Published in final edited form as:

Curr Opin Immunol. 2016 December ; 43: 39–45. doi:10.1016/j.coi.2016.09.003.

Targeting B cells in treatment of autoimmunity

S Elizabeth Franks¹, Andrew Getahun^{1,5}, P Mark Hogarth^{2,3,4}, and John C Cambier^{1,5}

¹Department of Immunology and Microbiology, University of Colorado School of Medicine, Denver, CO, USA

²Centre for Biomedicine, Burnet Institute, Melbourne, Vic., Australia

³Department of Immunology, Monash University, Melbourne, Vic., Australia

⁴Department of Pathology, University of Melbourne, Melbourne, Vic., Australia

⁵Department of Biomedical Research, National Jewish Health, Denver, CO, USA

Abstract

B cells have emerged as effective targets for therapeutic intervention in autoimmunities in which the ultimate effectors are antibodies, as well as those in which T cells are primary drivers of inflammation. Proof of this principle has come primarily from studies of the efficacy of Rituximab, an anti-CD20 mAb that depletes B cells, in various autoimmune settings. These successes have inspired efforts to develop more effective anti-CD20s tailored for specific needs, as well as biologicals and small molecules that suppress B cell function without the risks inherent in B cell depletion. Here we review the current status of B cell-targeted therapies for autoimmunity.

Introduction

Autoimmune diseases have been conveniently and often simplistically viewed as being of T cell origin wherein the T cell arm of adaptive immunity is directly responsible for executing pathological inflammation, as a B cell disease in which antibodies are the mediators of destructive inflammatory processes. However, the recent realization that B cells have a much broader role in the development and propagation of autoimmunity has raised the exciting prospect of therapeutic targeting of these cells, even in diseases considered as T cell in origin.

B cells are obvious therapeutic targets in diseases in which antibodies function as the primary effectors of pathology. This is especially the case in situations in which pathogenic antibodies are derived primarily from short-lived plasma cells that must be continuously replenished to sustain disease. Stemming the flow of B cells into this pool should, in principle, be an effective approach for temporary if not permanent elimination of disease.

The relative safety of therapeutic B cell targeting was established by the use of the B cell depleting therapy Rituximab for the treatment of lymphoma, where it became clear that with careful management, patients tolerate loss of the entire B cell compartment well. Of likely

importance in its safety profile is that Rituximab spares long-lived plasma cells that have developed as a consequence of earlier vaccination and infection, thereby allowing continued production of protective antibodies (Table 1).

Even more exciting developments are recent observations that B cells are also effective targets in autoimmune diseases, such as Type 1 Diabetes (T1D) and Multiple Sclerosis (MS), in which T cells, not B cells, function as executioners. In these situations B cells are presumed to function in an instructive role through the presentation of antigen to pathogenic T cells and/or production of cytokines. Indeed, studies in mouse models of T1D [1-3], MS [4], and Rheumatoid Arthritis (RA) [5,6] demonstrate protective effects of B cell depletion, consistent with the growing number of highly suggestive, though less well-developed studies in humans.

Here we review new strategies for the treatment of autoimmune diseases by targeting B cells using biologicals or small molecule drugs (Figure 1).

Biological therapeutics

The mAb targeting of B cell surface molecules for the treatment of autoimmunity was initially undertaken using mAbs employed for the destruction of B cell cancers, for example anti-CD20 mAbs [7-10]. More recent attempts have been directed at the avoidance of cell depletion and focus on manipulation of B cell biology, such as modulation of antigen receptor signaling.

Anti-CD20 cell-depleting strategies

Clear evidence that B cell depletion might be effective in treatment of autoimmunity came from a study of MS in which treatment with Rituximab was shown to increase remission rates and decrease development of new lesions [11]. New candidate therapeutic anti-CD20 mAbs that have subsequently been developed and engineered fall into two functionally distinct categories termed type I (TI) and type II (TII). TI mAbs recognize CD20 in lipid rafts, efficiently recruiting C1q, which on the one hand may hinder interactions with IgG Fc receptors limiting cell-mediated killing, but enables strong complement-dependent cytotoxicity (CDC) [12]. These antibodies appear not to be effective inducers of CD20 signaling-dependent death. TII mAbs bind CD20 outside of lipid rafts, recruit C1q poorly and induce little CDC, but are very strong inducers of CD20 signaling-dependent death [12-14].

The most commonly used TI mAb is Rituximab, which was originally approved for treatment of B cell cancers, non-Hodgkin's lymphoma and Chronic Lymphocytic Leukemia (CLL). This anti-CD20 mAb has recently been approved for treatment of RA in combination with methotrexate, as well as for granulomatosis and polyangiitis (GPA), and microscopic polyangiitis (MPA) in combination with glucocorticoids. Peripheral blood B cells disappear rapidly upon administration of Rituximab [8]; however organ resident B cells are not depleted because elimination may be dependent on interaction of antibody-coated cells with IgG Fc receptors on the surface of Kupffer cells in the liver [15-17].

