Targeting B Cells and Plasma Cells in Glomerular Diseases: Translational Perspectives

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ABSTRACT

The unique contributions of memory B cells and plasma cells in kidney diseases remain unclear. In this review, we evaluate the clinical experience with treatments directed at B cells, such as rituximab, and at plasma cells, such as proteasome inhibition, to shed light on the role of these two B lineage compartments in glomerular diseases. Specifically, analysis of these targeted interventions in diseases such as ANCA-associated vasculitis, SLE, and antibody-mediated transplant rejection permits insight into the pathogenetic effect of these cells. Notwithstanding the limitations of preclinical models and clinical studies (heterogeneous populations, among others), the data suggest that memory B and plasma cells represent two engines of autoimmunity, with variable involvement in these diseases. Whereas memory B cells and plasma cells appear to be key in ANCA-associated vasculitis and antibody-mediated transplant rejection, respectively, SLE seems likely to be driven by both autoimmune compartments. These conclusions have implications for the future development of targeted therapeutics in immune-mediated renal disease.


In health, memory B cells and plasma cells (PCs) comprise important but independently regulated compartments of our immunologic memory1 (Figure 1). Humoral immunity is key to the pathogenesis of many autoimmune renal diseases. Here B cells, when recognizing (auto)antigens and receiving appropriate T cell help, can differentiate into short-lived PCs, memory B or long-lived PCs also termed memory PCs2. However, their detailed contributions to different kidney diseases are not known.

Long-lived PCs are considered ultimately differentiated, bone marrow (BM) resident cells secreting high-affinity antibodies that disseminate through the body, whereas memory B cells3 can rapidly proliferate in a clonally and antigen specific manner, and differentiate and recirculate upon antigen re-exposure. Overall, memory B cells and PCs form two immune defense lines that allow preservation of previous immune encounters by antibodies produced by long-lived PCs and a dynamic component to adapt humoral immunity by memory B cells. The two cellular subsets ensure stability and flexibility to maintain a sufficient B cell lineage defense. The extent to which these “two engines” of B cell lineage memory contribute to different renal autoimmune diseases is not fully understood, but is likely to differ between disorders.

In this context, clinical experiences with therapeutics selectively targeting CD20+ memory B cells but not CD20− PCs provided interesting lessons (Figure 2A), e.g., the response to rituximab in ANCA-associated vasculitis (AAV) and idiopathic membranous nephropathy (IMN). In contrast, SLE did not show similarly convincing responses to CD20 targeting. In chronic antibody-mediated rejection (ABMR), the addition of PC targeting agents (e.g., the proteasome inhibitor bortezomib) appears to be beneficial, with less evidence for rituximab.4 Currently available data from more selective immune targeting suggests that the pathogenic relevance of memory B cells and PCs may vary between autoimmune diseases (reviewed recently5), improving our understanding of individual diseases.

Here we will take a reverse translational perspective to learn from the clinical use of B cell–directed therapies such as anti-CD20 or therapies that target the PC compartment in renal autoimmunity.

INDUCTION OF MEMORY B CELLS AND PCS

Distinct PC subsets can be induced via different pathways (Figure 1). First, B cells from the B1 cell lineage, which have been mainly studied in mice and

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lack a defined phenotype in humans, can form short-lived PCs, which produce polyreactive IgM for immediate defense. Second, B cells from the B2 cell lineage can form short-lived PCs in a T cell–independent manner (so-called marginal zone B2 cells), for example by stimulation with T cell-independent antigens such as pneumococcal capsular polysaccharides, and predominantly secrete low-affinity IgM antibodies.6,7 Moreover, B2 lineage cells are the main source of long-lived PCs and memory B cells upon activation by cognate T cells in germinal centers.8 The generation of long-lived PCs is the result of a two-step process. First, an extrafollicular response leads to the generation of short-lived activated B cells, of which some re-enter the B cell follicle and become plasmablasts in a T cell–dependent pathway. Subsequently, plasmablasts migrate through the blood stream and reside as long-lived PCs primarily in BM niches, possibly also in inflamed tissues.9,10 It is currently debated whether a small number of BM memory PCs can be induced independently of T cells.11,12 The germinal center response is a time-regulated developmental switch, first producing memory B cells and subsequently long-lived PCs13 with increasing affinity. It has long been suggested, but now experimentally proven, that memory B cells can also develop in a T cell–dependent, but germinal center–independent pathway.14 B cell lineage differentiation pathways are summarized in Figure 1. The bulk of the data reported above has been obtained from preclinical models, although it remains to be delineated which differentiation pathway(s) and B cell lineages are involved in certain autoimmune conditions.

