Kidney transplantation in highly sensitized patients

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Accepted 12 March 2015

Abstract

Background: Desensitization, a term loosely referring to a collection of antibody reduction and B-cell depletional therapies aimed at improving rates of transplantation in highly HLA and ABO-incompatible transplant recipients, has seen significant growth in the last decade. Advancements relate to an increasing unmet medical need for FDA-approved therapies, advancements in antibody detection methodologies and improved renal pathological assessments of antibody-mediated rejection (ABMR).

Sources of data, areas of agreement and controversy: Data reviewed include collective summaries of experience with high-dose intravenous immunoglobulin (IVIG), B-cell depletion with rituximab and the use of plasma exchange with low-dose IVIG. Consensus suggests that these protocols are the most commonly used while experiences with other agents (i.e. bortezomib) are evolving. Controversy exists as to the extent of resources required, expense and outcomes of desensitization protocols.

Growing points or areas timely for developing research: Here we review and synthesize data from evolving protocols and summarize developments of novel biologics aimed at modification of B-cells, antibodies and complement activation which will likely improve desensitization and treatment of ABMR.

Key words: desensitization, kidney transplant, IVIG, rituximab, antibody-mediated rejection, immunotherapy, complement, IL-6, eculizumab, C1-inhibitor

Introduction

Kidney transplantation is the preferred treatment for end-stage renal disease; however, rates of transplantation remain low for patients who become sensitized to human leukocyte antigens (HLA). Here, anti-HLA antibodies and memory B and T cells create an immunologic barrier, linked to an increased risk of antibody-mediated rejection (ABMR) and poorer
graft survival\textsuperscript{2,3} that remains a persistent and often impenetrable deterrent to transplantation. Approximately 30\% of the patients on the United Network for Organ Sharing (UNOS) wait list are considered highly sensitized. These patients have detectable alloantibodies generated through exposures to previous transplants, blood transfusions and pregnancy. Rates of transplantation remain low and have not improved for decades. Currently, only 6.5\% of patients with a panel reactive HLA antibody (PRA) levels above 80\% [i.e. highly sensitized (HS)] receive a transplant each year. Without the possibility of transplantation, these patients remain on dialysis and die at more than double the rate of sensitized patients who received transplants after desensitization.\textsuperscript{4-6} Clearly, providing life-saving transplants to HS patients is a significant and, as yet, unmet medical need. Recent attempts devoted to improving patient and graft survival in HS patients incorporate use of desensitization protocols combining B lymphocyte-depleting agents (rituximab, anti-CD20), intravenous immunoglobulin (IVIG) and plasmapheresis,\textsuperscript{5,7,8} along with better stratification of immunological risk using sensitive donor-specific anti-HLA antibody (DSA) screening and avoidance techniques. In addition, the use of desensitization with paired donor exchange may also improve rates of transplantation for sensitized patients. Despite reports of improved short-term graft survival, the impact of these strategies is unclear, since ABMR may occur in 30–40\% of cases, comprising the primary cause of early graft loss. In addition, the persistence of DSAs post-transplant can result in a chronic form of allograft injury termed transplant glomerulopathy (TG) that rapidly dissipates allograft function resulting in graft failure and return to dialysis with attendant emotional consequences for the patients and financial consequences for the health care system.\textsuperscript{3,9,10}

In this review, we discuss the current state of desensitization and identify novel and as yet emerging and untested approaches that are likely to improve access to life-saving transplants for these immunologically disadvantaged patients. We will also discuss how knowledge generated in the development of desensitization protocols has led to advancements in recognition and treatment of ABMR.

