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REVIEW

Prevention and treatment of alloantibody-mediated kidney transplant rejection

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Summary

Antibody-mediated rejection (AMR), which is commonly caused by preformed and/or *de novo* HLA alloantibodies, has evolved as a leading cause of early and late kidney allograft injury. In recent years, effective treatment strategies have been established to counteract the deleterious effects of humoral alloreactivity. One major therapeutic challenge is the barrier of a positive pretransplant lymphocytotoxic crossmatch. Several apheresis- and/or IVIG-based protocols have been shown to enable successful crossmatch conversion, including a strategy of peritransplant immunoadsorption for rapid crossmatch conversion immediately before deceased donor transplantation. While such protocols may increase transplant rates and allow for acceptable graft survival, at least in the short-term, it has become evident that, despite intense treatment, many patients still experience clinical or subclinical AMR. This reinforces the need for innovative strategies, such as complementary allocation programs to improve transplant outcomes. For acute AMR, various studies have suggested efficiency of plasmapheresis- or immunoadsorption-based protocols. There is, however, no established treatment for chronic AMR and the development of strategies to reverse or at least halt chronic active rejection remains a big challenge. Major improvements can be expected from studies evaluating innovative therapeutic concepts, such as proteasome inhibition or complement blocking agents.

Introduction

The challenge of antibody-mediated rejection

Antibody-mediated rejection (AMR) is a major cause of kidney allograft injury. Its prototype, hyperacute rejection, which is triggered by preformed complement-activating donor-specific antibodies (DSA), has already been described early in the beginnings of transplantation [1,2]. However, evidence has emerged that preformed or *de novo* DSA may also cause other much more common rejection forms, such as acute or chronic AMR [3,4].

The implementation of clear-cut diagnostic AMR criteria has now provided a solid basis for the establishment of effective therapeutic strategies to prevent and/or reverse AMR. According to the Banff classification of renal allograft pathology, the diagnosis of acute or chronic AMR relies on the presence of distinct morphological, immunohistological and/or serological diagnostic criteria, namely: typical histomorphological changes indicating alloantibody-triggered inflammation or injury [e.g. peritubular capillaritis and glomerulitis in acute AMR; transplant glomerulopathy (TG) in chronic AMR]; deposition of the classical complement split product C4d in peritubular capillaries; and circulating DSA detected at the time of rejection [5,6].

Anti-HLA DSA were shown to tightly correlate with features of AMR, and HLA antigens are considered to be the primary targets of rejection [7–10]. Nevertheless, there is now emerging evidence for a role also of antibodies against non-HLA antigens [11] and an involvement of a variety of distinct antigenic systems, including MHC class I chain-related gene A (MICA) antigens [12] or angiotensin II type 1 receptor [13,14] has been reported.

It is well established that recipient presensitization triggered by prior transplantation, pregnancies and/or transfusion represents a major risk factor for the development

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of early acute AMR [3,4]. A high clinical relevance of this rejection type was recognized in initial studies demonstrating high graft loss rates in the absence of specific treatment [15,16]. While a variety of strategies have been proven effective in treating acute AMR, chronic AMR still represents a major therapeutic challenge. The development of chronic AMR, which may culminate in irreversible structural damage, is considered to be a continuous process associated with fluctuating levels of (*de novo*) DSA, with or without detectable deposits of capillary C4d [17–23]. A particular clinical relevance of chronic AMR is underscored by studies suggesting a primary role of antibody-mediated injury as a major cause of kidney allograft loss in the long-term [24–26].

Anti-humoral treatment – general remarks

There are two well-established indications for anti-humoral treatment, namely: (i) desensitization of sensitized transplant candidates to increase transplant rates and prevent AMR, and (ii) treatment of acute AMR. Other less established indications are (iii) treatment of chronic AMR and (iv) early prevention of pending alloantibody-mediated injury in transplant recipients presenting with subclinical features of AMR.

As illustrated in Fig. 1, published protocols are based on two complementary therapeutic concepts: (i) removal of injurious alloantibodies from the circulation using apheresis [plasmapheresis (PP) or immunoadsorption (IA)]; and (ii) modulation of B cell immunity and other components of specific and innate immunity using intravenous immunoglobulin (IVIG), CD20 antibody rituximab, proteasome inhibitor bortezomib, anti-C5 antibody eculizumab, and/or splenectomy.

