy surgeon in theatre on release of vascular clamps with a blue, floppy, and unsalvageable kidney.

92.2.2 Acute T-Cell-Mediated Rejection (Cellular Rejection)

Acute cellular rejection represents the infiltration of T cells (CD3+) which include both cytotoxic ($CD8^+$) and T helper cells ($CD4^+$) that recruit the whole panoply of the immune system including macrophages, B cells, eosinophils and NK cells. It is associated with inflammation in three compartments, to a greater or lesser degree, namely tubules (Banff lesions score t1-3), interstitium (Banff lesions score i1-3), and arteries (Banff lesions score v1-3) (Fig. 92.1).

Immune invasion of the tubulointerstitium is the most common finding in early rejection, vascular involvement suggests more severe rejection and ranges from intimal arteritis (T cells invading the vascular intima, underneath the endothelium) (Fig. 92.2) to severe transmural inflammation and fibrinoid necrosis (Fig. 92.3).

92.2.3 Acute Antibody-Mediated Rejection

Acute antibody-mediated rejection (AMR) can occur in the context of a memory response if the patient is sensitized, so risk factors include a high CRF, previous positive cross-matches (CDC or flow) or simply as part of an unchecked primary alloimmune response. Rapid ("accel-

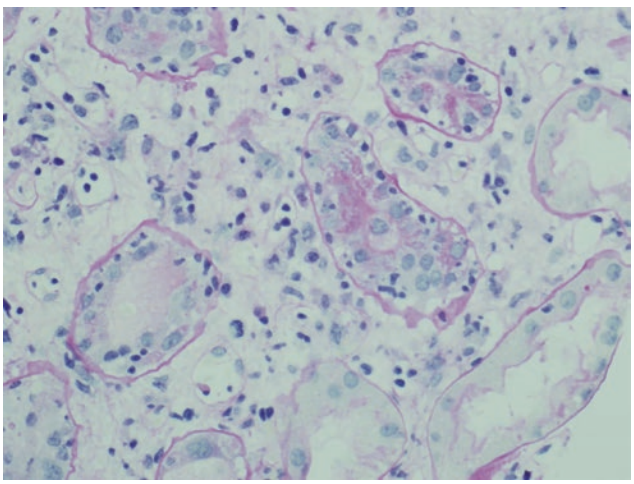

Fig. 92.1 T-cell mediated rejection, tubulointerstitial type (Banff 1): Lymphocytes and monocytes are present in the interstitium (Banff lesion score i) and within the confines of the tubular basement membranes (Banff lesion score t). PAS stain; tubular basement membranes stain bright pink

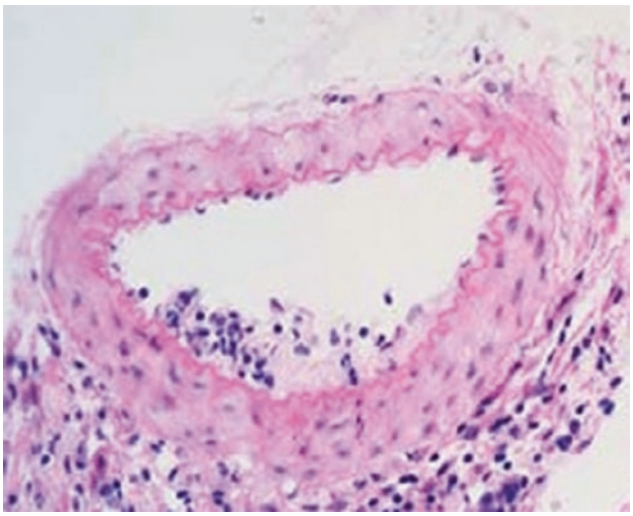

Fig. 92.2 T-cell mediated rejection, vascular type (Banff 2A): Lymphocytes and monocytes are present in the arterial intima, underneath the endothelium, occupying a limited circumference of the artery (Banff lesion score v1). H&E stain

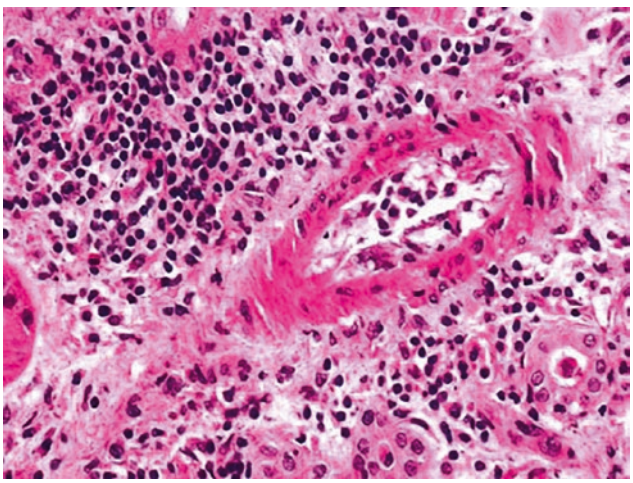

Fig. 92.3 T-cell mediated rejection, vascular type (Banff 2B): Lymphocytes and monocytes are present in the arterial intima, underneath the endothelium, occupying the full circumference of the artery (Banff lesion score v2). H&E stain

erated rejection") tends to occur in the context of a memory response to donor specific antibodies or non-HLA antibodies. The effector response to memory responses is faster than that of primary immune responses and as such acute rejection is more likely to occur in the setting of donor-specific sensitization, a positive CDC crossmatch, current or historical positive flow crossmatch and non-donor sensitization. The underlying disease process results from the fixation of donor-specific antibodies to the endothelium of renal arteries and microcirculation (glomerular and peritubular capillaries). This recruits leucocytes and NK cells with release of IFN-γ, and leads to activation of the endothelium and platelets with or without the fixation of complement which can be detected by the deposition of C4d in the peritubular capillaries. This can develop into a thrombotic microangiopathy initially indistinguishable from recurrent aHUS. Neutrophils in the peritubular capillaries are a useful early histological finding. The interstitial capillaritis may lead to interstitial haemorrhage (Fig. 92.4a–c).