**MEMORY B CELLS**

The definition of a memory B cell comprises an antigen-experienced, nonproliferating and, in the absence of antigen, persisting cell15 that responds more rapidly and efficiently when re-exposed to antigen. In contrast to sessile PCs, memory B cells recirculate and scan the body continuously. Their phenotype does not differ between certain lymphoid organs, and they do not depend on the presence of the spleen or tonsil.3 Most but not all memory B cells have undergone class-switch recombination, typically from IgM to IgG, and carry somatically hypermutated IgV gene rearrangements16,17 as one signature of previous T cell encounters. Loss of IgD expression (IgD−) identifies B cells that have undergone class-switch recombination. Switched memory B cells develop during T cell–dependent germinal center responses.13 In addition to Ig subclass B cell receptor (BCR) expression, other phenotypic B memory markers have been identified. Here, expression of CD27 serves as a universal marker for memory B cells in healthy donors. The BCR of memory B cells shows a high affinity to the immunizing antigen compared with naïve B cells caused by using affinity-matured and somatically hypermutated BCR genes.18 Memory B cells that emerge from the T cell–dependent but germinal center–independent pathway do not show somatic hypermutations, and thus are not affinity matured.19 After a given immune response, memory B cells must repress their activation program and rest in a quiescent state for a long period to maintain their
memory capacity. Upon antigen reactivation, memory B cells become activated and can differentiate either into PCs or further mature by re-entering the germinal center for additional affinity maturation. The involvement of memory B cells during resolution of an immune response and the mechanisms by which resident memory B cells are prevented from uncontrolled activation remain unclear for immunity as well as autoimmunity.

Notably, disturbances of circulating memory B cells in patients with chronic immune activation have been reported in chronic autoimmune conditions, such as SLE, rheumatoid arthritis (RA), or in HIV infection compared with healthy controls. In this context, enhanced CD27<sup>-</sup>IgD<sup>-</sup> B cells as well as CD27<sup>-</sup>IgD<sup>-</sup> coexpressing CD95<sup>+</sup>, CD21<sup>low</sup> or intracellular spleen tyrosine kinase Syk<sup>high</sup> were found. There is a possibility that these abnormalities result from an overly active immune system with enhanced B cell differentiation, but it is not clear if they result from incomplete germinal center responses, increased extrafollicular activity and/or enhanced T cell–independent B cell activation. Usually, these cells carry characteristics of B cell memory (e.g., mutated IgV genes), expressed costimulatory molecules, or lacked an active ABCB1 transporter as functional characteristic of memory B cells. It is currently not clear if these abnormalities of B cell memory are a source of autoimmunity or result of overly active immune activation.

The role of T cell–dependent memory B cell induction in SLE, AAV, and ABMR has been concluded from molecular data of Ig switched, hypermutated autoantibodies characteristic of these diseases, and histologic data of germinal center-like structures in affected tissues.

**PCS**

PCs are responsible for maintaining serum antibody levels by continuously producing high-affinity antibodies and reside preferentially in the BM. Only a few plasmablasts that arise from the germinal center response migrate through the blood stream to become memory PCs. Crucial for the survival of a plasmablast to become an ultimately differentiated, long-lived PC is that they find appropriate soluble and insoluble (niche) survival conditions, whereas no postgerminal center selection processes of PCs are known. Long-lived PCs, also called memory PCs, find optimal survival conditions in the BM in healthy individuals at the end of a competitive journey starting as an early B lineage cell.

As direct PC precursors, plasmablasts express the C-X-C chemokine receptor type 4 (CXCR4) and downregulate CXCR5 and CXCR7 when they start to migrate toward the BM. The BM survival niches are composed of stromal cells expressing C-X-C motif chemokine ligand 12 (CXCL12) (ligand of CXCR4) and vascular cell adhesion protein 1 (VCAM1). Other cell types such as megakaryocytes,
eosinophils, and basophils contribute to the production of survival factors such as a proliferation-inducing ligand (APRIL), B cell-activating factor (BAFF or BLYS), or IL-6, as well as adhesion molecules.37–39 The number of available niches in the BM or its capacity appears to be limited and serves as a regulatory factor for serum Ig levels. Recently, it has been debated if there is a physical or rather cell-type dependent functional BM niche.40

Besides the BM, other tissues can also offer survival niches in states of chronic inflammation. These niches disappear when the inflammation is resolved.41–43 Once it was thought that survival niches harbor almost exclusively highly-affinity matured PCs, but other studies revealed that immature PCs can access survival niches.44,45 Recent data suggest that there is heterogeneity of PCs in human BM. Here, a stable and highly differentiated CD19+PC fraction with a distinct phenotype (CD36+, HLA-DRnegative, CD44negative etc.) and a less mature CD19+PC fraction exist in human BM, which suggests that further differentiation even within the BM PCs is a possibility.46 These human PC subsets in the BM have been confirmed subsequently.47,48 Because CD19+PCs usually enriched in human BM were also found in autoimmune tissues including kidney transplant rejection,46 it needs to be further delineated what factors are involved in their induction and/or maintenance in autoimmunity. Notably, a potential role of T cells within the BM has been suggested recently. Here, regulatory T cells (Treg) cells share a particular niche with PCs in CD11c+BM cells and appear to regulate PC survival via CTLA-4.49 This suggests that T cells have distinct but largely unknown functions determining the lifestyle of PCs and mandates further studies.