A number of second generation anti-CD20 antibodies have been developed in an effort to achieve more efficient depletion or more targeted effects. Veltuzumab, originally developed for the treatment of blood cancers, is closely related to Rituximab [18] with CDRs differing from Rituximab by a single amino acid. This change alters the mAb's biophysical, pharmacokinetic and functional properties, importantly reducing off-rate and improving complement killing of target cells [19]. Veltuzumab depletes organ-resident as well as circulating B cells [20]. In Phase I/II studies of Veltuzumab for the treatment of immune thrombocytopenias, B cells were depleted and platelet numbers were rapidly restored. The B cell compartment normalized over seven months but autoimmunity recrudescence was delayed for two years [21]. Interestingly, both Rituximab [22] and Veltuzumab [23] have shown signs of efficacy for treatment of the skin blistering disease, pemphigus vulgaris (PV), and in 2015 Veltuzumab was granted orphan drug status in PV by the FDA.

Two new TI anti-CD20 antibodies that have recently undergone testing for treatment of MS are Ofatumumab and Ocrelizumab. Ofatumumab was originally approved for treatment of CLL and is in Phase II testing for relapsing-remitting MS (RRMS) [24] and Phase III testing for RA [25]. Like Rituximab, Ofatumumab triggers B cell killing via antibody-dependent cell-mediated cytotoxicity (ADCC) and CDC. Ofatumumab and Rituximab recognize distinct epitopes but both are thought to target CD20 located in lipid rafts. Ocrelizumab selectively targets mature B lymphocytes and was recently designated by the FDA as 'breakthrough therapy' for the treatment of MS. In clinical trials patients receiving Ocrelizumab had reduced relapse rates, decreased confirmed disability, and a reduction in brain lesions. Furthermore, the naïve B cell compartment returned, but the memory compartment did not, even 2 years following the last dose [26]. Ocrelizumab was also tested for treatment of primary-progressive MS (PPMS), a condition for which there is no approved, efficacious therapy. The study met its primary endpoints with a reduced risk of progression of clinical disability, and showed a reduction in whole brain loss. While the effects observed are modest, this is the first therapy to show efficacy for PPMS [27].

An example of a TII anti-CD20 mAb is Obinutuzumab, which is currently in Phase III clinical trials for patients with Lupus Nephritis [28]. Obinutuzumab is glycoengineered to interact 10-fold more strongly with Fc receptors, and thus mediates efficient ADCC [29,30]. B cell clearance following Obinutuzumab treatment does not require cell recirculation, presumably because it induces substantial CD20 signaling-mediated cell death, effectively killing organ-resident B cells.

Manipulation of B cell function

Exciting new strategies are being developed that seek to harness normal physiological regulation of B cell function. These include mimicking immune complex inhibition of B cell activation via Fc γ RIIB or the induction of anergic-like unresponsiveness of the B cells. These approaches silence B cells without causing their elimination, and thus may overcome safety concerns associated with B cell depletion [31-35]. Some of these approaches are described below.

Harnessing inhibitory IgG Fc receptor function—FcγRIIB is an Immunoreceptor Tyrosine-based Inhibitory Motif (ITIM)-containing inhibitory receptor expressed primarily by B cells that when coaggregated with the antigen receptor (BCR) is phosphorylated and recruits cytosolic phosphatases that suppress BCR signaling [36,37]. This inhibitory circuitry is critical for the control of pro-inflammatory immune responses, and limits the antibody response. The Fc engineered mAb XmAb5871 works by exploiting the regulation of BCR signaling by FcγRIIB1. XmAb5871 binds CD19 of the BCR complex and its Fc is engineered to increase its affinity for the inhibitory FcγRIIB. Since CD19 is associated with the BCR, XmAb5871 tethering of CD19 to FcγRIIB on the same cell poises the BCR complex for inhibition upon antigen-induced BCR aggregation. XmAb5871 may also have an improved safety profile compared to B cell depleting antibodies as it may not mediate B cell killing [36,38]. In pre-clinical, in vitro studies using B cells from RA patients [39*] and SLE patients [40] XmAb5871 inhibited B cell activation, including CD86 upregulation and humoral responses. XmAb5871 is in Phase II clinical trials for moderate to severe RA and a trial is currently recruiting patients to determine efficacy in SLE [41].

Bispecific antibodies have been developed that also invoke the inhibitory function of FcγRIIB to regulate BCR signaling. MGD010 pairs antibody fragments specific for FcγRIIB with those specific for CD79b, a signal-transducing component of the BCR [42], thereby tethering FcγRIIB to the BCR complex. In recent studies utilizing humanized mouse models, MGD010 inhibited the onset of autoimmunity. This bispecific antibody is non-depleting and has a favorable safety profile in non-human primates. MacroGenics is currently recruiting for Phase I trials to evaluate MGD010 efficacy and safety in healthy human subjects [43].

BAFF/Blys blockade—B cell activating factor (BAFF) is a member of the TNF superfamily that is critical for B cell differentiation and survival, and regulation of innate and adaptive immune responses [44-46]. There are three BAFF receptors, BAFF-R, TACI and BCMA, while the closely related proliferation-inducing ligand (APRIL) can interact with TACI and BCMA. Transgenic (Tg) mice overexpressing BAFF develop lupus-like disease with glomerulonephritis, suggesting that BAFF levels limit the activation of autoreactive B cells [47]. Interestingly, SLE and RA patients are characterized by an increase in serum BAFF levels [47-50]. These observations suggest that BAFF and APRIL antagonists may be therapeutic in lupus and perhaps other autoimmunities.