Current approaches to desensitization

Desensitization protocols emerged in the 1990s to address the increasing numbers of HS patients who waited years, often in futility, for a successful kidney transplantation.\textsuperscript{11} These approaches using combinations of IVIG, plasma exchange (PLEX) and rituximab have now gained acceptance. A recent publication summarized in the proceedings of the Food and Drug Administration (FDA) symposium on desensitization and ABMR treatment addressed the most important concern is the lack of any FDA-approved drugs for desensitization or treatment of ABMR.\textsuperscript{12} Despite these concerns, recent publications have summarized outcomes and what is considered ‘best practices’ for desensitization.\textsuperscript{13} Outcomes of desensitization have overall been good,\textsuperscript{5,14-16} but reports of desensitization failures are noted and controversy has arisen regarding the cost and efficacy of desensitization.\textsuperscript{17,18} A recent report by Orandi et al.\textsuperscript{19} evaluated outcomes of desensitization at 22 US centers. Assessments of patient and graft survival showed that attempts to desensitize patients with positive complement-dependent cytotoxicity crosshatches (CDC-CMX) showed poor long-term graft and patient survival compared with non-sensitized controls. Outcomes in patients with flow cytometry crossmatch (FCMX) positive (CDC-) and luminex single antigen bead DSA + (CDC- and FCMX-) were comparable or slightly less than non-sensitized patients. Progress in desensitization has been inconsistent due primarily to the complexity of creating a nexus of antibody reduction therapy, organ donor availability and acceptable crossmatch with timing of transplantation to avoid ABMR. Our group recently reported on risk factors associated with the development of ABMR after desensitization. One important finding of this investigation was that early and severe ABMR was difficult to treat, was associated with inferior 1-, 3- and 5-year graft survivals and was significantly associated with a higher incidence of death once patients returned to dialysis.\textsuperscript{20} These data and that of Orandi point out the importance of patient selection for desensitization protocols and the low likelihood of achieving good outcomes in patients who have positive CDC-CMXs at time of transplantation.
It is our opinion that, given the limitations of current desensitization protocols, this should be avoided. It is also important to recognize that outcome data compiled by the Scientific Registry for Transplant Recipients (SRTR) does not recognize highly HLA-sensitized patients as high risk; therefore, centers performing incompatible transplantation do so without risk adjustments and with possibility of being cited by Medicare for poor outcomes.20 Thus, the current system does not favor innovation in immune modulation for desensitization and treatment of ABMR.20 This has raised a number of concerns regarding the ability of transplant researchers and clinicians to provide innovative solutions to the vexing problem of sensitization through creative scholarship and clinical research. We recently reported on the efficacy, side effects and outcomes of desensitization with IVIG + rituximab.21 We compared outcomes to a similar group of age-matched and ESRD cause-matched patients with CPRA > 80% who remained on dialysis. This is important since dialysis is the only option for broadly sensitized patients. The mean wait time on dialysis for our sensitized patients was 114 ± 56 months before desensitization. After desensitization, the mean wait time to transplantation was 4.4 ± 4.9 months. We were able to transplant 146 of 207 patients treated. Most patients remained crossmatch and DSA positive at the time of transplantation. Thus, reduction in crossmatch and DSA levels is the goal since total elimination of DSAs is rarely possible. With this approach, outcomes similar to those seen with non-sensitized patients at 3 years were obtained.6 Figure 1 shows the overall approach to desensitization and management of DSAs post-transplant at Cedars-Sinai Medical Center.

We recently assessed the cost/benefit analysis of desensitization compared with dialysis. This study assessed all costs associated with transplantation after desensitization including medications, organ acquisition, treating rejection episodes and cost of returning to dialysis for those with failed allografts. We found the costs compared favorably with the costs of remaining on dialysis over the 3-year study period.16 The most critical issue was the patient survival benefit seen in transplanted patients. At 3 years, the transplanted patients had a 14.7–17.6% greater probability of survival than those remaining on dialysis. Thus, desensitization offers HS patients a significant improvement in transplant rates with improved outcomes in quality and length of life and reduced cost to the health care system.