**ANIMAL MODELS**

Different animal models for SLE, the two AAV conditions (myeloperoxidase [MPO] or proteinase 3), and ABMR could provide evidence for the role of B lineage cells. Here analyses of T cells and relevant autoantibodies50–52 provide the basis that activation of B cells has taken place. However, the particular role of B cells independent of PC producing autoantibodies has only been clearly demonstrated in a lupus model.52,53 Tables 1 and 2 summarize key features, advantages, and disadvantages of the most instructive animal models in which B cell and PC contributions have been studied within AAV, lupus nephritis, and ABMR.

**TARGETING MEMORY B CELLS VERSUS TARGETING MEMORY PCS IN HUMAN DISEASE**

Because expression of certain surface markers on memory B cells and PCs are distinct, selective targeting by using specific agents is a clinical possibility. Memory B cells show the phenotype of mature B cells expressing CD19, CD20, and CD22 besides the above discussed surface markers. PCs differ on the basis of their larger size and granularity, as well as loss of CD20 and CD22 expression, downregulation of CD19, and expression of CD38 and, to large extent, CD138.8 Memory B cells express three B cell cytokine receptors, BAFF receptor (BAFF-R), transmembrane activator and CAML interactor (TACI), and B cell maturation antigen (BCMA), whereas PCs have very diminished BAFF-R and TACI expression but receive survival signals through BCMA. There is differential signaling of BLYS/BAFF and APRIL through these receptors. We will briefly summarize certain agents that have an effect on PCs or (memory) B cells.

**CONVENTIONAL IMMUNOSUPPRESSANTS**

Although a number of agents have an effect on B cell subsets, nonproliferating memory B cells have been shown to be more resistant to conventional immunosuppressants such as cyclophosphamide,54 as well as to mycophenolate mofetil55 (Figure 2B). Further, generation of plasmablasts and short-lived PCs are sufficiently inhibited by conventional immunosuppressants, and nondividing PC memory is not affected by therapies dependent on proliferation.56

**CORTICOSTEROIDS**

Corticosteroids interrupt multiple steps of the immune response with genomic and nongenomic effects, but are mainly related to the inhibition of cytokine transcription via blocking transcription factors. This leads to the inhibition of certain ILs, thereby depleting T cells (IL-2) and eosinophils (IL-5), interrupting macrophage function (IL-1, TNFα), and increasing neutrophil release from the BM and neutrophil migration to the sites of inflammation.57 Less is known about the detailed effect of corticosteroids on B cells. It has been suggested that B cells are not significantly inhibited by steroids and Ig levels are only slightly decreased.58 However, high-dose glucocorticoids affects PCs and has been widely used in myeloma, especially dexamethasone. Recent data from children with steroid-responsive nephrotic syndrome show that B cell numbers decline under corticosteroid therapy and that especially peripheral memory B cells further decline after corticosteroid cessations.59 In patients with autoimmune hemolytic anemia, splenic germinal center B cells and circulating plasmablasts were largely suppressed in patients under long-term corticosteroid therapy.60

**ANTI-CD20 MONOCLONAL ANTIBODIES**

Anti-CD20 monoclonal antibodies, such as the type 1 antibodies rituximab, ofatumumab, and ocrelizumab, largely deplete peripheral B cells including memory B cells from the peripheral blood but not always in the BM,61 lymph nodes,62 or synovium.63 CD20+ pro-B cells and PCs are not targeted by these antibodies (Figure 2A), but they lead to impaired generation of plasmablasts and short-lived PCs. These monoclonals act by several mechanisms, including antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, and apoptosis induction.64 Novel type 2 monoclonal anti-CD20 antibodies,
Table 1. Indicators of the presumed pathogenic role of CD20\(^+\) B cells and CD20\(^-\) PCs on the basis of available data of therapeutic responses to different B lineage cell directed agents

