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REVIEW

The emerging role of rituximab in organ transplantation

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Summary

Long-term acceptance of solid organ allografts remains a challenge. While many acute rejection episodes can be treated, new mechanisms of allograft damage are now being defined especially in kidney transplantation. Unexpected clusters of $CD20^+$ cells have been discovered in renal biopsies performed for clinical rejection. C4d deposition is now routinely seen in refractory rejection. Despite the rapid introduction of new immunosuppressive agents in transplantation, the search for an efficacious anti-B-cell agent remains. With novel mechanisms of allograft damage now being defined, it is important to consider how an anti-B-cell agent might fit into an immunosuppressive regimen. Rituximab is a high-affinity CD20 specific antibody that depletes the B-cell compartment by inducing cellular apoptosis. Thus, it is a rational choice for therapy in transplantation to abrogate B-cell mediated events. In this review, we will discuss the mechanisms of action of rituximab, and its use in for a variety of indications in solid organ transplantation. There are emerging case reports that show that rituximab may be an effective agent to treat antibody-mediated rejection, and post-transplant lymphoproliferative disorder. Rituximab has been frequently cited as an important adjunct therapy in desensitization protocols for highly sensitized transplant recipients as well as recipients of ABO incompatible transplants. Rituximab demonstrates promise in this regard and warrants additional consideration in prospective clinical trials.

Introduction

While much of transplantation has focused on the role of T cells in allograft dysfunction, it is becoming increasingly clear that B-cell mediated events also play a vital role in long-term allograft outcomes. CD20 is a hydrophobic transmembrane protein that is located on pre-B and mature B cells [1]. Rituximab (Biogen-IDEC and Genentech pharmaceuticals, San Francisco, CA, USA) is a chimeric monoclonal antibody that reacts with the CD20 antigen. Rituximab was first approved in 1997 for use in treatment of relapsed or refractory B-cell non-Hodgkin's lymphoma [2,3].

Rituximab's structure is a key to its effects. It is a chimeric monoclonal antibody composed of human immunoglobulin (Ig) G_1 heavy chain and kappa light chain constant regions and variable light and heavy chain murine regions. Rituximab directly inhibits B-cell proliferation and induces cellular apoptosis [4,5], through the binding of complement. Complement, in turn, mediates antibody-dependent cell-mediated cytotoxicity and subsequent cell death.

The immunomodulatory effects of rituximab have been shown in nonhuman primate studies. Baboon $CD20^+$ B cells are effectively depleted by rituximab [6]. Both IgM and IgG responses were significantly blunted when compared with control baboons stimulated with dinitrophenol-KLH. In addition, baboons treated with rituximab did not mount a memory IgG response to a simple hapten. In humans, Bearden *et al.* [7] have shown that rituximab inhibits the primary and secondary antibody responses to a T-cell-dependent antigen bacteriophage. Viera and colleagues treated dialysis patients with rituximab to determine whether there was

an effect on panel reactive antibodies (PRA). A single dose of rituximab resulted in a decrease of PRA in one patient from 87% to 51% with a decrease in fluorescence intensity. Five patients had changes in histogram architecture consistent with a loss of antibody specificity [8]. While rituximab was first used for the treatment of lymphoma, clinicians quickly recognized that this therapy might be useful for autoimmune disorders. In 1999, Levine and Pestronk [9] treated five patients with IgM antibody-related polyneuropathy. All patients showed sustained improved strength. We first reported the use of rituximab in transplant patients in 2000 [10], successfully treating rejection episodes in five individuals who had not responded to other forms of treatment. We hypothesized that rituximab in targeting $CD20^+$ cells decreased B-cell proliferation and limited antibody production.

In studies of allosensitized dialysis patients, rituximab depletes $CD19^+$ and $CD20^+$ cells within 48 h post-therapy [11]. A reduction was sustained in follow-up studies for as long as 12 months. Importantly, immune reactivity as demonstrated by PRA levels is decreased in dose escalation studies of patients awaiting kidney transplantation. Pharmacokinetic studies by Pescovitz and colleagues have shown that nearly 80% of subjects studied had some change in PRA manifested by a decrease in donor-specific antibody titers or diminished fluorescence intensity [8]. In a single case report, rituximab was used to pretreat a highly sensitized pediatric patient awaiting cardiac transplantation. After two doses, the patient's PRA level decreased from 55% to 18% on the day of transplant [12].

Given the clinical responses of patients, rituximab has emerged as an effective therapy for a variety of antibody mediated events in allotransplantation. While the persistence of antibody-producing plasma cells and the early re-appearance of memory cells are problematic, abrogating the acute B-cell response is crucial. In this review, we summarize the uses of rituximab in transplantation. We will discuss strategies for the treatment of rejection, use in densensitization protocols, treatment of PTLD and future directions.

Discussion

Rituximab: mechanisms of action

Despite extensive empiric clinical trial experience in the treatment of hematologic malignancies, the mechanisms of action of rituximab continue to be the subject of debate. Until recently, our understanding of the mechanisms of action of rituximab has derived from *in vitro* studies of tumor cell killing, which have suggested that complement-dependent cytotoxicity (CDCC), drug-induced apoptotic death of B cells, and antibody-dependent cellular cytotoxicity (ADCC) were potential rituximab-induced pathways of depletion [13–16].

The recent generation of the human CD20 (hCD20) transgenic mouse [17], and the observation that the response rate to rituximab is better in patients with follicular lymphoma who have specific high affinity Fcγ receptor (Fc-γR) polymorphisms [18,19] have provided important *in vivo* insights about the selective depletional properties of this drug, and the role that ADCC at the level of effector cells [i.e. natural killer (NK) cells and macrophages (MACs)] play in the clinical effect of rituximab; and are likely to impact the future drug of this agent.

Insights from the hCD20 transgenic mice

hCD20 transgenic mice have been generated through the integration of bacterial artificial chromosomes encoding the hCD20 locus in FVB mice. In this murine model, hCD20 expression mimics that of humans yet the expression of the transgene occurs at a 50% level of that of circulating human B cells. As this model preserved the CD20 epitopes recognized by rituximab, it is an invaluable tool to study the *in vivo* mechanisms of action of this drug. [17]. Using this model, Gong *et al.* [17] have identified ADCC and CDCC as the most relevant *in vivo* depletional mechanisms of rituximab, and have shown that the susceptibility to depletion varies among the different lymphoid compartments.

While $CD20^+$ B cells in peripheral blood are rapidly depleted by rituximab [16,17], $CD20^+$ B cells homing in the lymphoid compartments are somewhat resistant to rituximab depletion, and longer and multiple-dose treatments are required to achieve an effective killing. Of particular interest is the resistance to rituximab exhibited by B cells in the marginal zone (MZ) and germinal centers (GC) of the spleen [17,20]. Depleting these cells is vital for the success of desensitization and antibody-mediated rejection protocols, as MZ and GC B cells are pivotal in the development of long-lived plasma cells and humoral responses against T-cell-dependent antigens [21,22]. Based on these observations, we must be cognizant that the use of peripheral blood CD19 and CD20 absolute counts may not accurately reflect rituximab-induced B-cell depletion. Although the assessment of lymphoid-bound B cells may be more accurate, this does not represent a clinically practical alternative.

Another interesting observation derived from this model focuses on the contribution of the complement system and B-cell survival factors to the depletional activity of rituximab [14,17,23]. Changes in the expression of complement regulatory proteins (e.g. CD55 and CD59) in the lymphoid microenvironment may increase the threshold for rituximab-mediated killing, whereas B-cell survival

factors such as BAFF/BLyS/TALL-1 may contribute to the resistance of MZ B cells to rituximab depletion [17,23,24].

Fc-γR polymorphisms: can we predict the efficiency and response to rituximab-induced B-cell depletion?

The observation made in the hCD20 transgenic model that ADCC is an important pathway of rituximab-induced depletion has been recently demonstrated in humans. In ADCC, the antibody binds to its cellular target (i.e. CD20) and then is engaged by effector cells such as NK cells and MACs via their receptors for IgG (Fc-γR). The bridge between antigen–antibody–effector cell results in the activation of NK and MACs, and results in the killing of antibody-coated targets (i.e. B cells). Subclasses of IgG display substantial differences in their ability to mediate ADCC and, as shown recently by Nimmerjahn and Ravetch [25], the selective Fc-γR-binding affinities for the IgG subclass determines the *in vivo* ADCC activity of cytotoxic antibodies. For a complete review of the biology of Fc-γR please refer to excellent reviews of Salmon and Pricop and Sautes-Fridman *et al.* [26,27].

It has been long recognized that the response to rituximab is variable among different lymphoma types and among different patients within each type. Recently, this variability in clinical response has been linked to specific Fc-γR polymorphisms [18,19].

In a study of 87 patients with follicular lymphoma [19], those with a specific polymorphism of Fc-γRIIIa (158 V/V genotype) showed a higher response rate to rituximab treatment, than patients who exhibited genotypes 158 V/F and F/F. Furthermore, patients with a high-affinity Fc-γRIIa polymorphism (131 H/H) had a better response to rituximab depletion than patients with 131 H/R or R/R genotypes. The association of Fc-γRIIIa with response to rituximab has been reported in autoimmune diseases [28], and it is not surprising as Fc-γRIIIa of 158 V allele binds human IgG1 better than the Fc-γRIIIa of 158F allele which translates into enhanced activation of NK cells and MACs and better ADCC [25–27,29,30]. The biology of the association between Fc-γRIIa 131 H/H polymorphism and rituximab response is less clear given that the allele of 131 H/H is known to bind better to IgG2 and no significant difference in the affinity of these two allelic forms has been noted for human IgG1 [19,31].

The likelihood that Fc-γR polymorphisms could be used in the near future to predict the *a priori* response to rituximab has relevance in the management of both solid organ transplant recipients with PTLD and antibody-mediated rejection as the efficacy of rituximab has been variable in PTLD trials as well as desensitization protocols [8,32–35]. The ability to predict response would not only allow for a rational patient selection and cost-effective therapy but also would enable us to choose alternative therapies for those patients with high probability of treatment failure.

Rituximab for treatment of rejection

The contributions of survival factors, complement regulatory proteins, ADCC, and integrin-mediated homeostasis to rituximab-mediated B-cell depletion in the hCD20 transgenic mice provide a mechanistic basis for potential combinatorial therapy aimed at enhancing the efficacy of rituximab in the treatment of antibody-mediated rejection, HLA-sensitization, malignancies, and autoimmune diseases.

Rejection is detrimental to long-term function of any organ transplant. Classic acute rejection is characterized by a T-cell-mediated process. Whether heart, lung, liver, or kidney transplant, cellular rejection can usually be treated effectively with bolus steroids. However, there is an increasing subset of patients that have rejection episodes that are resistant to traditional therapy. In cardiac transplantation, vascular rejection is diagnosed by the presence of IgG and complement in endomyocardial biopsies [36]. Hemodynamic compromise results and if humoral rejection is left unchecked, it can lead to patient death. Case reports of cardiac rejection treated with rituximab first appeared in 2002 [37–39]. In all cases, humoral rejection was diagnosed and therapy with plasmapheresis and cyclophosphamide was unsuccessful. Between one and four doses of rituximab at 375 mg/м^2 intravenously were given with resolution of clinical rejection and decreased IgG staining over the ensuing months. Given the success in the first case reports, Garrett *et al.* [36] reported on eight patients for whom rituximab was given a first-line therapy for humoral rejection. Rejection was reversed in all patients by immunoflourescent staining and clinical improvement of left ventricular ejection fraction (33% at the time of rejection back to baseline of 53% after therapy).

B cells have not been traditionally identified in association with acute rejection. However, Sarwal *et al.* [40] demonstrated unexpected large aggregates of CD20$^+$ staining B cells in kidney biopsies of patients with acute rejection episodes. The rejection was treated with a steroid pulse. Three of these patients also had antibody therapy. This cohort of patients had poor long-term graft survival following these rejection episodes. The authors went on to note that there was 'a strong association between the density of CD20$^+$ cells on immunostaining and the clinical phenotype of glucocorticoid resistance.'

We reported on 27 patients who were diagnosed with biopsy-confirmed rejection manifested by thrombotic

microangiopathy and/or endothelialitis. Twenty-four received initial steroid bolus therapy while 22 of the 27 patients were also treated with plasmapheresis and antithymocyte globulin with no clinical improvement in creatinine. These individuals were then treated with a single dose of rituximab, in addition to other therapies in an effort to reverse their rejection episodes. Only three patients experienced graft loss not associated with patient death during the follow-up period (605 ± 335.3 days). In the 24 successfully treated patients, the serum creatinine at the time of initiating rituximab therapy was 5.6 ± 1.0 mg/dl and decreased to 0.95 ± 0.7 mg/dl at discharge [41].

The presence of $CD20^+$ cells in biopsy specimens may explain the clinical improvement seen in patients treated with rituximab for renal rejection. It has also been shown that the presence of $CD20^+$ cells within renal biopsy specimens is associated with reduced graft survival compared with CD20 negative controls [42]. As rituximab has no effect on plasma cells and little effect on circulating antibody, it is likely to be most effective for the treatment of rejection in combination with other strategies that include plasmapheresis and/or intravenous immunoglobulin (IVIg) therapy. Long-term follow-up studies of patients with $CD20^+$ rejection treated with rituximab should be pursued.

Rituximab and its side-effect profile

In lymphoma patients, rituximab has caused a cytokine release syndrome felt to be secondary to tumor burden [43]. This syndrome has also been described in transplant patients with PTLD [33]. However, the side-effect profile in nonlymphoma patients receiving rituximab for other indications in transplant has been minimal and mainly associated with first dose reactions. The reactions include transient hypotension responsive to fluids, low-grade fever, mild tachycardia, and arthralgias. When rituximab is given in combination with high-dose steroids (as in treatment for rejection), these reactions were completely abrogated [8]. Rituximab related late-onset neutropenia has been reported, but no increases in infectious complications have been clearly linked to rituximab alone [44]. Concerning bone marrow suppression, the majority of reports are limited to single patients or small case series. Persistent hypogammaglobulinemia has been shown in patients with autoimmune hemolytic anemia and bone marrow transplant recipients [45].

Rituximab for desensitization

Highly sensitized patients pose many challenges for the transplant community. Individuals with elevated PRA levels suffer from increased rejection rates despite aggressive induction protocols and complex maintenance immunosuppression regimens [46,47]. In addition, it is difficult to identify crossmatch-negative organs for transplantation. Up to 25% of patients awaiting kidney transplant have significant levels of anti-HLA antibodies. A patient with pre-existing high antibody levels waits at least 3 years before receiving a transplant [8]. However, of the 5000 patients with PRA levels above 80%, <300 are transplanted each year making calculation of mean waiting time exceedingly difficult [48]. Even after the transplant event, there is an increased risk of rejection, be it cellular or antibody-mediated. This can have significant consequences with regards to allograft function and survival. Plasmapheresis and immunoabsorption protocols have been used to decrease HLA antibodies in the pretransplant period with a modicum of success. They certainly have abrogated the incidence of hyperacute rejection episodes. Yet, nearly 90% of highly sensitized patients still develop rejection in the first 3 months following transplantation if no desensitization protocol is initiated [49].

In combination with strategies including plasmapheresis and IVIg, rituximab has become an important adjunct in desensitization protocols. Desensitization has been primarily employed in kidney transplantation. However, there are case reports of successful transplantation across ABO-incompatible barriers in liver and lung transplant [50–52].

Historically, splenectomy has been a necessary component of ABO incompatible transplant. Patients who did not undergo splenectomy experienced aggressive antibody-mediated rejection and graft loss. However, splenectomy is associated with significant risk of infectious complications and usually is performed as a procedure separate from the transplant. Performing ABO-incompatible transplants without splenectomy is controversial [53]. Nevertheless, several centers now report that the use of rituximab in effect provides a 'chemical' splenectomy and that the short-term protection preventing antibody-mediated rejection can be accomplished without an additional surgical procedure [54,55]. Sonnenday *et al.* [56] showed that with the addition of rituximab, six patients safely underwent ABO-incompatible kidney transplant and no episodes of rejection had occurred at 12 months median follow up.

A subset of patients who had high titers of antidonor blood type antibody (>1:256) did not achieve adequate clearance (titer <1:16 prior to transplant) with plasmapheresis. These four patients were treated with rituximab and had a drop in their titers to 1:16 allowing for transplant [57].

While hyperacute rejection can be averted by desensitization protocols, low levels of donor-specific antibody may persist post-transplant. It is possible that the expres-

sion of antibody allows the endothelium to develop resistance to antibody-mediated damage through accommodation [58]. Whether rituximab contributes to the development of accommodation has not been studied.

Rituximab and PTLD

Post-transplant lymphoproliferative disorders are a relatively rare complication. The incidence ranges from 1% to 10% [33] depending on organ type transplanted. The risk of development depends on a variety of factors including prior Epstein–Barr virus status, immunosuppressive load and type of organ transplanted. Reduction of immunosuppression is the first-line therapy. Chemotherapy with a variety of agents including cyclophosphamide, doxorubicin, vincristine, etoposide is associated with significant morbidity and outcomes are poor. Many of these lymphomas are aggressive $CD20^+$ B-cell clones. As such, investigators have used rituximab with success [59,60]. A European multicenter trial has been conducted using rituximab as first line therapy for PTLD [32]. In this phase II trial, 43 patients were analyzed with the primary endpoint being response at day 80. The overall response rate was 44.2%. Some patients who had a partial response at day 80, had complete clinical response by day 180. The authors noted that treatment of PTLD with rituximab was well tolerated and patients had a low-relapse rate when compared with chemotherapy. In the USA, a smaller phase II trial involving 11 patients revealed a 64% response rate [35]. However, the response of PTLD to rituximab alone can be variable as pointed out earlier.

Rituximab and recurrent disease in kidney allografts

Although rituximab has been used increasingly for the treatment of primary and secondary antibody-mediated glomerular diseases of the kidney [61–65], the experience on the use of B-cell depletion in recurrent disease of the allograft is limited to few case reports. In a review of the Medline publication database using the MESH terms recurrent disease and transplantation, they were only able to identify four reports of the use of rituximab in recurrent disease of the allograft of which three pertained to kidney transplants [60,66,67] and one to patients with peripheral blood stem transplants [68]. In a recent case study by Nozu *et al.*, a patient with nephrotic syndrome and a history of focal segmental glomerulosclerosis (FSGS) was treated with rituximab post-transplant for PTLD. Coincident with the treatment, the nephrotic syndrome resolved [60]. In another case, presented at the American Society of Nephrology meeting, November 2005, Gossmann and colleagues reported on the successful treatment of a second relapse of FSGS in a renal transplant patient (abstract no. SA-FC031, November 12, 2005). Although the largest experience on the use of rituximab in native glomerular diseases has been reported in patients with lupus nephritis and membranous nephropathy (MN) [61,62], no experience with B-cell depletion has been reported in recurrent lupus or MN of the allograft. Clearly, this is an area in which the use of rituximab should be actively pursued given the lack of effective therapy and the poor outcome of patients with recurrent or de novo glomerular disease of the allograft [69,70]. Given the paucity of cases, studies will require the design of multicenter studies to reach the critical sample size needed to yield meaningful results.

Conclusions

We have reviewed the mechanisms of action of rituximab and how these actions may contribute to the role rituximab can play in solid organ transplantation. The effects of rituximab in the B-memory cell/plasma cell compartment continue to be problematic. The mechanism(s) by which rituximab reduces antibody levels remains unclear given that its target is not expressed by mature plasma cells. Although the depletion of the memory B-cell compartment may indirectly result in reduced antibody production, only one study has demonstrated the effect of rituximab in B-memory cells of potential transplant candidates [11]. Rituximab-induced B-memory cell depletion is usually short-lived as these cells are the first to re-populate the lymphoid compartment and their re-appearance may correlate with rituximab failure [71]. Nonetheless, we cannot ignore the multiple observations in small case series that show that rituximab is an effective B-cell depletional agent and has been clinically effective in the treatment of antibody-mediated rejection and antibody-induced diseases in solid organ transplant recipients.

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