**IVIG for desensitization**

IVIG can be referred to as a natural modulatory of inflammation and immunity.22 Immunomodulatory activities were first noted in the 1980s when patients with immune thrombocytopenia normalized platelet counts after IVIG infusions.23 Over the years, numerous explanations regarding the mechanism(s) of action of IVIG have emerged. These include inhibition of T-cell proliferation, inhibition of inflammatory cytokine synthesis and action and inhibition of complement activation and anti-idiotypic blockade of alloantibodies. Immunomodulation by IVIG was recently reviewed.24 Recent data from humans receiving high-dose IVIG are strongly supportive of an interaction of IVIG with macrophages and dendritic cells (DCs) which suppresses DC maturation in vivo. IVIG infusions result in an upregulation of the Th2 cytokine, IL-33 from DCs and lymph node cells which subsequently increases IL-4 and IL-13 production. These Th2 cytokines decrease expression of the inflammatory FcγRIIA resulting in a net upregulation of the inhibitory receptor FcγIIB. FcγIIB is the only FcR on B cells and plasma cells. Cross linking results in plasma cell apoptosis and reduced B-cell activation and antigen-presenting cell (APC) activity.25 IVIG also inhibits expression of the activating receptor IFN-γR2, reducing IFN-γ signal transduction. Overall, these observations may explain why IVIG has significant anti-inflammatory activities and inhibits B cell and regulates antibody production.26 Furthermore, recent data25 suggest an expanding role for IL-33 in curbing inflammation by promoting a potent expansion of FoxP3+ regulatory T cells. IVIG infusions have also been associated with FoxP3+ T-cell expansion in humans.

The only randomized control trial of IVIG as a desensitization agent was conducted by our team (1997–2002).14 This was a multicenter, placebo-controlled trial and showed efficacy of IVIG as a
A desensitization agent resulting in improved transplantation rates for highly HLA-sensitized patients (35% IVIG vs. 17% in placebo; \( P < 0.05 \)). The deceased donor (DD) transplantation rates were 31 versus 12% for placebo (\( P = 0.0137 \)). This group was matched for all significant parameters. Graft survival for the IVIG group was 80 and 75% for the placebo group at 30 months (\( P = \text{NS} \)). Adverse events were also assessed and were minimal and similar to placebo.\(^{14,16}\) Numerous studies have been performed using high-dose IVIG for desensitization and have been summarized in other publications.\(^ {27} \) However, reports by our group and others suggest that high-dose IVIG alone is not sufficient to render enduring desensitization and is often associated with antibody rebound and ABMR.\(^ {5} \) Data recently published by our group suggest that long-term outcomes of patients desensitized with IVIG alone are inferior to patients receiving desensitization with IVIG + rituximab.\(^ {21} \)

**Rituximab for desensitization**

B cells are significant contributors to the pathogenesis of allograft rejection, both T cell mediated (TCMR) and ABMR. B cells present antigen to CD4+ T cells and collaborate with DCs and other APCs.\(^ {28-30} \) B cells express co-stimulatory molecules (B7, CD40) that co-stimulate activated T cells and enhance progression to full effector and cytotoxic

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**Fig. 1** Cedars-Sinai Medical Center algorithm for management of highly sensitized patients. Briefly, this figure shows the overall approach of desensitization at Cedars-Sinai Medical Center. Highly HLA-sensitized patients referred to the program are screened for HLA antibodies and wait time. When appropriate, desensitization with IVIG + rituximab is initiated. If an acceptable crossmatch is not achieved within 6 months, PLEX + IVIG + rituximab is performed and HLA antibodies assessed. Once transplanted patients have DSA determinations monthly times 3M then every 3 M for the first year, then yearly if stable. If patients present with rising DSAs, biopsies are performed and if ABMR is present, treatment is commenced as shown. Chronic ABMR (TG) unresponsive to treatment with IVIG + rituximab may be treated with tocilizumab.
functions. In addition, alloantigen-specific B cells progress to plasma cells and produce DSAs that can activate complement and induce antibody-dependent cellular cytotoxicity (ADCC)\textsuperscript{28–30} with allograft injury. B-effector cells produce pro-inflammatory cytokines such as lymphotokinin-α, IFN-γ and IL-6.\textsuperscript{31–33} IL-6 producing B cells are associated with relapse in multiple sclerosis patients with remission induced by B-cell depletion using rituximab.\textsuperscript{34} B-regulatory subsets (Bregs) have the capacity to modify inflammation and autoimmune. Here, total B-cell depletion may actually increase immune activation\textsuperscript{35}