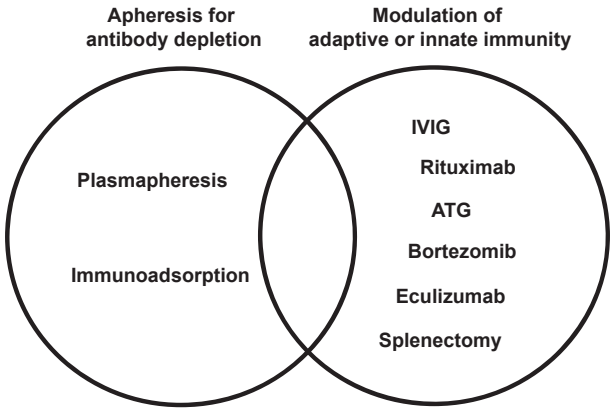

Figure 1 Anti-humoral treatment concepts: Most protocols combine two major therapeutic principles: (a) apheresis for antibody depletion and (b) modulation of B cell immunity and other components of adaptive and innate immunity.

Most published protocols include a combined application of two or more different treatment modalities. Accordingly, it is often difficult to dissect the individual contribution of a specific modality to treatment success. A representative example is the use of proteasome inhibitor bortezomib to directly affect alloantibody-producing plasma cells. In many case series, this agent was applied together with PP, IVIG, rituximab, and/or high-dose steroids to reverse severe AMR episodes and decrease DSA levels [27–32]. However, two recent observational studies have suggested that bortezomib as a sole treatment may not or only modestly reduce alloantibody levels [33,34].

Our current knowledge is based upon a large number of anecdotal reports and uncontrolled studies. However, only few randomized controlled trials (RCT) are available. There is one placebo-controlled RCT, a National Institute of Health (NIH)-sponsored multicenter trial, which has provided evidence for efficiency of IVIG in recipient desensitization [35]. Regarding rejection therapy, early controlled studies have evaluated the impact of PP-based treatment; however, all of them have been designed in the 1980s when clear-cut criteria of AMR have not yet been defined [36–39]. Only one small RCT has been designed to assess the efficiency of apheresis in the treatment of AMR defined according to modern diagnostic criteria [40].

Desensitization for AMR prevention

In recent years, several groups have pioneered protocols for recipient desensitization both in living and deceased donor kidney transplantation. There are three major strategies: (i) high-dose IVIG or (ii) PP plus IVIG for desensitization with or without XM conversion prior to living or deceased donor transplantation, and (iii) peritransplant IA for rapid XM conversion immediately before deceased donor transplantation (Table 1).

High-dose IVIG

A variety of studies have shown that high-dose IVIG is capable of reducing levels of allosensitization and of increasing the chance to receive a suitable XM-negative kidney transplant [35,41–44]. As detailed in a recent excellent review article, IVIG exerts its effects via a multiplicity of different mechanisms [45]. IVIG may affect a variety of components of adaptive and innate immunity and this may culminate in a modulation of B cell-mediated immunity including alloantibody responses [45]. In an effort to enhance treatment efficiency, some authors have combined IVIG with the CD20 antibody rituximab [43,44]. In addition, IVIG-based desensitization was commonly combined with depleting [anti-thymocyte globulin

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Table 1. Major indications and strategies for desensitization of allosensitized recipients.

Indications	Donor type	Published treatment principles: PP + low-dose IVIG	High-dose IVIG ± Rituximab	IA with protein A
XM conversion	LD	yes	yes*	no
Desensitization on the waiting list	DD	yes	yes*	yes
Rapid XM conversion	DD	yes	no	yes

PP, plasmapheresis; IVIG, intravenous immunoglobulin; IA, immunoadsorption; XM, crossmatch; LD, living donor; DD, deceased donor.
*Proof of efficiency in a randomized controlled trial [35].

(ATG); alemtuzumab] or non-depleting (IL-2 receptor antibody) anti-lymphocyte antibody induction therapy [35,41–44].

Glotz *et al.* [41] evaluated the impact of IVIG-based desensitization in 15 sensitized kidney transplant candidates [≥ 50% complement-dependent cytotoxicity (CDC) panel-reactive antibody (PRA) levels or positive CDCXM with a potential living donor]. Recipients awaiting a deceased donor allograft were subjected to three monthly courses of IVIG (2 g/kg over 48 h). Living donor transplant candidates received a single IVIG course for XM conversion. Eleven deceased donor (mean decrease of CDC-PRA by 80%) and two living donor transplant recipients (XM conversion) were successfully desensitized and were transplanted under ATG induction and further IVIG treatment. The authors reported two early graft losses (one from rejection). The other 11 patients had an uneventful course for the first year [41].

Jordan *et al.* [42] applied a protocol of IVIG-based desensitization in 45 highly sensitized recipients of a living or a deceased donor kidney or heart allograft. All included patients had a positive initial CDCXM with their potential donor. Patients were selected according to *in vitro* prescreening to assess the capability of IVIG to inhibit CDC test results. Twenty-six recipients of a living donor kidney were subjected to a single infusion of IVIG (2 g/kg). In 24 of these patients the CDCXM was rendered negative and transplantation was performed. Patients awaiting a deceased donor transplant ($n = 17$) were subjected to 2 g/kg IVIG at monthly intervals. Upon follow-up, 16 of these recipients received a kidney transplant. In the overall cohort, favorable 24-month patient and graft survival rates were reported (97.6% and 89.1%, respectively). However, the authors noted a high rejection rate (31%) [42].