92.2.4 Chronic Antibody-Mediated Rejection

Chronic antibody-mediated rejection (CAMR) has perhaps belatedly been recognized as the commonest cause of renal allograft loss. While the mechanisms of acute rejection are reasonably well defined those underlying CAMR remain less so and histological definitions have changed over time to reflect an improved understanding in this field. It is broadly accepted, largely from published data correlating the development of chronic rejection with acute rejection episodes, that CAMR results from recurrent "waves" of acute/sub-acute/chronic donor-specific (usually anti-HLA) antibody deposition and injury. Although, the lack of observed response to treatment with immunotherapies (as discussed later in this chapter) is strongly suggestive that additional cellular and molecular mechanisms are at play.

Endothelial cell activation resulting from deposition of immunoglobulin and complement is thought to lead to lying down of new basement membrane layers around peritubular capillaries and glomerular capillary loops. In glomeruli, this leads to double contouring on light microscopy and the pathognomonic multilayering of basement membranes seen on electron microscopy (Fig. 92.5a, b)—referred to as transplant glomerulopathy, which results in progressive occlusion of the vascular lumen and ischemia of the remaining nephrons. This is associated with progressive fibrosis and chronic inflammation of the interstitium as well as tubular atrophy causing a relentless fall in GFR usually accompanied by significant proteinuria.

92.3 Epidemiology and Risk Factors for Rejection

While rejection has always been an issue the clinical presentation and timeline has changed significantly since the dawn of transplantation (Fig. 92.6). The avoidance of unintentional blood group incompatible transplantation and CDC crossmatch incompatible transplantation has largely eliminated hyper-acute rejection and diminished acute antibody-mediated rejection.

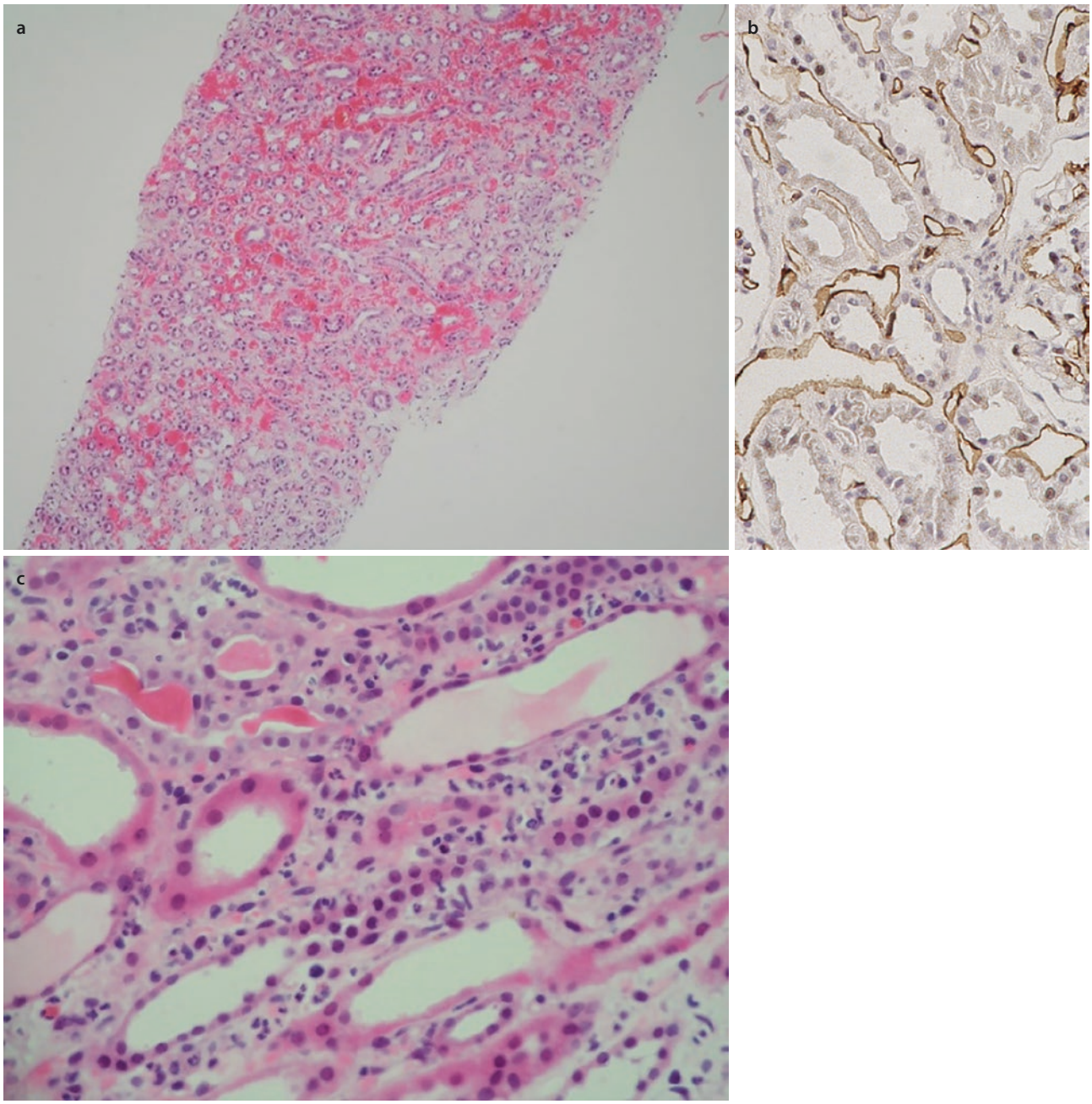

Fig. 92.4 (**a**, **b**) Acute antibody-mediated rejection with interstitial haemorrhage (H&E, on the left) and positive staining for complement factor 4d along the peritubular capillary endothelium on the right (C4d immunoperoxidase). (**c**) H&E section showing neutrophils in the capillaries between the tubules (peritubular capillaritis)

Since the symphony Elite study, acute rejection rates with tacrolimus, mycophenolate mofetil, and anti-CD25 mAb regimens, are in the mid-teens, units using depleting antibodies routinely achieve acute rejection rates in single figures. However, early success has left us with a larger proportion of chronic antibody-mediated rejection (CAMR).

With modern tissue-typing, regular antibody screening and good governance around blood group cross-matching hyperacute rejection should now be a "never event" and fortunately this irredeemable complication is extremely rare now. The role of antibody pre-screening and risk is nicely reviewed by Gebel et al. [6].