Belimumab is a therapeutic mAb that inhibits the activity of soluble BAFF homotrimers as well as 60-mers [51] and was approved for the treatment of autoantibody positive SLE in 2011, after Phase III trials showed SLE responder index was higher following treatment [52]. A recent study suggests that Belimumab may restore a peripheral B cell tolerance checkpoint, as indicated by the observed restoration of anergy of SLE patient B cells [53*]. However, recent evidence indicates that the loss of TACI protects BAFF Tg mice from lupus-like disease, without impacting B cell survival [54**]. Thus, effective therapies targeting this family may require blocking of both BAFF and April. For example, Atacicept binds both soluble BAFF and APRIL, blocking the activity of both ligands [55]. Unfortunately, Phase II/III Atacicept trials for SLE were terminated prematurely due to two fatal infections wherein a role of Atacicept could not be excluded [56,57].

Other strategies to inhibit B cell activation—Additional therapeutic strategies that seek to harness inhibitory signaling are under development for treatment of autoimmune diseases. Epratuzumab targets CD22, an inhibitory C-type lectin expressed by B cells, and has completed Phase III trials for the treatment of SLE [58]. Findings indicate that it decreases B cell activation and induces only partial depletion of peripheral B cells [59,60].

Still another approach involves targeting aspects of the unique biology of the BCR using receptor-desensitizing CD79a/b mAbs. Recombinant anti-CD79 antibodies engineered to remove ADCC or CDC functions, induce anergy-like B cell unresponsiveness to antigen, and prevent lupus-like disease and collagen-induced arthritis [61*,62, 63] in animal models. Additionally, this treatment does not deplete B cells but rather results in the short-term sequestration of the cells in organs. Unresponsiveness is sustained only during the in vivo persistence of the antibody. Suspension of treatment leads to reappearance of the B cells and the rapid restoration of immune competence [62**].

Small molecule therapeutics

Whilst biologicals have become many of the leading autoimmunity drugs, there is resurgence of interest in the development of small molecule therapeutics for autoimmunity and a number of these target B cell function.

BCR signaling inhibitors

Following BCR stimulation, the Tyrosine-based Activation Motifs (ITAMs) in the cytoplasmic tails of CD79 become phosphorylated, leading to sequestration and activation of kinases (Lyn and Syk) and adaptor molecules, for example BLNK, and initiation of signaling involving, among other things, production of PI(3,4,5)P₃ by PI3 kinase (PI3K) [64]. PI(3,4,5)P₃ accumulation is crucial for membrane recruitment and activation of Btk and Akt, leading to downstream B cell activation, antigen presentation, cytokine production, proliferation and differentiation.

The PI3K pathway is negatively regulated by the inositol phosphatases SHIP-1 and PTEN. Recently SHIP-1 activity and PTEN levels were shown to be upregulated in anergic B cells and are critical for maintenance of their unresponsiveness [65,66**,67,68]. Importantly, this anergic state is reversible, making anergic B cells a likely target for environmental triggers of autoimmunity [69]. B cells from SLE patients express reduced PTEN, which is consistent with their reduced ability to maintain anergy [70**]. Indeed changes in the anergic B cell population precedes development of T1D [71*], and SLE [53*,72].

PI3Kδ has emerged as a possible new target for autoimmunity therapy. In situations in which autoimmunity is associated with reduced regulation of the PI3K pathway it may be possible to treat disease by enforcing B cell unresponsiveness via inhibition of PI3K. Multiple forms of PI3K exist but PI3Kδ is the predominant isoform in B cells and its genetic ablation results in defective BCR signaling. Moreover the partial blockade of PI3K in mouse models of autoimmunity reduces autoantibody production and associated pathology [73,74].

Idelalisib is a small molecule inhibitor of PI3K δ . It has a 40-300 fold greater selectivity for PI3K δ than for other class I PI3K isoforms and its use leads to a reduction in cellular viability [75]. It is approved for use in a number of B cell cancer indications [76] and has completed Phase I clinical trials in allergic rhinitis [77].

Bruton's tyrosine kinase (Btk) has also emerged as an attractive therapeutic target in autoimmunity. Btk is a Tec-family kinase that is most highly expressed in B cells and is of central importance in BCR signaling. Its importance in B cells is underscored by the fact that inactivating mutations in Btk lead to X-linked agammaglobulinemia (XLA) in the human [78-80] and X-linked immunodeficiency (XID) in the mouse [81,82]. Btk is activated during BCR signaling by the concerted action of Syk and Lyn kinases, BLNK and PI(3,4,5)P₃ [64]. The selective expression of Btk in B cells and myeloid cells makes it an attractive target treatment of autoimmunity.

The small molecule Btk inhibitor Ibrutinib forms a covalent bond with cysteine 481 in the ATP binding site leading to kinase inactivation, preventing its phosphorylation of PLC γ 2, and downstream BCR signaling [83]. Treatment of MRL/*lpr* mice with Ibrutinib abrogated development of lupus-like disease [84]. Treatment led to severe nodal reduction, as well as a reduction in lymphocytes that returned to baseline over time [85]. Another Btk inhibitor, CGI-1746, is a highly specific small molecule inhibitor which binds Btk in a reversible manner, stabilizing it in an inactive conformation. The molecule has 1000-fold selectivity for Btk relative to other kinases screened [86]. CGI-1746 is currently in Phase I study for the treatment of RA following an observed reduction in BCR-mediated B cell proliferation and reduction in autoantibody levels in an RA model [86].

