Published in final edited form as:

Curr Opin Pharmacol. 2007 August ; 7(4): 426–433. doi:10.1016/j.coph.2007.05.006.

B cell modulation in rheumatology

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Summary of recent advances

While evidence of dysregulation of the B-cell compartment was first demonstrated with the identification of autoantibodies, other functional roles of B lymphocytes in autoimmune pathogenesis have generally been underappreciated or completely overlooked. With the recent approval of the first B-cell targeting agent in rheumatoid arthritis, new strategies are being developed to target B cells through a range of membrane-associated lineage-specific molecules, and also by interfering with B-cell specific pro-survival signals. B-cell directed agents are therefore providing an effective new mechanistic approach to treatment, and also enabling new perspectives from the dissection of the contributions of B cells in physiologic and pathologic immune responses.

I. Introduction

In a reiteration of a now familiar tale, rheumatologists have begun to embrace the first in a new class of agents that was originally developed to treat diseases with very different pathogenesis [1]. The anti-CD20 antibody, rituximab (RituxanTM, MabTheraTM), was initially approved for the treatment of CD20+ B-cell, low grade or follicular non-Hodgkin's lymphoma after relapse or refractory to conventional treatment. In early 2006 the FDA approved Rituximab for patients with moderate to severe rheumatoid arthritis (RA) with inadequate clinical response to TNF-blocking agents. Interim experience indicates that B-cell specific therapy may provide a desirable safety profile in many patients, especially in comparison to the toxicities and adverse events associated with standard immunosuppressives, cytotoxic and biologic therapeutic agents.

The therapeutic targeting of B lymphocytes in RA also provides a conceptually new approach for the treatment of chronic inflammatory diseases that was unexpected for many physicians. Despite the known prevalence and utility in confirming diagnosis the actual direct contributions of disease-associated autoantibodies (such as rheumatoid factors, RF and autoantibodies to citrullinated cyclic peptide, CCP) to pathogenesis have long been debated. While autoantibody-containing immune complexes can trigger and perpetuate inflammatory responses through recruitment of complement and Fc γ receptor mediated pathways, which are often prominent pathogenetic features in RA, there are also patients that are seronegative for these autoantibodies who still fulfill clinical diagnostic criteria.

A secondary but not insignificant consequence of the demonstrated success of B-cell deletion therapy in this common autoimmune disease is a reconsideration of how B lymphocytes may

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be enmeshed in pathogenesis in roles beyond their contributors of secreted antibodies. B-lineage cells also potentially have the capacity to contribute to immune defenses as cytokine producers, antigen-presenting cells and T-cell stimulators. It is therefore now being questioned whether clinical benefits of B-cell targeted therapy may result from removal of non-immunoglobulin (Ig) B-cell activities from the self-perpetuating pathways, even in autoimmune diseases in which circulating autoantibodies are uncommon or even unknown [2,3].

II. Ligands for B-cell antigen receptors as immune modulating agents

Each clonally related set of B cells shares an antigen receptor (i.e., B-cell antigen receptor, BCR), which is encoded by paired somatically generated antibody gene rearrangements. There may be special advantages for targeting via the cell membrane-associated BCR, which distinguish defined related sets of B cells, as it may be possible to delete B cells that express only certain binding (auto) specificities or that use only certain variable region elements, without affecting other B cells that are required for immune defenses. For example, it may be possible to generate variations of an antigen that soak up binding antibodies, and thereby block their contribution to pathogenesis and tissue injury. Moreover, some therapeutic BCR ligands may selectively induce the clonal deletion or functional inactivation of autoreactive B cells.

The synthetic oligomeric DNA agent, termed LJP394 (Abetimus, Riquent™) was developed as a means to counter pathogenic anti-DNA responses in SLE patients [4]. While it has been argued that treatment with this agent correlates with reduction in circulating levels anti-DNA antibodies, the actual molecular/cellular mechanism of action has not yet been clearly elucidated.