<table>
<thead>
<tr>
<th>Glomerular Disease</th>
<th>AAV</th>
<th>SLE</th>
<th>ABMR</th>
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<tbody>
<tr>
<td>Response to...</td>
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<tr>
<td>Anti-CD20 (rituximab)</td>
<td>Experience from RCTs leading to approval and several observational studies.</td>
<td>Experience from two RCTs and several observational studies.</td>
<td>Experience from one RCT and several cohort studies.</td>
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<td></td>
<td>Effective in remission induction in and noninferior to cyclophosphamide,(^{106,107})</td>
<td>RCTs failed to reach primary end points in renal(^{140}) and nonrenal SLE,(^{188})</td>
<td>No difference in RCT of acute ABMR,(^{189})</td>
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<td></td>
<td>Superior to azathioprine in maintenance of remission,(^{108})</td>
<td>Meta-analysis of published data and EULAR and ERA-EDTA recommendations warrant the use of rituximab,(^{142,144})</td>
<td>Partial success in retrospective cohort studies of acute ABMR,(^{171})</td>
</tr>
<tr>
<td></td>
<td>Meta-analysis of uncontrolled studies support data from RCTs. Rituximab might be superior in relapsing disease,(^{190})</td>
<td></td>
<td>No effect of rituximab in chronic ABMR,(^{171})</td>
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<tr>
<td>Proteasome inhibition (bortezomib)</td>
<td>Very limited experience.</td>
<td>Limited experience from small case series.</td>
<td>Limited experience from small cohort studies.</td>
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<td></td>
<td>One patient with AAV refractory to rituximab successfully treated with bortezomib,(^{119})</td>
<td>Report of five patients with lupus nephritis, three had complete remission after 6–12 mo.(^{156})</td>
<td>Bortezomib is able to reduce DSA in most patients but graft survival remains very poor,(^{181})</td>
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<td>Pathogenic relevance of...</td>
<td></td>
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<tr>
<td>(Memory) B cells</td>
<td>Regulatory B cells, but not memory B cells predict relapse after rituximab therapy,(^{104,105})</td>
<td>Antibody-independent mechanisms e.g., autoantigen presentation, costimulation of T cells, production of regulatory cytokines,(^{192})</td>
<td>Memory B cells can be reactivated in secondary transplantation upon antigen re-exposure,(^{191})</td>
</tr>
<tr>
<td>Long-lived PCs/ autoantibodies</td>
<td>Materno-fetal transfer of anti-MPO antibodies led to vasculitis with pulmonary renal syndrome(^{12}) in neonates and can cause murine necrotizing crescentic GN,(^{193})</td>
<td>Reconstitution of memory B cell subset can predict disease flares after rituximab therapy,(^{136,137})</td>
<td>Responsible for the maintenance of serum DSA that cause transplant glomerulopathy,(^{164})</td>
</tr>
<tr>
<td></td>
<td>Pathogenicity of anti-PR3 antibodies is not entirely proven,(^{194})</td>
<td>Involved in the formation of immune complexes that lead to nephritis,(^{192})</td>
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</table>

RCT, randomized, controlled trial; EULAR, European League Against Rheumatism; ERA-EDTA, European Renal Association/European Dialysis and Transplant Association.
<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Kind of Intervention</th>
<th>Key Feature</th>
<th>Advantages and Disadvantages</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAV Mpo−/− mice/(Rag2−/−) mice</td>
<td>Transfer of anti-MPO antibodies or splenocytes, generated by MPO immunization of MPO deficient mice.</td>
<td>Induces necrotizing pauci-immune GN and, in some cases, pulmonary capillaritis. Transfer of splenocytes: more severe disease with immune complex deposition in the glomeruli. Transfer of antibody alone: mild disease but pauci-immune.</td>
<td>Mainly B cell but not T cell–dependent, neutrophils are required. Only mild disease without granuloma formation. Disease induction strongly depends on genetic background. Depends on the passive transfer of antibodies. No cellular immunity.</td>
<td>Xiao et al.193</td>
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<tr>
<td>Wistar-Kyoto rats</td>
<td>Immunization of Wistar–Kyoto rats with human MPO and adjuvants.</td>
<td>Leads to crescentic nephritis and lung hemorrhage in all immunized rats.</td>
<td>Stable and severe disease induction. Depends on the passive transfer of antibodies. No cellular immunity. Disease induction strongly depends on genetic background. Very mild disease. Technically challenging. Low level neutrophil reconstitution and absence of human T cells due to mixed chimerism.</td>
<td>Little et al.196, Chavelel et al.197</td>
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<tr>
<td>Humanized NOD-SCID-IL-2Rγ−/− mice</td>
<td>Transfer of patients’ PR3-ANCA IgG into endotoxin-primed human-hematopoietic stem cell (HSC) mice, created using healthy donor (PR3) HSC.</td>
<td>Induction of hematuria, glomerular hypercellularity, and pulmonary capillaritis in a some of mice.</td>
<td>Very mild disease. Technically challenging. Low level neutrophil reconstitution and absence of human T cells due to mixed chimerism.</td>
<td>Little et al.198</td>
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<td>Lupus nephritis</td>
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<td></td>
<td>Difficult to use as a genetically modified model. Alleles have to be matched to NZB and NZW backgrounds. No sex difference.</td>
<td>Dixon et al.199, Andrews et al.200, Rudofsky et al.201, More202</td>
</tr>
<tr>
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<td>MRL/MpJ-FAS/pr (MRL/lpr) mice</td>
<td>MRL is a polygenetic mouse model. FAS/lpr mutation accelerates the time course of murine disease.</td>
<td>Production of class-switched autoantibodies (anti-DNA, Sm), end-organ disease including dermatitis, GN cardiovascular and lung disease. Mice that expressed a mutant transgene encoding surface Ig but are unable to secrete Ig developed nephritis in the absence of soluble autoantibodies. B cells with cognate BCR are essential for lupus nephritis via antigen-presentation to cognate T cells or other functions (cytokine, chemokine production, formation of germinal center).</td>
<td>Model meets all of the ACR criteria for SLE. Rapid and stable onset of disease.</td>
<td>Dixon et al.199 Cohen et al.203 Andrews et al.200 Herlands et al.204 Chan et al.53</td>
</tr>
<tr>
<td>ABMR</td>
<td>Mouse heterotopic cardiac allografts</td>
<td>Animals develop chronic transplant arteriopathy. In B cell–deficient mice it was shown that chronic allograft vasculopathy was only observed in the presence of alloantibodies.</td>
<td>Depends on the passive transfer of antibodies. No cellular immunity. No insights into the mechanism of antibody formation. Offers the possibility to study outcomes of animals with continuous de novo.</td>
<td>Uehara et al.205</td>
</tr>
<tr>
<td>Humanized CD52Tg (H-2K) mice</td>
<td>Transgenic mouse model in which CD52 is only expressed on T cells. T cells are depleted by alemtuzumab (anti-CD52) and de novo allospecific B cells and DSA but not alloreactive T cells arise in some animals.</td>
<td>Animals show cardiac allograft vasculopathy, whereas animals without DSA show normal grafts.</td>
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<td>Kwun et al.206</td>
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</table>
such as obinutuzumab (GA 101), are primarily inducing cell death with a more profound and lasting B cell depletion and are currently under investigation in autoimmune diseases.65