Prior to the "modern immunosuppressive era" acute rejection (AR) remained very common with rates of up to 50% biopsy proven rejection in the USA in the 1980s and early 1990s. Three large RCTs published in the

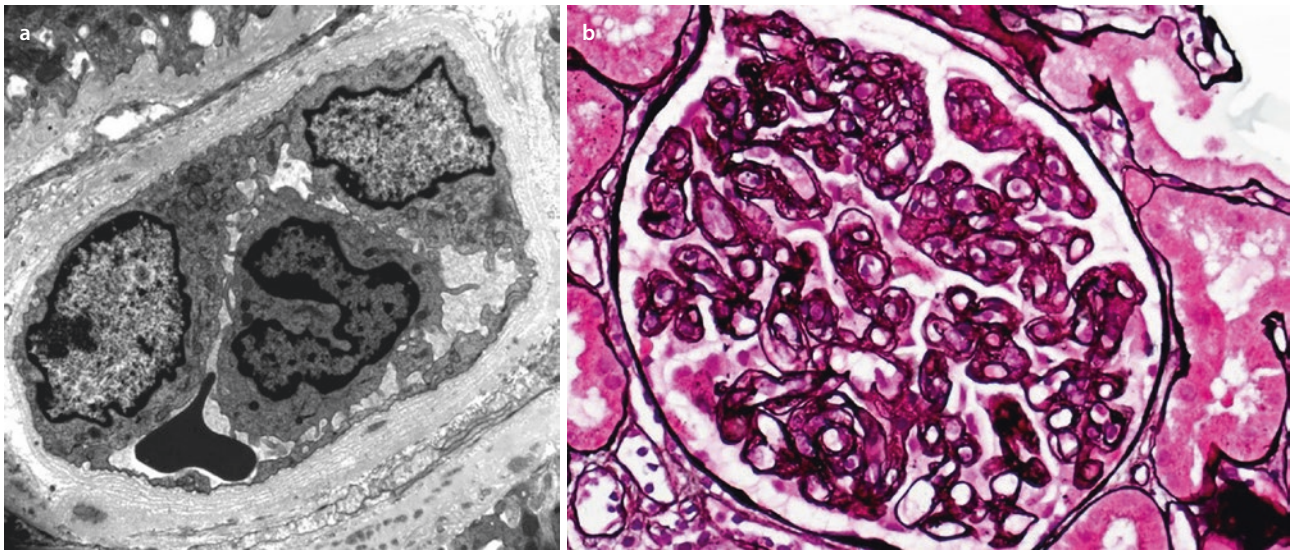

Fig. 92.5 (**a**) Electron micrograph of a peritubular capillary showing multiple layers of basement membrane, a typical finding in chronic antibody-mediated rejection (top). (**b**) Glomerulus with double contours along capillary walls, typical appearances of transplant glomerulopathy (right, silver stain)

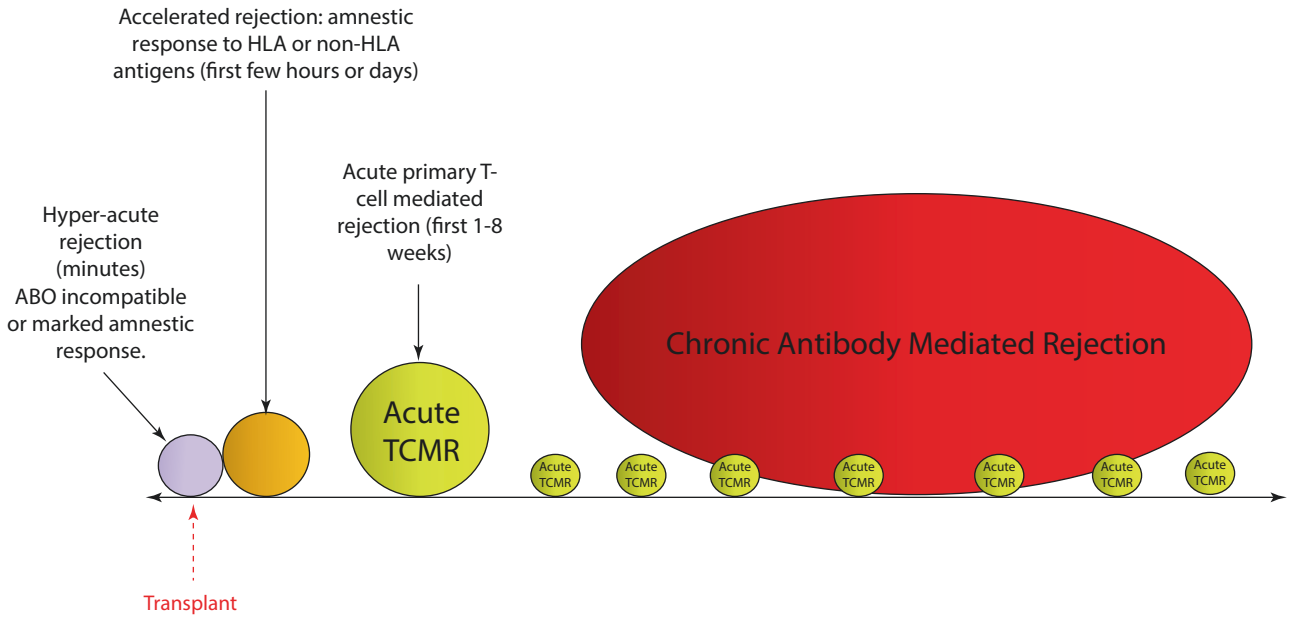

Fig. 92.6 Timeline of rejection. Hyperacute rejection when the transplant goes black on the operating table should now be a "never event". Accelerated rejection is still a useful term in that early aggressive rejection within the first few days is likely to represent a memory or amnestic response to HLA or non-HLA antigens. The majority of T-cell mediated rejection (TCMR) occurs within the first two months of an uncomplicated transplant. However, late acute TCMR and occur at any stage of a stable transplant, this may be obvious or subclinical but almost invariably results from underdosing of immunosuppression from non-adherence or medical error. Chronic antibody-mediated rejection is now thought to be the commonest cause of graft loss and can occur and progress at any stage of the transplant

1990s demonstrated reduction in AR with MMF versus AZA (for review, see reference [7]. Registry data from over 66,000 transplant in the USA seemed to confirm the benefit of mycophenolate mofetil over azathioprine in terms of acute rejection (15.5% versus 24.7%, respectively) and a further study in the elderly demonstrated reduced early AR with MMF (24%) versus AZA (28%) but also a significant reduction in late rejection after 12 months (2.3% versus 12.6%) [8].