Subsequently, Jordan *et al.* [35] published the results of an NIH-sponsored multicenter RCT. In this study, 98 sensitized transplant candidates (CDC-PRA ≥ 50%) were randomized to receive placebo or IVIG. IVIG was administered at four monthly intervals and 12 and 24 months after inclusion. Major results of this study were a significant reduction in CDC-PRA reactivity, an increase in transplant rates, a shortened time to transplantation, and a transplant survival comparable with that of placebo-treated recipients. Reductions in PRA levels, however, were rather modest and transient, and IVIG-treated patients experienced a considerable rate of rejection (9 of 17 IVIG-treated patients) [35].

More recently, Vo *et al.* [43] reported a protocol combining IVIG with CD20 antibody rituximab. Twenty highly sensitized transplant candidates were included. IVIG at 2 g/kg was given twice on days 0 and 30. Rituximab (1 g) was applied on days 7 and 22. The protocol led to a significant reduction of panel reactivity and 16 patients were offered a suitable transplant within a short period of time (5 ± 6 months). One-year patient and graft survival rates were 100% and 94%, respectively. However, despite intense immunosuppressive treatment (including alemtuzumab induction), a 50% rejection rate (C4d-positive AMR: 31% of rejections) was reported [43]. Comparable results were obtained in a subsequent study of 76 kidney transplant recipients subjected to a slightly modified protocol [44].

In conclusion, IVIG-based desensitization may allow for considerable XM conversion rates and satisfactory short-term survival. However, complete downregulation of circulating alloreactivity was rarely observed, and in some subjects antibody levels were not or only modestly affected. Another concern is a substantial rejection rate despite intense treatment including rituximab or alemtuzumab. The impact of the observed high rates of (humoral) rejection on long-term outcomes is currently unknown and will have to be evaluated in long-term studies.

PP plus IVIG

In recent years, several groups have published their experience with XM conversion in living donor kidney transplantation using serial pre- and/or post-transplant PP plus low-dose IVIG [20,22,46–51]. Schweitzer *et al.* [46] applied a protocol of pretransplant PP/IVIG in 15

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positive XM [anti-human globulin (AHG)-enhanced CDCXM] living donor transplant recipients, of whom two received simultaneous deceased donor pancreas allograft. Eleven recipients were successfully desensitized and transplanted under OKT3 induction. The authors reported reversible rejection episodes in four patients (three patients with suspected AMR). The two pancreas allografts, however, failed [46]. At the same time, the Johns Hopkins group published a small initial cohort of four living donor recipients subjected to PP/IVIG [47]. All recipients had a positive flow cytometry crossmatch (FCXM) one a positive AHG-CDCXM. Remarkably, three recipients developed AMR, which could be reversed by additional treatment courses [47]. Several other groups have subsequently adopted and modified PP-based desensitization. While all these studies revealed considerable rejection rates, satisfactory short-term transplant outcomes were reported [49–51].

Recent protocol biopsy studies have revealed a considerable rate of subclinical rejection following XM conversion [20–22]. For example, in a cohort of 50 XM-incompatible kidney transplant recipients, the Johns Hopkins group reported a 39.7% subclinical cell-mediated rejection rate. Capillary C4d deposits were detected in 20–30% of the recipients [22]. One can argue that subclinical rejection following recipient desensitization could culminate in chronic injury. However, long-term clinical data are lacking and it remains unclear whether the reported high clinical and subclinical rejection rates definitely lead to a higher incidence of premature graft loss. In this respect, it is important to note that recent studies have suggested a causal relationship between clinical or subclinical AMR and the subsequent development of features of chronic rejection. In a nonhuman primate renal transplant model, Smith *et al.* [18] found that the late development of TG was preceded by alloantibody formation and capillary C4d deposition. Similarly, in a study of renal allograft recipients who underwent indication biopsies, capillary C4d deposits in biopsies with normal glomerular morphology turned out to be associated with the finding of TG in late follow-up biopsies [17]. A major role of alloantibodies in the pathogenesis of characteristic lesions in the microcirculation was confirmed in subsequent large studies [19] and associations between clinical/subclinical AMR and the subsequent development of TG were described also for desensitized XM-incompatible renal transplant recipients [20,21].

Up to now, there is no RCT available designed to directly compare the efficiencies of different desensitization strategies and it is still unclear which protocol offers the best balance of risk and benefit. However, some evidence suggests superiority of protocols including antibody-depletion by apheresis. In an observational study, Stegall *et al.* [48] reported lower XM conversion rates and more rejections in patients subjected to IVIG-based desensitization without apheresis, while superior outcomes were reported for recipients subjected to PP/IVIG together with rituximab and/or ATG induction.

