Novel B Cell Therapeutic Targets in Transplantation and Immune-Mediated Glomerular Diseases

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B cells and antibodies play an important role in the alloresponse to renal grafts as well as in immune-mediated glomerular diseases. In transplantation, greater recognition and improved diagnosis of antibody-mediated rejection have been a catalyst to the introduction of newer drugs and regimens that target B cells, plasma cells, and donor-specific antibodies to improve the outcome associated with antibody-mediated rejection. In immune-mediated renal disease, novel and more selective B cell therapies are gradually modifying the traditional therapeutic approach that consists of steroids and other immunosuppressants. A new era of selective and more effective immunosuppression agents that target the humoral response is finally emerging in transplantation and renal diseases.

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Figure 1: Schematic model for B cell biology. B cells and antibodies play a critical role both in the alloresponse after renal transplantation and in immune-mediated glomerular diseases. In transplantation, B cells, in addition to their role in the humoral response to alloantigens, act as efficient antigen-presenting cells and thus participate in the activation of T cells (1). The development of more sensitive solid phase–based serologic assays for detecting anti-HLA antibodies and the routine use of C4d (a complement fragment considered a marker of antibody deposition) staining in kidney biopsies have led to better recognition of the importance of both acute and chronic antibody-mediated rejection on graft outcome (2,3). The role of B cells in generating and perpetuating alloantibody production has provided the rationale for the use of anti–B cell therapies in desensitization of highly sensitized patients, decreasing the titer of donor-specific antibodies (DSA) and reversing acute antibody-mediated rejection after transplantation (4).

In glomerular diseases that are suspected to be mediated by B cells and antibodies, novel and more selective targeting of B cells is gradually altering the traditional therapeutic approach that consists of steroids and other immunosuppressive drugs (Table 1). In this review, we summarize clinical trials in renal transplantation and immune-mediated glomerular diseases using novel therapies that target B cells and antibodies.

Novel Anti–B Cell Therapies

The anti–B cell agent most frequently used, albeit off-label, in renal transplantation and in glomerular diseases is rituximab. Rituximab, a chimeric monoclonal antibody (mAb) that is approved for use in B cell lymphoma and rheumatoid arthritis, binds to the CD20 receptor of B cells and depletes them through complement-dependent cytotoxicity, antibody-dependent cytotoxicity, and stimulation of B cell apoptosis (4,5). B cell depletion with rituximab may persist for ≥6 mo and is followed by a repopulation of B cells that are deficient of the CD27 receptor, a marker for memory B cells. Repopulation of naive B cells and the persistence of normal Ig levels impart to rituximab both its efficacy and safety (5). For decreasing the potential immunogenicity that is associated with rituximab (30% of its framework is murine) and improving CD20 affinity and B cell depletion, several humanized/fully human anti-CD20 mAbs are in clinical development (Figure 1) (6). Both ocrelizumab, a humanized anti-CD20 mAb with lower risk for immunogenicity and less complement activation than rituximab, and ofatumumab (HuMax-CD20), a fully human CD20 mAb, are in clinical development for lymphoma and systemic lupus erythematosus (SLE). AME-133, a humanized anti-CD20 mAb, shows higher affinity and potency in depleting B cells as compared with rituximab and is in preclinical studies.

Epratuzumab is a humanized mAb that targets the CD22 receptor on B cells, which remains expressed on the B cells as they mature, while losing their CD20 receptors, and become committed to antibody secretion. Thus, targeting CD22 may be a more effective strategy than using anti-CD20 mAbs.

An important pathway for B cell differentiation, survival, and activation is two ligands of the TNF superfamily, BlyS (also known as Baff) and April and their respective B cell receptors BCMA, TACI, and BAFF-R (the last only for BlyS) (7). All three receptors increase NF-κB, which stimulates activation and differentiation of B cells. BlyS and April enhance B cell survival
because B lymphocyte apoptosis is decreased by signaling through BCMA and BAFF-R through an increase in BCL-2, a key antiapoptotic mediator. Two therapeutic agents that target this important network are in clinical development (6). Belimumab is a fully human mAb to BlyS, binding and neutralizing its activity (7). The second agent is atacicept, a recombinant fusion protein that consists of the extracellular binding portion of TACI bound to the Fc portion of IgG1. TACI-Ig inhibits both BlyS and April, resulting in a decrease in Ig levels and B cell count (8).