Rituximab is a monoclonal antibody specific for CD20, a member of the membrane-spanning-4-domain A family of proteins. CD20 is highly expressed on B cells, but CD20 is lost with transition to plasma cells. CD20 is closely associated with the B-cell receptor (BCR) and is vital in controlling BCR-activated calcium influxes.\textsuperscript{35,36} Rituximab has now shown efficacy in a number of B-cell malignancies and autoimmune diseases. Two recent publications helped illuminate the role of CD20 in B-cell immunity. First, a child with common variable immune deficiency was found to have CD20 mutations resulting in absent CD20 protein expression.\textsuperscript{36} This patient demonstrated hypogammaglobulinemia and was found to have a defect in immune responses to T-dependent antigens. Similar findings in a murine model were reported by Morsy et al.\textsuperscript{35} in CD20-deficient mice. The authors conclude that CD20 has a critical role in B-cell activation and T-dependent humoral immunity. These observations suggest that absence of CD20 significantly impairs APC activity of B cells. Recent data from Kamisawa et al. showed that B-cell depletion with rituximab in patients with IgG4-related disease is associated with rapid depletion of IgG4+ plasma cells and resolution of T-cell infiltrates of affected tissues. These investigators suggest that depletion of activated B cells removes important co-stimulation from T cells and cytokines that drive plasmablast to IgG4-secreting plasma cells.\textsuperscript{37}

There have been a number of interesting advancements in understanding the role of B cells and alloantibodies in kidney transplantation. Zachary et al.\textsuperscript{38} assessed allo-reactive antigen-specific B cells in DSA-negative patients with historic DSA positivity after transplantation. Two groups of patients were identified. The first were those who did not receive prior rituximab therapy and a similar group of patients who received rituximab prior to transplantation. Analysis showed that the post-transplant emergence of allo-reactive B cells was common in those patients not receiving rituximab but was usually inhibited with rituximab. The authors’ conclude that early treatment with rituximab abrogates anamnestic responses. A more recent study by Jackson et al. from the Hopkins group suggests that with longer follow-up patients treated with rituximab do develop DSAs.\textsuperscript{39} Kohei et al.\textsuperscript{40} reported that treatment of ABOi transplant patients with rituximab resulted in long-term prevention of de novo DSA development and reduced ABMR episodes compared with a cohort of non-ABOi living donor (LD) transplant recipients not treated with rituximab. They concluded that B-cell depletion prior to transplant prevents B-cell activation, de novo DSA production and chronic ABMR. Lynch et al.\textsuperscript{41} reported that non-sensitized LD transplant recipients rapidly developed detectable allo-reactive B cells-secreting HLA Class I-specific DSAs within 1 month post-transplant. The authors conclude that cryptic B-cell responses to allografts are common and may have long-term implications for graft survival.

Van Den Hoogen et al. reported on a placebo-controlled trial of rituximab as an induction agent for kidney transplant recipients.\textsuperscript{42} Two hundred and eighty patients were examined (138 randomized to rituximab and 142 placebo). After 6 months, there was no difference in graft rejection rates. However, assessment of high-risk patients (repeat transplants and HLA sensitized) showed a significant reduction in rejection episodes in the rituximab group (17.9 vs. 41.1%, \(P = 0.039\)). The authors conclude that a single dose of rituximab given as an induction agent significantly reduces rejection rates in sensitized patients. Data from a recent blinded, placebo-controlled trial of IVIG + placebo vs. IVIG + rituximab for desensitization performed by our group also demonstrated a critical role for rituximab in prevention of DSA rebound, acute and chronic ABMR and graft loss post-transplant.\textsuperscript{21} Taken together, these studies
reinforce the importance of both DSAs and B cells in mediation of allograft rejection and suggests that improved techniques for monitoring B-cell responses may aid in prevention of acute and chronic ABMR.