IA for rapid XM conversion

An interesting option for high risk recipients awaiting a deceased donor transplant may be the rapid removal of DSA immediately prior to transplantation, without a period of extended desensitization on the waiting list. A major challenge is the short interval between an organ offer and transplant surgery, a situation necessitating the use of a highly efficient regimen for rapid and extensive antibody removal. Semiselective IA may have the major advantage over PP that three or more plasma volumes can be easily processed during a single session, without a considerable loss of essential plasma constituents. This may allow for efficient and selective antibody depletion within a couple of hours.

In an initial study, Higgins *et al.* [52] evaluated a protocol of pretransplant IA with protein A for XM conversion (FCXM; in some patients CDCXM). In this study one or two extended treatment sessions with up to 40 l processed plasma volume were applied immediately before transplantation (''one shot'' IA). Recipients received OKT3 induction and cyclophosphamide-based maintenance therapy. A major finding was the complete prevention of hyperacute rejection. Nevertheless, the authors reported a considerable early graft loss rate (five losses among nine CDCXM-positive recipients) and a high rejection rate. The actual rate of AMR in this early study, however, was not reported. Moreover, prolonged pre-operative IA led to a marked extension of cold ischemia times [52].

The Vienna group has established a protocol of peritransplant IA to decrease levels of allosensitization immediately before transplantation [53–55]. The principle of this strategy is illustrated in Fig. 2. In a recent update the clinical course of 68 broadly sensitized recipients subjected to peritransplant IA (transplantation between 1999 and 2008) was described in detail [55]. Twenty-one recipients had a positive CDCXM which could be converted by a single pretransplant (pre-Tx) IA session. All CDCXM-positive recipients had DSA uncovered by Luminex-based HLA antibody detection. Among CDCXM-negative recipients, 30 recipients were identified to have preformed DSA. There was no difference between CDCXM-positive and -negative patients, with or without Luminex DSA, regarding AMR rates, cellular rejection rates, occurrence of delayed graft function, allograft function and protein excretion, and long-term graft survival. Major outcomes are illustrated in Fig. 3. Interestingly,

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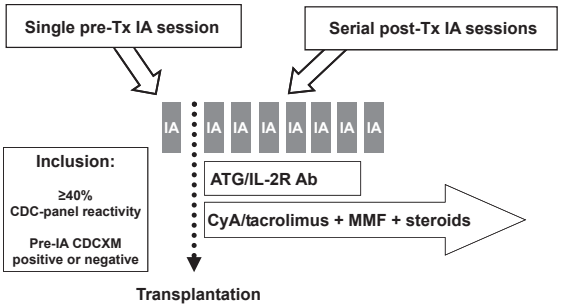
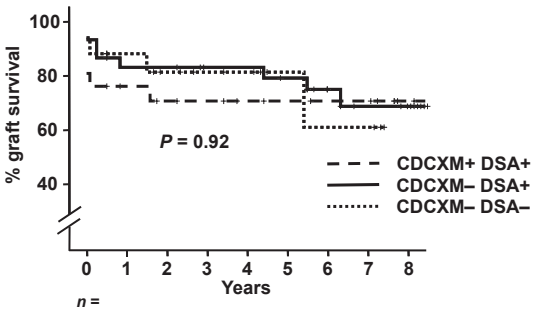


Figure 2 Vienna protocol of peritransplant immunoadsorption (IA) for recipient desensitization immediately before deceased donor transplantation. Broadly sensitized kidney transplant candidates [complement-dependent cytotoxicity (CDC) panel reactivity ≥ 40%; CDC crossmatch (XM) positive or negative] are subjected to a single pre-transplant (pre-Tx) IA session. CDCXM-positive patients proceed to transplantation if the XM can be converted to negative upon 6l plasma treatment. After transplantation patients are subjected to serial IA sessions (until stabilization of allograft function) to prevent alloantibody rebound. In addition, patients receive anti-thymocyte globulin (ATG) or IL-2 receptor antibody (IL-2 R Ab) induction, together with cyclosporine A (CyA) or tacrolimus, mycophenolate mofetil (MMF) and steroids.