Activation of the co-stimulation pathway is critical for both T and B cells (9). The most important ligand receptor pairs within this pathway are CD80/CD86-CD28, and CD40-CD40L (CD154; Figure 1). Abatacept (CTLA4Ig) is a fusion receptor protein that consists of the extracellular domain of CTLA4 combined with the Fc portion of IgG1 that binds with higher affinity to CD80/CD86 than to CD28, thereby inhibiting co-stimulatory signals through CD28 (10). Abatacept is Food and Drug Administration approved for use in rheumatoid arthritis and is being developed for treatment of several autoimmune diseases. Belatacept, a second-generation CTLA4Ig, has even greater affinity to the CD80/CD86 ligands and is in clinical trials for organ transplantation (11). Hu5c, IDEC-131, and BG9588 are anti-CD40L (CD154) mAbs that were promising in experimental studies but failed in clinical trials because of thrombotic complications (likely related to the presence of CD40L on platelets) and/or lack of efficacy (6,12,13). More recently, inhibiting the CD40-CD40L pathway through direct targeting of CD40 has drawn interest from both investigators and biopharma. Although CD40 has more widespread expression than CD40L, it is not present on platelets. Thus, therapy against CD40 is not likely to result in thrombotic events. There are five anti-CD40 mAbs at various stages of clinical development. 4D11, a fully human IgG4 anti-CD40 from Astellas Pharma, is being developed specifically for organ transplantation. The other anti-CD40 mAbs are being tested in lymphoma and autoimmune diseases.

Another co-stimulation receptor ligand pair that may effect B cells and antibody production is the ICOS-Bh7 (COSL) pathway. ICOS is expressed on B cells and provides signaling for Ig production by activated B cells (6). Anti-ICOS mAbs are not yet in clinical trials. Abetimus (LJP394) targets B cells specifically for SLE by cross-linking anti-double-stranded DNA (dsDNA) Ig receptors (BCR), leading to B cell apoptosis and reduction of anti-DNA antibodies (14).

Proteasome inhibitors are emerging as promising therapeutic agents in antibody-mediated rejection and potentially in some renal diseases (15,16). Proteasomes are large, barrel-like protein complexes that are located in the nucleus and cytoplasm of eukaryotic cells and contain proteases that degrade misfolded or unneeded proteins. The proteasomal pathway is essential for cellular function, including the cell cycle, apoptosis, response to cellular stress, and class I HLA peptides. Proteasome inhibitors have effective antitumor activity by inducing apoptosis of tumor cells and have a similar effect on other cells with high metabolic activity, such as plasma cells. Bortezomib is the first proteasome inhibitor approved for the treatment of multiple myeloma and is being used off-label in renal transplantation.

Table 1. Novel B cell therapies in glomerular disease

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mechanism of Action</th>
<th>Clinical Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab</td>
<td>Chimeric anti-CD20 mAb (B cell depletion)</td>
<td>SLE, ANCA (vasculitis), Wegener’s, membranous, IgA, and FSGS</td>
</tr>
<tr>
<td>Ocrelizumab</td>
<td>Humanized anti-CD20 mAb with decreased immunogenicity and complement activation (B cell depletion)</td>
<td>SLE</td>
</tr>
<tr>
<td>Epratuzumab</td>
<td>Humanized anti-CD22 mAb (B cell depletion)</td>
<td>SLE</td>
</tr>
<tr>
<td>Belimumab</td>
<td>Humanized anti-BlyS mAb (inhibitor of B cell activation)</td>
<td>SLE</td>
</tr>
<tr>
<td>Atacicept</td>
<td>Fusion receptor protein (inhibitor of B cell activation)</td>
<td>SLE</td>
</tr>
<tr>
<td>Abatacept</td>
<td>Co-stimulation blockade (binds CD80/CD86 ligands)</td>
<td>SLE</td>
</tr>
<tr>
<td>Abetimus</td>
<td>Cross-links dsDNA on B cells</td>
<td>SLE</td>
</tr>
</tbody>
</table>

Figure 1. Novel agents targeting receptors and ligands on B cells.
Although many of these novel agents offer exciting treatment avenues, the failure rate of novel agents remains high as they advance through clinical development.

Renal Transplantation

In the past quarter century, the emphasis in the clinical development of immunosuppression has been to target the T cell, which play a preeminent role in the response to alloantigens. Therapeutic advances in blocking B cells and humoral immunity have lagged behind the advance achieved in depleting and/or modulating T cell function. Recognition of the important role of alloantibodies in graft dysfunction was made possible by the use of more sensitive serologic testing for DSA and the staining of kidney biopsies for C4d. Current therapies for patients with preexisting sensitization, DSA, and acute and chronic antibody-mediated rejection consist of plasmapheresis, intravenous Ig (IVIg), and rituximab (17,18). These therapies target the humoral response at different levels and seem most effective when used in combination. Plasmapheresis produces acute depletion of anti-HLA antibodies but is also followed with a rapid rebound of the antibody titers and thus is best used in conjunction with other therapies. IVIg is used extensively in desensitization protocols and in the therapy of antibody-mediated rejection (19). IVIg is approved for use for Ig replacement therapy but is useful in a broad range of autoimmune and inflammatory diseases. The exact mechanism of action of IVIg in autoimmune disease and transplantation remains unclear. There is evidence that IVIg mediates its effects through inhibition of multiple pathways. The most obvious beneficial effect of IVIg is attributed to its neutralizing effects in circulating autoantibodies or HLA antibodies; however, Jordan et al. (19) observed that the effect of IVIg on HLA antibodies extended beyond the half-life and presence of IVIg in circulation. This prolonged effect of IVIg is attributed to its ability to modulate cell-mediated immunity through Fc receptors. IVIg can also block the effector mechanisms that mediate antibody-associated tissue injury by inhibiting complement activation and scavenging anaphylatoxins, as well as activated complement fragments. In addition to plasmapheresis and IVIg, rituximab is frequently used in desensitization protocols as well as in antibody-mediated rejection to deplete CD20+ B cells that ultimately differentiate into antibody-secreting plasma cells.