Tacrolimus also resulted in better AR rates than cyclosporin (4–17% versus 14–20%, respectively). The requirement of ATG to treat rejection the first year was lower with tacrolimus and mycophenolate mofetil (6%) compared to Tacrolimus and azathioprine (13%) or

cyclosporine and mycophenolate (12.6%). A meta-analysis of 30 studies and over 4000 patients in 2005 showed a RR of AR of 0.69 and steroid resistant AR 0.49 with tacrolimus compared with cyclosporine [9].

The use of tacrolimus and mycophenolate mofetil was embedded in practice following the Elite Symphony Study [10] which demonstrated an acute rejection rate of 17% in patients receiving anti-CD25mAb induction and triple therapy with steroids, mycophenolate, and "low dose" tacrolimus versus other regimens with rates of 29–43%. Nonetheless, even in this "modern era," early acute rejection has clear risk factors which relate broadly to:

1. Immunological barriers (mismatch and sensitization)
2. Host factors
3. Immunosuppression

92.3.1 Immunological Barriers

European registry data from 1995 to 2004 showed five-year outcomes of 80% for 000 mismatched grafts compared with 70% for 222 mismatched transplants although the difference was less significant for live donor transplants [11]. The implication here is that the worst outcomes are related to alloimmune damage.

It is not just the degree of mismatch but also the specific HLA antigens involved that determines outcome; the immunological hierarchy is broadly accepted to run DR > B > A in terms of outcome. In a retrospective review of over 1300 patients treated with anti-CD25mab, tacrolimus and mycophenolate mofetil and early steroid withdrawal showed that there was a significant difference in acute biopsy-proven rejection between those with no DR mismatches and no or only one B mismatch and those with a DR and/or two B mismatches (Henry Stephens, Personal communication) (Table 92.1).

Table 92.1 Acute renal transplant rejection rates based on mismatch

Mismatch	ACR
000 mm	7%
0DR ± 0|1Bmm (100, 010, 110, 200, 210)	11.7%
0DR ± 2B or 1DR ± 0|1Bmm (020, 120, 220, 001, 101, 201, 011, 111, 211)	15.3%
1DR ± 2B or 2DRmm (021, 121, 221, 002, 102, 202, 012, 112, 212, 022, 122, 222)	17.7%

NHSBT organ matching scheme 2006–2019. (*ACR* acute rejection rates)

92.3.2 Sensitization

The rules that have evolved on what is a negative or safe crossmatch, what is intermediate and what is high risk or unsafe derive entirely from the predicted allo-immune response to the donor antigens. Thus, by definition, patients with positive crossmatches and high levels of DSA are at very high risk of rejection and patients who are highly sensitized may find themselves effectively precluded from transplantation because the risk of rapid and severe rejection is unacceptably high.

Patients with lower levels of DSA that do not result in a positive crossmatch may be transplanted but significantly higher rates of acute antibody rejection (AMR) have been reported in retrospective analyses of patient cohorts, with detrimental effects on overall rates of allograft survival [12–14]. One such study reported more than double the incidence, 63% versus 26%, of AMR in those with low level DSA as compared to those without. At five years posttransplantation, allograft survival in those with DSA who experienced AMR was significantly inferior to those with DSA who experienced no AMR (68% versus 87%, $p = 0.002$). Interestingly, allograft survival in those without DSA was comparable to those with DSA but no AMR (89% versus 87%) [12].

Those who do not have DSA but who are highly sensitized to many other HLA antigens also tend to do less well in terms of both rejection episodes and long-term allograft survival when compared to unsensitized counterparts but outcomes are reported to be better when compared to those sensitized by DSA [13]. The accepted theory underlying this pertains to the notion that HLA antigens share epitopes which allows binding, albeit with variable avidity, of nonspecific HLA antibodies to multiple antigenic epitopes expressed on the renal allograft with resultant immune activation and allograft injury.

92.3.3 Host Factors

Outside the setting of sensitization, older patients have a lower rate of acute rejection, and increased rate of CMV reactivation and reduction in vaccine response, consistent with the idea that the reduction in rejection rates in older patients is secondary to immune senescence. Conversely, a young patient with a low immunosuppressive burden has a much higher relative risk of rejection. High intra-patient tacrolimus trough level variation in young adults seems to be particularly hazardous (see below).

Despite initial concerns that patients with controlled HIV would be over-immunosuppressed with transplant immunosuppression, HIV consistently confers a near

doubling of acute rejection rates, somewhere in the region of around 30–40%. The reason for this is intriguing but not clear; it may be a combination of chronic immune dysregulation and low exposure to tacrolimus in patients on protease inhibitors.

While there is an increased incidence of acute rejection associated with CMV viremia and attributed to an antiviral pro-inflammatory milieu (interferon-γ treatment is contra-indicated in renal transplantation for this reason), the majority of CMV viremia follows, not proceeds treatment for rejection and is likely a consequence of an escalation in immunosuppression. However, it is clear that rejection is sometimes precipitated by immunosuppression reduction (ISR) in the face of troublesome primary CMV infection, as is the case with ISR to treat BK virus nephropathy.

92.3.4 Immunosuppression

Induction immunotherapy serves largely to ameliorate T-cell alloresponses involved in direct allorecognition encountered early in the post-transplant period. Randomized control trials and meta-analyses indicate that induction therapy plus conventional oral therapies offer superior outcomes to conventional therapies alone [14–16]. Published data regarding the optimal prophylactic induction therapy to prevent rejection remains controversial.

Induction agents can be broadly divided into cell depleting and non-depleting agents. Lymphocyte depleting agents including rabbit anti-thymocyte globulin—rATG, a polyclonal antibody targeted against numerous human T-cell antigens including MHC, alemtuzumab—Campath-1H, a monoclonal anti-CD52 antibody which is pan-lymphocyte depleting, belatacept, a CTLA-4-Ig which inhibits CD80/CD86 co-stimulation required for T-cell activation and rituximab a monoclonal anti-CD20 antibody which targets B-cells. Non-depleting agents include basiliximab and daclizumab both monoclonal antibodies which act as antagonists to CD25, the IL-2 receptor, activation of which is critical to T-cell expansion.