	Pre-IA serology		
Rejection type	**CDCXM+ (*n* = 21)**	**CDCXM– DSA+ (*n* = 30)**	**CDCXM– DSA– (*n* = 17)**
ACR	**14%**	**24%**	**18%**
Acute C4d+ AMR	**19%**	**23%**	**24%**
Chronic AMR	**5%**	**10%**	**6%**

Figure 3 Transplant outcomes in 68 sensitized transplant recipients subjected to peritransplant immunoadsorption (IA). Patients were grouped according to pre-IA serology [complement-dependent cytotoxicity (CDC) crossmatch (XM); Luminex-based detection of donor-specific antibodies (DSA)]. There were no significant differences between patient groups with respect to death-censored graft survival, acute cell-mediated rejection (ACR), acute antibody-mediated rejection (AMR), and chronic AMR, respectively.

applying IA-based desensitization, no qualitative or quantitative pre- or post-transplant serological parameter (e.g. HLA class specificity, number of targeted antigens, binding strength) could be identified to predict early and/or late C4d-positive AMR [55].

Rapid XM conversion immediately before transplantation may be a domain of semiselective IA. Currently, there is only scarce experience with the use of other apheresis modalities, such as PP, in this particular context [56].

Role of depleting anti-lymphocyte antibody therapy

There is some controversy in the literature regarding the role of polyclonal anti-lymphocyte antibody therapy in the context of humoral rejection. There is even data suggesting that ATG itself may occasionally trigger AMR episodes. In a small observational study, Colovai *et al.* [57] reported three cases of hyperacute or acute C4d-positive AMR following ATG induction. According to a detailed serological work-up, the authors suggested a causative role of passively transferred anti-lymphocyte and anti-endothelial xenoantibodies [57]. Other studies have suggested that ATG could favor DSA formation post-transplantation, presumably a result of its action on regulatory T cells, and this could at least in part explain differences regarding DSA persistence between desensitization protocols using ATG versus IL-2 receptor antibody induction [58,59]. In this respect, however, it is important to note that anti-lymphocyte antibody preparations may cause false-positive results using conventional XM testing or even solid phase assays [60]. In a retrospective cohort study of renal transplant recipients, no associations between ATG induction and capillary C4d deposition were noted [61]. Moreover, Nickeleit *et al.* [62] suggested that their failure to detect outcome differences between C4d-positive and -negative patients could have been a result of intensified rejection treatment using depleting anti-lymphocyte antibodies (ATG, OKT3). Indeed, there is experimental data suggesting potent anti-humoral efficacy of ATG, including induction of apoptosis of B cells and bone marrow resident plasma cells, triggered by binding and crosslinking of a variety of surface molecules [63]. One can argue that such effects could enhance the efficiency of anti-humoral regimens. Indeed, several authors have reported the successful use of ATG as an adjunct to desensitization protocols and anti-rejection treatments [41,48,55,64,65].

Innovative HLA serology and AMR prevention

In recent years, highly sensitive HLA antigen-specific techniques for solid phase alloantibody detection have

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been established and are now increasingly used by HLA laboratories [66]. Applying Luminex-based bead array technology, a detailed analysis of reactivity patterns to defined HLA antigens can be easily accomplished and with this method it is possible to distinguish between donor- and nondonor-specific HLA reactivity by comparing test results with the donor HLA type (''virtual crossmatch'') [67–69]. Currently, there is an intense discussion regarding the actual clinical value of such innovative methodology and there is still no consensus regarding definitions of clinically relevant test thresholds. Moreover, as described for cell-based antibody detection techniques, some test results may be affected false positive (e.g., binding to irrelevant epitopes exposed on the surface of microbeads [70]) and false negative results (e.g., interference by IgM binding [71]).

As a valuable adjunct to cell-based XM testing (CDCXM, FCXM), Luminex-based bead array technology may provide important information to identify compatible donors and immunological risks and may provide a useful basis for the implementation of individualized desensitization protocols. In the context of recipient desensitization, a potential advantage over cell-based assays may be that test results are less affected by therapeutic antibody treatment (e.g. ATG, CD20 antibody rituximab).

Several cohort studies have suggested that preformed HLA-DSA uncovered by bead array technology (positive virtual XM), in the absence of a positive current XM, may pose a considerable risk of AMR and/or graft loss [72–75]. A caveat remains that, despite a good predictive value of low level DSA for AMR, there is still a considerable proportion of DSA-positive recipients who do not experience rejection. The value of additional qualitative parameters, such as antibody strength, to improve test performance is currently a matter of discussion [72,74,76]. In XM-positive recipients subjected to desensitization, several studies have suggested that DSA strength could be useful for further risk stratification [75,77,78].

Preformed DSA and targeted pre-emptive therapy

Recent studies have now provided evidence that solid phase HLA alloantibody detection before transplantation (''virtual-XM'') could provide a useful basis for targeted anti-humoral treatment [65,79,80]. In a study by Bächler *et al.* [65], 37 DSA-positive (CDCXM-negative) renal allograft recipients were subjected to combined induction therapy with IVIG and ATG. A comparison to the outcomes observed in a historical group of 67 nontreated DSA-positive recipients suggested that such treatment may substantially decrease rejection rates (e.g., clinical AMR: 46% vs. 11%) [65]. Very recently, the same authors have extended their observations to a larger cohort of 233 renal transplant recipients (43 DSA-positive subjects) [80]. This analysis further supported reduction of rejection rates by pre-emptive anti-humoral treatment. Nevertheless, also in this analysis, higher rates of clinical/subclinical AMR (42% vs. 8% in DSA-negative patients) and graft loss because of AMR (7% vs. 1%) were noted, despite intensified treatment.