Potentially important mechanistic insights on BCR targeting have come from studies of the active moiety in the Prosorba™ apheresis column, which is approved for the treatment of autoimmune thrombocytopenia and RA [5]. The active moiety in this column is staphylococcal protein A (SpA), a microbial immunoglobulin (Ig)-binding protein. The column contains 200 mg of SpA, which is inadequate to completely deplete the IgG content of even 60 ml of blood. Due to the many liter volume of distribution of IgG in the human body such treatments are also unable to induce a measurable change in circulating IgG levels. However, during pheresis treatments patients are reported to be infused with doses of SpA that leach off the column, which are comparable to those shown to induce quantitative in vivo B-cell deletion in mice [6,7]. SpA can act as a superantigen with the capacity to specifically target large clonal sets of B cells, which express BCR from the large VH3 gene family, by a mitochondrial pathway of activation-associated apoptotic B-cell death [8]. Induced B cell death had an absolute requirement for Bim, a prodeath Bcl2 family member, which appeared to play a central role in integrating BCR and B cell survival factor signals to determine clonal fate [8]. Significantly, SpA treatments can ameliorate murine SLE [9]. It has therefore been proposed that SpA, or therapeutic analogues with similar BCR-binding/cross-linking activity, may provide therapeutic benefits in autoimmune diseases with prominent autoreactive B-cell populations [10].

III. Agents that target B-cell membrane-associated molecules

Therapeutic agents have been developed against a range of B-cell associated molecules (Figure 1 & 2). While in several cases these have proven to be practical lineage-specific targets, our knowledge of the natural functional roles of these molecules is currently incomplete. Importantly, there are likely immunobiologic differences between these gene products in humans and in the murine and primate species routinely used for early development, as well as their potentially different roles in health and disease.

CD20

CD20 is a 33–37 kDa non-glycosylated transmembrane protein that is expressed on B-lineage cells from the pre-B cell stage to the mature B-cell stage. While it is debated whether it is expressed on memory B cells, it is not displayed on early B-cell progenitors or end-differentiated plasma cells. The functional roles of CD20 remain controversial. CD20 has been implicated to form calcium channels in cell membranes that may be involved in B-cell activation, proliferation and differentiation [11]. However, engineered CD20-deficient mice have intact B-cell compartments and are not immunocompromised, which suggests that CD20 may be functionally redundant with other proteins, even though CD20 is lineage-specific [11].

The prototypic therapeutic anti-CD20 agent, rituximab, is a chimeric (i.e., of more than one species) antibody, as the variable regions involved in antigen recognition are of mouse origin. As sequences of the mouse proteins are foreign to the human body, they can act as an antigen itself (Figure 3). Therefore the constant regions responsible for antibody effector functions were replaced with human constant regions to reduce the potential for immunogenicity [1], which potentially can induce immune responses that attenuate anti-CD20 biologic activity or even result in allergic reactions.

Rituximab is an example of what has been termed a naked antibody, as it is devoid of an associated toxin. The biologic activity of rituximab derives from the binding to CD20-bearing B cells, which serves to form immune complexes that focus host IgG effector functions that otherwise defend from exogenous threats. In different experimental systems, the B-cell deleting activity of rituximab has been shown to reflect diverse antibody-based mechanisms: hypercross-linking of membrane-associated CD20 molecules induction of apoptosis; antibody dependent cytotoxicity (ADCC) evoked through FcγR-mediated interactions with adjacent mononuclear cells; and complement-dependent cytotoxicity (CDC) [12]. While earlier studies primarily used in vitro studies of lymphoma cell lines, more recent reports of in vivo murine models have confirmed earlier findings, and also implicated a special role of hepatic macrophages of the reticuloendothelial system in the clearance of CD20-targeted B cells [13, 14,15]. These in vivo systems have also suggested that some CD20-bearing B cells may be resistant to therapeutic deletion, either due to ineffective tissue penetration or cell-mediated or soluble factor induced pro-survival influences.

Anti-CD20 therapeutic targeting can provide an interval of significant clinical improvement in patients with RA, a systemic autoimmune disease with chronic symmetric polyarticular inflammatory arthritis that is associated with systemic immune abnormalities leading to progressive joint destruction and disability. While the joint lining of affected rheumatoid joints often include pathologic cellular infiltrates with prominent B cells and plasma cells, the pioneering randomized controlled trial of Edwards and coworkers, which showed the significant clinical responses at 6 month after an intravenous 1 gram infusions of rituximab, also repeated 14 days later, provided direct evidence that B-lineage cells play central roles in pathogenesis [16]. Although better than rituximab as monotherapy, the clinical response in a treatment arm of rituximab with i.v. cyclophosphamide was shown to be no better than after co-treatment with rituximab along with weekly oral methotrexate, the benchmark disease-modifying agent for RA [16]. FDA approval was based on the significant clinical responses to rituximab and methotrexate demonstrated in a randomized controlled trial in RA patients with past TNF inadequate responses [17]. Depletion of circulating B cells (up to 98% reductions) were detectable within hours to days of the first infusion of rituximab, which generally persisted for 4 to 6 months, or longer. Only one limited dose ranging study has been reported (DANCER) [18], which suggests that treatment regimens may be improved for safety or efficacy.