**Bortezomib**

Bortezomib (Velcade®, Millenium pharmaceuticals, Johnson & Johnson) was FDA approved in 2003 for treatment of refractory multiple myeloma. Bortezomib inhibits the 26 s proteasome that ultimately leads to plasma cell apoptosis. Bortezomib has been used as an adjunctive agent with rituximab and PLEX, demonstrating significant decrease in HLA antibodies—a thorough review of the efficacy of bortezomib as a desensitization, and agent to treat ABMR is highlighted in a recent review by Abu Jawdeh et al.27

**PLEX + low-dose IVIG**

Probably, the most popular desensitization protocol is low-dose IVIG + PLEX. Removal of alloantibody by PLEX followed by IVIG (100–150 mg/kg) has yielded excellent results with >90% success in transplanting LD HLA-sensitized and ABOi patients. Montgomery et al. published the most definitive assessments of outcomes from the PLEX + low-dose IVIG protocol.8 Outcomes in 211 patients followed for up to 8 years showed a significant reduction in risk of mortality for HS patients who underwent desensitization using PLEX + low-dose IVIG and transplantation compared with patients who remained on dialysis or received dialysis and HLA-compatible transplants. Data from Orandi et al.19 also suggest that good long-term patient and graft survival can be achieved if proper patient selection is used before attempting desensitization and transplantation. Despite all advances, ABMR, both acute and chronic, remains a significant obstacle to the long-term success of incompatible transplants.19,27

Other authors43,44 have developed novel approach to desensitization of crossmatch-positive DD recipients. Bohmig et al.43 describe the use of plasma exchange immediately before transplant to remove DSAs. In their hands, this has yielded good results. Loupy et al. also described a post-transplant approach to desensitization using high-dose IVIG, rituximab and PLEX.44 Both approaches appear to be useful in desensitization patients with low to moderate DSA levels and preventing early ABMR.

**Defining acceptable crossmatch and DSA parameters for HLA incompatible transplantation**

What is clear from our experience and that of others19 is one should avoid transplanting patients who are CDC-CMX positive at time of transplantation. However, we can create ‘acceptable’ CMX parameters that allow for incompatible transplantation with low risk for ABMR.45

Briefly, unacceptable antigens are assigned as those expected to produce a positive CDC-CMX. Also excluded are those antigens in the high-binding Luminex-platform single antigen assay range 10 000 mean fluorescent intensity (MFI). These antigens were entered into UNet to optimize identification of a compatible DD. Our previous reports indicate that DSA levels below these threshold criteria minimize the risk for ABMR after transplantation.45 Negative CMX was defined by a FCMX <130 mean channel shift (MCS) for B cell and <70 MCSs for T-cell pronase. Pronase treatment was used to remove CD20 from B cells and non-HLA antigens from T and B cells, allowing more precise determinations of HLA specificity and eliminating rituximab effect. DSA binding was determined by the multianalyte bead assay performed on the Luminex platform.45 The strength of the reactions was graded as weak (<5000 MFI), moderate (5000–10 000 MFI) and strong (>10 000 MFI). Antibody specificities and strengths are compared with those obtained before desensitization. To simplify analysis, we created a scoring system to represent MFI intensity of DSAs. The DSA-RIS gave 0 points for no DSA, 2 points for each weak DSA (MFI <5000), moderate (5000–10 000 MFI) and strong (>10 000 MFI). Patients with a DSA score of >17 had ~91% chance of developing ABMR. Thus, patients with scores >17 did not proceed to transplant.

Desensitization was considered successful if post-therapy donor-specific CMX was acceptable, as
determined by negative CDC in 1:2 or higher dilution, or FCMX with a shift of < 225 MCSs and DSA scores \( \leq 17 \). Using this approach, we have reduced our ABMR rate to \(~16\%\) in the first-year post-transplant. See Figure 1.

### New advances

There are a number of novel agents currently FDA approved for use in oncologic or immunologic disease states that have shown potential for use as desensitization agents and have potential for post-transplant allograft maintenance. These agents target cytokines, B-cell growth factors and plasma cells and IgG molecules as well as the complement system (Table 1).