The hierarchical therapeutic importance of plasmapheresis, IVIg, and rituximab in desensitization and the treatment of antibody-mediated rejection is not clear. The majority of clinical trials are single-center, nonrandomized studies that use a combination of these therapies. The only exception is the National Institutes of Health–sponsored IGO2 trial, a multicenter, double-blind, placebo-controlled study of IVIg in highly sensitized patients who are waiting for kidney transplantation (20). In that trial, IVIg was shown to be superior to placebo in reducing anti-HLA antibody levels and improving the rate of transplantation. Two single-center trials reported the effects of different therapies in reducing anti-HLA antibodies in patients with high levels of sensitization and/or DSA (17,18). The first study used an open-label design to examine whether human polyclonal IVIg (10% formulation) given twice (2 g/kg body wt on days 0 and 30), in addition to rituximab given twice (1 g on days 7 and 22), could reduce the rate of or eliminate a positive cross-match in 20 highly sensitized patients who were awaiting transplantation at Cedars-Sinai Medical Center (17). The mean panel-reactive antibody level was 77 ± 19 before treatment and 44 ± 30% after the second infusion of IVIg (P < 0.001 for the comparison with the pretreatment level). At study entry, the mean time on dialysis among recipients of a transplant from a deceased donor was 144 ± 89 mo (range 60 to 324). After desensitization, the time to transplantation was shortened to 5 ± 6 mo (range 2 to 18). Sixteen (80%) of the 20 patients received a transplant. Although 50% of the patients had a rejection episode at 12 mo, the mean serum creatinine level was 1.5 ± 1.1 mg/dl (133 ± 97 μmol/L), and the mean survival rates of patients and grafts were 100 and 94%, respectively. The second trial compared three different desensitization regimens in sequentially treated patients with high levels of DSA (18). With IVIg therapy alone, only 38% achieved a negative cross-match and after transplantation, the patients had a very high rate of rejection (80%). In contrast, the addition of plasmapheresis and rituximab with and without splenectomy increased the rate of transplantation to >80% with a reduction in the incidence of acute rejection. Similar therapeutic approaches have been used in reversing acute antibody-mediated rejection. Again, the relative benefit of each of the therapeutic modalities (plasmapheresis, IVIg, and rituximab) is not clear because of a lack of controlled trials. In a recent study reported by Lefaucheur et al. (21), two sequential regimens (A and B) were used in patients with documented acute antibody-mediated rejection. Group A patients were treated with high-dosage IVIg, 2g/kg over 2 d every 3 wk for four doses, and group B patients were treated with plasmapheresis daily for 4 d followed by one high dose of IVIg and rituximab. Three months after treatment of the rejection, group B patients had significantly lower levels of DSA than group A patients and by 36 mo had significantly higher graft survival (92 versus 50%). That study suggests that the addition of plasmapheresis and rituximab to IVIg may be required to more fully reverse acute antibody-mediated rejection. It is clear that improving the outcome of patients with pretransplantation anti-HLA antibodies and patients who experience antibody-mediated rejection will require regimens with novel agents that neutralize anti-HLA antibodies, deplete the cells that secrete these antibodies (i.e., plasma cells), and inhibit the effector mechanisms that result in tissue injury.

The new generation of humanized anti-CD20 antibodies may prove to be more effective than rituximab in depleting B cells in transplantation. The fusion receptor protein atacicept, which blocks the activity of BlyS and April, and the monoclonal antibody belimumab, are already being tested in SLE and are in preclinical development in transplant models. On the basis of its mechanism of action on plasma cells, bortezomib has been used to reverse refractory antibody-mediated rejection in patients after renal transplantation (15,16). Perry et al. (15) successfully treated two patients with bortezomib for antibody-mediated rejection after kidney transplantation and demonstrated a decrease in bone marrow plasma cells in vivo and persistent alteration in alloantibody specifici-
ties. Total IgG were unchanged, suggesting that quiescent plasma cells were not depleted, thereby reducing the potential risk for infectious complications. Everly et al. (16) treated six transplant recipients who had acute antibody-mediated rejection with bortezomib, resulting in prompt reversal of the rejection and prolonged reduction in DSA levels associated with improvement in renal function. Although these patients were reported to have tolerated the therapy without major adverse events, bortezomib can be associated with a number of adverse effects, including severe neuropathy. Bortezomib and a newer generation of more selective and less toxic proteasome inhibitors may emerge as important therapeutic agents in controlling humoral alloimmunity in organ transplantation.