A cautionary note; Btk is extremely important in B cell central tolerance [87], and therefore blocking this kinase could increase the number of autoreactive B cells that reach the periphery. Such repertoire changes could also be an issue with PI3K inhibitors as recent work indicates that reduced negative regulation of this pathway augments central B cell tolerance [88,89*].

Conclusions

While B cells have emerged, in some cases unexpectedly, as effective targets for the treatment of autoimmune diseases, currently approved therapies are not without safety concerns. The increase in research and development of non-depleting therapies that target inhibitory signaling pathways, as well as BCR signal transducing intermediaries, seek to circumnavigate this problem. An added exciting possibility is that these therapies may reset the repertoire obviating need for lifelong treatment. These are certainly exciting times with great promise for the future of autoimmune disease therapy.

Acknowledgements

We thank Sandra Duran for assistance in preparing this manuscript. This work was completed under NIH grants 5R01DK096492-05, 1R21AI124488-01, 1R01AI1244887-01, 5T32AR007534-29, NHMRC grant 1079946 and the Victorian Operational Infrastructure Grant.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Noorchashm H, Noorchashm N, Kern J, Rostami SY, Barker CF, Naji A. B-cells are required for the initiation of insulinitis and sialitis in nonobese diabetic mice. *Diabetes*. 1997; 46:941–946. [PubMed: 9166663]
2. Serreze DV, Fleming SA, Chapman HD, Richard SD, Leiter EH, Tisch RM. B lymphocytes are critical antigen-presenting cells for the initiation of T cell-mediated autoimmune diabetes in nonobese diabetic mice. *J Immunol*. 1998; 161:3912–3918. [PubMed: 9780157]
3. Yang M, Charlton B, Gautam AM. Development of insulinitis and diabetes in B cell-deficient NOD mice. *J Autoimmun*. 1997; 10:257–260. [PubMed: 9218752]
4. Matsushita T, Yanaba K, Bouaziz JD, Fujimoto M, Tedder TF. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. *J Clin Invest*. 2008; 118:3420–3430. [PubMed: 18802481]
5. Svensson L, Jirholt J, Holmdahl R, Jansson L. B cell-deficient mice do not develop type II collagen-induced arthritis (CIA). *Clin Exp Immunol*. 1998; 111:521–526. [PubMed: 9528892]
6. Yanaba K, Hamaguchi Y, Venturi GM, Steeber DA, St Clair EW, Tedder TF. B cell depletion delays collagen-induced arthritis in mice: arthritis induction requires synergy between humoral and cell-mediated immunity. *J Immunol*. 2007; 179:1369–1380. [PubMed: 17617630]
7. Looney RJ. Treating human autoimmune disease by depleting B cells. *Ann Rheum Dis*. 2002; 61:863–866. [PubMed: 12228152]
8. Looney RJ, Anolik JH, Campbell D, Felgar RE, Young F, Arend LJ, Sloand JA, Rosenblatt J, Sanz I. B cell depletion as a novel treatment for systemic lupus erythematosus: a phase I/II dose-escalation trial of rituximab. *Arthritis Rheum*. 2004; 50:2580–2589. [PubMed: 15334472]
9. Patel DD. B cell-ablative therapy for the treatment of autoimmune diseases. *Arthritis Rheum*. 2002; 46:1984–1985. [PubMed: 12209499]
10. Kneitz C, Wilhelm M, Tony HP. Effective B cell depletion with rituximab in the treatment of autoimmune diseases. *Immunobiology*. 2002; 206:519–527. [PubMed: 12607727]
11. Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, Fox RJ, Bar-Or A, Panzara M, Sarkar N, Agarwal S, et al. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N Engl J Med*. 2008; 358:676–688. [PubMed: 18272891]
12. Klein C, Lammens A, Schafer W, Georges G, Schwaiger M, Mossner E, Hopfner KP, Umana P, Niederfellner G. Epitope interactions of monoclonal antibodies targeting CD20 and their relationship to functional properties. *MAbs*. 2013; 5:22–33. [PubMed: 23211638]
13. Awan FT, Lapalombella R, Trotta R, Butchar JP, Yu B, Benson DM Jr, Roda JM, Cheney C, Mo X, Lehman A, et al. CD19 targeting of chronic lymphocytic leukemia with a novel Fc-domain-engineered monoclonal antibody. *Blood*. 2010; 115:1204–1213. [PubMed: 19965644]
14. Niederfellner G, Lammens A, Mundigl O, Georges GJ, Schaefer W, Schwaiger M, Franke A, Wiechmann K, Jenewein S, Sliotstra JW, et al. Epitope characterization and crystal structure of GA101 provide insights into the molecular basis for type I/II distinction of CD20 antibodies. *Blood*. 2011; 118:358–367. [PubMed: 21444918]
15. Glennie MJ, French RR, Cragg MS, Taylor RP. Mechanisms of killing by anti-CD20 monoclonal antibodies. *Mol Immunol*. 2007; 44:3823–3837. [PubMed: 17768100]
16. Hamaguchi Y, Uchida J, Cain DW, Venturi GM, Poe JC, Haas KM, Tedder TF. The peritoneal cavity provides a protective niche for B1 and conventional B lymphocytes during anti-CD20 immunotherapy in mice. *J Immunol*. 2005; 174:4389–4399. [PubMed: 15778404]
17. Gong Q, Ou Q, Ye S, Lee WP, Cornelius J, Diehl L, Lin WY, Hu Z, Lu Y, Chen Y, et al. Importance of cellular microenvironment and circulatory dynamics in B cell immunotherapy. *J Immunol*. 2005; 174:817–826. [PubMed: 15634903]