TARGETING B CELL SURVIVAL FACTORS

There are several approaches indirectly targeting B cell and PC survival via cytokine targeting. One is blocking BAFF or the related cytokine APRIL (Figure 2B). These cytokines bind to three different receptors expressed on B cells: BAFF-R, TACI, and BCMA. BAFF-R exclusively binds BAFF,66 whereas both cytokines can bind to TACI and BCMA.67 APRIL, binding to BCMA is crucial for the survival of PCs,68 whereas memory B cells do not depend on these survival factors.69,70 Several biologic drugs targeting the BAFF/BLyS and APRIL axis have been studied over the last years, including belimumab, tabalumab, atacicept, and blisibimod. Belimumab binds to soluble BAFF and has been approved for the treatment of SLE.71 Tabalumab is a human mAb whereas blisibimod is a fusion polypeptide protein. Both block biologically active BAFF72–74 but development programs of tabalumab in SLE have been cancelled after poor efficacy results in clinical trials. Atacicept is a TACI-Ig fusion protein that blocks both BAFF and APRIL.75 So far, the effect of these compounds on PCs becomes evident by the substantial reduction of all Ig classes to different degrees, whereas the effect on memory B cells is largely confined to the initial increase in peripheral blood likely related to their mobilization.76,77

PROTEASOME INHIBITORS

Proteasome inhibitors are prototypic for their effect on highly differentiated PCs with substantial endoplasmic reticulum stress. They selectively target the 26S proteasome,78,79 which is needed for the degradation of ubiquitinated proteins.80 Their cytotoxic effect causes accumulation of misfolded proteins in the endoplasmic reticulum, leading to the activation of the terminal unfolded protein response causing apoptosis81 and prevention of the activation of NF-κ light chain enhancer of activated B cells (NF-κB), which is crucial for PC survival.82 Initial data were obtained for multiple myeloma but proteasome inhibitors are toxic to non-neoplastic cells as well.83 Further, cells with high protein production, such as PCs, are susceptible to proteasome inhibition.84 Bortezomib is the most widely used proteasome inhibitor approved for the treatment of mantle cell lymphoma and myeloma.85 The main limitation of bortezomib is severe neurotoxicity that occurs in up to 30% of all patients, resulting in therapy discontinuation,86 which is less with the new generation compounds carfilzomib87 and delanzomib.88

ANTI-CD38

The monoclonal daratumumab targets CD38, which is highly expressed on the surface of short-lived PCs, long-lived PCs, and myeloma cells,89 but only expressed at low levels on lymphoid or myeloid cells and some other tissues.90 Daratumumab monotherapy was recently approved for the treatment of refractory multiple myeloma on the basis of two studies.91–93 Also, the addition of daratumumab to standard care has shown favorable results.94,95 with an overall acceptable safety profile.96 Nonetheless, a high number (71% of patients) of adverse reactions of cough, bronchospasm, and dyspnea were observed upon infusion of daratumumab.93 Patients with autoimmune diseases have not yet been treated with this antibody but it appears to lead to a profound depletion of plasma cells.

TARGETING PC HOMING AND SURVIVAL

A number of recent efforts have been made to target certain factors involved in PC homing/migration or interfere with their survival in order to affect long-lived PCs otherwise refractory to treatment (reviewed recently2), including autologous stem cell transplantation.97 Clinical experiences are very limited. In contrast, the extrinsic and intrinsic characteristics of memory B cell maintenance are far less understood.