**Immune-Mediated Glomerular Disease**

B cells play a critical role in the pathogenesis of glomerulonephritis (GN) (22–24). Targeted B cell–depleting therapies are increasingly being studied for the treatment of a number of glomerular diseases (25). Older, nonspecific forms of immunosuppression, including corticosteroids and alkylating agents, have been associated with significant adverse events, which may limit their effectiveness, particularly in vulnerable patient populations including the elderly and those with a variety of systemic disorders. The most commonly used anti–B cell therapy in the treatment of glomerular disorders is rituximab (25). Rituximab has been tried in the treatment of a wide variety of glomerular disorders, including SLE nephritis, membranoproliferative GN (MPGN), pauci-immune GN, anti–glomerular basement membrane disease, fibrillary GN, membranous nephropathy (MN), FSGS, and minimal-change disease (MCD; Table 1). More novel targeted B cell therapies that have been used in far more limited experimental settings for the treatment of glomerular disease include ocrelizumab, epratuzumab, abetimus, abatacept, IDEC-131, BG9588, atacicept, and belimumab (Table 1). There have recently been a number of case series and observational studies supporting a role for rituximab in the treatment of refractory GN (25). With the exception of two recent randomized, controlled trials, none of these studies were robust in numbers and well controlled. The specific role of B cell–depleting agents for the treatment of glomerular diseases remains unclear and awaits the results of further prospective, randomized, controlled clinical trials. Table 2 summarizes published and ongoing clinical trials with anti–B cell therapies in glomerular diseases.

**Lupus Nephritis**

B cells play a significant role in the pathogenesis of autoimmune diseases including SLE (26). As new bioengineered monoclonal anti–B cell antibodies have been developed, there has been significant interest in the past decade in studying the effects of specific B cell–depleting agents for the treatment of SLE.

Data on the use of rituximab for SLE nephritis is largely mixed. Observational data support the use of rituximab particularly in cases of refractory SLE nephritis. Controlled, randomized trials have not yet proved a role for the drug (27,28).

Sfikakis et al. (29) studied the use of rituximab in combination with corticosteroids in 10 patients with class III or IV SLE nephritis. Rituximab was dosed at 375 mg/m² per wk for four doses along with corticosteroids 0.5 mg/kg per d for 10 wk and decreased by 4 mg every 2 wk thereafter. Eight of 10 patients reached a partial remission within a median period of 2 mo of follow-up. By month 6, complete remission was achieved in five of the 10 patients. At month 12, four patients remained in complete remission.

Lindholm et al. (30) studied 17 patients with active SLE nephritis that was refractory to standard therapies. The mean estimated GFR was 55 ml/min in this cohort. Six to 12 mo after therapy, two patients achieved a complete remission and nine of the 17 patients had a partial remission. Therapy with rituximab allowed for the discontinuation of cyclophosphamide in 10 patients because of a positive renal response.

Perez et al. (31) studied the effects of rituximab in a prospective, uncontrolled, open-label trial of 22 patients with active classes III and IV SLE nephritis that was refractory to standard immunosuppression. Rituximab was dosed 500 to 1000 mg on days 1 and 15 (rheumatoid arthritis dosing regimen). There was a significant reduction in proteinuria and disease activity in the cohort. A majority of the patients also showed a significant improvement in creatinine clearance. Other nonrandomized, uncontrolled trials have shown similar beneficial effects (32). It is unclear whether B cell depletion must be maintained to continue in remission. Moreover, adverse effects such as infusion reactions to the chimeric molecule and development of human antichimeric antibodies (HACA) may limit the long-term repeated use of rituximab in these patients.