18. Stein R, Qu Z, Chen S, Rosario A, Shi V, Hayes M, Horak ID, Hansen HJ, Goldenberg DM. Characterization of a new humanized anti-CD20 monoclonal antibody, IMMU-106, and its use in combination with the humanized anti-CD22 antibody, epratuzumab, for the therapy of non-Hodgkin's lymphoma. *Clin Cancer Res*. 2004; 10:2868–2878. [PubMed: 15102696]
19. Goldenberg DM, Rossi EA, Stein R, Cardillo TM, Czuczman MS, Hernandez-Ilizaliturri FJ, Hansen HJ, Chang CH. Properties and structure-function relationships of veltuzumab (hA20), a humanized anti-CD20 monoclonal antibody. *Blood*. 2009; 113:1062–1070. [PubMed: 18941114]
20. Immunomedics I. Study of Veltuzumab (hA20) at Different Doses in Patients With ITP (NCT00547066). 2016.
21. Liebman HA, Saleh MN, Bussel JB, Negrea OG, Horne H, Wegener WA, Goldenberg DM. Low-dose anti-CD20 veltuzumab given intravenously or subcutaneously is active in relapsed immune thrombocytopenia: a phase I study. *Br J Haematol*. 2013; 162:693–701. [PubMed: 23829485]
22. Colliou N, Picard D, Caillot F, Calbo S, Le Corre S, Lim A, Lemerrier B, Le Mauff B, Maho-Vaillant M, Jacquot S, et al. Long-term remissions of severe pemphigus after rituximab therapy are associated with prolonged failure of desmoglein B cell response. *Sci Transl Med*. 2013; 5:175ra130.
23. Ellebrecht CT, Choi EJ, Allman DM, Tsai DE, Wegener WA, Goldenberg DM, Payne AS. Subcutaneous veltuzumab, a humanized anti-CD20 antibody, in the treatment of refractory pemphigus vulgaris. *JAMA Dermatol*. 2014; 150:1331–1335. [PubMed: 25133328]
24. GlaxoSmithKline. Ofatumumab Dose-finding in Relapsing Remitting Multiple Sclerosis (RRMS) Patients NCT00640328. 2010.
25. GlaxoSmithKline. Investigating Clinical Efficacy of Ofatumumab in Adult Rheumatoid Arthritis (RA) Patients Who Had an Inadequate Response to MTX Therapy NCT00611455. 2009.
26. Genentech, I. A Study of Ocrelizumab in Participants With Relapsing Remitting Multiple Sclerosis (RRMS) Who Have Had a Suboptimal Response to an Adequate Course of Disease-Modifying Treatment (DMT) (NCT02637856). 2016.
27. Hoffmann-La, R. A Study of Ocrelizumab in Patients With Primary Progressive Multiple Sclerosis NCT01194570. 2017.
28. Hoffmann-La, R. A Study to Evaluate the Safety and Efficacy of Obinutuzumab, an Antibody Targeting Certain Types of Immune Cells, in Participants With Lupus Nephritis (LN) NCT02550652. 2017.
29. Mossner E, Brunker P, Moser S, Puntener U, Schmidt C, Herter S, Grau R, Gerdes C, Nopora A, van Puijenbroek E, et al. Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. *Blood*. 2010; 115:4393–4402. [PubMed: 20194898]
30. Umana P, et al. Novel 3rd generation humanized type II CD20 antibody with glycoengineered Fc and modified elbow hinge for enhanced ADCC and superior apoptosis induction. *Blood*. 2006; 108:229.
31. Kontermann RE. Dual targeting strategies with bispecific antibodies. *MAbs*. 2012; 4:182–197. [PubMed: 22453100]
32. Nelson AL, Dhimolea E, Reichert JM. Development trends for human monoclonal antibody therapeutics. *Nat Rev Drug Discov*. 2010; 9:767–774. [PubMed: 20811384]
33. Stohl W, Gomez-Reino J, Olech E, Dudler J, Fleischmann RM, Zerbini CA, Ashrafzadeh A, Grzeschik S, Bieraugel R, Green J, et al. Safety and efficacy of ocrelizumab in combination with methotrexate in MTX-naïve subjects with rheumatoid arthritis: the phase III FILM trial. *Ann Rheum Dis*. 2012; 71:1289–1296. [PubMed: 22307942]
34. Aksoy S, Dizdar O, Hayran M, Harputluoglu H. Infectious complications of rituximab in patients with lymphoma during maintenance therapy: a systematic review and meta-analysis. *Leuk Lymphoma*. 2009; 50:357–365. [PubMed: 19263297]
35. Aksoy S, Harputluoglu H, Kilickap S, Dede DS, Dizdar O, Altundag K, Barista I. Rituximab-related viral infections in lymphoma patients. *Leuk Lymphoma*. 2007; 48:1307–1312. [PubMed: 17613758]

36. D'Ambrosio D, Hippen KL, Minskoff SA, Mellman I, Pani G, Siminovitch KA, Cambier JC. Recruitment and activation of PTP1C in negative regulation of antigen receptor signaling by Fc gamma RIIB1. *Science*. 1995; 268:293–297. [PubMed: 7716523]
37. Ono M, Bolland S, Tempst P, Ravetch JV. Role of the inositol phosphatase SHIP in negative regulation of the immune system by the receptor Fc(gamma)RIIB. *Nature*. 1996; 383:263–266. [PubMed: 8805703]
38. Kontermann RE, Brinkmann U. Bispecific antibodies. *Drug Discov Today*. 2015; 20:838–847. [PubMed: 25728220]
39. Chu SY, Yeter K, Kotha R, Pong E, Miranda Y, Phung S, Chen H, Lee SH, Leung I, Bonzon C, et al. Suppression of rheumatoid arthritis B cells by XmAb5871, an anti-CD19 antibody that coengages B cell antigen receptor complex and Fcgamma receptor IIb inhibitory receptor. *Arthritis Rheumatol*. 2014; 66:1153–1164. [PubMed: 24782179] SCID mice engrafted with PBMCs from RA patients produce less antibody when treated with XmAb5871.
40. Horton HM, Chu SY, Ortiz EC, Pong E, Cemerski S, Leung IW, Jacob N, Zalevsky J, Desjarlais JR, Stohl W, et al. Antibody-mediated coengagement of FcgammaRIIb and B cell receptor complex suppresses humoral immunity in systemic lupus erythematosus. *J Immunol*. 2011; 186:4223–4233. [PubMed: 21357255]
41. Xencor, I.; Ppd, W.; plc, I. A Study of the Effect of XmAb® 5871 in Patients With Systemic Lupus Erythematosus NCT02725515. 2016.
42. Veri MC, Burke S, Huang L, Li H, Gorlatov S, Tuailon N, Rainey GJ, Ciccarone V, Zhang T, Shah K, et al. Therapeutic control of B cell activation via recruitment of Fcgamma receptor IIb (CD32B) inhibitory function with a novel bispecific antibody scaffold. *Arthritis Rheum*. 2010; 62:1933–1943. [PubMed: 20506263]
43. MacroGenics: Phase 1 Study of MGD010 in Healthy Subjects NCT02376036. 2016.
44. Cancro MP, D'Cruz DP, Khamashta MA. The Role of B lymphocyte stimulator (BLyS) in systemic lupus erythematosus. *J Clin Invest*. 2009; 119:1066–1073. [PubMed: 19411764]
45. Mackay F, Schneider P. Cracking the BAFF code. *Nat Rev Immunol*. 2009; 9:491–502. [PubMed: 19521398]
46. Moore PA, Belvedere O, Orr A, Pieri K, LaFleur DW, Feng P, Soppet D, Charters M, Gentz R, Parmelee D, et al. BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator. *Science*. 1999; 285:260–263. [PubMed: 10398604]
47. Bosello S, Youinou P, Daridon C, Tolusso B, Bendaoud B, Pietrapertosa D, Morelli A, Ferraccioli G. Concentrations of BAFF correlate with autoantibody levels, clinical disease activity, and response to treatment in early rheumatoid arthritis. *J Rheumatol*. 2008; 35:1256–1264. [PubMed: 18528969]
48. Cheema GS, Roschke V, Hilbert DM, Stohl W. Elevated serum B lymphocyte stimulator levels in patients with systemic immune-based rheumatic diseases. *Arthritis Rheum*. 2001; 44:1313–1319. [PubMed: 11407690]
49. Stohl W, Metyas S, Tan SM, Cheema GS, Oamar B, Xu D, Roschke V, Wu Y, Baker KP, Hilbert DM. B lymphocyte stimulator overexpression in patients with systemic lupus erythematosus: longitudinal observations. *Arthritis Rheum*. 2003; 48:3475–3486. [PubMed: 14673998]
50. Zhang J, Roschke V, Baker KP, Wang Z, Alarcon GS, Fessler BJ, Bastian H, Kimberly RP, Zhou T. Cutting edge: a role for B lymphocyte stimulator in systemic lupus erythematosus. *J Immunol*. 2001; 166:6–10. [PubMed: 11123269]
51. Baker KP, Edwards BM, Main SH, Choi GH, Wager RE, Halpern WG, Lappin PB, Riccobene T, Abramian D, Sekut L, et al. Generation and characterization of LymphoStat-B, a human monoclonal antibody that antagonizes the bioactivities of B lymphocyte stimulator. *Arthritis Rheum*. 2003; 48:3253–3265. [PubMed: 14613291]
52. Furie R, Petri M, Zamani O, Cervera R, Wallace DJ, Tegzova D, Sanchez-Guerrero J, Schwarting A, Merrill JT, Chatham WW, et al. A phase III, randomized, placebo-controlled study of belimumab, a monoclonal antibody that inhibits B lymphocyte stimulator, in patients with systemic lupus erythematosus. *Arthritis Rheum*. 2011; 63:3918–3930. [PubMed: 22127708]
53. Malkiel S, Jeganathan V, Wolfson S, Orduno NM, Marasco E, Aranow C, Mackay M, Gregersen PK, Diamond B. Checkpoints for autoreactive B cells in peripheral blood of lupus patients