B-cell depletion using rituximab has not been shown to offer effective induction of immunosuppression over standard therapies and head-to-head comparison of T-cell depleting versus non-depleting agents have revealed conflicting results. In high immunologic risk renal transplants and ABOi, rATG proved superior when compared to anti-IL2 agents in preventing rejection at 1 year [17]. Early outcomes comparing alemtuzumab and rATG show no difference in rejection rates between these two agents. However, late acute rejection is more common with alemtuzumab (although not significantly different) and it appears that the beneficial effects may be lost over time with an increased risk of death and allograft failure at five years post-transplant in alemtuzumab treated patients (25.9% versus 22.9%) [18]. The evidence for low immunologic risk transplants is less clear. Some studies demonstrate a lower incidence of acute rejection, death and graft loss with rATG but at the expense of increased infection rates, others show no superiority of rATG over IL-2 antagonists and a similarity in adverse events.

In steroid-free protocols, at 12 months alemtuzumab was demonstrated to be superior to IL-2 antagonists (5% versus 17%, $P = <0.001$) in preventing rejection episodes. At 3 years, in the low risk groups treated with alemtuzumab, significant reduction in the incidence of acute rejection, graft loss and death was observed (10% versus 22%, $P = 0.003$) when compared to basiliximab but in high-risk patients there was no differences observed between rATG and alemtuzumab (18% versus 15%,) [19].

CNIs are difficult to beat in their ability to suppress rejection. CNI-free initial regimens are associated with high acute rejection rates that have, in the case of mTOR inhibitors precluded their de novo use. Belatacept (CTLA4-Ig competitive agonist for CD28, blocking co-stimulation via CD80 and CD86) in combination with MMF and prednisolone incurred rejection rates of 22–17% compared to those on a CNI with 7%. The CTOT-16 trial with belatacept and MMF but no steroids or CNI was stopped early due to rejection rates of >30% in the non-CNI arm [20].

One other compelling piece of data relates to transplant outcomes based on immunosuppression levels at one year. In a retrospective study of registry data, those patients with tacrolimus levels of ≤ 5 at one year had significantly worse outcome at five years and this was exacerbated by low MPA doses and improved by higher MPA doses implying that failure to control the alloimmune response is highly dependent on background immunosuppression levels (Fig. 92.7).

An important caveat is that while acute rejection rates are undoubtedly lower with induction agents Tacrolimus and MPA there is little evidence of long-term benefit from these regimens in low or standard risk recipients [21].

92.3.5 Non-adherence

The commonest cause of late rejection is undoubtedly non-adherence which may either manifest as an abrupt, often irreversible deterioration in renal function in the context of acute rejection, unrecordable CNI levels, secondary to complete discontinuation of immunosuppres-

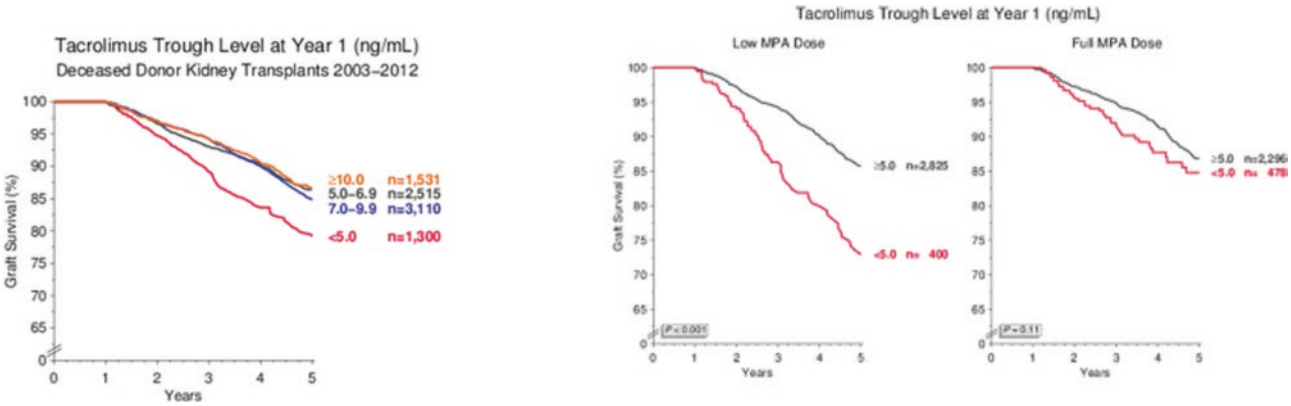

Fig. 92.7 Outcome data from the collaborative transplant study, based on trough tacrolimus level at one year. Patients with a tacrolimus level <5 have a worse outcome in terms of graft survival (left hand graph) which is worse still if combined with low Mycophenolic acid (MPA) doses (middle graph). High doses of MPA had a protective effect for those patients with a low tacrolimus trough levels. (Thanks to G Opelz for permission to reproduce this data)

sion. Possibly much more frequent than catastrophic abrupt cessation is the chronic intermittent subtherapeutic exposure due to multiple and frequent missed doses. Patients with high trough tacrolimus variability fit this pattern and have been shown to have worse rejection-free survival (78% vs 90% at 8 years) [20] or a combination of adverse outcomes [22].

92.4 Diagnosis

Historically in the early days of transplantation patients may notice a tender swollen kidney, oliguria or a temperature but these days this is pretty unusual unless there has been a complete withdrawal of immunosuppression. A version of this occurs in patients with a failed transplant who return to dialysis and have a wean of immunosuppression. This may result in disproportionate anaemia, an unexplained CRP and tender ("hot") kidney. More frequently the diagnosis is suggested simply by a rising creatinine or delayed graft function and failure of creatinine to fall, unexplained hypertension, proteinuria or perhaps the appearance of a new donor-specific antibody. A Doppler ultrasound may show a deterioration in perfusion index and reverse diastolic flow but this is not specific for rejection. A variety of biomarkers have been mooted to avoid the need for biopsy but as yet, none have made it to clinical practice. Ultimately, the definitive diagnosis rests on a renal biopsy although it is not infrequent to for patients to be treated blindly for rejection on best-guess speculation if a biopsy is not feasible. The disadvantage of this approach is that it is not clear that rejection was the correct diagnosis and the patient has been committed to a substantial increase in immunosuppression, moreover, a biopsy after high dose steroids is often much more difficult to interpret. Additionally, renal biopsy allows for the identification of immunosuppression-related toxicity such as that caused by CNIs as well as pathologies other than rejection such as recurrent disease and infection including BK nephropathy. It is also beneficial from a prognostic point of view allowing assessment of the degree of established scarring which often informs treatment decisions.