### Interleukin-6 (IL-6) receptor antagonist (Tocilizumab)

Tocilizumab (Actemra\textsuperscript{®}, Genentech-Roche) is an IL-6 antagonist for both soluble and membrane-bound IL-6 receptors. This humanized monoclonal antibody was FDA approved in January 2010 for moderate to severe rheumatoid arthritis, in April 2011 for systemic juvenile idiopathic arthritis (SJIA), and April 2013 for polyarticular juvenile idiopathic arthritis (PJIA). Subcutaneous formulation was FDA approved in October 22, 2013 with recent data revealing that subcutaneous infusion has more injection site reactions vs. intravenous administration.\textsuperscript{46} Animal models reveal that anti-IL-6 receptor therapy weakens alloantibody responses by modulating T-regulatory, B-effector and plasma cells in bone marrow.\textsuperscript{47} In addition, IL-6 stimulates Th17 cells that increase inflammation and allograft rejection.\textsuperscript{48} IL-6 is one of the major cytokines involved in progression of B cells to IgG-secreting plasmablast and finally to plasma cells. By targeting the IL-6/IL-6R pathway, reduction in antibody production and increases in Treg cells are likely. We recently completed a Phase I/I trial of anti-IL-6R therapy for HS patients who failed standard desensitization (NCT01594424).

<table>
<thead>
<tr>
<th>Medication (generic/trade name)</th>
<th>Mechanism of action</th>
<th>Potential use in kidney transplant (clinical trial reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tocilizumab (Actemra\textsuperscript{®})</td>
<td>Soluble and membrane-bound IL-6 receptor antagonist</td>
<td>• Desensitization (NCT01594424) • Treatment of antibody-mediated rejection</td>
</tr>
<tr>
<td>Belimumab (Benlysta\textsuperscript{®})</td>
<td>Prevents B-lymphocyte stimulator protein from stimulating B-cell activation and differentiation</td>
<td>• Desensitization (NCT01025193, terminated) • Prevention of kidney transplant rejection (NCT01536379)</td>
</tr>
<tr>
<td>C1 esterase inhibitor (Berinert\textsuperscript{®})</td>
<td>C1 inhibitor inactivates both C1r and C1s of the complement pathway</td>
<td>• Prevention of antibody-mediated rejection (NCT01134510) • Delayed graft function and ischemic reperfusion injury (NCT02134314)</td>
</tr>
<tr>
<td>C5 inhibitor (Eculizumab\textsuperscript{®})</td>
<td>C5 inhibitor preventing cleavage to C5a and C5b preventing formation of C5b-9, terminal complement complex</td>
<td>• Desensitization (NCT01567085) • Delayed graft function and ischemic reperfusion injury (NCT01756508, NCT0919346) • Kidney Transplantation in Catastrophic Antiphospholipid Antibody Syndrome (NCT01029587) • Antibody-mediated rejection (NCT01327573, NCT02113891) • Desensitization (NCT 02224820)</td>
</tr>
<tr>
<td>IgG Endopeptidase (Ides\textsuperscript{®})</td>
<td>Cleavage of all four classes of Human IgG</td>
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Table 1 Novel therapeutic agents for kidney transplantation

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Anti-B-cell activating factor (Belimumab)

Belimumab (Benylsta®, GlaxoSmithKline) inhibits growth and differentiation of B cells by blocking B lymphocyte stimulator (also known as BlyS) and is FDA approved for treatment of adults with active, autoantibody positive, systemic lupus erythematosus. Belimumab has been studied as a desensitization agent in kidney transplantation (NCT01025193); however, the study was terminated early for reported lack of efficacy. Currently, a Phase 2 double-blinded, randomized, placebo-controlled trial of Belimumab plus standard of care is being examined for prevention of allograft rejection in renal allograft recipients (NCT01536379).

IgG endopeptidase

IgG endopeptidase (Idex®, Hansa Medical) is a bacterial enzyme selected from Streptococcus pyogenes that cleaves all four human subclasses of IgG at positions 236 and 237 of the lower hinge region of IgG heavy chains yielding F(ab’)2 and Fc. The precise cleaving of the IgG molecule may be helpful in lowering antibody levels in the highly sensitized kidney transplant recipients prior to receiving kidney transplantation. Phase 2 trials are currently taking place in Europe focusing on safety, tolerability, pharmacokinetics and efficacy of use in chronic kidney disease patients (NCT02224820). Safety information from previous studies revealed no adverse events of concern.

Complement: an important mediator of kidney allograft injury

Activation of the complement system

A summary of the complement cascade activation pathways are shown in Figure 2A. There are >30 soluble and membrane-bound proteins that are activated by three separate activation systems. Initiation of all three pathways results in activation of C3 and formation of C3a and C3b. C3a is an anaphylatoxin while C3b combines with the C3 convertase (C4b2C3b) to form C5 convertase. C5 is cleaved to C5a (potent anaphylatoxin) and C5b. C5b activated C6–9 forming the C5b-C9 membrane attack complex (MAC). Activation of the cascade results in the activation of T effector cells, B-cells (with increased antibody production) polymorphonuclear leukocytes and as well as endothelial cell injury and formation of microthrombi. The highly HLA-sensitized patient is prone to complement-mediated injury that is initiated by C1q-activating DSAs. ABMR has very distinctive features that often include the deposition of C4d complement fragments at the site of injury. If unmitigated, ABMR results in rapid destruction of the allograft. More chronic forms of ABMR also exist and may be less dependent on CDC and more likely mediated ADCC. Figure 2B shows the complete complement activation cascade and the points of intervention by current anti-complement therapies.

Complement and antibody-mediated rejection

Current concepts suggest that the pathophysiology of ABMR depends on antibodies. However, a growing body of experimental and clinical evidence suggests that other effector mechanisms, especially the complement system, participate in tissue damage and graft dysfunction/loss induced by antibodies. These studies point to additional potential targets that might help to ameliorate ABMR. Despite the success of current therapies, post-transplant ABMR and chronic ABMR (CABMR) remain significant problems. Antibody- and complement-mediated injury to allografts accounts for the majority of TG and late renal graft loss. Recent data suggest that complement inhibition using anti-C5 (Eculizumab®, Alexion Pharmaceuticals) was highly effective in preventing ABMR in HS patients desensitized with PE + IVIG. The expected rate of ABMR in this patient group was 42% based on historical controls, but only 7% actually experienced ABMR in that single-arm study. However, preliminary reports of a placebo-controlled trial of Eculizumab® for prevention of ABMR in sensitized transplant patients failed to meet the composite statistical end point.

Papers from the Paris Necker group have also described important role of eculizumab in prevention
Fig. 2 (A) This figure depicts the complement activation pathways with known consequences. Briefly, DSA binding to HLA or other autoantigens on allograft endothelium activates the classic complement pathway (CP) by interacting with C1qrs. The lectin pathway is activated by mannose-binding serine proteases (MAS) which binds to mannose-binding lectin on bacteria cell walls. The MBL pathway is also activated by I/R injury. Activated C1qrs activate C2 and C4 that form C3 convertase, converting C3 to C3a and C3b. Subsequent activation of the terminal complement cascade then proceeds with attendant inflammatory and lytic events mediated by C5a and C5b-C9 MAC. The alternative pathway (AP) is activated by a tick-over mechanism when C3 is spontaneously bound to H2O. C3(H2O) then

A

Complement Activation Pathways

CP

DSA/HLA Complexes

C1q C1r/s

C2 C4

C3 Convertase

fB/fD

C3b-dimer

C5 Convertase

C5b C6-C9

LP

Carbohydrate Manose

MBL/Ficolins MASP

C3 Convertase

fB/fD

C3b-dimer

C5 Convertase

C5b C6-C9

AP

Tick-over

C3(H2O)

MAC

B

Complement Activation Pathways

CP

C1INH

C1q C1r/s

C2 C4

C3 Convertase

fB/fD

C3b-dimer

C5 Convertase

C5b C6-C9

LP

Carbohydrate Manose

MBL/Ficolins MASP

C3 Convertase

fB/fD

C3b-dimer

C5 Convertase

C5b C6-C9

AP

Tick-over

C3(H2O)

MAC

Proinflammatory Signals

Amplification Phagocytosis

Cell Lysis

Proinflammatory Signals

Amplification Phagocytosis

Cell Lysis
of recurrence of atypical hemolytic uremic syndrome (aHUS) and antiphospholipid syndrome (APLS) post-transplant. Indeed in our own anecdotal experience, we have seen excellent results in reversing kidney injury in three patients with catastrophic APLS. Thus, the benefits of C5 inhibition extend beyond the pathogenic effects of DSAs, especially in patients with aHUS and APLS.

C1 esterase inhibitor (Berinert®, C1-INH, CSL-Behring) is a multi-functional member of the serpin family of protease inhibitors. C1-INH inactivates both C1r and C1s and is the only plasma protease that regulates the classic complement pathway. C1-INH can also inhibit the analogous serine proteases in the lectin pathway of C activation. During C1qrs activation by antibody/immune complexes, C1-INH can dissociate C1r and C1s from the activated C1 macromolecule, thus preventing proteolytic activation of C4 and C2 which forms C3 convertase. However, there is very little information regarding the effects of C1-INH on ABMR in humans, and no data on the safety or efficacy of C1-INH in prevention or treatment of ABMR.

We recently completed a blinded, placebo-controlled trial of C1-INH for prevention of ABMR in highly HLA-sensitized patients. Twenty patients were enrolled in this Phase I/II trial and results showed that no patient in the C1-INH group developed ABMR during the 1-month study period. Analysis of complement levels during the study suggested an important inhibitory effect on systemic complement activation after transplant surgery and incompatible kidney transplantation. Complement-activating antibodies were also strongly inhibited in the C1-INH treatment group where no effect was seen in the placebo group. These results are also encouraging and support the need for larger studies of C1-INH in the prevention and treatment of ABMR.

Complement and ischemia/reperfusion injury

There is now a growing body of evidence that suggests that innate complement activation occurs in the kidney with generation of C3 convertase, C3a, C5a and the MAC, C5b-C9 after ischemia/reperfusion injury (I/R). In addition, Damman et al. performed microarray analysis of >588 biopsies from high-risk extended criteria and donors (ECD) after cardiac death (DCD). These investigators found significant increases in gene signals for hypoxia, complement activation and coagulation. Their work suggests that inhibition of complement activation may be an important strategy for prevention of (I/R) injury in kidney allografts. Both anti-C5 and C1-INH are now being investigated in larger clinical trials for prevention of I/R-induced delayed graft function (Eculizumab NCT01756508, C1 Esterase Inhibitor NCT02134314).

Conclusions

Much progress has been made over the last decade in removing immunologic barriers to transplantation through desensitization. The nexus of significant advances in antibody detection methods, pathological classification of antibody-mediated allograft injury and development of therapeutics aimed at antibody reduction and modification of effector functions such as complement activation has helped establish desensitization as a more robust therapeutic entity in transplant medicine. Advancements in desensitization will also have significant implications for transplantation.
for treatment of ABMR and for prevention of allograft loss from chronic antibody-mediated TG. Clinical trials of novel agents are essential for improving the access to and success of kidney transplantation in the future.

The future of desensitization

From our standpoint, we feel the therapy with the greatest potential for impacting desensitization and treatment of ABMR will likely emerge from novel antibody reduction therapies (i.e. IdeS and anti-IL-6R) and inhibition of complement activation (i.e. anti-C5 and C1INH). Attention to both is required since antibody reduction therapies to date have not been sufficient to always prevent ABMR, and early results with complement inhibitors show that chronic non-complement-dependant pathways of injury result in TG.

However, these are exciting times for transplant physicians and immunologists who have long struggled with the immunologic barriers to transplantation created by B-cell antibodies and complement. There is much work to be done. It will be important to create a nexus among immunologists, clinicians and the biotech industries to define and implement the best way forward.

Acknowledgements

We thank the members of the Kidney Transplant Program, HLA Laboratory and Transplant Immunology Laboratory for their untiring efforts on behalf of our patients.

Conflict of Interest statement

A.V. and J.C. report no conflict of interest.

Funding

S.C.J. has research grants from CSL-Behring and Genentech and has served as a consultant for CSL-Behring, Genentech and Bristol Myers Squib, Inc.

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