- assessed by flow cytometry. *Arthritis Rheumatol.* 2016 Using a novel flow-cytometric assay, the authors demonstrate SLE patients fail to properly anergize B cells reactive with nuclear antigens.
- 54••. Figgett WA, Deliyanti D, Fairfax KA, Quah PS, Wilkinson-Berka JL, Mackay F. Deleting the BAFF receptor TACI protects against systemic lupus erythematosus without extensive reduction of B cell numbers. *J Autoimmun.* 2015; 61:9–16. [PubMed: 26027434] This paper demonstrated deletion of the BAFF receptor TACI prevents the development of lupus-like disease in BAFF-Tg mice, suggesting Belimumab may not be functioning via B cell depletion, but rather by inhibiting B cell activation through TACI.
 55. Dall'Era M, Wallace CE, Genovese D, Weisman M, Kavanaugh M, Kalunian A, Dhar K, Vincent P, Pena-Rossi E, Wofsy D. Reduced B lymphocyte and immunoglobulin levels after atacicept treatment in patients with systemic lupus erythematosus. *Arthritis Rheum.* 2007; 56:4142–4150. [PubMed: 18050206]
 56. Cogollo E, Silva MA, Isenberg D. Profile of atacicept and its potential in the treatment of systemic lupus erythematosus. *Drug Des Devel Ther.* 2015; 9:1331–1339.
 57. Isenberg D, Gordon C, Licu D, Copt S, Rossi CP, Wofsy D. Efficacy and safety of atacicept for prevention of flares in patients with moderate-to-severe systemic lupus erythematosus (SLE): 52-week data (APRIL-SLE randomised trial). *Ann Rheum Dis.* 2015; 74:2006–2015. [PubMed: 24951103]
 58. Pharma UCB: Study of Epratuzumab Versus Placebo in Subjects With Moderate to Severe General Systemic Lupus Erythematosus NCT01408576. 2015.
 59. Wallace DJ, Kalunian K, Petri MA, Strand V, Houssiau FA, Pike M, Kilgallen B, Bongardt S, Barry A, Kelley L, et al. Efficacy and safety of epratuzumab in patients with moderate/severe active systemic lupus erythematosus: results from EMBLEM, a phase IIb, randomised, double-blind, placebo-controlled, multicentre study. *Ann Rheum Dis.* 2014; 73:183–190. [PubMed: 23313811]
 60. Sieger N, Fleischer SJ, Mei HE, Reiter K, Shock A, Burmester GR, Daridon C, Dorner T. CD22 ligation inhibits downstream B cell receptor signaling and Ca(2+) flux upon activation. *Arthritis Rheum.* 2013; 65:770–779. [PubMed: 23233360]
 - 61•. Bruhl H, Cihak J, Talke Y, Rodriguez Gomez M, Hermann F, Goebel N, Renner K, Plachy J, Stangassinger M, Aschermann S, et al. B-cell inhibition by cross-linking CD79b is superior to B-cell depletion with anti-CD20 antibodies in treating murine collagen-induced arthritis. *Eur J Immunol.* 2015; 45:705–715. [PubMed: 25471597] In an induced model of arthritis, the authors show increased efficacy with anti-CD79b treatment over anti-CD20 therapy in preventing development of CIA.
 - 62••. Hardy IR, Anceriz N, Rousseau F, Seefeldt MB, Hatterer E, Irla M, Buatois V, Chatel LE, Getahun A, Fletcher A, et al. Anti-CD79 antibody induces B cell anergy that protects against autoimmunity. *J Immunol.* 2014; 192:1641–1650. [PubMed: 24442438] This paper showed that treatment of mice with non-B cell-depleting antibodies to CD79b can induce an anergic-like state and prevent development of collagen induced arthritis.
 63. Li Y, Chen F, Putt M, Koo YK, Madaio M, Cambier JC, Cohen PL, Eisenberg RA. B cell depletion with anti-CD79 mAbs ameliorates autoimmune disease in MRL/lpr mice. *J Immunol.* 2008; 181:2961–2972. [PubMed: 18713966]
 64. Packard TA, Cambier JC. B lymphocyte antigen receptor signaling: initiation, amplification, and regulation. *F1000Prime Reports.* 2013; 5:40. [PubMed: 24167721]
 65. Browne CD, Del Nagro CJ, Cato MH, Dengler HS, Rickert RC. Suppression of phosphatidylinositol 3,4,5-trisphosphate production is a key determinant of B cell anergy. *Immunity.* 2009; 31:749–760. [PubMed: 19896393]
 - 66••. Getahun A, Beavers NA, Larson SR, Shlomchik MJ, Cambier JC. Continuous inhibitory signaling by both SHP-1 and SHIP-1 pathways is required to maintain unresponsiveness of anergic B cells. *J Exp Med.* 2016; 213:751–769. [PubMed: 27114609] Authors showed continuous, B cell-intrinsic negative regulation of the PI3K pathway by inositol lipid phosphatases SHIP-1 and PTEN, and tyrosine phosphorylation by the tyrosine phosphatase SHP-1, is required to maintain B cell anergy.