Table 92.2 Banff classification of renal allograft rejection

1.	Normal
2.	Antibody mediated rejection
3.	Borderline
4.	T cell-mediated rejection
5.	Interstitial Fibrosis and Tubular Atrophy
Other	E.g. BK virus

A single core has a sensitivity of around 91% whereas 2 cores, closer to 99% sensitivity. Clearly the size of the core and while the diagnosis may be made on a fragment >10 glomeruli and >2 vessels (as endarteritis is a focal process) is thought to be an adequate sample [23]. Given the importance of peritubular capillary basement membrane multilayering for a diagnosis of CAMR, a sample for electron microscopy should be standard or considered for biopsies beyond the acute first few months of a transplant.

However, in the context of an adequate biopsy prior to treatment the diagnosis is usually fairly clear. The Banff Classification for Allograft Pathology has evolved with the science and offers a standardization that facilitates an assessment of all compartments and comparative research. The Banff Classification comprises six categories shown in Table 92.2:

The rejection categories are established by specific thresholded combinations of individual Banff lesion scores, which are graded zero (if normal) to 3. Activity scores grade degrees on inflammation present: Tubulitis (t), arteritis (v), glomerulitis (g),peritubular capillaritis (ptc), interstitial inflammation (i, ti, ifta). Chronicity scores establish degree of scarring/new basement membrane present: tubular atrophy (ct), interstitial fibrosis (ci), intimal fibrosis (cv), glomerular basement membrane double contouring (cg),, peritubular capillary basement membrane lamellation (PTCML). There are also Banff lesion scores for extent of C4d staining (C4d), mesangial matrix expansion (mm) and degree of arteriolar hyalinosis (ah).

It is important to consider the differential diagnosis of a cellular infiltrate which includes:

1. BK virus nephropathy,
2. Other viral nephropathies (rarely CMV or adenovirus),
3. Lymphoma (monotonous infiltrate, may stain for EBV antigens)
4. Allergic tubulointerstitial nephritis (perhaps eosinophil rich but not specific)
5. Recurrent disease (recurrent TIN rare)
6. Pyelonephritis (common)
7. Tuberculosis (granulomas)

Rejection-mediated glomerulopathy has the important differential diagnosis of recurrent disease or de novo primary or secondary glomerulonephritis.

92.5 Treatment

92.5.1 Acute Rejection

The treatment of rejection episodes focusses largely on augmentation of immunotherapies in one form or another. High dose, often intravenous steroids are the backbone of treatment with variable use of T-cell depleting agents and implementation of antibody removal techniques such as plasma exchange and ivIg in the case of AMR but the evidence base for the best treatment is very poor.

Our policy is to obtain a transplant biopsy if at all possible prior to initiating treatment in order to avoid unnecessary IS acutely and a long-term escalation in the absence of rejection, to exclude BKV nephropathy and other potential causes of graft dysfunction such as pyelonephritis that are unlikely to benefit from a huge increase in IS.

Treating sub-clinical rejection remains controversial, the largest RCT involving contemporary IS (tacrolimus and MMF) in over 200 patients showed no benefit from treating sub-clinical rejection found on protocol biopsy [24].

For biopsy-proven acute TCMR and AMR high-dose steroids are most commonly used and work by blocking the synthesis and release of pro-inflammatory cytokines (IL-1, TNF-α) and inhibit IL-2 production by T cells. Steroids are usually given as IV bolus doses (typically 3–5 mg/kg or more typically as 500 mg daily for 3–5 days of methylprednisolone, or as high-dose oral 100–200 mg tapering over days). The evidence base for dose, route, and duration is very weak. Steroids are typically accompanied by an increase in antiproliferative (usually MMF) and CNI dose (usually tacrolimus) and long-term low-dose oral steroids. High dose steroids successfully treat acute rejection in roughly 60–70% of cases.

Thymoglobulin (ATG) is the most commonly used alternative treatment for rejection. Doses range from 1.5–3 mg/kg from 5–10 days (intention to treat). It has to be given via a central line and can be associated with significant allergy (particularly those who have had exposure/allergic to rabbits).

A meta-analysis of 11 studies comparing polyclonal and monoclonal antibodies for the treatment of AR concluded that polyclonal antibodies (ATG, ALG and OKT3) are probably better than steroids at treating acute TCMR (RR 0.5) and preventing subsequent rejection (RR 0.7) but the studies were of poor quality [25]. Neither anti-CD-25mAb nor Rituximab have any benefit in the setting of TCMR but Alemtuzumab (humanized anti-CD52 mAb) has been used as rescue therapy, is simpler to give than ATG; it does not require a central line (indeed can be given subcutaneously) and is a shorter course. To date there is not convincing prospective RCT demonstrating a benefit of depleting antibodies over high-dose steroids for the treatment of AMR. Even with access to high-dose steroids and ATG in the 1990s acute rejection had a significant impact on outcome with US registry date of over 63,000 transplants showing that AR conferred a relative risk for graft loss of 5.2.

In the setting of acute antibody mediated rejection plasma exchange and IVIg are both commonly used. It may seem counterintuitive (and expensive) to administer both IVIg and plasma exchange which removes Ig but it is important to note that lowering plasma IgG (either via plasma exchange or IgG endopeptidase (Imlifidase)) results in increased production of antibody so any ben-

efit of plasma exchange alone is short-lived. IvIg suppressed the upregulated production, alternatively depleting remove B lymphocytes and further production, while plasma exchange removed preexisting donor-specific antibodies that would persist despite depleting antibodies or increased IS. It is important to note that plasma exchange can be associated with post-biopsy bleeding if clotting not supported and IVIg can result in arterial thrombosis if given in high concentrations. An important and noble attempt was made to address the potential for CD20 depletion in the setting of acute antibody-mediated rejection in the RITUX ERAH study. This French multicenter double-blind placebo-controlled phase III study compared steroids, plasma exchange and IVIg against this treatment plus rituximab for acute AMR. There were 19 patients in each group and there was no benefit at one year with the addition of rituximab [26].