One can argue that modifications of the protocol could increase efficiency of the desensitization protocol. In this respect, a small study by Loupy *et al.* [79] has to be mentioned, where DSA-positive renal allograft recipients were subjected to two different subsequent pre-emptive treatment protocols: a first cohort of 36 patients was subjected to pre-emptive high-dose IVIG together with ATG or IL-2 receptor antibody therapy; a second subsequent cohort of 18 recipients were treated with additional PP (immediately after transplantation and three times per week during 3 weeks) and rituximab (day 4; second administration according to B cell counts). Groups did not differ regarding acute AMR rates, however, in the second group less glomerulitis and capillaritis, a lower rate of transplant glomerulopathy, less frequent chronic AMR, and superior graft function were noted [79].

DSA persistence after XM-incompatible transplantation

Several authors have reported persistent levels of DSA following successful desensitization and transplantation [21,55,59,81]. There is some controversy regarding the actual relevance of such post-transplant reactivity. For example, Haas *et al.* [21] found an association of post-transplant DSA detection with subclinical AMR features and they suggested that antibody persistence could predict subsequent chronic injury. In contrast, in a smaller study of 12 patients by Gloor *et al.* [81] low levels of persistent DSA were not associated with the development of clinical AMR. In an elegant study, Zachary *et al.* [59] characterized distinct variables predicting the persistence of DSA following desensitization, such as HLA antigen specificity or antibody strength at the time of initiation of treatment [59].

Recipient allocation

Broadly sensitized recipients may strongly benefit from inclusion into specific allocation programs, either in the context of living (kidney paired donation) or deceased donor transplantation (e.g. Eurotransplant acceptable mismatch program) [82–86]. Such allocation strategies may help expand donor pools and increase the chance for successful transplantation in recipients otherwise unlikely to receive a transplant within an acceptable period of time [82–87].

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The Eurotransplant acceptable mismatch (AM) program was implemented to select compatible (XM-negative) donors for highly sensitized patients on the kidney waiting list [85,87]. In the AM program, acceptable mismatches are defined according to a subtle characterization of alloreactivity patterns using cell-based and solid phase assays in the context of HLA typing results. After identification of a suitable donor with a high probability of a negative XM, the organ is shipped immediately to the recipient unit, where the decisive XM is performed. Priority allocation via the AM program was shown to increase the chance to receive a compatible organ (6.6 months in 2008) and to enable excellent transplant outcomes [85,87].

A valuable tool for a more accurate assessment of permissible mismatches may be an analysis of HLA incompatibilities on the basis of a detailed characterization of immunogenic epitopes corresponding to amino acid polymorphisms on the surface of HLA antigens [88]. Using a computer algorithm (HLAMatchmaker), the epitope load of a given HLA mismatch can be individually calculated and predict immunological risks. For sensitized transplant candidates, epitope analysis of alloantibodies was shown to facilitate the identification of acceptable mismatches [88]. This approach has been incorporated as a valuable tool for identifying acceptable mismatches in the AM program [85,87].

Evaluating the results of the Eurotransplant AM program, it was noted that apparently because of unique HLA phenotypes, some patients failed to receive an organ within an acceptable waiting time and thus did not profit from this allocation approach. Such patients may benefit from desensitization prior to transplantation [89].

Recently, the Heidelberg group reported an impressive example of an integrative approach for high risk patients [86]. Their algorithm included a subtle evaluation of individual immunological risks using CDC- as well as solid phase HLA antibody testing and recipient desensitization with the option of CDCXM conversion using PP or IA and rituximab. In this study, every second patient was included in the Eurotransplant acceptable mismatch program to optimize matching and increase the chance of a compatible transplant offer. In a cohort of 34 deceased or living donor transplant recipients categorized as high immunological risk, excellent graft outcome comparable with those in nonsensitized patients was reported [86].

Treatment of acute AMR

A variety of anti-humoral treatment protocols were reported to effectively reverse acute AMR episodes. However, interpretation of available intervention studies, most of them uncontrolled series, may be impeded by small sample sizes, a marked heterogeneity of applied protocols, differences regarding the severity of treated AMR episodes or timing of treatment (early versus rescue therapy). Most authors have analyzed short-term outcomes and there are only scarce data available regarding long-term graft performance following acute AMR treatment [90]. The majority of published protocols are based on the application of serial apheresis therapy (PP or IA) for depletion of circulating alloantibodies and there is strong support for a primary role of antibody depletion by extracorporeal therapy as a critical cornerstone of AMR treatment [91].