CLINICAL EXPERIENCES SUGGEST DISTINCT ROLE OF MEMORY B AND PCS IN DIFFERENT GLOMERULAR DISEASES

AAV

Small-vessel vasculitis, which is associated with autoantibodies against neutrophil cytoplasmic antigens, manifests as a necrotizing inflammation of vessels with little or no Ig and complement deposition in the vessel wall. This lack of Ig deposit distinguishes ANCA-associated GN (directed against MPO or proteinase 3) from immune complex-mediated GN, e.g., found in lupus nephritis.88 In human kidney specimens of patients with AAV, B cell clusters with IgD+B cells forming germinal center-like structures were found, whereas CD138-positive plasma was almost absent.99 T cells that are present in the inflamed kidney tissue mostly belong to a senescent memory T cell population, whereas the number of memory T cells in circulation is decreased, indicating their migration as effectors into the kidney.100,101

Disturbances of peripheral B cell subsets have been described in patients with AAV. Here memory B cells are reduced in patients with active disease,102 possibly by recruiting B cells to the sites of inflammation or their differentiation into PCs. During remission memory B cell levels are restored.102 An imbalance of regulatory B cells, a B cell fraction that is commonly defined by the production of IL 10, a cytokine able to suppress T cells, has been reported.103 These regulatory B cells are decreased in patients with AAV. Diminished regulatory B cells were found to predict relapse after rituximab therapy.103,104 The overall memory B cell population did not correlate with disease activity or the time to flare after B cell depletion,104 whereas CD38+ peripheral PC and B cell activation have been reported to correlate with active ANCA vasculitis.105

Anti-CD20 therapy with rituximab has been approved on the basis of clinical
studies for remission induction, and is also a valuable maintenance therapy, especially in refractory disease or patients not responding to cyclophosphamide. Because the ANCA titers typically, but not always correlate with disease activity and are reliably reduced by rituximab to a greater extent than seen with cyclophosphamide, they appear to be the product of short-lived PCs, which provides the basis for the key role of autoreactive B cells in AAV. However, rituximab has also been effective in ANCA-negative patients with granulomatosis and polyangiitis, and is also a valuable maintenance therapy. Studies for remission induction, as well as supportive by a case report in which febrile neutrophil activation with neutrophil extracellular traps were seen after rituximab, due to the reduction of B cell BAFF-R clearance, promotes autoreactivity in the reconstituting B cell repertoire. BLyS/BAFF inhibition is also effective in IMN, where it significantly reduces anti-PLA2R antibodies within 28 weeks (Clinicaltrials.gov identifier NCT01610492).

There is little experience with agents targeting PCs in AAV. Bontscho et al. reported that bortezomib is able to inhibit anti-MPO-mediated necrotizing crescentic GN in mice by depleting MPO-specific PCs in the spleen and BM. A favorable effect of proteasome inhibition was also reported in one patient with AAV, but precludes any firm conclusion in the absence of larger experiences.

SLE
Lupus nephritis is the most common severe organ manifestation in SLE, seen in up to 50% of cases. Histologically, it can present with a spectrum of glomerular, vascular, and tubulointerstitial lesions, including germinal center-like structures in the interstitium. Cytokine-mediated activation of glomerular mesangial cells and in situ tubulointerstitial macrophages promotes the deposition of anti-dsDNA, anti-C1q, anti-nucleosome, and anti-glomerular antibodies recognizing glomerular structures—the formation of immune complexes with complement deposition initiating renal damage. Circulating neutrophil activation with neutrophil extracellular trap deposition in the glomeruli may also be an initiating event in renal inflammation. Anti-dsDNA antibodies represent a risk factor for lupus nephritis, with negative predictive value for renal outcome in some studies. In addition to the typical presence of autoantibodies, antibody-independent mechanisms contribute to lupus pathogenesis. Lupus nephritis biopsies contain interstitial B cell lymphocyte infiltrates that are organized along with T cells and dendritic cells, and undergoing further differentiation as in germinal centers. Within these aggregates, T follicular helper cells are important for establishing germinal centers to promote activation and differentiation of B cells. T follicular helper cells are expanded in patients with SLE and the expression of programmed death 1 correlates with circulating plasma-blasts and anti-dsDNA antibodies.

The presence of these tertiary lymphoid structures is associated with the formation of autoantibodies and tubular basement membrane immune complexes in more active disease.