Other agents such as Bortezomib Eculizumab, anti-IL-6 and IgG endopeptidase have all been promoted as treatments for acute rejection but to date none have been proven in prospective RCTs.

Our practice is to treat all but the most severe grades of acute rejection with three pulses of methylprednisolone initially, along with an increase in MPA and Tacrolimus dose. If there is a high level of DSA or evidence of AMR then the steroids are often combined with plasma exchange. In the setting of AMR or failure of TCMR to respond on repeat biopsy we have a low threshold for a treatment course of ATG. ATG is withheld if total lymphocyte count is ≤0.1 or given at half-dose if 0.2 but with an intention to treat for 7–10 days. Response to treatment is followed closely with renal biopsy unless rapid improvement. All patients return to PCP prophylaxis following treatment of acute rejection for a minimum of six months (or until CD4 count ≥200), enhanced BKV monitoring (PCR fortnightly) and CMV PCR monitoring (minimum of weekly if not receiving prophylaxis).

92.5.2 Chronic Antibody-Mediated Rejection

While steroids, depleting antibodies and ivIg/plasma exchange are commonly used and perceived to have some efficacy in the setting of acute rejection the therapeutic options for chronic antibody-mediated rejection are largely nonexistent. A thoughtful and comprehensive consensus document on the treatment of chronic (and acute) AMR, in 2019 summed the current situation thus:

> Currently, there are no approved therapies and treatment guidelines are based on low-level evidence. The number of prospective randomized trials for the treatment of AMR is small, and the lack of an accepted common standard for care has been an impediment to the development of new therapies". Furthermore, "there was no conclusive evidence to support any specific therapy. As a result, the treatment recommendations are largely based on expert opinion [27].

This reflects rather badly on the transplant community in terms of decent RCTs although there have been several noble attempts to address treatment options in a scientific manner. It is clear that this is a critically important issue. The RituxiCAN-C4 Trial (see ClinicalTrials.gov for study design) aimed to evaluate the effectiveness of anti-CD20 therapy in those with biopsy proven C4d+ CAMR. An interim analysis of the data revealed it to be underpowered for measurement of the primary outcome and as such recruitment to the trial was halted. Exploratory analyses revealed that optimization of immunosuppression with tacrolimus, MMF, and prednisolone resulted in favourable outcomes, although ***not*** with use of rituximab [28]. The OuTSMART Trial (► trialsjournal.biomedcentral.com) has been designed to test whether a routine screening programme for HLA antibody in all kidney transplant recipients is useful by comparing blinding versus unblinding of HLA antibody status. Additionally, it is designed to test whether those found to be HLA antibody positive experience a reduction in graft failure rates with the introduction of a standard optimization treatment protocol. The trial data is yet to be reported.

Graft survival of patients with and without DSA has been reported at 63% and 83% ($P = 0.0001$), respectively, and the hazard ratio for graft loss after developing de novo DSA =7.7 resulting in a 10-year graft survival of 27% versus 80%. Transplant glomerulopathy (TG) has a particularly poor prognosis; a recent meta-analysis of studies comprising 6783 patients gave a medium graft survival of 3.11 years following the diagnosis of TG, 15 years less than those patients without (18.82 years) [29]. In short, trying to combat a memory response to highly expressed HLA is very difficult and currently there is no effective treatment. The trick is prevention, that is, avoiding pre-sensitization and posttransplant sensitization. A unit that achieves low sensitization rates will have significantly better outcomes for their patients.

92.6 Summary

Despite advances in immunosuppression and immune-histocompatibility graft rejection remains a significant clinical issue, both for those who are highly sensitized and for the significant number of patients who lose transplants prematurely from CAMR. There remain a few absolutes: the quest for a clinically relevant alternative test to a diagnostic renal biopsy remains a quest; good biopsies with experienced interpretation remain essential for patient management. Prevention is better than cure for all forms of rejection but most particularly those forms with little effective treatment. Finally, it is likely that inadequate immunosuppression is the principle cause of late acute rejection and CAMR so identifying and tackling causes of non-adherence or inadequate immunosuppression should be a major focus of clinical care.

Tips and Tricks

Take a sample for electron microscopy when performing a biopsy on an established renal transplant; it will help to establish a diagnosis of recurrent GN or a definitive diagnosis of CAMR.

Establishing sampling for DSA at the time a renal transplant biopsy performed is very helpful to ensure the diagnosis of AMR or CAMR can be advanced if suggested by the biopsy.

High tacrolimus trough level variance (and unrecordable levels), as well as missed clinics offer important and stark warnings of impending CAMR or late T-cell mediated rejection and it is important to try and work with the patient to alter behaviour to prevent this outcome. While changing behaviour is often difficult it is likely to be more impactful and less dangerous than trying to treat transplant glomerulopathy or severe late TCMR.

Case Study

A 43-year-old man with ESRD secondary to IgA nephropathy and a stable renal transplant nine years earlier presented having not attended clinic for a year. His creatinine had gone from a baseline of 105 to 400 (see Fig. 92.8) with an undetectable tacrolimus level and urine PCR of 420. He admitted that he had missed his IS for the preceding two weeks. He received pulsed methylprednisolone on the basis of a biopsy that demonstrated an intense cellular infiltrate with tubulitis, interstitial oedema, acute tubular injury, and moderate chronic damage (IFTA 55%), but no obvious signs of AMR and C4d were negative. Tacrolimus and MPA doses were increased. However there was a new DSA to a class II antigen with an MFI of 18 000. There was some initial improvement in function followed by a drift upwards. Plasma exchange was initiated and a discussion was had about repeating three pulses of methylprednisolone or the use of ATG. Given the DSA and in the context of another biopsy demonstrating on-going cellular rejection (but 65% IFTA) he was treated with a 10-day course of ATG.

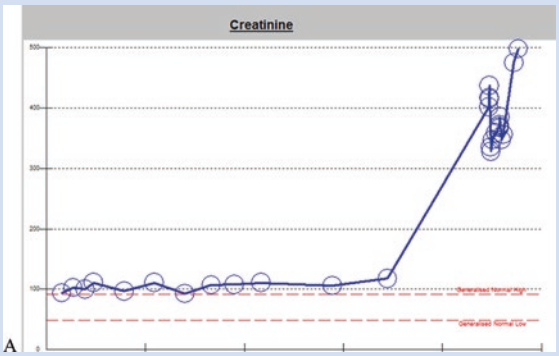

Fig. 92.8 Creatinine time plot

There was no further improvement in transplant function and he required dialysis within three weeks. On dialysis he developed high-level CMV viremia and was admitted with colitis and pneumonitis followed by two episodes of shingles.