Anti-CD20 in SLE

In lupus pathogenesis, B cells have also been implicated due to their roles in autoantibody production, and for the recruitment and stimulation of T lymphocytes and of cells of the innate immune system [19]. However, clinical progress has been slowed by difficulties in the design of SLE trials, including issues involved in developing validated clinical disease activity endpoints [20]. While Rituximab treatment results in almost uniform induction of blood B-cell depletion in RA patients, the responses to Rituximab as a monotherapy have been reported to be more heterogeneous in SLE patients [21,22], although this may not be common after co-treatment with cyclophosphamide [23,24]. Initial phase I/II rituximab open trials in SLE patients have shown efficacy an attractive safety profile [21,23,24]. Larger randomized controlled trials are currently in progress.

Anti-CD20 in other rheumatic diseases

In case reports and small open trials, rituximab is also reported to be well tolerated and provide benefits in primary Sjögren's Syndrome (pSS). Most patients were reported to experience significant improvement in sicca symptoms, along with an increase in salivary gland function [25,26,27]. Clinical responses in pSS have also been associated with a reduction in corticosteroid usage. Initial trials have shown that rituximab has been well-tolerated and induced effective remission in some patients with severe refractory anti-neutrophil cytoplasmic autoantibody (ANCA) associated vasculitis [28,29,30].

New anti-CD20 agents

Several other anti-CD20 targeting biologic agents (ocrelizumab, ofatumumab, TRU-015), which represent variations in monoclonal antibody engineering techniques, are also in clinical development (Figure 3). To overcome species related immunogenicity, ocrelizumab was engineered from rituximab without residual mouse amino acids in the variable regions [31]. Ofatumumab was made from mice engineered to express large transgenes of human Ig variable regions. These mice were then challenged cells bearing human CD20 antigen, to enable selection of the parental B-cell lines expressing human anti-CD20 antibodies [32]. Another antibody-based agent, TRU-015 represents what has been termed a small modular immunopharmaceutical (SMIP) as it has engineered to be smaller than a naturally occurring IgG, by omitting the constant region domain of the light chain and the first constant region domain of the heavy chain. To hold the variable regions of the heavy and light chains together and link the remaining constant regions that are responsible for antibody effector functions, a small linking peptide was also inserted into this construct [33]. All of these new anti-CD20 agents have shown good safety profiles, especially with the use of corticosteroid pretreatments to decrease infusion reactions, and larger trials are currently planned.

CD22

CD22 is a 135 kDa B-cell specific sialoglycoprotein, which can bind 2–6-linked sialic acid residue containing oligosaccharides, acts as an adhesion molecule and can also provide both stimulatory and inhibitory influences on B-cell activation. CD22 is widely expressed on the membranes of B-lineage cells, including immature, transitional and activated B-cells [34]. Epratuzumab, a humanized IgG1 anti-CD22 monoclonal antibody, is currently in early clinical trials for B-cell lymphomas, and autoimmune diseases including SLE and Sjögren's syndrome [35,36]. In contrast to the cytotoxic anti-CD20 agents, epratuzumab acts as an immunomodulatory agent [37]. In vitro studies have shown that combination treatment with epratuzumab did not decrease the capacity of rituximab to induce apoptosis, or for complement- or antibody-mediated cytotoxicity. In the presence of a cross-linking second antibody, this combination also enhanced inhibition of the proliferation of lymphoma B cells [37].

In an open-label, single-center study, 14 patients with moderately active SLE who received 360 mg/m² epratuzumab intravenously every 2 weeks for 4 doses showed significant decreases in SLE activity (measured by British Isles Lupus Activity BILAG). These patients displayed a mean 35% decrease in blood B-cell numbers that persisted 6 months post treatment, while there were no significant changes in T cells, Ig or autoantibody levels [36]. In another open-label, phase I/II study of active pSS, 14/16 patients completed the infusion regimen without significant reactions, and 53% achieved a clinical response (as measured by Schirmer test of tear production), unstimulated whole salivary flow, as well as improvements of the acute phase reactant, erythrocyte sedimentation rate at 6 weeks, with comparable improvements lasting up to 32 weeks. While blood B-cell levels were reduced a mean 54% and 39% at 6 and 18 weeks, T-cell and Ig levels did not significantly change [35].

Other membrane-associated B-lineage targets, such as an antibody to CD79a [38], the transmembrane molecules expressed broadly during B cell development, as well as the CD19 [39], are also being evaluated.

VI. Agents specific for B-cell survival factors

In the absence of appropriate cell-mediated or soluble pro-survival factors, B lymphocytes spontaneously undergo apoptotic cell death. These survival signals extend the lifespan of B-lineage cells at local sites in lymphoid tissues, and are likely at times to also convey resistance of B cells to therapeutic CD20-targeted interactions [13]. Among the many soluble factors that can affect B-cell survival during their differentiation, much interest has recently been focused on two related members of the TNF family, termed B-cell activating factor (BAFF) and also called B lymphocyte stimulator (BLySTM) or TALL-1, THANK, and zTNF-4), and the related factor, A Proliferation Inducing Ligand (APRIL) [40,41]. BAFF is a type II transmembrane protein expressed on various cell types including dendritic cells, neutrophils, monocytes and lymphoid stromal cells, especially when they have been activated. While active as a membrane-bound trimer, a soluble protein fragment is released after cleavage by a furin-like convertase. BAFF binds with different affinities and with different stimulatory outcomes to receptors that include BAFF receptor (BR3), B-cell maturation antigen (BCMA), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) and to proteoglycans [42]. While the expression of these different receptors is debated and there may be species-specific variations, BR3 is expressed on mature B cells, BCMA is expressed on mature B cells and plasma cells, whereas TACI is expressed on B cells and reportedly on some T cells. BCMA and TACI also bind to APRIL and heterotrimers of BAFF/APRIL [43], which can act as a co-stimulator along with a BCR signal that results in B-cell proliferation. BAFF can also prevent death of auto-reactive B cells from death, presumably by providing a second signal after encounter with endogenous self-antigenic ligand. BR3 ligation yields up-regulation of Bcl-2 expression and activation of NF- κ B, that also increases B-cell survival [44]. Hence, BAFF and/or APRIL may be essential to physiologic immune homeostasis for the survival and selection of B-cell clones at critical points, termed checkpoints in their differentiation/maturation.

The pathologic autoimmunity in RA may in part reflect the dysregulated expression and cellular responses to BAFF and APRIL. It has been postulated that TNF, locally produced in rheumatoid synovium, leaks into the bloodstream. When it enters the bone marrow, it mediates the early release of B-cell precursors as they follow gradients of B-cell chemotactic factors inappropriately produced by inflammatory cells into the joints. Immune complex-mediated triggering of mast cells and release of humoral factors induce the release of neutrophils from the bone marrow, which directly exacerbate local tissue injury through the release of proteases and related factors, and are major sources of the TNF family B-cell pro-survival factors, BAFF. The lining fibroblast-like synoviocytes can express high levels of BAFF in presence of TNF and interferon- γ [45]. High locally produced BAFF and APRIL may also allow the survival,

proliferation and differentiation of autoreactive B-lineage cells that enter the joint, by potentially bypassing clonal selection mechanisms that are otherwise required to maintain immune tolerance.

After rituximab treatment, the effects on BAFF and APRIL levels appear quite different in RA and SLE patients. In a limited study with 10 SLE patients and 9 RA patients treated with rituximab, blood BAFF levels were reported to rise after rituximab treatment in both patient groups. However, in SLE patients the BAFF levels declined close to pre-treatment levels upon B-cell repopulation. APRIL levels which were normal at baseline, and decreased during depletion in SLE patients, whereas patients with RA had APRIL levels 10-fold higher than normal, which did not change during depletion [46].

As an alternate strategy to target B cells, therapeutic agents have been developed to interfere with B-cell specific survival factors (Figure 3). Belimumab (Lympho-Stat-BTM), a fully human IgG antibody developed to antagonize BAFF activity in autoimmune diseases and B-cell malignancies. In non-human primates, belimumab was shown to be safe and well tolerated at 5–50 mg/kg for up to 26 weeks, with potent biologic activity as demonstrated by significant decreases in peripheral blood and tissue B lymphocytes by 13 weeks of exposure while other leukocyte lineages were unaffected. Moreover, B-cell depletion was generally reversible within a 34-week recovery period [47].

In recent preliminary report on phase II studies In SLE patients, belimumab demonstrated a high level of safety and although it did not meet its primary clinical end-point, it has been argued that subset analysis revealed some efficacy [48].

Other B-cell targeting agents have been generated by the expression of human cellular receptors for B-cell survival factors, act as decoy receptor fusions by soaking up local levels of B-cell survival factors. TACI-Ig (AtaciceptTM) is a decoy receptor that fuses the two human ligand-binding domains of the TACI natural cellular receptor for BAFF and APRIL molecules, to human IgG heavy-chain constant region domains that increases its circulating half-life. BR3-Fc is similar to Atacicept except it contains a human ligand-binding region of the natural receptor, BR3 [49]. Phase I studies for both of these agents have demonstrated safety for limited therapeutic exposures, but will require further trials to better understand the fuller implications for longer term treatment and their clinical utility as therapeutic agents.

Signalling through the lymphotoxin (LT) pathway has been shown to be a crucial element in the maintenance of the organized microenvironment of peripheral lymphoid tissues. As another means for directly or indirectly affecting B-cell responses, inhibitors of the LT pathways have been shown to reduce disease in a wide range of autoimmune models [50]. Although it has pleiotropic properties with effects on a range of cell types, as well as being a primary contributor to acute phase responses, IL-6 has important pro-survival effects on B-lineage cells, and in particular end-differentiated Ig-secreting cells [51]. Significantly, early controlled studies have shown significant clinical efficacy with an antibody that blocks interactions with the IL-6 receptor [52].

IV. Conclusions

Recent progress in the development of B-cell targeting therapy has demonstrated utility in RA, a common autoimmune inflammatory disease, and many other autoimmune rheumatic diseases, as well as those seen by a wide range of medical subspecialists are also currently being evaluated in clinical trials. Even more exciting, these new agents provide a practical means for dissecting the contribution of different B-cell subsets to pathogenesis. Initial reports have also begun to describe the immunobiological implications for anti-CD20 mediated B-cell depletion and subsequent regeneration and repopulation [53,54]. This new field of clinical

investigation promises to reveal how B-cell responses are regulated in health and disease, and potentially how B-cell immunologic memory is maintained [55]. both for protective immune defenses and autoimmunity that drives pathogenesis.

Acknowledgments

Efforts in our research group are supported by grants AI40305, AR47360, AR50659 and AI46637 from NIH and the Alliance for Lupus Research (to GJS).

Ethics in Publishing: General Statement. GJS has been a consultant for Genentech.

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Figure 1. Survival factors for B-lineage cells

There are several cytokine and chemokine factors that can enhance survival of B-lineage cells, as primarily demonstrated from in vitro studies, but are believed to potentially also act in physiologic lymphocyte development, and dysregulated expression may contribute to autoimmune pathogenesis. Stem cells in the bone marrow can replace peripheral B cells. These factors have different levels of activity for B-lineage cells at different stages of differentiation. These factors may also affect certain non-B cells as well. IL-7 is primarily made by mesenchymal stromal cells in the bone marrow, while other factors may be made by a range of cells that differ in health and disease. Adapted from G. Silverman *Arthritis Rheum.* 2006

Figure 2. Potential Targets on B-lineage cells

Throughout life, B cells pass through sequential stages of differentiation that can be distinguished based on the expression of diagnostic patterns of surface molecules. CD20, which is expressed during intermediate stages of differentiation, has been used as a B-cell-specific target or new therapeutic agents. Other membrane-associated molecules, which may provide alternative targets for new therapies, are differentially expressed during B cell maturation and differentiation. While CD19, CD20 and CD22 are B-lineage specific, CD27 is also expressed on some T cells. Adapted from Silverman G. *Arthritis Rheum.* 2006. 54:2356–2367.

Figure 3. Construction of B-cell targeting therapeutic agents

The range of types of recombinant protein-based agents that target B cells. The first antibody in the series was rituximab. It was originally all mouse (red). It was engineered to replace both light-chain and heavy-chain constant regions with human constant regions (green). The variable regions involved in antigen recognition are all mouse (red). Several human monoclonal antibodies are now available. It is believed that ocrelizumab was an engineered version of rituximab that attempted to remove any residual mouse amino acids in the variable regions. Ofatumumab were made by immunization of mice that had been given large transgenes of human Ig variable regions, so that the mouse made a human antibody that was then cloned and expressed as a recombinant human antibody. Belimumab is a human antibody. TRU-015 is somewhat smaller than a natural IgG because two constant region domains were omitted. To hold the variable regions of the heavy and light chains together, a small linking peptide is included. Atacicept is the two human binding domains on TACI fused to human heavy-chain constant region domains. It binds/blocks both APRIL and BAFF/BLyS. The natural ligands are different homotrimers or heterotrimers of these molecules. BR3-Fc is similar except only one binding region was required. It binds/blocks only BAFF/BLyS. The natural ligand forms a trimer that is required for interactions. The IgG heavy chain constant regions greatly improve circulating half-life of the molecule by interacting with FcRn. Any engineered protein with an unnatural protein sequence may still be recognized by the human immune system.