All these data show that the interaction of B and T cells is critical in lupus nephritis. In addition to the footprints of B cell abnormalities (autoantibodies and immune complexes), continuous activation of innate and the adaptive immunity is operative. Here, T cells provide help to B cells or are cytotoxic to glomeruli or tubular cells. A key characteristic of SLE T cells is their reduced IL-2 production, which inversely correlates with enhanced IL-17 regulated by the transcription factor CREM-α. Moreover, IL-2 as an essential growth and survival factor for Treg cells has been found to be functionally deficient in patients with SLE. A number of genetic risk alleles and epigenetic modifications altering the gene expression and the function of certain T cell subsets in SLE appear to be involved. These abnormal T cell responses can result in disturbances of B cells in SLE. Because SLE pathology apparently comprises pathogenetic contributions of memory B cells and PCs dependent on T helper cells, abnormal T cell activation may represent a critical link. Proof for this idea is very small, but a recent phase 2 trial is using voclosporine in lupus nephritis. Alternatively, therapies with an effect on T and B cell interaction (Figure 3) are attractive. In addition, low-dose IL-2 has been studied in non-renal SLE and demonstrated some clinical efficacy, leading to an increase of Treg cells in vitro and in vivo.

The contribution of CD20+ and especially memory B cells became apparent by experience with B cell-depleting agents, such as rituximab. In several reports the reconstitution of the memory B cell subset after rituximab predicted recurrence of active disease. This phenomenon has not exclusively been observed in SLE but also in patients with rheumatoid arthritis and
IMN, and suggests that memory B cells contribute to the pathogenesis of these diseases. A reduction in autoantibody titers, in contrast to AAV, did not always predict the effectiveness of rituximab therapy.\textsuperscript{139} Rituximab did not meet the primary efficacy end point in a lupus nephritis trial (LUNAR).\textsuperscript{140} Partly due to aspects of the trial design. Clinical experiences\textsuperscript{141,142} in otherwise refractory patients support a clinical value that has led to recommendations as a second line agent by the American College of Rheumatology\textsuperscript{143} and European League Against Rheumatism-European Renal Association/European Dialysis and Transplant Association.\textsuperscript{144} Rituximab, in combination with mycophenolate mofetil, has been reported in newly diagnosed patients with lupus nephritis in a UK center,\textsuperscript{145} with a substantial rate of patients achieving complete remission without use of oral glucocorticoids. This study requires confirmation in treatment-naïve patients, whereas the currently available data suggest that anti-CD20 depletion alone may not be sufficient in patients with established disease. In this population, the additional role of PCs likely requires cotargeting.

Belimumab has been approved for general SLE on the basis of the successful phase 3 studies BLISS-52\textsuperscript{146} and BLISS-76.\textsuperscript{147} However, there are conflicting data about the interrelation of the cytokine levels of BAFF and APRIL with lupus activity.\textsuperscript{148} Notably, these studies found an association between response to belimumab and reductions in autoantibody titers, suggesting successful targeting of PCs. Although other compounds targeting BAFF, such as tabalumab\textsuperscript{149,150} or atacicept\textsuperscript{151} cotargeting APRIL, did not provide convincing clinical data, a more profound effect on PCs reflected by Ig reductions was noted. Selective inhibition of the PC-relevant cytokines without sufficient treatment of CD20\textsuperscript{+} memory B cells may hold more promise. In this context, a sequential approach of B cell depletion and BlyS/BAFF inhibition to influence PC activity is being evaluated in lupus nephritis, the Rituximab and Belimumab for Lupus Nephritis (CALIBRATE) study (clinicaltrials.gov identifier NCT02260934) and in extrarenal lupus, the BEAT-LUPUS study.\textsuperscript{152}

Efforts to preferentially target the PC compartment have been undertaken. In a murine model of lupus-like nephritis, short- and long-lived PCs were depleted by bortezomib and lupus-like nephritis improved remarkably.\textsuperscript{83} These results were confirmed by Ichikawa et al.\textsuperscript{153} and Seavey et al.,\textsuperscript{154} using the less toxic proteasome inhibitors carfilzomib\textsuperscript{67} and delanzomib.\textsuperscript{88} Clinical experiences with proteasome inhibition in lupus nephritis rely only on bortezomib. Alexander et al.\textsuperscript{155} reported 12 patients with refractory SLE including six patients with nephritis. Musculoskeletal and mucocutaneous manifestations improved in all patients, and proteinuria significantly decreased in patients with nephritis. A recently published study reports a beneficial effect with a decrease in creatinine in three of five patients with lupus nephritis.\textsuperscript{156} A phase 2 study is currently investigating the safety and efficacy of bortezomib in patients with SLE (Clinicaltrials.gov identifier NCT02102594). A phase 1 study in lupus nephritis with the oral proteasome inhibitor ixazomib is underway (Clinicaltrials.gov identifier NCT02176486). Although proteasome inhibition is often considered a PC-targeted therapy, it has the advantage to cotarget PCs but also affect other immune cells.\textsuperscript{155} A potential difference of lupus nephritis versus AAV is that effective B lineage therapy requires cotargeting of memory B cells and PCs.