Late rejection invariably results from inappropriately reduced immunosuppression and probably due to delayed diagnosis has a poor outcome. Making an assessment of whether treatment will be successful or not and whether to escalate further or stop is very tricky but it was clear that this patient had missed two previous clinic appointments and it is likely that he had missed IS for longer than two weeks. He had high level DSA, significant proteinuria and IFTA, all giving a poor medium-term prognosis for the transplant. What is clear is that a marked escalation of IS caries a significant risk of infectious complications and is the commonest cause of death on returning to dialysis, furthermore, ATG is not a treatment for non-compliance.

A 67-year-old woman with ESKD secondary to diabetic nephropathy received a DBD kidney transplant. She

had been previously sensitized due to pregnancy with a historic DQ class II DSA which was not detectable at the time of transplantation. Flow and CDC crossmatch were both negative prior to transplantation. She received IL-2 induction therapy followed by standard triple immunotherapy with Tacrolimus, MMF, and corticosteroids on a tapering regimen. She achieved immediate graft function and was discharged on day 6 with a serum creatinine of close to 100umol/L. She suffered superficial wound infection and received a prolonged course of antibiotics. Her renal function remained stable on serial monitoring for a number of weeks with consistently therapeutic Tacrolimus levels.

At four weeks post-transplant, she was noted to have a slightly elevated creatinine from her baseline measuring 120umol/L and a rise in her CRP from <5 to 25. Urine dip was positive for leucocytes and protein. Pending urine culture and quantification of urinary protein excretion she underwent ureteric stent removal following concerns regarding early urinary tract infection. Her serum creatinine continued to increase 30–50 points on each serial measurement thereafter. Urine PCR was recorded at 220 and culture was negative for organisms. She was referred for an USS of her transplant kidney following concerns regarding ureteric outflow obstruction following the stent removal. Her USS showed no evidence of hydronephrosis and no extension of her wound infection.

However, concurrent measurement of class I and class II DSA revealed a recurrence of her class II DQ antibody with an MFI >16000 and well as de novo class I (B) and class II (DP) DSA. Urgent renal transplant biopsy revealed C4d+ and peritubular capillaritis consistent with AMR. She received three pulses of high-dose methylprednisolone, followed by up-titration in the dose of her oral corticosteroids as well as plasma exchange and iv Ig. She recovered her function to baseline with largely undetectable DSA thereafter.

In her case, acute rejection resulted from a memory response to class II HLA antigen the detection of de novo DSA likely resulting from recognition of shared antigenic epitopes. The question here is whether she would have benefitted from induction with a lymphocyte depleting agent such as ATG to prevent the aforementioned. This can often be a difficult decision to make. In her case, older age and underlying diabetes already predispose her to increased risk of infection related to immunosuppression. Furthermore, she suffered complications related to wound infection and as such lymphocyte depleting agents were also avoided as part of her treatment of AMR. Augmentation of immunosuppression in any case was necessary to treat rejection. Some may argue that the long-term burden of high-dose steroids, plasma exchange and IVIg is less than that imposed by lymphocyte depleting agents. Others would disagree and as we have discussed the evidence base for existing approaches is weak. The debate goes on but clearly highlights the need for individualized approaches to immunotherapy in transplantation.

Chapter Review Questions

1. What are the features of chronic antibody-mediated rejection?
2. What are the features of acute antibody rejection?
3. What are the risk factors for late rejection and chronic antibody-mediated rejection?
4. What are the risk factors for acute antibody-mediated rejection?
5. What treatment strategies are employed to treat chronic antibody-mediated rejection?

Answers

1. Thickening of the peritubular capillary and glomerular basement membranes—manifesting as double contouring on light microscopy and the pathognomonic multilayering and double contouring seen on electron microscopy. As such, examination of renal tissue by electron microscopy is critical to diagnosis. Endovascular neo-intimal thickening of renal vasculature and subsequent interstitial fibrosis and tubular atrophy.
2. Intimal or transmural arteritis. Inflammatory cell infiltration into peritubular capillaries—so-called peritubular capillaritis. Mononuclear cell infiltration within glomerular capillaries and endothelial cell enlargement—so-called glomerulitis. Positive C4d staining identified either by immunoperoxidase or immunofluorescence within peritubular and glomerular capillaries. It should be noted that the Banff Classification of transplant rejection also recognizes C4d negative AMR as a pathologic entity. In severe cases thrombotic microangiopathy (TMA) and interstitial haemorrhage may be evident.
3. The number and severity of acute rejection episodes. Sub-therapeutic immunosuppression as a result of non-adherence to immunotherapies is the leading cause. High variability in Tacrolimus trough levels suggestive of long-standing inter-

mittent non-adherence as opposed to total cessation of immunotherapy is most common in clinical practice. Immunosuppression reduction in the face of viral infection such as BK viral nephropathy or CMV infection is also contributory in this context.

4. Number of donor/recipient HLA mismatches as well as the specific antigens that are mismatched (DR > B > A). Sensitization and subsequent immunologic memory to previously encountered HLA are widely accepted to increase risk. Both donor-specific- and non-donor-specific HLA antibody confer a higher risk of rejection. The use of non-depleting anti-lymphocyte induction agents have been associated with a higher incidence of rejection at one year in high immunological risk groups.
5. Optimization of oral immunosuppression is often trialled but with little or no effect on graft survival. Most, if not all, would balance the risk of augmented immunotherapy against the risk of infection and/or malignancy while considering the degree of currently irreversible vasculopathy and parenchymal fibrosis. Control of secondary risk factors such as BP and urinary protein excretion is aimed at prolonging the time taken to reach end-stage disease. Some exciting preclinical data is emerging that focuses on inhibition of the pro-fibrotic pathways that contribute to the development of this pathology but are a long way from implementation into clinical practice. Importantly no treatment has been shown to be effective and as such prevention rather than cure remains the focus for specialists in this field.

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