Until recently, no prospective, randomized, controlled trial had evaluated the potential role for rituximab in SLE nephritis. The Explorer trial of patients with SLE and without nephritis did not document a benefit of additional rituximab compared with standard therapy; however, the results of the LUNAR (A Study to Evaluate the Efficacy and Safety of Rituximab in Subjects With ISN/RPS Class III or IV Lupus Nephritis) trial were recently made available (25). In that randomized, placebo-controlled trial, rituximab was studied as an induction therapy for 140 patients with classes III and IV SLE nephritis. All patients received standard induction therapy with mycophenolate mofetil up to 3 g/d and steroids, and half of the patients were randomly assigned to rituximab therapy superimposed on standard therapy for 12 mo. There was no significant difference in the primary outcomes of complete or complete and partial remissions between the groups at 12 mo of follow-up. Rituximab did have a greater effect in normalizing lupus serology, anti-DNA antibody titer, and serum complement. It is unclear whether further analysis will define benefits of therapy in this study (e.g., partial remissions, role in high-risk black patients). On the basis of the available data, rituximab still remains an option for some patients who either have refractory disease or are intolerant to conventional immunosuppressive therapies.

Ocrelizumab is being studied in a prospective, randomized, placebo-controlled trial of patients with proliferative classes III and IV SLE nephritis (33). Because of the nature of this antibody, there should be no HACA formation or adverse infusion...
Table 2. Anti–B cell therapies in clinical trials in immune-mediated glomerular disease

<table>
<thead>
<tr>
<th>Reference</th>
<th>Glomerular Disease</th>
<th>N</th>
<th>Treatment Protocol</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sfikakis et al. (26), 2005</td>
<td>Class III/IV SLE nephritis</td>
<td>10</td>
<td>Rituximab + prednisolone</td>
<td>8/10 PR, 5/10 CR at month 6</td>
</tr>
<tr>
<td>Lindholm et al. (27), 2008</td>
<td>Class III/IV SLE nephritis</td>
<td>17</td>
<td>Rituximab + standard immunosuppression regimen</td>
<td>2/17 CR, 9/17 PR at month 6</td>
</tr>
<tr>
<td>Perez et al. (28), 2006</td>
<td>Class III/IV SLE nephritis</td>
<td>22</td>
<td>Rituximab for nephritis refractory to standard immunosuppression</td>
<td>Proteinuria and disease activity decreased; creatinine clearance improved</td>
</tr>
<tr>
<td>LUNAR (25), 2009*</td>
<td>Class III/IV SLE nephritis</td>
<td>140</td>
<td>Rituximab + MMF + steroids versus MMF + steroids</td>
<td>No significant difference in CR or PR versus placebo</td>
</tr>
<tr>
<td>Dorner et al. (31), 2006</td>
<td>Moderately active SLE</td>
<td>14</td>
<td>Epratuzumab every 2 wk intravenously for 4 doses</td>
<td>35% decrease in B cell level after 32 wk of follow-up</td>
</tr>
<tr>
<td>Strand et al. (14), 2003a</td>
<td>Class III, IV, V nephritis</td>
<td>230</td>
<td>Abetimus versus placebo</td>
<td>Decrease in anti-DNA titer and increase in C3 level, but no difference in number of renal exacerbations</td>
</tr>
<tr>
<td>Boumpas et al. (13), 2003</td>
<td>Class III/IV SLE nephritis</td>
<td>28</td>
<td>BG9588 biweekly for 3 doses then monthly for 4 doses</td>
<td>50% decrease in proteinuria, increased incidence of thrombosis</td>
</tr>
<tr>
<td>Kalunian et al. (12), 2002</td>
<td>Mild to moderately active SLE</td>
<td>85</td>
<td>IDEC-131 versus placebo for 6 doses over 16 wk</td>
<td>No difference versus placebo</td>
</tr>
<tr>
<td>Keogh et al. (37), 2005</td>
<td>ANCA GN</td>
<td>11</td>
<td>Rituximab + cyclophosphamide/steroids</td>
<td>100% CR</td>
</tr>
<tr>
<td>Keogh et al. (28), 2006</td>
<td>Wegener’s GN</td>
<td>10</td>
<td>Rituximab weekly for 4 doses + prednisone</td>
<td>100% CR by month 3</td>
</tr>
<tr>
<td>Stasi et al. (39), 2006</td>
<td>ANCA GN</td>
<td>10</td>
<td>Rituximab weekly for 4 doses, resistant or relapsed patients</td>
<td>9/10 CR by month 6</td>
</tr>
<tr>
<td>Jones et al. (40), 2008a</td>
<td>ANCA GN</td>
<td>44</td>
<td>Rituximab weekly for 4 doses + intravenous methylprednisolone for 1 dose + cyclophosphamide intravenously for 1 dose versus cyclophosphamide/ AZA + steroids</td>
<td>Sustained remissions equivalent</td>
</tr>
<tr>
<td>Ruggenenti et al. (44), 2003</td>
<td>Membranous Nephropathy</td>
<td>8</td>
<td>Rituximab weekly for 4 doses</td>
<td>At month 12, proteinuria decreased by 66% and renal function stabilized in 8/8</td>
</tr>
<tr>
<td>Fervenza et al. (45), 2008</td>
<td>Membranous Nephropathy</td>
<td>15</td>
<td>Rituximab days 1 and 15 and at 6 mo redosed if B cells recovered</td>
<td>At month 12, 2/15 CR, 6/16 PR</td>
</tr>
<tr>
<td>Yabu et al. (51), 2008</td>
<td>Recurrent FSGS</td>
<td>4</td>
<td>Rituximab in patients who were plasmapheresis dependent or refractory to treatment</td>
<td>3/4 no response, 1/4 graft failure</td>
</tr>
<tr>
<td>Peters et al. (54), 2008</td>
<td>MCD, FSGS</td>
<td>4</td>
<td>Rituximab</td>
<td>3/4 CR</td>
</tr>
<tr>
<td>Collins et al. (72), 2008</td>
<td>Fibrillary GN</td>
<td>3</td>
<td>Rituximab</td>
<td>4/4 showed a decrease in proteinuria to &lt;1.5 g/d by 27 mo, and kidney function was preserved</td>
</tr>
</tbody>
</table>