**Kidney Transplantation**

In the transplant setting, donor-specific antibodies (DSA) are likely the main biological reason for allograft injury and loss.\textsuperscript{157} Their detection either pre- or post-transplant increases the risk of graft loss.\textsuperscript{158,159} DSA directly bind to the grafts and cause local inflammation and tissue damage through complement activation and human Fc gamma-mediated cytotoxicity, and also serve as opsonins that facilitate the activation of alloreactive T cells.\textsuperscript{160–162} These mechanisms lead to the development of transplant glomerulopathy, the histologic feature of chronic ABMR.\textsuperscript{163} Long-lived PCs are responsible for the maintenance of long-term circulating DSA, which are detectable in solid-organ transplant recipients and are associated with worse transplant outcomes.\textsuperscript{164} Quiescent memory B cells appear to be reactivated in cases of secondary transplantation in presensitized individuals. The best risk management to avoid transplant glomerulopathy comprises transplantation without preexisting DSA\textsuperscript{159} and avoidance of transplantation with HLA mismatches, especially...
as class 2 HLA mismatches are a strong predictive biomarker for DSA. The induction of DSA requires interaction between B cells and antigen-specific CD4 T helper cells. The presence of memory T cells before transplantation is associated with a poor transplant outcome. ABMR and the underlying importance of pathogenically relevant PCs producing DSA are a key target of immunotherapy to eradicate DSA. These strategies include plasmapheresis and immunoadsorption for the direct removal of DSA, intravenous Igs for immunomodulation, rituximab to deplete PC precursors, and targeting PCs by proteasome inhibition. Combined renal and allogeneic BM transplantation have also aimed to deplete PCs and induce tolerance without the need for antirejection therapy.

B cell–directed therapy by rituximab seems to be partially effective in acute ABMR but ineffective in chronic ABMR. Jackson et al. provided interesting evidence about long-lived PCs in humans and the capacity of rituximab to interfere with relevant B cells as PC precursors when they compared DSA levels in patients who have been desensitized with or without rituximab. They found a greater reduction of DSA but a similar rate of DSA persistence, most likely explained by refractory long-lived PCs, whereas the difference in DSA titers can be ascribed to memory B cells undergoing differentiation into PCs.

Kidney transplantation permits comprehensive corresponding insights in tissue resident immunology. Just as in other glomerular diseases, tertiary lymphoid structures have been identified in kidney grafts. Within the tertiary lymphoid structures an increase of activated memory CD4 T cells and a decrease in T regulatory subsets (IL-10–producing Tr1 cells and Foxp3–positive Tregs) has been observed. Also PC–rich infiltrates have been found in renal allografts and their occurrence was associated with an increased risk of allograft failure in the pediatric population. However, data on PC–rich infiltrates in the adult population are limited. To target the PC compartment, proteasome inhibition has been included in the treatment repertoire. In a model of chronic allograft nephropathy, bortezomib reduced antibody titers and depleted PCs in the BM. The reduction of PCs was also observed in BM aspirates of two patients treated with bortezomib for humoral rejection. Several small case series report the effect of bortezomib treatment on graft survival, kidney function, and DSA levels. Overall, patients responded together with a reduction in DSA levels and a stabilization of kidney function in refractory patients. The BORTEJECT (Clinicaltrials.gov identifier NCT01873157), the TRIBUTE study (Clinicaltrials.gov identifier NCT02201576), and the Effect of BMMSCs on Chronic AMR after Kidney Transplantation study (Clinicaltrials.gov identifier NCT02563340), will evaluate the clinical value of bortezomib on chronic ABMR.

Notably, levels of BAFF were found to be associated with the occurrence of transplant glomerulopathy and enhanced ABMR. thus BAFF targeting appeared an attractive option. However, a study of tabalumab for desensitization did not lead to clinically significant reduction of DSA, which may be taken as another indicator that outstanding long-lived PCs are a key challenge in this clinical setting. Studies of belimumab for pretransplant desensitization and at the time of transplantation are ongoing (clinicaltrials.gov identifier NCT01025193). An innovative approach to reduce HLA antibodies in the setting of desensitization of highly sensitized patients is the use of the endopeptidase derived from Streptococcus pyogenes (IdeS), which cleaves IgG into F(ab′)2 and Fc fragments. IdeS leads to a complete IgG deficiency what makes it less attractive for long-term use in chronic ABMR. However, early clinical experiences with IdeS in renal transplants provide additional proof that targeting autoreactive PCs is key in targeting ABMR.

CONCLUDING REMARKS

A number of preclinical models have supported the role of B lineage cells in glomerulopathies, but do not reflect the complexity of human diseases. Interventions in human disease have inspired a revision of the role of B cells and long-lived (memory) PCs in these entities. Here, currently available data support a key role of B cells (likely memory and activated B cells) in AAV and IMN, and long-lived PCs as a pathogenic driver in chronic ABMR, whereas lupus nephritis appears to be under the influence of both B cells and PCs (Figure 4). As a consequence, the idea that distinct immunopathogenic contributions by memory B cells and PCs is also probably related to distinct T helper cell abnormalities requires consideration.
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