PP-based AMR therapy

Before establishment of clear-cut diagnostic criteria for AMR, several prospective controlled studies have been conducted to analyse the efficiency of PP in the treatment of severe episodes of kidney transplant rejection. However, presumably as a result of varying criteria for patient inclusion, such studies have provided conflicting results [36–39]. More recently, uncontrolled studies have suggested efficiency of PP-based therapy in the treatment of biopsy-proven acute AMR. Some authors have applied PP as a sole anti-humoral strategy [92–94]. However, there is evidence suggesting that additional modalities may be necessary to improve success rates. For example, a recent observational study has suggested superior efficiency of combined treatment (PP plus IVIG) when compared with a historical control group subjected to PP alone [95]. As part of polypragmatic treatment protocols, PP was commonly combined with one or more additional measures, such as tacrolimus/MMF [96,97], deoxyspergualin [98,99], IVIG at low [47] or high dosage [100], ATG [64], or rituximab [101,102]. Overall, response rates between 70% and 100% have been reported. However, some severe AMR episodes may not adequately respond to treatment. A variety of small case series or anecdotal reports have now suggested reversal of refractory AMR episodes by rescue treatment with IA [103], bortezomib [27,28], anti-C5 antibody eculizumab [104,105], or splenectomy [106,107], respectively.

IVIG-based AMR therapy

Early studies have suggested efficiency of high-dose IVIG in the treatment of rejection. In a study of 10 rejecting renal or heart allograft recipients, IVIG treatment was found to effectively reduce anti-HLA antibody levels and reverse AMR [108]. In a randomized study, IVIG was evaluated in direct comparison with anti-lymphocyte antibody therapy (OKT3) in the treatment of steroid-resistant kidney allograft rejection [109]. Remarkably, IVIG was found to be as effective as OKT3 regarding response rates,

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course of allograft function, or transplant and patient survival [109]. Nevertheless, there is some evidence for limited efficiency of IVIG as a sole treatment. In a study of 24 renal allograft recipients with AMR, IVIG turned out to be less effective than a combined regimen consisting of PP, IVIG, and rituximab [91]. In this study, patients receiving IVIG alone ($n = 12$) had significantly worse graft survival (50% vs. 91.7%). Moreover, DSA levels were significantly lower following combined therapy [91]. In further support of superior efficiency of combined treatment regimens, Kapotsztas *et al.* [110] reported on better 2-year graft survival following addition of rituximab to a treatment protocol consisting of PP with or without IVIG. Importantly, in this study, a multivariate model also revealed a significant benefit from the use of IVIG [110].

IA-based AMR therapy

Earlier studies have suggested efficacy of IA with protein A in the treatment of severe refractory rejection episodes [111–113]. In these studies, IA was commonly initiated after other treatments, such as depleting anti-lymphocyte antibody, high-dose steroids, or even PP, have failed. More recently, anecdotal reports and small uncontrolled series have suggested efficiency of IA in the treatment of acute AMR defined according to modern biopsy- and serology-based criteria [103,114–117]. Initial results were promising and have prompted the design of an RCT to assess the efficiency of IA in the early treatment of severe C4d-positive AMR [40]. According to study design, all rejecting patients were converted to tacrolimus and received standard anti-rejection treatment (steroids and/or ATG) if additional cellular rejection was diagnosed. Patients randomized to IA received serial IA therapy with protein A columns. In the control group, no initial treatment was applied, however, patients had the option of rescue IA treatment after 21 days nonresponsiveness. After inclusion of 10 patients, the study was prematurely terminated for ethical reasons because of a high graft loss rate in the control group (four of five patients), while all five patients allocated to IA responded to treatment. Even though limited by small sample sizes, this study may strongly support efficiency of IA in the treatment of AMR [40]. To our knowledge, there is no RCT available or under way comparing the efficiency of IA with that of PP-based protocols in the context of AMR prevention and treatment.

Treatment and prevention of chronic AMR

In recent years, it has become evident that chronic AMR represents a leading cause of allograft injury and transplant loss [24–26]. However, there is currently only scarce data regarding treatment of this rejection type. A small initial case series (four kidney transplant recipients with chronic AMR) has suggested downregulation of alloantibodies and stabilization of graft function following conversion to tacrolimus/mycophenolate mofetil [118]. However, the results of this report could not be confirmed by a subsequent study including 11 rejecting recipients [119]. Notably, one of these patients was subjected to an extended course of serial IA. This treatment, however, failed to prevent progression of graft dysfunction [119]. There are now recent uncontrolled studies suggesting downregulation of alloantibody levels and stabilization of graft function by high-dose IVIG with or without rituximab [120–122]. However, available studies are limited by small sample sizes and the efficiency of such promising protocols will have to be proven in a larger controlled study.