AZA, azathioprine; CR, complete remission; MMF, mycophenolate mofetil; PR, partial remission

* Randomized, controlled clinical trial.
reactions to a mouse protein, although it is unclear whether development of HACA limits the ability of rituximab to deplete B cells.

Other anti–B cell therapies that have been tried in experimental settings for SLE nephritis include epratuzumab, abetimus, abatacept, belatacept, BG9588, IDEC 131, and belimumab (26). An open-label pilot study of epratuzumab in 14 patients with SLE showed safety and was effective in reducing B cell levels by approximately 35% during a 32-wk follow-up period (34). Again, the goal for specific B cell elimination and its relationship to therapeutic efficacy is unclear. A prospective, multicenter, randomized, placebo-controlled trial is being conducted to evaluate epratuzumab further.

Abetimus acts by cross-linking anti-dsDNA Ig receptors that are present on B cells, leading to apoptosis and reduction of anti-dsDNA antibody production (14). A randomized, placebo-controlled trial of abetimus in 230 patients with class III, IV, or V SLE nephritis showed a decreased in anti-dsDNA titers and increased C3 levels but no difference in the time or number of renal exacerbations (14). A multicenter, randomized, controlled trial of >700 patients with lupus was recently discontinued because of the futility of achieving clear superiority over placebo.

Abatacept (CTLA4Ig) represents a novel and promising therapy for SLE (26). A mouse model of SLE nephritis treated with CTLA4-Ig had decreased anti-dsDNA titers, decreased proteinuria, and increased survival (35). A pilot study of abatacept in humans with SLE nephritis is under way to investigate the effects of this molecule (23).

IDEC-131 and BG9588 are anti-CD40L (anti-CD154) mAbs that inhibit the interaction between CD40 and CD40L (12). BG9588 was studied in an open-label trial of 28 patients with proliferative SLE nephritis and led to a 50% decrease in proteinuria (13); however, there was an increased incidence of thrombosis in the treated patients, leading to early termination of the study. IDEC-131 was evaluated in a multicenter trial of 85 patients with SLE nephritis, and there was no difference in outcomes compared with placebo (12). Concerns about the link between anti-CD40L therapy and thromboembolism have discouraged further clinical development of antibodies against this target. A possible safer alternative is to block CD40 directly, and several mAbs to CD40 are being initially tested in lymphoma (6).

Belimumab is a humanized mAb directed against BlyS (BAFF), a critical factor for the development and proliferation of B cells (36). A total of 449 patients who had SLE and were given this antibody showed a decrease in disease activity (37). Two Phase III clinical trials have been successfully completed and demonstrate significant efficacy of belimumab (data not yet published). Atacicept, a fusion receptor protein, also blocks both BlyS and APRIL and inhibits both B cells and plasma cells. It is undergoing a Phase II trial in SLE. Prospective, randomized, controlled trials are needed to evaluate further the effects of these novel therapeutic agents for the treatment of SLE nephritis.

**Rapidly Progressive GN**

Cyclophosphamide and corticosteroids have formed the mainstay of induction therapy for pauci-immune GN (25). In specific clinical circumstances, including pulmonary hemorrhage and advanced renal insufficiency, there is evidence supporting a role for plasmapheresis as well (38). Given the potential pathogenicity of anti-neutrophil cytoplasmic antibodies (ANCA) in the development of this disease, specific B cell–depleting agents may have a role in the therapy of this potentially life-threatening form of vasculitis (39).

Keogh et al. (40) studied the use of rituximab in 11 patients who had active ANCA vasculitis and were either intolerant of or resistant to cyclophosphamide therapy. All patients received treatment with corticosteroids up to 1 mg/kg per d and four weekly doses of rituximab at 375 mg/m². Complete remission was achieved in all patients and correlated with depletion of peripheral B lymphocyte cell counts.