67. Maxwell MJ, Duan M, Armes JE, Anderson GP, Tarlinton DM, Hibbs ML. Genetic segregation of inflammatory lung disease and autoimmune disease severity in SHIP-1^{-/-} mice. *J Immunol*. 2011; 186:7164–7175. [PubMed: 21572033]
68. O'Neill SK, Getahun A, Gauld SB, Merrell KT, Tamir I, Smith MJ, Dal Porto JM, Li QZ, Cambier JC. Monophosphorylation of CD79a and CD79b ITAM motifs initiates a SHIP-1 phosphatase-mediated inhibitory signaling cascade required for B cell anergy. *Immunity*. 2011; 35:746–756. [PubMed: 22078222]
69. Cambier JC, Gauld SB, Merrell KT, Vilen BJ. B-cell anergy: from transgenic models to naturally occurring anergic B cells? *Nat Rev Immunol*. 2007; 7:633–643. [PubMed: 17641666]
70. Wu XN, Ye YX, Niu JW, Li Y, Li X, You X, Chen H, Zhao LD, Zeng XF, Zhang FC, et al. Defective PTEN regulation contributes to B cell hyperresponsiveness in systemic lupus erythematosus. *Sci Transl Med*. 2014; 6:246ra299. Phenotypic analysis of peripheral B cells showed decreased PTEN expression in SLE patients. PTEN levels were inversely correlated with disease score.
71. Smith MJ, Packard TA, O'Neill SK, Henry Dunand CJ, Huang M, Fitzgerald-Miller L, Stowell D, Hinman RM, Wilson PC, Gottlieb PA, et al. Loss of anergic B cells in prediabetic and new-onset type 1 diabetic patients. *Diabetes*. 2015; 64:1703–1712. [PubMed: 25524915] Authors report that while anergic (B_{ND}) insulin-reactive B cells are present in peripheral blood of healthy individuals, they leave this compartment in some first-degree relatives and all prediabetic and new onset patients, suggesting that a breach in B cell anergy is associated with development of T1D.
72. Quach TD, Manjarrez-Orduno N, Adlowitz DG, Silver L, Yang H, Wei C, Milner EC, Sanz I. Anergic responses characterize a large fraction of human autoreactive naive B cells expressing low levels of surface IgM. *J Immunol*. 2011; 186:4640–4648. [PubMed: 21398610]
73. Maxwell MJ, Tsantikos E, Kong AM, Vanhaesebroeck B, Tarlinton DM, Hibbs ML. Attenuation of phosphoinositide 3-kinase delta signaling restrains autoimmune disease. *J Autoimmun*. 2012; 38:381–391. [PubMed: 22537464]
74. Winkler DG, Faia KL, DiNitto JP, Ali JA, White KF, Brophy EE, Pink MM, Proctor JL, Lussier J, Martin CM, et al. PI3K-delta and PI3K-gamma inhibition by IPI-145 abrogates immune responses and suppresses activity in autoimmune and inflammatory disease models. *Chem Biol*. 2013; 20:1364–1374. [PubMed: 24211136]
75. Lannutti BJ, Meadows SA, Herman SE, Kashishian A, Steiner B, Johnson AJ, Byrd JC, Tyner JW, Loriaux MM, Deininger M, et al. CAL-101, a p110delta selective phosphatidylinositol-3-kinase inhibitor for the treatment of B-cell malignancies, inhibits PI3K signaling and cellular viability. *Blood*. 2011; 117:591–594. [PubMed: 20959606]
76. Gilead, S. A Randomized, Double-Blind, Placebo-Controlled Study of Idelalisib in Combination With Rituximab for Previously Treated Chronic Lymphocytic Leukemia (CLL) NCT01539512. 2013.
77. Gilead, S. Study to Investigate Effects of CAL-101 in Subjects With Allergic Rhinitis Exposed to Allergen in an Environmental Chamber NCT00836914. 2009.
78. Mattsson PT, Lappalainen I, Backesjo CM, Brockmann E, Lauren S, Vihinen M, Smith CI. Six X-linked agammaglobulinemia-causing missense mutations in the Src homology 2 domain of Bruton's tyrosine kinase: phosphotyrosine-binding and circular dichroism analysis. *J Immunol*. 2000; 164:4170–4177. [PubMed: 10754312]
79. Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Klisak I, Sparkes RS, Kubagawa H, Mohandas T, Quan S, et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell*. 1993; 72:279–290. [PubMed: 8425221]
80. Vihinen M, Brandau O, Branden LJ, Kwan SP, Lappalainen I, Lester T, Noordzij JG, Ochs HD, Ollila J, Pienaar SM, et al. BTKbase, mutation database for X-linked agammaglobulinemia (XLA). *Nucleic Acids Res*. 1998; 26:242–247. [PubMed: 9399844]
81. Khan WN, Alt FW, Gerstein RM, Malynn BA, Larsson I, Rathbun G, Davidson L, Muller S, Kantor AB, Herzenberg LA, et al. Defective B: cell development and function in Btk-deficient mice. *Immunity*. 1995; 3:283–299. [PubMed: 7552994]

82. Rawlings DJ, Saffran DC, Tsukada S, Largaespada DA, Grimaldi JC, Cohen L, Mohr RN, Bazan JF, Howard M, Copeland NG, et al. Mutation of unique region of Bruton's tyrosine kinase in immunodeficient XID mice. *Science*. 1993; 261:358–361. [PubMed: 8332901]
83. Pan Z, Scheerens H, