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REVIEW ARTICLE

Georg Böhmig
Heinz Regele

Diagnosis and treatment of antibody-mediated kidney allograft rejection

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G. Böhmig
Department of Internal Medicine III,
Division of Nephrology and Dialysis,
University of Vienna, Vienna, Austria

H. Regele (✉)
Clinical Institute of Pathology,
University of Vienna, AKH,
Währinger Gürtel 18–20,
1090, Vienna, Austria
E-mail: heinz.regele@akh-wien.ac.at
Tel.: +43-1-404005176
Fax: +43-1-404005193

Abstract Evidence of a significant pathogenetic role of donor-reactive antibodies (DRA) in kidney allograft rejection is accumulating. At least, partially owing to the recent discovery of the complement split product C4d as a valuable rejection marker, antibody-mediated rejection (AMR) has regained increasing attention. We review here the value of various diagnostic criteria, including immunohistochemistry (C4d staining), histomorphology and posttransplant serology, for the diagnosis of AMR. Furthermore, the mechanisms underlying alloantibody/complement-mediated allograft injury are discussed in detail. Finally, a thorough discussion of recently proposed "anti-humoral" therapeutic strategies is provided.

Keywords Antibody-mediated rejection · Apheresis · C4d · Immunoadsorption · Kidney · Transplantation

Abbreviations *AHR* Acute humoral rejection · *AMR* Antibody-mediated rejection · *DARC* Duffy antigen/receptor for chemokines · *DRA* Donor-reactive antibodies · *FCXM* Posttransplant flow cytometry · *IA* Immunoadsorption · *Ig* Immunoglobulin · *iTCC* Inactive terminal complement complex · *IVIG* Intravenous immunoglobulin · *mAb* Monoclonal antibodies · *MAC* Membrane attack complex · *PTC* Peritubular capillaries · *PP* Plasmapheresis · *vWF* von Willebrand factor ·

Introduction

In kidney transplantation, potential deleterious effects of donor-reactive alloantibodies (DRA) have been identified as early as the 1960 s, when it became obvious that preformed antibodies directed against polymorphic (primarily HLA) antigens can mediate severe and often irreversible graft damage [1, 2]. Moreover, a critical role of antibody-dependent immunity as mediator of graft rejection is well established for AB0-incompatible transplantation and xenotransplantation [3, 4]. Nevertheless, in contrast to the enormous scientific, diagnostic and therapeutic efforts devoted to cellular (T-cell-mediated) alloimmunity, for more than two decades, comparatively little attention has been paid to antibody-mediated rejection (AMR) [5, 6, 7, 8, 9, 10]. Data are accumulating that humoral immune mechanisms may not only cause hyperacute rejection, a rejection type now virtually eliminated by routine pretransplant crossmatch testing, but may also mediate other types of rejection, such as acute and chronic rejection [5, 6, 7, 8, 9, 10]. We would like to emphasize that already in 1970, Jeannet and co-workers [11] suggested a pathogenetic role of posttransplant DRA in acute kidney allograft rejection. In earlier reports, the diagnosis of AMR was mainly based on posttransplant serology (testing for circulating alloantibodies) and/or detection of particular histomorphologic lesions putatively pathognomonic for

DRA-positive rejection [11, 12, 13, 14, 15, 16]. Diffuse capillary deposition of immunoglobulin (Ig) turned out to be a rare finding in rejecting allografts, possibly as a result of rapid shedding of bound antibodies from the endothelial surface [5]. Recently, a decisive improvement in the diagnosis of AMR was enabled by Feucht's discovery of a novel rejection marker, i.e. C4d [17, 18]. This complement split product is stably bound to capillary walls, presumably as a result of classical pathway complement activation, and can easily be detected immunohistochemically. Numerous subsequent reports have confirmed the high diagnostic value of C4d as a marker of AMR in kidney transplantation. Very recently, a standardization of diagnostic criteria for AMR, including capillary C4d staining, was proposed in an addition to the Banff 97 Classification of Renal Allograft Rejection [19].

The complement split product C4d, a specific marker of acute AMR

Diffuse C4d deposition along peritubular capillaries (PTC), a major surface of contact between the recipient's immune system and the transplanted organ, may represent a valuable marker for alloantibody-mediated classical complement activation and, thus, AMR. Feucht and co-workers [17, 18] were first to demonstrate peritubular capillary C4d deposits in a subset of kidney allografts. Furthermore, the authors demonstrated a remarkable association between C4d deposition in PTC and poor allograft survival [18]. Even though serologic testing for posttransplant DRA was not performed in this analysis, the association of recipient pre-sensitization with positive C4d staining suggested a pathogenetic role of humoral immunity in C4d-positive recipients [18]. Subsequent studies confirmed the presence of capillary C4d deposits in a substantial proportion of kidney allograft biopsies [20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32]. In addition, many of these reports confirmed the previously reported relationship between capillary C4d staining and recipient pre-sensitization. In 1999, Collins et al [21] were first to demonstrate a strong correlation between C4d deposits in cortical peritubular capillaries and the detection of de novo anti-HLA DRA in recipient sera. This observation was critical in triggering a renewed interest in the field of humoral allograft rejection [10]. In subsequent studies, associations between C4d staining and posttransplant serology were investigated in larger patient cohorts [22, 27, 29] (Table 1). These studies revealed circulating alloantibodies (posttransplant crossmatching with donor cells or panel reactive antibody (PRA) testing) in the majority (78–90%) of C4d-positive cases, suggesting a high specificity of C4d for the presence of DRA [22, 27, 29]. Data on the sensitivity of C4d staining, however, are controversial. Using

Table 1 Prevalence and clinical impact of C4d staining in PTC. *BX* biopsy, *Clin* BX performed due to renal dysfunction, *Cr* serum creatinine, *DRA* donor-reactive antibodies, *f* C4d staining on frozen sections (monoclonal antibody), *nr* not reported, *p* staining with C4dpAb on paraffin sections, *Pat* patient, *Prot* protocol biopsy, *Rej* BX with morphologic evidence of (cellular) rejection, *TX* transplantation

Author, year	No of BX/Pat	Timing of BX: months post TX (median)	Indication for BX (staining)	C4d + %BX/ %Pat	Post-Tx DRA %BX/%Pat	DRA + BX %C4d +	Graft loss %C4d + /%C4d -(months post-TX)	Cr (mg/dl) C4d + /C4d- (months post TX)
Feucht, 1993	93/93	first 4 weeks	Clin (f)	46 (9[a])/ 46 (9[a])	nr	nr	43 (37[a])/10 (12)	2.3(1.6[a])/1.9 (12[b])
Lederer, 2001	310/218	nr	Clin (f)	nr/46[c](72[d])	38/38[e]	78[e]	50(16)/25(38)[f]	nr
Regele, 2001	102/61	0.1–18 months (0.5)	Clin (p)	41/51	nr	nr	13/0 (18)	2/1.5 (18)
Böhmig, 2002	113/58	0.1–23 months (0.6)	Clin (p)	21/28	60/60	88	25/2 (< 24)	2.8/1.6 (12)
Nickeleit, 2002	398/265	0.1–239 months (4.4)	Clin (f)	30/35	nr	nr	14/10 (12[g])	1.8/1.7 (12[g])
Herzenberg, 2002	126/93	nr	Rej (f)	nr/37	nr	nr	29/5 (12)	1.5/1.9 (> 24[b])
Mauiyyedi, 2002	67/67	first 3 months	Clin (f)	30/30	28/28	90	30/4 (12)	1.6/1.3 (12)
Regele, 2002	213/213	12–211 months (59)	Clin (p)	34/34	nr	nr	nr	nr
Sund, 2003	37/37	5–11 days (0.25)	Prot (f)	30/30	nr	nr	20/8 (12)	1.7/1.9 (12)
Mengel, 2003	467/nr278/nr	> 6 weeks > 6 weeks	Prot (p)Clin (p)	18/nr[h]45/nr[h]	nrnr	nrnr	nrnr	nrnr

[a]Evaluation of 8 patients with focal C4d staining
[b]Creatinine levels evaluated for functioning grafts
[c]C4d deposition in first grafts
[d]C4d deposition in regrafts
[e]48 patients analysed
[f]Calculated for biopsies < 6 months post-TX
[g]12 months after BX
[h]Definition of C4d positivity included trace amounts of C4d deposits

posttransplant flow cytometry (FCXM) or lymphocytotoxic crossmatch testing, Mauiyyedi et al [29] calculated a 95% sensitivity of C4d staining. In contrast, Böhmig et al [27] described positive FCXM and FlowPRA test results also in a considerable number of C4d-negative patients. Accordingly, compared to posttransplant FCXM, a low sensitivity was calculated for peritubular C4d staining (31%). This finding may be explained by the particularly high sensitivity of serologic tests employed in this analysis. Indeed, in the study by Böhmig et al [27], DRA were detectable in as many as 60% of patients studied, compared to only 28% DRA-positive patients reported by Mauiyyedi et al [29]. The reported high specificity of capillary C4d staining stresses its value as a marker for AMR. Nevertheless, the occasional finding of capillary C4d deposition in DRA-negative patients raises the probability of antibody-independent C4d deposition. Indeed, in a study evaluating cardiac allograft biopsies, Baldwin et al [33] suggested C4d deposition as a result of ischemic injury. This may be in accordance with experimental studies demonstrating ischemia/reperfusion-induced classical complement activation [34]. For kidney transplants, however, there are no indications for ischemia/reperfusion-induced C4d deposition. Most significantly, Haas et al [32], who investigated 47 1-h postreperfusion allograft biopsies, found capillary C4d deposits in only two patients. Both recipients subsequently developed AMR [32]. In addition, recent studies did not reveal associations of capillary C4d staining with acute tubular necrosis (ATN) [23, 30] or cold ischemia time [23, 28].

C4d staining properties in renal allograft biopsies

To date, various anti-C4d antibodies are commercially available. Numerous studies have been performed with monoclonal antibodies (mAb) supplied by Quidel (San Diego, Calif., USA) [28, 30, 32, 35, 36] or Biogenesis (Sandown, N.H., USA) [21, 25, 26, 29]. These, and the mAb produced by Feucht and colleagues [17, 18, 37, 38, 39] have in common that they can only be used on frozen, but not on paraffin sections. A novel polyclonal anti C4d antibody termed C4dpAb (Biomedica, Vienna, Austria; http://www.biomedica.co.at) was reported to be applicable to frozen sections as well as to formalin fixed paraffin embedded tissue [23, 24, 27, 31, 40, 41, 42]. All above mentioned reagents nearly identically reveal the diagnostically relevant linear staining pattern along the walls of peritubular capillaries (Fig. 1). This linear staining pattern is exclusively found in a subset of allograft biopsies. Since staining signals in other locations (glomeruli, arteries, veins) may also be observed in native kidneys and even in extrarenal tissues, they do not seem be of diagnostic relevance for AMR [9]. Immunofluorescence and immunoperoxidase can equally be employed for detection of bound anti-C4d antibodies. In addition, C4dpAb has also been used for immune electron microscopy [31]. So far, no systematic comparative analysis of staining sensitivity of frozen versus paraffin sections has been published. In a small series of 12 biopsies stained in parallel on frozen and on paraffin sections, in all five biopsies with positive staining on frozen sections, C4d deposits were also detectable on

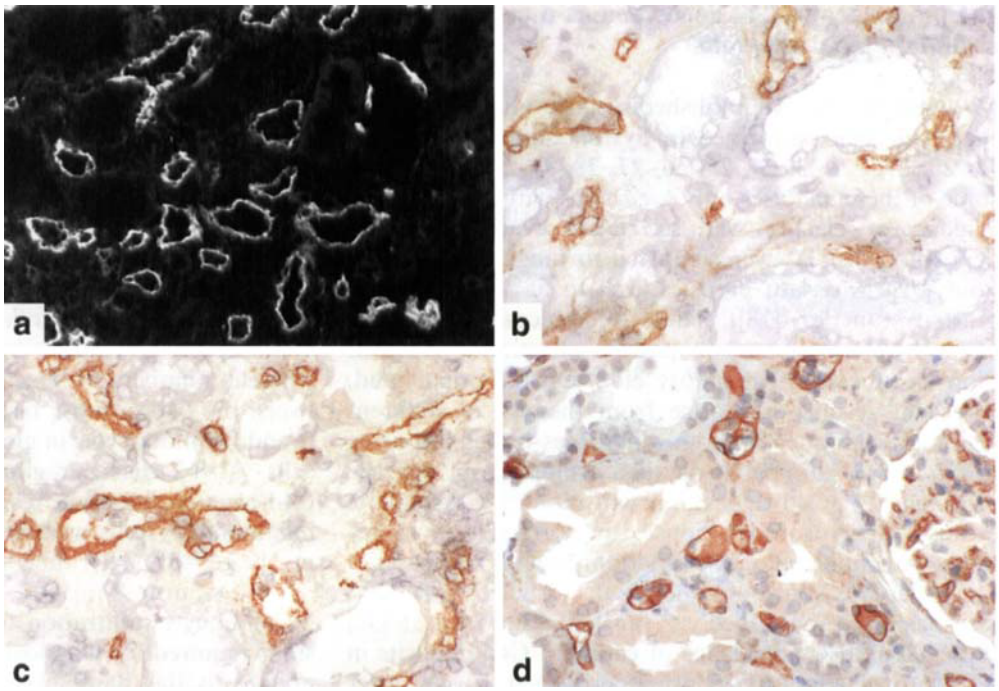

Fig. 1 Linear C4d staining in PTC on frozen- (A-C) and paraffin sections (D) of an allograft biopsy. Immunofluorescence (A) and immunoperoxidase (B) stain employing the monoclonal anti-C4d antibody (Quidel). Immunoperoxidase stain on a frozen section (C) and on a paraffin section (D) with the polyclonal anti-C4d antibody (Biomedica). All stainings have been performed on sections from the same allograft biopsy of a patient with severe acute humoral rejection [42]

paraffin sections [23]. A similar result was reported by Smith and co-workers at the Seventh Banff Conference on Allograft Pathology (Aberdeen, Scotland, 2003) who could demonstrate positive C4d staining both in the frozen and the paraffin sections of 11 of 34 cardiac biopsies. Immunofluorescent double staining for C4d employing CD31 and *Ulex europaeus lectin* as endothelial cell (EC) markers and Collagen type IV for identification of basement membranes revealed C4d being predominantly bound to EC with only focal adherence to basement membranes of PTC [21, 26, 31]. Staining signals are invariably present in the mesangium and along basement membranes of glomeruli on frozen sections, but, interestingly, they cannot be observed in these locations on paraffin sections [23]. These different staining properties might result from an alteration of C4d, bound to extracellular matrix during fixation and processing of tissue. Hence, using C4dpAb on paraffin sections, it is not possible to use the glomerular staining as "internal" positive control. However, on paraffin sections, endothelial C4d deposits are readily detectable, whereas in frozen sections such deposits might be hidden by prominent constitutive glomerular staining. Glomerular endothelial C4d staining on paraffin sections was demonstrated to be always associated with C4d deposition in PTC and can be observed in 18 to 36% of C4d-positive biopsies [23, 31]. Remarkably, glomerular endothelial staining was described to be associated with glomerulitis [23]. An independent diagnostic or prognostic significance of glomerular endothelial C4d deposits, however, could not be established so far.

The prevalence of C4d deposition as a marker of AMR in renal allografts

Most studies on C4d published so far evaluated biopsies that had been performed in dysfunctioning grafts [17, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32]. In some of these analyses only specimens with morphologic evidence of rejection were selected. Prevalences of C4d deposition in PTC were reported to range from 4% (1-hour posttransplant biopsies [32]) to 55% (acute early graft dysfunction [18]). These differences may in part result from differences in patient selection (timing of biopsy, indication for biopsy etc.). On the other hand, divergent results may arise from the use of different staining techniques or from differences in definitions of positive C4d staining. A detailed evaluation of the true prevalence of C4d deposition will require a systematic evaluation of protocol biopsies performed also in patients with stable allograft function. Several such studies are under way, but only one regular article on the subject has been published so far [35]. Sund et al [35] reported a 30% prevalence of capillary C4d deposits in 37 protocol allograft biopsies performed 1 week after transplantation. Only 2 of 11 C4d-positive patients had stable renal function at the time of biopsy, and in one of them acute rejection occurred 11 days after protocol biopsy. C4d deposits were not found in 13 patients without clinical or morphologic evidence of rejection. In a much larger multi-center study reported by Mengel and co-workers at the Seventh Banff Conference on Allograft Pathology, only 16/467 (3.4%) protocol biopsies (performed > 6 weeks post TX) exhibited C4d staining in > 25% of PTC. The prevalence rose to 18% if even trace amounts of C4d were considered. In the same study, the prevalence for > 25% C4d staining was approximately 20% in 278 clinical biopsies (performed for renal dysfunction) and reached 45% if cases with minimal C4d staining were also included. Both studies suggest a low incidence of C4d deposition in grafts with stable function, but analysis of clinical follow-up data is required to reveal the significance of C4d in well-functioning allografts.

Histomorphologic features of AMR

Analyzing rejecting kidney allograft specimens obtained from recipients developing posttransplant DRA (HLA-class-I antibodies) Halloran et al [13, 14] and later Trpkov et al [15] could demonstrate the frequent finding of severe vasculitis, capillary fibrin thrombi and accumulation of granulocytes in PTC and glomeruli. These histomorphologic lesions, however, were not invariably present in antibody-positive rejection and could also be found in some DRA-negative cases, suggesting that a histomorphologic work-up alone may not be sufficient for a reliable diagnosis of AMR. Nevertheless, some authors reported frequent findings of distinct morphologic lesions in C4d-positive biopsies, including capillary margination of inflammatory cells [21, 24, 25, 28, 29, 36]. Very recently, Magil et al [36] analyzed the numbers of monocytes (CD68+) and of granulocytes in various compartments of 23 C4d-positive and 28 C4d-negative biopsies with evidence of acute cellular rejection. All biopsies had been performed within 6 months after transplantation. The numbers of interstitial and glomerular mononuclear inflammatory cells and granulocytes were significantly higher in C4d-positive grafts, whereas numbers of T-cells ($CD3^+$) did not differ between the two groups. The most marked difference was found for monocytes in glomeruli with an average of 3.4 cells vs. 0.2 cells per glomerulus in C4d-positive and C4d-negative biopsies, respectively. Whether interstitial inflammatory cells were also located within PTC could not be reliably determined in this study. Based on these data, the authors proposed the addition of glomerulitis by monocyte infiltration to the list of morphologic lesions required for diagnosis of AMR by the most recent update to the Banff classification (see below) [19]. A

previous study by Regele et al [31] investigating allograft biopsies performed >12 months post TX, found a strong association of C4d deposits with an accumulation of mononuclear cells in PTC and also with glomerulitis. These data suggest that the presence of mononuclear cells in PTC may indicate a more subacute or chronic form of AMR.

Immunotyping of cells was not performed in this study. Even in studies that did not reveal a significant accumulation of granulocytes in PTC, glomerulitis was found to be a prominent feature of AMR [23, 30, 31]. In some cases, however, acute tubular damage was described to be the only morphologically detectable injury [8, 23, 35, 43]. Recently, a case of severe C4d-positive graft dysfunction in the complete absence of histomorphologic signs of rejection was reported [41]. Simultaneous occurrence of capillary C4d deposits with signs of cellular rejection, however, is not uncommon, and Nickeleit et al [30] even observed a significant association of C4d with intimal arteriitis and features of interstitial rejection including enhanced MHC-class II expression in tubular epithelial cells. In another analysis, however, no association of peritubular C4d deposits with signs of cellular rejection could be demonstrated [23].

Humoral alloreactivity and chronic rejection

Two recent studies analyzing C4d deposition in late allograft biopsies suggest that humoral immune mechanisms might also be relevant in chronic rejection [26, 31]. Regele et al [31] detected C4d deposits in 34% of late allograft biopsies and found a strong association of C4d deposition with transplant glomerulopathy, reduplication of basement membranes of PTC and the above mentioned accumulation of mononuclear cells in PTC. Previously, Mauiyeddi et al [26] reported C4d deposits in 61% of cases with histomorphological evidence of chronic rejection (i.e. transplant glomerulopathy or arteriopathy), which is quite similar to C4d deposits in 53% of biopsies with transplant glomerulopathy observed in the study by Regele and co-workers. Transplant glomerulopathy and reduplication of peritubular capillary basement membranes are commonly regarded as signs of chronic allograft rejection [44, 45, 46, 47, 48]. These lesions, being characterize d by similar patterns of basement membrane thickening and reduplication, tend to occur simultaneously [44, 45, 46, 47, 49] and are believed to result from endothelial injury [45, 46, 47]. Putatively, antibody-mediated complement activation is likely to play a major role in endothelial injury. Complement-induced injury therefore might represent the common pathogenic mechanism underlying basement membrane changes in glomeruli and PTC, thereby explaining the frequently observed simultaneous occurrence of these lesions. Given the close relation of endothelial C4d deposition with circulating alloantibodies reported for acute AMR, it is very likely that endothelial-bound alloantibodies (inducing transplant glomerulopathy and reduplication of peritubular capillary basement membranes) are a powerful stimulant also in chronic rejection. The assumption of chronically active AMR is further supported by numerous serologic studies reporting an association of posttransplant DRA with chronic allograft dysfunction [50, 51, 52, 53, 54]. These studies, however, do not provide information on morphologic features of chronic humoral rejection [50, 51, 52, 53, 54].

Diagnostic criteria for acute AMR

Successful clinical management of humoral rejection may critically depend on standardized diagnostic criteria. Therefore, at the Sixth Banff Conference on Allograft Pathology in 2001, an effort was made to establish diagnostic criteria for acute AMR. Since serology, histomorphology or immunohistochemistry, if applied individually, might be insufficient for unequivocal identification of acute AMR (false-positive and/or false-negative results), it was felt that a combination of all three diagnostic procedures could compensate the perhaps insufficient sensitivity and specificity of any individual technique. The diagnostic criteria for AMR, which are now incorporated into the Banff classification [19], are summarized in Table 2. The methods for serologic alloantibody detection, however, are not specified in this proposal.

In previous studies, definitions of positive C4d staining were quite variable, with some investigators requiring diffuse (present in all high power fields) staining of PTC [9, 21, 25] and others also accepting focally accentuated staining [23, 24, 27, 28, 30]. However, there is general agreement that only linear (not coarsely granular) and circumferential staining of PTC (not of arteries or veins) is diagnostically significant (Fig. 1). According to the Banff classification scale, C4d staining of more than 50% of PTC is required to be classified as positive. As already mentioned above, C4d staining may coincide with signs of acute cellular rejection indicating the simultaneous presence of cellular and humoral mechanisms of immunologic injury.

The role of complement as a mediator of graft injury in AMR

Deposition of C4d has turned out to be a specific marker of humoral allograft injury. The functional role of this complement split product on its own, however, has still to be established. Nevertheless, C4d deposition may be a

Table 2 Criteria for acute AMR in renal allografts—3 cardinal diagnostic features (addition to the Banff 97 classification of renal allograft rejection [19])

1. Morphologic evidence of acute tissue injury
   (a) Acute tubular injury
   (b) Neutrophils and/or mononuclear cells in peritubular capillaries and/or glomeruli, and/or capillary thrombosis; or
   (c) Intimal arteritis/fibrinoid necrosis/intramural or transmural inflammation in arteries
2. Immunopathologic evidence for antibody action
   (a) C4d and/or (rarely) immunoglobulin in peritubular capillaries
   (b) Immunoglobulin and complement in arterial fibrinoid necrosis
3. Serologic evidence of circulating antibodies to donor HLA or other anti-donor endothelial antigens

good indicator of classical complement activation at the surface of the capillary endothelium. There is now increasing evidence that alloantibody-triggered classical complement activation may critically contribute to tissue injury in rejecting allografts [55, 56]. One can speculate that the typical morphologic features described for AMR, such as margination of inflammatory cells (monocytes/macrophages, granulocytes) or the occurrence of capillary microthrombi are, at least in part, due to effects of complement activation products on the integrity and function of EC (Fig. 2). The membrane attack complex (MAC), a multimer formed by the terminal components C5b-C9 may thereby play a central role as a trigger of endothelial injury. Indeed, in a series of studies, defective MAC formation in C6-deficient rats was demonstrated to prevent hyperacute, acute or chronic rejection of solid organ allografts [57, 58, 59]. Furthermore, sublytic MAC concentrations or cytolytically inactive terminal complement complex (iTCC) were found to induce a variety of proinflammatory stimuli by EC activation, including enhanced expression of specific adhesion molecules, cytokines/chemokines or growth factors [60, 61, 62, 63].

Several reports indicate that complement activation on EC might play a role in inflammatory cell margination, a typical sign of humoral rejection [28, 29, 36, 42, 64]. Recently, Segerer and co-workers [42] proposed a contribution of the chemokine-binding protein, Duffy Antigen/Receptor for Chemokines (DARC), which was found to be expressed at high levels in PTC in C4d-positive renal allograft specimens, to leukocyte recruitment in AMR. DARC might bind and concentrate various chemokines and could therefore contribute to recruitment of inflammatory cells and tissue injury [42]. In a recent experimental study, induction of leukocyte adherence and transmigration across rat mesenteric postcapillary venules was demonstrated by topical application of iTCC [63]. Similar effects were observed after treatment with C5a, reinforcing a significant role of this anaphylatoxin in leukocyte recruitment [63]. In addition, cell transmigration experiments revealed that stimulation of EC with iTCC or C5a led to increased adhesiveness and diapedesis of granulocytes [63].

Activation of EC by alloantibody-dependent classical complement activation may also promote a procoagulant response resulting in the formation of capillary microthrombi. Thrombotic microangiopathy represents one of the morphologic signs of anti-HLA antibody- and/or C4d-positive renal allograft dysfunction [15, 24, 29]. In an experimental study, Wasowska et al [65] reported that passive transfer of complement-fixing alloantibodies in immunoglobulin knock-out mice restores rejection of cardiac allografts associated with extensive vascular platelet aggregates and von Willebrand factor (vWF) release. In-vitro studies suggest that induction of a procoagulant state could be mediated by a MAC-induced release of high molecular weight multimers of EC-derived vWF, exposure of factor Va binding sites on EC, or endothelial expression of tissue factor [66, 67]. A critical role of MAC in the induction of platelet thrombi is reinforced by the observation that in C6-deficient rats, delayed rejection is associated with decreased vascular platelet aggregation [57]. In a recent experimental study, Ren and co-workers [68] reported a rat model of anti-endothelial antibody-induced thrombotic microangiopathy showing features of thrombotic thrombocytopenic purpura. In this model, depleting animals of complement with cobra venom factor prevented thrombopenia, suggesting complement-dependency of experimental thrombotic microangiopathy [68]. Sumitran-Holgersson et al [69] reported an association of kidney allograft rejection with the occurrence of alloantibodies directed against the polymorphic, non-classical HLA class I molecule major histocompatibility complex I-related chain A (MICA). Interestingly, MICA antibodies were found to induce a prothrombotic phenotype in renal microvascular EC in-vitro. This phenotype was also induced by sera obtained from three kidney allograft recipients, who had lost their graft due to vascular thrombosis [69].

There is increasing evidence of a significant role of the complement system as a regulator of specific immune responses. Crucial interaction between complement and cellular immunity was suggested by experimental models of allotransplantation [70, 71, 72], demonstrating that T and B cell immunity critically depend on distinct complement proteins, such as C3 and C4. We can speculate that the commonly observed simultaneous occurrence of

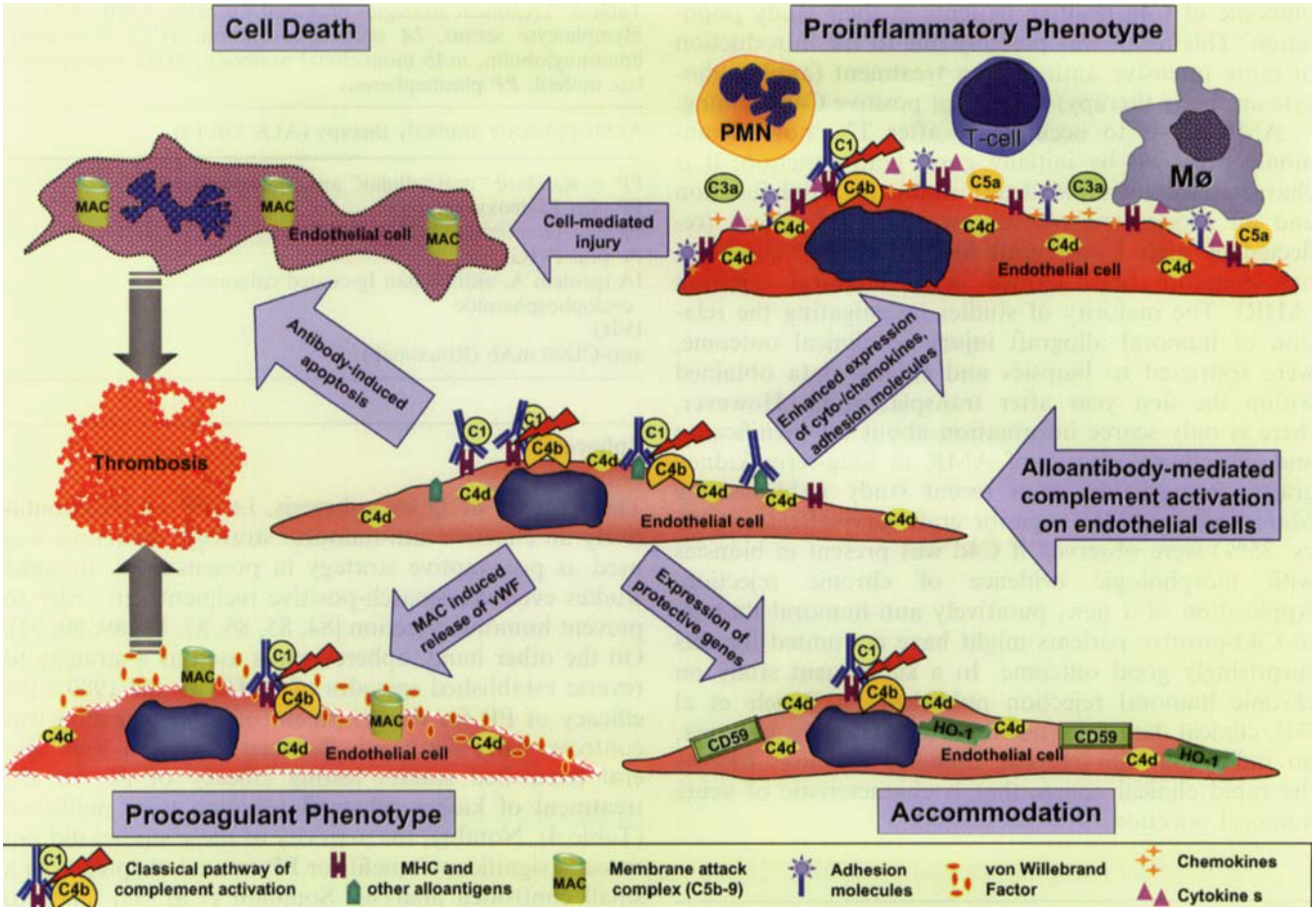

Fig. 2 Alloantibody-mediated complement activation may induce various phenotypic and functional alterations of endothelial cells. Antibody-dependent complement activation may cause loss of endothelial cells (most likely by apoptosis) or alternatively result in activation of endothelial cells, associated with enhanced expression of adhesion molecules, and release of mediators inducing recruitment of inflammatory cells. Enhanced release of von-Willebrand-Factor from activated endothelial cells or endothelial cell loss may trigger thrombosis. Protective mechanisms, however, may induce resistance to antibody-mediated endothelial cell injury resulting in so-called accommodation. Even though C4d per se, does not seem to play an active role in any of these processes, it represents a useful diagnostic marker of complement activation. *C1, C3a, C4b, C4d, C5a* components of the complement system, *CD59* complement regulatory protein, *HO-1* heme-oxygenase, *MAC* membrane attack complex (C5b-9), *Mø* monocyte/macrophage, *PMN* polymorphonuclear neutrophil granulocyte

C4d deposition with signs of cellular rejection in renal allografts might, at least in part, result from a subtle interaction between humoral and cellular immunity.

The clinical course of AMR

Numerous studies indicate an unfavourable influence of antibody-mediated alloimmunity on kidney allograft outcome. Recipient pre-sensitization uncovered by PRA- and crossmatch testing is well known to be associated with inferior kidney graft survival [40, 73, 74, 75, 76] and, in numerous reports, the occurrence of alloantibodies during the posttransplant period was shown to predict poor graft outcome [11, 12, 14, 22, 50, 51, 77, 78, 79, 80, 81, 82]. In recent studies, capillary C4d deposition as presumed marker of AMR, was demonstrated to be associated with inferior renal function and graft survival (Table 1). Feucht et al [18] observed a 1-year graft survival rate of only 57% in patients with diffuse and 63% in patients with focal C4d deposits, as opposed to 90% in C4d-negative recipients. In a subsequent publication, the same group calculated a mean graft survival of only 4 years for C4d-positive recipients vs. 8 years for grafts without C4d deposition [22]. Similarly, other studies confirmed an association of C4d deposition with inferior graft outcome [25, 27, 28, 29]. The high rate of steroid- and antibody-resistant rejection episodes in patients with evidence of AMR [14, 23, 25, 27] may suggest limited anti-humoral efficiency of drugs primarily directed at cellular immune mechanisms and further stresses the need for a specific anti-humoral therapy. In contrast to the above mentioned studies, Nickeleit et al [30] did not observe a significantly inferior

outcome of C4d-positive patients in their study population. This result was possibly due to the introduction of more intensive antirejection treatment (antilymphocyte antibody therapy) in cases of positive C4d staining.

AMR tends to occur early after TX, not uncommonly preceded by initially good graft function, it is characterized by rapid deterioration of renal function and an accelerated course towards renal failure (reflected by high 1-year graft loss rates). The condition has therefore been termed acute humoral rejection (AHR). The majority of studies investigating the relation of humoral allograft injury to clinical outcome, were restricted to biopsies and clinical data obtained within the first year after transplantation. However, there is only scarce information about the significance and the clinical course of AMR in long-term kidney grafts. Remarkably, in a recent study published by Mauiyyedi et al [26], superior graft survival rates (62% vs. 25%) were observed if C4d was present in biopsies with morphologic evidence of chronic rejection. Application of a new, putatively anti-humoral therapy to C4d-positive patients might have accounted for this surprisingly good outcome. In a subsequent study on chronic humoral rejection published by Regele et al [31], clinical data were not reported. There is, however, no indication that chronic humoral rejection follows the rapid clinical course that is characteristic of acute humoral rejection.

Treatment of acute AMR

Mainly based on anecdotal reports or uncontrolled case series, various therapeutic strategies have been proposed for the treatment of acute AMR (Table 3). These "anti-humoral" strategies include apheresis, i.e. plasmapheresis (PP) and immunoadsorption (IA) therapy, intravenous immunoglobulin (IVIG), or the use of anti-CD20 mAb Rituximab. The true effectiveness of these modes of treatment has still to be established in controlled randomized trials. Due to the low incidence of acute AMR, these will have to be designed as multicenter studies. In addition to these above-mentioned therapeutic strategies, immunosuppressive agents with primarily "anti-cellular" (anti-T-cell) action were recently suggested to exert also anti-humoral activity. Pascual and co-workers [83] suggested anti-humoral effects of tacrolimus and/or MMF rescue therapy as an adjunct to PP. Furthermore, antilymphocyte antibody therapy was recently discussed to effectively reverse AMR [30]. Nevertheless, in a number of studies, many episodes of acute AMR were reported to be refractory to antilymphocyte agents, suggesting the necessity of more effective and favourably more selective anti-humoral treatment modalities.

Table 3 Treatment strategies proposed for acute AMR. *ALS* antilymphocyte serum, *IA* immunoadsorption, *IVIG* intravenous immunoglobulin, *mAb* monoclonal antibody, *MMF* mycophenolate mofetil, *PP* plasmapheresis

Antilymphocyte antibody therapy (ALS, OKT3)
PP ± standard "anti-cellular" antirejection therapy
PP plus 15-deoxyspergualin
PP plus tacrolimus/MMF
PP plus IVIG
IA (protein A, anti-human Ig-coated columns) ± cyclophosphamide
IVIG
anti-CD20 mAb (Rituximab)

Apheresis

The removal of Ig by apheresis, i.e. PP or IA is putatively an effective anti-humoral strategy. Apheresis was used as pre-emptive strategy in presensitized, in some studies even crossmatch-positive recipients, in order to prevent humoral rejection [84, 85, 86, 87, 88, 89, 90, 91]. On the other hand, apheresis was used as a strategy to reverse established episodes of AMR. In the 1980 s the efficacy of PP for the treatment of acute rejection was controversially discussed. Between 1981 and 1990 several controlled studies testing efficacy of PP for the treatment of kidney allograft rejection were published (Table 4). Notably, the majority of these studies did not reveal a significant benefit for PP-treated recipients. In a small controlled analysis, Soulillou et al [92] failed to demonstrate effectiveness of PP for the treatment of DRA-positive rejection. In this study, ten rejecting kidney allograft recipients underwent either the conventional treatment (corticosteroid/azathioprine) alone or PP in addition. For both study groups, poor graft survival rates were reported with only one patient per group with a graft survival exceeding 6 months. Similarly, in two other controlled trials testing the effectiveness of PP, no significant improvement of outcome was reported for PP-treated kidney allograft recipients with severe vascular rejection [93, 94]. In a randomized study testing 44 kidney transplant recipients, Bonomini et al [95], however, demonstrated effective reversal of steroid-resistant acute vascular rejection by PP-treatment of. Of control patients who were treated with a second steroid bolus and cyclophosphamide, 81% lost their graft due to uncontrolled rejection. Remarkably, PP-treatment in addition to cyclophosphamide, was reported to decrease graft loss rates to 30%. Differences in allograft survival were reported to achieve statistic significance [95].

In more recent studies (all of them uncontrolled) testing small patient cohorts, PP as part of combined anti-humoral regimens was suggested effectively treat AMR [83, 96, 97, 98, 99, 100]. These reports are listed in Table 5. Gannedahl and co-workers [96] reported

Table 4 Plasmapheresis as antirejection treatment—controlled studies. *ALS* antilymphocyte serum, *CP* cyclophosphamide, *d* days, *DSA* donor-specific antibodies, *HD* hemodialysis, *n* number (of included patients), *PP* plasmapheresis

Author, year	*N*	Rejection episode	Treatment	Clinical outcome
Kirubakaran, 1981	24	Severe vascular rejection	Control ($n=12$): steroid bolus	No PP: Improvement in 6/12 patients on day 28 (3 patients on dialysis)
			PP group ($n=12$): PP + steroid bolus	PP: Improvement in 3/12patients on day 28 (8 patients on dialysis)
Soullilou, 1983	10	Early (within 1 month) DSA-positive rejection	Control ($n=5$): standard (steroids ± ALS)	No PP: 1, 2, 2, 6, 12 months graft survival
			PP group ($n=5$): standard + PP	PP: 1, 2, 2, 3, 13 months graft survival
Bonomini, 1985	44	Steroid-resistant acute vascular rejection (most episodes DSA +)	Control ($n=21$, 71% DSA): steroids, CP	No PP: 81% graft loss due to rejection
			PP group ($n=23$, 82% DSA): PP, CP	PP: 30% graft loss due to rejection ($P<0.02$)
Allen, 1990	27	Steroid-resistant acute vascular rejection	Control ($n=14$): standard	No PP: 4/14 (29%) patients at HD after 6 days
			PP group ($n=13$): PP in addition	PP: 3/13 (23%) patients at HD after 6 days Comparable actuarial graft survival

Table 5 Plasmapheresis as treatment for acute AMR—recent (uncontrolled) studies and case reports. *ACR* acute cellular rejection, *ALS* antilymphocyte serum, *AMR* antibody-mediated rejection, *CDC* complement-dependent cytotoxicity, *CX* crossmatch, *FCXM* flow cytometry crossmatch, *IVIG* intravenous immunoglobulin, *n* number of patients, *PP* plasmapheresis

Author, year	*N*	Definition of acute AMR	Treatment	Outcome
Gannedahl, 1992	2	Histology, CDC-CX	PP + 15-deoxyspergualin	Reversal in 2/2 patients
Pascual, 1998	5	Histology, C4d+, CDC-CX	PP + MMF/Tacrolimus (IVIG)	Reversal in 5/5 patients
Montgomery, 2000	3	Histology, C4d+, CDC-CX	PP + IVIG	Reversal in 3/3 patients
Rocha, 2003	16	Histology, C4d+, FlowPRA	PP ± IVIG, steroid pulses, OKT3/ALS in patients with ACR	Reversal in 15/16 patients 81% 1-year graft survival
Shimizu, 2002	1	Histology, C4d+, FCXM	PP	Reversal of AHR
Sayegh & Colvin, 2003	1	Histology, C4d+, FCXM	PP + IVIG + MMF/Tacrolimus	Reversal of AHR

reversal of AMR by PP combined with 15-deoxyspergualin in two recipients of a renal allograft. These interesting data suggest anti-humoral efficiency of 15-deoxyspergualin. Unfortunately, however, this agent has not been approved so far for clinical use. Pascual et al [83] demonstrated effective reversal of refractory AMR by PP combined with tacrolimus and/or mycophenolate mofetil (IVIG in addition) in five tested kidney transplant recipients. In a subsequent report, the entire Boston series (ten patients with refractory AMR, reversal of the rejection process in nine of ten patients) was reported [25]. Furthermore, Montgomery and co-workers [97] reported effective reversal of AMR in a small study group by means of PP plus IVIG. Similarly, in a very recent report, Rocha et al [100] suggested high efficacy of PP and/or IVIG for the treatment of acute AMR. It has to be mentioned that also in these two studies, most patients underwent immunosuppression with tacrolimus and MMF, substances that might be important to maintain DRA suppression. In this study, AMR was primarily defined by histology. Notably, some recipients showed negative peritubular C4d staining. Regarding the rather low specificity of capillary margination of inflammatory cells, the use of this sign as major inclusion criterion may have led to the inclusion of patients without AMR, especially in cases of negative C4d staining [100].

In contrast to PP, IA therapy allows selective and nearly complete antibody removal. Notably, this strategy, renders concomitant substitution of albumin solutions and fresh frozen plasma unnecessary. These advantages make the use of IA therapy especially attractive for the treatment of AMR. Indeed, in a number of uncontrolled analyses, IA was suggested to effectively reverse a substantial proportion of DRA-positive rejection episodes resistant to standard antirejection therapy (Table 6) [24, 41, 43, 101, 102, 103]. In these uncontrolled trials, IA was initiated as rescue therapy late during the course of rejection, after other immunosuppressive strategies, such as high dose steroids, antilymphocyte antibody therapy or even PP had failed. Based on a timely diagnosis of AMR, early initiation of IA may further improve its therapeutic efficacy. Recently, we reported the clinical course of a mother of five children who received a kidney transplant from her husband [43]. An episode of refractory AMR presumably caused by preformed alloantibodies against the spousal alloantigens triggered by the previous pregnancies, turned

Table 6 Immunoadsorption as anti-rejection treatment—recent (uncontrolled) studies and case reports. *ACR* acute cellular rejection, *AMR* antibody-mediated rejection, *ALS* antilymphocyte serum, *CDC* omplement-dependent cytotoxicity, *CX* crossmatch, *FCXM* flow cytometry crossmatch, *IA* immunoadsorption, *IVIG* intravenous immunoglobulin, *n* number of enrolled patients, *PRA* panel reactive antibody

Author, year	*n*	Definition of acute AMR	Treatment	Outcome
Persson, 1995	12	Histology, CDC-CX	IA ± IVIG	Reversal in 6/12 patients
Pretagostini, 1996	23	Histology, CDC-CX	IA + steroids + ALS or OKT3	Reversal in 16/23 patients
Hickstein, 1998	11	Histology, CDC-PRA, ELISA	IA + cyclophosphamide	Reversal in 7/11 patients
Böhmig, 2000	1	Histology, C4d, CDC-CX/-PRA, FCXM	IA	Reversal of AMR
Böhmig, 2001	10	Histology, C4d, CDC-CX/-PRA, FCXM	IA ± Tacrolimus (ACR: steroids, ATG)	Reversal in 9/10 patients 80% actuarial graft survival (14 months)
Habicht, 2002	1	C4d, FCXM, FlowPRA	IA	Reversal of AMR

out to be resistant to tacrolimus rescue therapy and antilymphocyte antibody, but was reversible by IA [43]. Subsequently, two further cases of humoral rejection of spousal donor kidney allografts, which responded well to IA or PP, were reported [41, 99]. Prompted by our first case of IA-treated acute AMR we instituted routine staining for C4d at our unit in December1998 and introduced IA therapy for severe C4d-positive rejection. Of 352 patients who had received a kidney allograft at our unit between November 1998 and September 2000, 10 recipients had severe acute AMR. All ten patients were subjected to IA with protein A. Seven recipients with additional histological signs of cellular rejection also underwent antilymphocyte antibody therapy. Acute AMR was successfully reversed in nine of ten patients. A 1-year graft survival rate of 80% was reported [24].

Remarkably, in many sensitized or humorally rejecting recipients, temporary removal of alloantibodies over a short period, either to treat an established rejection or to prevent humoral rejection, was found to enable long-term allograft survival without recurrence of humoral rejection. At least two mechanisms, i.e. transplant accommodation and humoral tolerance, could account for this phenomenon. Recent studies indicate that donor-specific alloantibodies may not only occur without causing graft injury, but even induce resistance of grafts to humoral injury, a phenomenon termed transplant accommodation. The mechanisms underlying graft accommodation have been investigated in animal models of xenotransplantation, where in accommodating xenografts, EC were found to express products of distinct survival genes including products of the Bcl family or heme oxygenase 1 (HO-1) [104]. In clinical transplantation, the occurrence accommodation was discussed for AB0-incompatible transplantation [105]. Furthermore, a potential protective role of endothelially expressed survival genes has recently been demonstrated for highly sensitized kidney allograft recipients subjected to IA prior to transplantation. In these patients, Salama et al [106] demonstrated recurrence of alloantibodies without causing rejection. Interestingly, accommodating grafts showed up-regulation of Bcl-xL in glomerular and peritubular capillaries [106]. Alternatively, however, temporary removal of alloantibodies by apheresis can be speculated to promote a state of immunologic tolerance, as supported by the reported prolonged donor antigen-specific downregulation of humoral immunity after a 3-week course of IA therapy in a case of severe AMR [43].

IVIG

It is well established that high doses of IVIG effectively modulate immune responses in a variety of pathologic conditions [107]. Numerous mechanisms have been proposed to underly IVIG-induced immune modulation [107]. Recent reports suggest an immunosuppressive potential of IVIG also in the setting of kidney transplantation. In a recent uncontrolled analysis, Luke et al [108] reported reversal or improvement of refractory rejection episodes by IVIG treatment in a cohort of 17 kidney transplant recipients. Furthermore, in a controlled randomized study analyzing 30 kidney transplant recipients, IVIG was reported to treat steroid-resistant renal allograft rejection as effectively as monoclonal anti-CD3 antibody OKT3 [109]. In addition, IVIG combined with PP was suggested to be highly effective in reversing humoral rejection [97, 100]. Very recently, high doses of IVIG were reported to desensitize a substantial proportion of sensitized allograft recipients and thus to enable successful transplantation in most of these patients [97, 110, 111]. Remarkably, despite the use of very high doses of IVIG exceeding 2 g/kg in some reports, no substantial side effects were reported in these studies. Nevertheless, the possibility of severe IVIG-induced side effects including acute renal failure, a complication particularly described for the use of sucrose-containing preparations, has to be taken into account [112, 113].

Rituximab

Anti-CD20 mAb Rituximab may represent a promising therapeutic option for the treatment of AMR. Ritux-

imab leads to sustained elimination of circulating B cells and was reported to (incompletely) suppress antibody formation. This mAb is well established in the therapy of various types of lymphoid malignancies [114]. Furthermore, there is evidence for efficacy of Rituximab for the treatment of various autoimmune disorders including rheumatoid arthritis and autoimmune thrombocytopenia [115]. Recently, Rituximab was also tested as rejection treatment in allograft recipients. Aranda et al [116] reported a patient with humoral cardiac allograft rejection refractory to steroids, cyclophosphamide, and PP who showed a sustained response to the addition of anti-CD20 mAb therapy. Similarly, Garret et al [117] reported that this antibody effectively cured a case of humoral cardiac allograft rejection refractory to PP. Rituximab has further been used in AB0 incompatible transplantation. In a case of erronous AB0-incompatible lung allograft rejection, anti-A antibodies in a blood type B patient were successfully reduced by IA, Rituximab and recombinant soluble complement receptor type 1. This treatment was suggested to have effectively prevented humoral rejection in this patient [118]. Furthermore, Sawada et al [119] reported effectiveness of a preconditioning regimen including Rituximab, PP, and splenectomy in a recipient of an AB0 incompatible kidney with anti-A1 antibody titers not responding to PP alone. At present, effectiveness of Rituximab as first line therapy in humoral renal allograft rejection has not been published. Considering the use of Rituximab in transplant recipients, one has to keep in mind that elimination of B cells from the periphery may not allow a complete downregulation of alloantibodies, as plasma cells are not targeted by this strategy. In a recent experimental study, Alway et al [120] reported that in baboons anti-CD20 antibody treatment effectively depleted blood, bone marrow or lymph nodes of B cells, but failed to change titers of xenoreactive anti-alphaGal Ab antibodies. The authors concluded that future efforts will have to be directed towards suppression of plasma cell function [120].

Treatment of chronic humoral rejection

There is now accumulating evidence that alloantibodies induce or contribute to chronic renal allograft rejection [26, 31, 50, 51, 52, 53, 54]. However, optimal treatment for humoral (C4d-positive) chronic rejection has still to be established. The use of apheresis in this setting has, to our knowledge, so far not been reported. Recently, Theruvath et al [121] suggested anti-humoral activity of tacrolimus plus MMF in the setting of chronic rejection. In this analysis, four patients on CyA and azathioprine maintenance immunosuppression were selected who showed progressive deterioration of graft function, the pathologic features transplant arteriopathy and glomerulopathy, peritubular C4d deposition, and de-novo occurrence of donor antigen-specific antibodies. The major finding was a rapid and sustained decrease in antibody titers after rescue therapy with tacrolimus and MMF, whereby in two patients, DRA became undetectable after 9 months, and a biopsy performed 12 months later revealed a decrease in C4d deposition [121]. These results may suggest that in patients, who are on CyA and/or azathioprine, tacrolimus and/or MMF rescue therapy could inhibit antibody formation. However, the true effectiveness of this treatment on long-term kidney allograft outcome will have to be established in a prospective controlled study. In a recent retrospective study, Lee et al [54] demonstrated that in all tested kidney transplant recipients with chronic rejection, the occurrence of alloantibodies preceded the diagnosis of rejection. Similarly, Regele et al [31] found that C4d deposition in early biopsies without chronic histologic changes is a substantial risk factor for the development of chronic transplant glomerulopathy. According to these results, we can presume that early introduction of anti-humoral therapy is necessary to prevent chronic rejection. Detection of early stages of chronic humoral rejection would require serial serologic evaluation and/or performance of protocol biopsies.

Conclusion

In recent years, the diagnosis of AMR, a rejection type with unfavorable prognosis, has been significantly improved by the establishment of capillary C4d staining as a marker for alloantibody-triggered complement activation within allografts. In an addition to the Banff 97 scheme, a novel classification scheme has now been proposed for AMR. A standardized definition AMR is undoubtedly a prerequisite for the design of clinical trials aimed to evaluate the effectiveness of anti-humoral therapeutic strategies. Future prospective randomized studies will have to clarify the true effectiveness of proposed strategies, including distinct immunosuppressive drug combinations (tacrolimus, MMF, or other new drugs such as sirolimus), apheresis, IVIG and Rituximab.

References

1. Kissmeyer-Nielsen F, Olsen S, Petersen VP, Fjeldborg O. Hyperacute rejection of kidney allografts associated with preexisting humoral antibodies against donor cells. Lancet 1966; 1: 662–665.
2. Patel R, Terasaki PI. Significance of the positive test in kidney transplantation. N Engl J Med 1969; 280: 735–739.
3. Wu A, Bühler LH, Cooper DKC. ABO-incompatible organ and bone marrow transplantation: current status. Transplant Int 2003; 16: 291–299.
4. Samstein B, Platt J. Physiologic and immunologic hurdles to xenotransplantation. J Am Soc Nephrol 2001; 12: 182–193.
5. Feucht HE, Opelz G. The humoral immune response towards HLA class II determinants in renal transplantation. Kidney Int 1996; 50: 1464–1475.
6. Baldwin III WM, Halloran PF. Clinical syndromes associated with antibody in allografts. In: Racusen LC, Solez K, Burdick JF (eds) Kidney transplant rejection. Marcel Dekker, New York 1998; pp 127–147.
7. Crespo M, Delmonico F, Saidman S, et al. Acute humoral rejection in kidney transplantation. Graft 2000; 3: 12–17.
8. Mauiyyedi S, Colvin RB. Humoral rejection in kidney transplantation: new concepts in diagnosis and treatment. Curr Opin Nephrol Hypertens 2002; 11: 609–618.
9. Böhmig GA, Exner M, Watschinger B, Regele H. Acute humoral renal allograft rejection. Curr Opin Urol 2002; 12: 95–99.
10. Watschinger B, Pascual M. Capillary C4d deposition as a marker of humoral immunity in renal allograft rejection. J Am Soc Nephrol 2002; 13: 2420–2423.
11. Jeannet M, Pinn VW, Flax MH, Winn HJ, Russel PS. Humoral antibodies in renal allotransplantation in man. New Engl J Med 1970; 282: 111–117
12. Martin S, Dyer PA, Mallick NP, Gokal R, Harris R, Johnson RWG. Post-transplant antidonor lymphocytotoxic antibody production in relation to graft outcome. Transplantation 1987; 44: 50–53.
13. Halloran PF, Wadgymar A, Ritchie S, Falk J, Solez K, Srinivasa NS. The significance of the anti-class I response: I. Clinical and pathologic features of anti-class I-mediated rejection. Transplantation 1990; 49: 85–91.
14. Halloran PF, Schlaut J, Solez K, Srinivasa NS. The significance of the anti-class I response: II. Clinical and pathologic features of renal transplants with anti-class I-like antibody. Transplantation 1992; 53: 550–555.
15. Trpkov K, Campbell P, Pazderka F, Cockfield S, Solez K, Halloran PF. Pathologic features of acute renal allograft rejection associated with donor-specific antibody: Analysis using the Banff grading schema. Transplantation 1996; 61: 1586–1592.
16. McKenna RM, Takemoto SK, Terasaki PI. Anti-HLA antibodies after solid organ transplantation. Transplantation 2000; 69: 319–326.
17. Feucht HE, Felber E, Gokel MJ, et al. Vascular deposition of complement-split products in kidney allografts with cell-mediated rejection. Clin Exp Immunol 1991; 86: 464–470.
18. Feucht HE, Schneeberger H, Hillebrand G, et al. Capillary deposition of C4d complement fragment and early renal graft loss. Kidney Int 1993; 43: 1333–1338.
19. Racusen LC, Colvin RB, Solez K, et al. Antibody-Mediated Rejection Criteria – an Addition to the Banff '97 Classification of Renal Allograft Rejection. Am J Transplant 2003; 3: 708–714.
20. Lederer SR, Schneeberger H, Albert E, et al. Early renal graft dysfunction. The role of preformed antibodies to DR-typed lymphoblastoid cell lines. Transplantation 1996; 61: 313–319.
21. Collins AB, Schneeberger EE, Pascual MA, et al. Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries. J Am Soc Nephrol 1999; 10: 2208–2214.
22. Lederer SR, Kluth-Pepper B, Schneeberger H, Albert E, Land W, Feucht HE. Impact of humoral alloreactivity early after transplantation on the long-term survival of renal allografts. Kidney Int 2001; 59: 334–341.
23. Regele H, Exner M, Watschinger B, et al. Endothelial C4d deposition is associated with inferior kidney allograft outcome independently of cellular rejection. Nephrol Dial Transplant 2001; 16: 2058–2066.
24. Böhmig GA, Regele H, Exner M, et al. C4d-positive acute humoral renal allograft rejection: effective treatment by immunoadsorption. J Am Soc Nephrol 2001; 12: 2482–2489.
25. Crespo M, Pascual M, Tolkoff-Rubin N, et al. Acute humoral rejection in renal allograft recipients: I. Incidence, serology and clinical characteristics. Transplantation 2001; 71: 652–658.
26. Mauiyyedi S, Pelle PD, Saidman S, et al. Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. J Am Soc Nephrol 2001; 12: 574–582.
27. Böhmig GA, Exner M, Habicht A, et al. Capillary C4d deposition in kidney allografts: a specific marker of alloantibody-dependent graft injury. J Am Soc Nephrol 2002; 13: 1091–1099.
28. Herzenberg AM, Gill JS, Djurdjew O, Magil AB. C4d deposition in acute rejection: an independent long-term prognostic factor. J Am Soc Nephrol 2002; 13: 234–241.
29. Mauiyyedi S, Crespo M, Collins AB, et al. Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification. J Am Soc Nephrol 2002; 13: 779–787.
30. Nickeleit V, Zeiler M, Gudat F, Thiel G, Mihatsch MJ. Detection of the complement degradation product C4d in renal allografts: diagnostic and therapeutic implications. J Am Soc Nephrol 2002; 13: 242–251.
31. Regele H, Böhmig GA, Habicht A, et al. Capillary Deposition of Complement Split Product C4d in Renal Allografts is Associated with Basement Membrane Injury in Peritubular and Glomerular Capillaries: A Contribution of Humoral Immunity to Chronic Allograft Rejection. J Am Soc Nephrol 2002; 13: 2371–2380.
32. Haas M, Ratner LE, Montgomery RA. C4d staining of perioperative renal transplant biopsies. Transplantation 2002; 74: 711–717.
33. Baldwin III WM, Samaniego-Picota M, Kasper EK, et al. Complement deposition in early cardiac transplant biopsies is associated with ischemic injury and subsequent rejection episodes. Transplantation 1999; 68: 894–900.
34. Barrington R, Zhang M, Fischer M, Carroll MC. The role of complement in inflammation and adaptive immunity. Immunol Rev 2001; 180: 5–15.
35. Sund S, Hovig T, Reisaeter AV, Scott H, Bentdal O, Mollnes TE. Complement activation in early protocol kidney graft biopsies after living-donor transplantation. Transplantation 2003; 75: 1204–1213.
36. Magil AB, Tinckam K. Monocytes and peritubular capillary C4d deposition in acute renal allograft rejection. Kidney Int 2003; 63: 1888–1893.
37. Behr TM, Feucht HE, Richter K, et al. Detection of humoral rejection in human cardiac allografts by assessing the capillary deposition of complement fragment C4d in endomyocardial biopsies [In Process Citation]. J Heart Lung Transplant 1999; 18: 904–912.

38. Zwirner J, Felber E, Burger R, Bitter-Sauermann D, Riethmüller G, Feucht HE. Classical pathway of complement activation in mammalian kidneys. Immunology 1993; 80: 162–167.
39. Zwirner J, Felber E, Herzog V, Riethmüller G, Feucht HE. Classical pathway of complement activation in normal and diseased human glomeruli. Kidney Int 1989; 36: 1069–1077.
40. Karpinski M, Rush D, Jeffery J, et al. Flow cytometric crossmatching in primary renal transplant recipients with a negative anti-human globulin enhanced cytotoxicity crossmatch. J Am Soc Nephrol 2001; 12: 2807–2814.
41. Habicht A, Regele H, Exner M, et al. A case of severe C4d-positive kidney allograft dysfunction in the absence of histomorphologic features of rejection. Wien Klin Wochenschr 2002; 114: 945–948.
42. Segerer S, Böhmig GA, Exner M, et al. When allografts turn DARC. Transplantation 2003; 75: 1030–1034.
43. Böhmig GA, Regele H, Säemann MD, et al. Role of humoral immune reactions as target for antirejection therapy in recipients of a spousal-donor kidney graft. Am J Kidney Dis 2000; 35: 667–673.
44. Drachenberg CB, Steinberger E, Hoehn-Saric E, et al. Specificity of intertubular capillary changes: comparative ultrastructural studies in renal allografts and native kidneys. Ultrastruct Pathol 1997; 21: 227–233.
45. Gough J, Yilmaz A, Miskulin D, et al. Peritubular capillary basement membrane reduplication in allografts and native kidney disease: a clinicopathologic study of 278 consecutive renal specimens. Transplantation 2001; 71: 1390–1393.
46. Lajoie G. Antibody-mediated rejection of human renal allografts: an electron microscopic study of peritubular capillaries. Ultrastruct Pathol 1997; 21: 235–242.
47. Monga G, Mazzucco G, Messina M, Motta M, Quaranta S, Novara R. Intertubular capillary changes in kidney allografts: a morphologic investigation on 61 renal specimens. Mod Pathol 1992; 5: 125–130.
48. Yilmaz A, Yilmaz S, Kallio E, Rapola J, Hayry P. Evolution of glomerular basement membrane changes in chronic rejection. Transplantation 1995; 60: 1314–1322.
49. Takeuchi O, Oikawa T, Koyama K, et al. Multilayering of peritubular capillary is a specific diagnostic criterion for immunologic chronic rejection: does a humoral factor contribute to the pathogenesis of peritubular capillary lesions in chronic rejection? Transplant Proc 2000; 32: 306–307.
50. Abe M, Kawai T, Futatsuyama K, et al. Postoperative production of anti-donor antibody and chronic rejection in renal transplantation. Transplantation 1997; 63: 1616–1619.
51. Kerman RH, Susskind B, Kerman DH, et al. Anti-HLA antibodies detected in posttransplant renal allograft recipient sera correlate with chronic rejection. Transplant Proc 1997; 29: 1515–1516.
52. El Fettouh HA, Cook DJ, Bishay E, et al. Association between a positive flow cytometry crossmatch and the development of chronic rejection in primary renal transplantation. Urology 2000; 56: 369–372.
53. Piazza A, Poggi E, Borrelli L, et al. Impact of donor-specific antibodies on chronic rejection occurrence and graft loss in renal transplantation: posttransplant analysis using flow cytometric techniques. Transplantation 2001; 71: 1106–1112.
54. Lee PC, Terasaki PI, Takemoto SK, et al. All chronic rejection failures of kidney transplants were preceded by the development of HLA antibodies. Transplantation 2002; 74: 1192–1194.
55. Baldwin III WM, Samaniego M, Qian Z, et al. Complement as a mediator of allograft injury: an inflammatory view. Transplantation Rev 2000; 14: 41–51.
56. Regele H, Böhmig GA. Tissue injury and repair in allografts: novel perspectives. Curr Opin Nephrol Hypertens 2003; 12: 259–266.
57. Brauer RB, Baldwin WM, 3rd, Ibrahim S, Sanfilippo F. The contribution of terminal complement components to acute and hyperacute allograft rejection in the rat. Transplantation 1995; 59: 288–293.
58. Qian Z, Hu W, Liu J, Sanfilippo F, Hruban RH, Baldwin WM, 3rd. Accelerated graft arteriosclerosis in cardiac transplants: complement activation promotes progression of lesions from medium to large arteries. Transplantation 2001; 72: 900–906.
59. Nakashima S, Qian Z, Rahimi S, Wasowska BA, Baldwin WM, 3rd. Membrane attack complex contributes to destruction of vascular integrity in acute lung allograft rejection. J Immunol 2002; 169: 4620–4627.
60. Benzaquen LR, Nicholson-Weller A, Halperin JA. Terminal complement proteins C5b-9 release basic fibroblast growth factor and platelet-derived growth factor from endothelial cells. J Exp Med 1994; 179: 985–992.
61. Kilgore KS, Schmid E, Shanley TP, et al. Sublytic concentrations of the membrane attack complex of complement induce endothelial interleukin-8 and monocyte chemoattractant protein-1 through nuclear factor-kappa B activation. Am J Pathol 1997; 150: 2019–2031.
62. Tedesco F, Pausa M, Nardon E, Introna M, Mantovani A, Dobrina A. The cytolytically inactive terminal complement complex activates endothelial cells to express adhesion molecules and tissue factor procoagulant activity. J Exp Med 1997; 185: 1619–1627.
63. Dobrina A, Pausa M, Fischetti F, et al. Cytolytically inactive terminal complement complex causes transendothelial migration of polymorphonuclear leukocytes in-vitro and in vivo. Blood 2002; 99: 185–192.
64. Colvin RB. Transplant Mac attack: humor the macrophages. Kidney Int 2003; 63: 1953–1954.
65. Wasowska BA, Qian Z, Cangello DL, et al. Passive transfer of alloantibodies restores acute cardiac rejection in IgKO mice. Transplantation 2001; 71: 727–736.
66. Hamilton KK, Hattori R, Esmon CT, Sims PJ. Complement proteins C5b-9 induce vesiculation of the endothelial plasma membrane and expose catalytic surface for assembly of the prothrombinase enzyme complex. J Biol Chem 1990; 265: 3809–3814.
67. Saadi S, Holzknecht RA, Patte CP, Stern DM, Platt JL. Complement-mediated regulation of tissue factor activity in endothelium. J Exp Med 1995; 182: 1807–1814.
68. Ren G, Hack BK, Minto AW, et al. A complement-dependent model of thrombotic thrombocytopenic purpura induced by antibodies reactive with endothelial cells. Clin Immunol 2002; 103: 43–53.
69. Sumitran-Holgersson S, Wilczek HE, Holgersson J, Soderstrom K. Identification of the nonclassical HLA molecules, mica, as targets for humoral immunity associated with irreversible rejection of kidney allografts. Transplantation 2002; 74: 268–277.
70. Marsh JE, Farmer CK, Jurcevic S, Wang Y, Carroll MC, Sacks SH. The allogeneic T and B cell response is strongly dependent on complement components C3 and C4. Transplantation 2001; 72: 1310–1318.
71. Hancock WW, Gao W, Shemmeri N, et al. Immunopathogenesis of accelerated allograft rejection in sensitized recipients: humoral and nonhumoral mechanisms. Transplantation 2002; 73: 1392–1397.
72. Pratt JR, Basheer SA, Sacks SH. Local synthesis of complement component C3 regulates acute renal transplant rejection. Nat Med 2002; 8: 582–587.
73. Opelz G. Collaborative transplant study - 10-year report. Transplant Proc 1992; 24: 2342–2355.
74. Cecka JM. The UNOS scientific renal transplant registry. Clin Transpl 1998; 1–16.

75. Cook DJ, Fettouh HI, Gjertson DW, Cecka JN. Flow cytometry crossmatching (FCXM) in the UNOS Kidney Transplant Registry. Clin Transpl 1998; 413–419.
76. Kerman RH, Susskind B, Buelow R, et al. Correlation of ELISA-detected IgG and IgA anti-HLA antibodies in pretransplant sera with renal allograft rejection. Transplantation 1996; 62: 201–205.
77. Scornik JC, Salomon DR, Lim PB, Howard RJ, Pfaff WW. Posttransplant antidonor antibodies and graft rejection. Transplantation 1989; 47: 287–290.
78. Lobo PI, Spencer CE, Stevenson WC, Pruett TL. Evidence demonstrating poor kidney graft survival when acute rejections are associated with IgG donor-specific lymphocytotoxin. Transplantation 1995; 59: 357–360.
79. Monteiro F, Buelow R, Mineiro C, Rodriguez H, Kalil J. Identification of patients at high risk of graft loss by pre- and posttransplant monitoring of anti-HLA class I IgG antibodies by enzyme-linked immunosorbent assay. Transplantation 1997; 63: 542–546.
80. Christiaans MHL, Overhof-de Roos R, Nieman F, van Hooff JP, van den Berg-Loonen EM. Donor-specific antibodies after transplantation by flow cytometry. Transplantation 1998; 65: 427–433.
81. Kimikawa M, Tojimbara T, Nakajima I, et al. Posttransplant antidonor antibodies and chronic rejection in renal transplantation. Transplant Proc 1999; 31: 2872–2873.
82. Worthington JE, Martin S, Al-Husseini DM, Dyer PA, Johnson RW. Post-transplantation production of donor HLA-specific antibodies as a predictor of renal transplant outcome. Transplantation 2003; 75: 1034–1040.
83. Pascual M, Saidman S, Tolkoff-Rubin N, et al. Plasma exchange and tacrolimus-mycophenolate rescue for acute humoral rejection in kidney transplantation. Transplantation 1998; 66: 1460–1464.
84. Alarabi A, Backman U, Wikstrom B, Sjoberg O, Tufveson G. Plasmapheresis in HLA-immunosensitized patients prior to kidney transplantation. Int J Artif Organs 1997; 20: 51–56.
85. Haas M, Böhmig GA, Leko-Mohr Z, et al. Peri-operative immunoadsorption in sensitized renal transplant recipients. Nephrol Dial Transplant 2002; 17: 1503–1508.
86. Hickstein H, Korten G, Bast R, Barz D, Nizze H, Schmidt R. Immunoadsorption of sensitized kidney transplant candidates immediately prior to surgery. Clin Transplant 2002; 16: 97–101.
87. Hiesse C, Kriaa F, Rousseau P, et al. Immunoadsorption of anti-HLA antibodies for highly sensitized patients awaiting renal transplantation. Nephrol Dial Transplant 1992; 7: 944–951.
88. Higgins RM, Bevan DJ, Carey BS, et al. Prevention of hyperacute rejection by removal of antibodies to HLA immediately before renal transplantation. Lancet 1996; 348: 1208–1211.
89. Kupin WL, Venkat KK, Hayashi H, Mozes MF, Oh HK, Watt R. Removal of lymphocytotoxic antibodies by pretransplant immunoadsorption therapy in highly sensitized renal transplant recipients. Transplantation 1991; 51: 324–329.
90. Schweitzer EJ, Wilson JS, Fernandez-Vina M, et al. A high panel-reactive antibody rescue protocol for cross-match-positive live donor kidney transplants. Transplantation 2000; 70: 1531–1536.
91. Taube D. Immunoadsorption in the sensitized transplant recipient. Kidney Int 1990; 38: 350–358.
92. Soulillou JP, Guyot C, Guimbretiere J, et al. Plasma exchange in early kidney graft rejection associated with anti-donor antibodies. Nephron 1983; 35: 158–162.
93. Allen N, Smith J, Tate D, Slapak M. Intensive plasma exchange in acute renal allograft rejection. Transplant Proc 1983; 15: 1061–1063.
94. Kirubakaran MG, Disney AP, Norman J, Pugsley DJ, Mathew TH. A controlled trial of plasmapheresis in the treatment of renal allograft rejection. Transplantation 1981; 32: 164–165.
95. Bonomini V, Vangelista A, Frasca GM, di Felice A. Effects of plasmapheresis in renal transplantation. Trans ASAIO 1985; 31: 698–701.
96. Gannedahl G, Ohlmann S, Persson U, et al. Rejection associated with early appearance of donor-reactive antibodies after kidney transplantation treated with plasmapheresis and administration of 15-deoxyspergualin. Transplant Int 1992; 5: 189–192.
97. Montgomery RA, Zachary AA, Racusen LC, et al. Plasmapheresis and intravenous immune globulin provides effective rescue therapy for refractory humoral rejection and allows kidneys to be successfully transplanted into cross-match-positive recipients. Transplantation 2000; 70: 887–895.
98. Sayegh MH, Colvin RB. Case record of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 8–2003. A 35-year-old man with early dysfunction of a second renal transplant. N Engl J Med 2003; 348: 1033–1044.
99. Shimizu T, Tokiwa M, Yamaguchi Y. A case of acute antidonor antibody-mediated humoral rejection after renal transplantation with specific consideration of serial graft biopsy histology. Clin Transplant 2002; 16 (Suppl 8): 62–67.
100. Rocha PN, Butterly DW, Greenberg A, et al. Beneficial effect of plasmapheresis and intravenous immunoglobulin on renal allograft survival of patients with acute humoral rejection. Transplantation 2003; 75: 1490–1495.
101. Hickstein H, Korten G, Bast R, et al. Protein A immunoadsorption (IA) in renal transplantation patients with vascular rejection. Transfus Sci 1998; 19: 53–57.
102. Persson NH, Bucin D, Ekberg H, et al. Immunoadsorption in acute vascular rejection after renal transplantation. Transplant Proc 1995; 27: 3466.
103. Pretagostini R, Berloco P, Poli L, et al. Immunoadsorption with protein A in humoral rejection of kidney transplants. ASAIO J 1996; 42: M645–648.
104. Wang N, Lee JM, Tobiasch E, et al. Induction of xenograft accommodation by modulation of elicited antibody responses. Transplantation 2002; 74: 334–345.
105. Platt JL. C4d and the fate of allografts. J Am Soc Nephrol 2002; 13: 2417–2419.
106. Salama AD, Delikouras A, Pusey CD, et al. Transplant accommodation in highly sensitized patients: a potential role for Bcl-xL and alloantibody. Am J Transplant 2001; 1: 260–269.
107. Kazatchkine MD, Kaveri SV. Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin. N Engl J Med 2001; 345: 747–755.
108. Luke PP, Scantlebury VP, Jordan ML, et al. Reversal of steroid- and anti-lymphocyte antibody-resistant rejection using intravenous immunoglobulin (IVIG) in renal transplant recipients. Transplantation 2001; 72: 419–422.
109. Casadei DH, del CRM, Opelz G, et al. A randomized and prospective study comparing treatment with high-dose intravenous immunoglobulin with monoclonal antibodies for rescue of kidney grafts with steroid-resistant rejection. Transplantation 2001; 71: 53–58.
110. Glotz D, Antoine C, Julia P, et al. Desensitization and subsequent kidney transplantation of patients using intravenous immunoglobulins (IVIg). Am J Transplant 2002; 2: 758–760.

111. Al-Uzri AY, Seltz B, Yorgin PD, Spier CM, Andreoni K. Successful renal transplant outcome after intravenous gamma-globulin treatment of a highly sensitized pediatric recipient. Pediatr Transplant 2002; 6: 161–165.
112. Zhang R, Szerlip HM. Reemergence of sucrose nephropathy: acute renal failure caused by high- dose intravenous immune globulin therapy. South Med J 2000; 93: 901–904.
113. Tsinalis D, Dickenmann M, Brunner F, Gurke L, Mihatsch M, Nickeleit V. Acute renal failure in a renal allograft recipient treated with intravenous immunoglobulin. Am J Kidney Dis 2002; 40: 667–670.
114. Plosker GL, Figgit DP. Rituximab: a review of its use in non-Hodgkin's lymphoma and lymphocytic leukemia. Drugs 2003; 63: 803–843.
115. Edwards JC, Leandro MJ, Cambridge G. B-lymphocyte depletion therapy in rheumatoid arthritis and other autoimmune disorders. Biochem Soc Trans 2002; 30: 824–828.
116. Aranda JM, Jr., Scornik JC, Normann SJ, et al. Anti-CD20 monoclonal antibody (rituximab) therapy for acute cardiac humoral rejection: a case report. Transplantation 2002; 73: 907–910.
117. Garrett HE, Jr., Groshart K, Duvall-Seaman D, Combs D, Suggs R. Treatment of humoral rejection with rituximab. Ann Thorac Surg 2002; 74: 1240–1242.
118. Pierson RN, 3rd, Loyd JE, Goodwin A, et al. Successful management of an ABO-mismatched lung allograft using antigen- specific immunoadsorption, complement inhibition, and immunomodulatory therapy. Transplantation 2002; 74: 79–84.
119. Sawada T, Fuchinoue S, Teraoka S. Successful A1-to-O ABO-incompatible kidney transplantation after a preconditioning regimen consisting of anti-CD20 monoclonal antibody infusions, splenectomy, and double-filtration plasmapheresis. Transplantation 2002; 74: 1207–1210.
120. Alwayn IP, Xu Y, Basker M, et al. Effects of specific anti-B and/or anti-plasma cell immunotherapy on antibody production in baboons: depletion of CD20- and CD22-positive B cells does not result in significantly decreased production of anti- alphaGal antibody. Xenotransplantation 2001; 8: 157–171.
121. Theruvath TP, Saidman SL, Mauiyyedi S, et al. Control of antidonor antibody production with tacrolimus and mycophenolate mofetil in renal allograft recipients with chronic rejection. Transplantation 2001; 72: 77–83.