In a follow-up study, Keogh et al. (41) performed a prospective, open-label trial in 10 patients using rituximab for the induction treatment of Wegener’s granulomatosis. All patients were either resistant or intolerant of previously dosed cyclophosphamide. Rituximab was dosed at 375 mg/m² per wk for 4 wk along with oral prednisone at 1 mg/kg per d for 5 mo. Once again, rituximab was well tolerated and resulted in complete remission in all 10 patients by 3 mo after therapy. Five of the 10 patients had rising cANCA titers and were retreated prophylactically.

Stasi et al. (42) evaluated the long-term outcomes of rituximab for the treatment of ANCA vasculitis in 10 patients who were refractory to cyclophosphamide and corticosteroid therapy. Eight patients in the cohort had Wegener’s granulomatosis and two had microscopic polyangiitis. Nine of the 10 patients had a complete remission and one of the patients had a partial remission at 6 mo of follow-up. The patients were then followed for a median period of 33.5 mo. During this time, there were three relapses, and repeat courses of rituximab in these patients led to complete remission again. The authors concluded that rituximab is a potential option for the treatment of refractory ANCA vasculitis and for patients who are unable to tolerate the use of traditional alkylating agents because of significant comorbidity.

In the Rituxivas trial (43), patients with ANCA and rapidly progressive GN were randomly assigned in a 2-to-1 manner to therapy with one initial dose of intravenous cyclophosphamide, one dose of intravenous methylprednisolone, and four weekly doses of rituximab versus repeated intravenous doses of cyclophosphamide along with steroids as the standard treatment arm. Although relatively small in numbers, this trial was well controlled and dealt with a high-risk population. Sustained remissions were equivalent between the two groups. Given the preliminary studies and the results of the Rituxivas trial, a role for rituximab is clearly emerging in some patients with ANCA-associated rapidly progressive GN.

There have also been two isolated case reports discussing the successful use of rituximab for the treatment of anti–glomerular basement membrane disease (44,45). In both settings, rituximab was combined with other immunosuppressive therapy
including corticosteroids; therefore, the independent role for B cell–depleting therapy in this disease remains unclear and requires further study in a randomized, placebo-controlled trial.

Membranous Nephropathy

Several clinical trials have evaluated the effect of rituximab for the treatment of MN. As with other immune-modulating therapies, rituximab seems to have the greatest beneficial effect on patients with less established chronic tubulointerstitial fibrosis (46).

Ruggenenti et al. (47) studied the effects of rituximab in an observational study of eight patients with idiopathic MN and proteinuria >3.5 g/24 h. By month 12, proteinuria had decreased by approximately 66% and renal function had stabilized in the entire cohort. No adverse reactions were reported. This study is limited by its observational nature and small sample size.

A larger and more recent study of patients with idiopathic MN by Fervenza et al. (48) also showed a positive effect of rituximab on remission of the nephrotic syndrome. Fifteen patients with nephrotic syndrome unresponsive to renin-angiotensin-aldosterone system blockade were evaluated in an open-label trial and followed for 12 mo. Mean proteinuria decreased from 13.0 ± 5.7 to 6.0 ± 7.3 g/24 h. At 12 mo of follow-up, complete remission was obtained in two patients (defined as proteinuria <0.3 g/24 h) and partial remission was found in six patients (defined as proteinuria <50% peak value and proteinuria <3 g/24 h).

Bomback et al. (49) recently completed a meta-analysis of 21 case reports and uncontrolled case series that evaluated the use of rituximab for idiopathic MN. There was a 15 to 20% complete remission rate and 35 to 40% partial remission rate after treatment with rituximab; however, only prospective, randomized, controlled trials can truly establish the role for rituximab in the treatment of idiopathic MN. Because first-line therapy of MN with severe or persistent proteinuria has already been established by randomized, controlled trials, rituximab use should be limited to patients whose therapies fail or are intolerable.

FSGS and MCD

There are limited data and mixed outcomes on the use of B cell–depleting therapy for the treatment of steroid-dependent or -resistant MCD and FSGS. For patients for whom an elevated permeability factor is believed to play a prominent role in the pathogenesis of disease (especially in the recurrent form after transplantation), use of agents such as rituximab may be of benefit. There have been several case reports of clinical remission after use of rituximab for the treatment of recurrent posttransplantation FSGS (50–53); however, Yabu et al. (54) recently reported a case series of four patients who had early recurrence of FSGS and whose disease did not respond to rituximab treatment despite B cell depletion. No patient experienced a decrease in proteinuria, and one of the four patients went into graft failure during the observation period.