One may argue that implementation of treatment at a stage of irreversible chronic graft injury may be too late to reverse ongoing injury and prevent graft loss. Hence, it may be critical to define early predictors of subsequent humoral graft injury, for example, on the basis of protocol biopsies or systematic post-transplant anti-HLA monitoring. However, there is increasing evidence suggesting that detection of circulating alloantibodies in patients with normal graft function may not necessarily predict inferior allograft performance [123–125]. These data warrant careful interpretation of AMR features detected in stable patients and potential benefits of pre-emptive treatment for the prevention of graft injury remain speculative.

Innovative therapeutic concepts

Proteasome inhibition

The proteasome inhibitor bortezomib is a selective inhibitor of the 26S proteasome that has proven highly effective in the treatment of malignant plasma cell disorders [126,127]. Proteasome inhibition was shown to cause a variety of cellular effects, such as inhibition of NF-κB activity, induction of endoplasmatic reticulum stress because of the accumulation of unfolded or misfolded proteins, and induction of cell cycle arrest and apoptosis [128]. Moreover, proteasome inhibitors were shown to affect antigen presentation by impaired peptide loading to MHC molecules, presumably a result of inhibition of short peptide synthesis [129]. Such effects may culminate in a modulation of several components of adaptive immunity, including T-cells [130–132] and dendritic cells [133]. In the context of prevention and treatment of AMR, a particularly relevant mode of action could be a direct effect on antibody-secreting plasma cells. Indeed,

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there is increasing evidence suggesting that, in contrast to other available anti-humoral modalities (e.g. IVIG), bortezomib has the potential to interfere with the integrity and function of nonmalignant auto- or alloantibody-producing plasma cells [28,134–137].

Recent clinical studies have provided evidence that bortezomib has the capability to downregulate circulating DSA and/or reverse (refractory) AMR episodes in organ transplant recipients [27–32]. However, most published protocols are polypragmatic and bortezomib was commonly administered together with one or more other treatment strategies, such as PP or rituximab. In this respect, two recent studies have to be noted, where bortezomib was applied without additional anti-humoral measures [33,34]. Wahrmann *et al.* [33] reported two sensitized dialysis patients, where two subsequent bortezomib cycles, the second combined with steroids, did not or only moderately affect levels of allosensitization. Sberro-Soussan *et al.* [34] reported that bortezomib applied as a sole treatment did not affect post-transplant alloantibody levels in kidney transplant patients with subclinical AMR. Such data reinforce the need for a prospective (controlled) study to clarify the true impact of bortezomib on sensitization and rejection.

Currently, several prospective studies evaluating the efficiency of bortezomib-based protocols are under way. Recently, a multicenter experience evaluating bortezomib-based treatment of AMR was initiated by the University of Cincinnati group (START Collaborative). In addition, further trials have been initiated designed to assess the impact of bortezomib-based regimens on recipient desensitization (ClinicalTrials.gov identifier: NCT00908583), on the course of mixed acute rejection (NCT00771875), or on antibody secreting cells in sensitized transplant candidates (NCT00722722).

Complement inhibition

Recent data suggest that inhibition of complement activation could be an effective means to prevent or reverse episodes of acute AMR. There is now promising data regarding the use of eculizumab, a humanized anti-C5 monoclonal antibody approved for the treatment of paroxysmal nocturnal hemoglobinuria. Binding of C5 may inhibit its cleavage to C5a and C5b and halts the formation of the membrane attack complement. In the context of kidney transplantation, eculizumab was recently shown to be effective in the prevention and treatment of recurrent atypical hemolytic uremic syndrome [138,139].

In a recent case report, eculizumab applied together with PP, IVIG and rituximab, was reported to effectively reverse an episode of severe AMR [104]. In a second report, eculizumab was shown to reverse AMR in a highly sensitized recipient of a kidney paired donor desensitized with bortezomib, IVIG, and rituximab [105]. These initial data are promising and future studies will have to assess the efficiency of C5 blockade or of other strategies, such as complement inhibition at the level of C1 [140] in the prevention and treatment of AMR.

Conclusion

The implementation of innovative tools for assessment of presensitization and diagnosis of AMR, and the establishment of an efficient repertoire of anti-humoral treatment strategies have now led to considerable improvements in the management of sensitized and/or rejecting kidney allograft recipients. Nevertheless, there is still place for further improvement. In this respect, innovative treatment concepts, such as proteasome inhibition or complement blockade, may be of particular interest. Future systematic studies will have to clarify the actual contribution of such treatments to the success of recipient desensitization or treatment of acute and/or chronic AMR.

Authorship

GB, ES, and GAB: critically collected and reviewed the literature and wrote the review article.

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