In the nontransplantation setting, multiple case reports document a positive response to rituximab; however, once again, the data are mixed (55,56). In one case series, four patients with MCD, FSGS, or recurrent FSGS after transplantation were treated with rituximab (57). In three of the four patients, there was a positive response with remission of proteinuria, although the response rate was variable among the patients. All four cases of disease were resistant to multiple other immunosuppressive therapies, including prednisone, calcineurin inhibitors, and mycophenolate mofetil, supporting a potential adjunctive role for rituximab in patients with refractory disease. Recent preliminary studies of 31 patients (including 27 children and four adults) showed a high response to rituximab, allowing almost all patients to discontinue therapy; however, patients who received only one course of therapy had a high relapse rate (58–60).

Other Glomerulonephritides

A recent trial by Yuling et al. (61) supported a potential role for B cells in the pathogenesis of IgA nephropathy (IgAN). Thirty-six patients with primary IgAN were evaluated and compared with five control subjects and 10 patients with active SLE. CD19+CD5+ B cell numbers from the peripheral blood, peritoneal fluid, and kidney were significantly higher in the IgAN group compared with control subjects and with patients who had SLE. After treatment with immunosuppression including corticosteroids, 33 of the 36 patients with IgAN had a positive response and also showed a significant decrease in the number of CD19+CD5+ B cells in the peritoneal tissue, peripher al blood, and kidney. Three patients did not show a response to therapy and also did not show a decrease in the number of circulating CD19+CD5+ B cells. On the basis of this and other preliminary evidence, a prospective, randomized, placebo-controlled trial of rituximab for the treatment of IgAN is under way.

There is increasing literature supporting a role for rituximab in the treatment of chronic hepatitis C virus (HCV) infection associated with cryoglobulinemia (62,63). MPGN associated with underlying HCV infection has also been treated successfully with rituximab (64–68). HCV infection promotes the proliferation of B lymphocytes with subsequent production of autoantibodies and the formation of mixed polyclonal types II and III cryoglobulinemia. In conjunction with disappearance of cryoglobulins and reduction of HCV antibody, patients have experienced reductions in proteinuria and stabilization of the GFR; however, there are no prospective, randomized, controlled clinical trials supporting a role for rituximab in this setting because there have been reports of reactivation of both hepatitis B and C infection after immunosuppression with rituximab. Caution is advised until more evidence is available. Antiviral prophylaxis is recommended if rituximab is to be administered to patients with underlying hepatitis infection. Cryoglobulinemic GN has also been reported in HCV-negative patients. Several case reports have shown a positive outcome with the use of rituximab in this clinical setting (69,70).

There have also been recent reports of positive outcomes using rituximab for other causes of secondary type I MPGN. Bhat et al. (71) reported three patients who had GN associated with monoclonal Ig deposits on renal biopsy and were treated with rituximab. Two patients had a light microscopy pattern of
MPGN, and the third patient had diffuse proliferative GN with IgG A deposits. All three patients had a partial remission after therapy. Mutluay et al. (72) reported a case of MPGN and light chain nephropathy associated with chronic lymphocytic leukemia and nephrotic syndrome. The patient was treated with rituximab, cyclophosphamide, vincristine, and prednisolone and went into a partial remission of both the chronic lymphocytic leukemia and the MPGN. Proteinuria decreased by 50% after the chemotherapy. There was also a recent case report of a patient with low-grade B cell lymphoma and nephrotic syndrome caused by secondary type I MPGN (73). After six cycles of rituximab and four cycles of bendamustine, the nephrotic syndrome went into a complete remission. There is limited evidence for the use of specific monoclonal B cell–depleting agents for the treatment of idiopathic type I MPGN, although anecdotal cases exist.

The treatment of fibrillary GN remains controversial. Treatment that is based on the light microscopy pattern on renal biopsy has been recommended (74). Collins et al. (75) reported three patients who had fibrillary GN and were treated with rituximab and went into a partial remission. Proteinuria decreased to <1.5 g/d, and kidney function was preserved during the 27 mo of follow-up.

Conclusions
In renal transplantation, the recognition of the role of B cells and anti-HLA antibodies in rejection and late graft loss has been a catalyst for innovative therapeutic strategies to deplete B cells and plasma cells and to decrease DSA. In many forms of glomerular diseases, data support a role for B cells and antibodies in their pathogenesis. The availability of Food and Drug Administration–approved and experimental forms of B cell–depleting or –modulating agents has led to animal studies, anecdotal reports, and many small series of their use in glomerular diseases. Unfortunately, only a few randomized, controlled trials have been performed. Thus, although the literature supports a specific role for rituximab in the treatment of some refractory cases of glomerular diseases, conclusive proof of efficacy must await additional controlled clinical trials in each specific disease. The newer anti–B cell/plasma cell drugs look promising in SLE but are still in early clinical development for other glomerular diseases.

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References
7. Schneider P: The role of APRIL and BAFF in lymphocyte activation. Curr Opin Immunol 17: 282–289


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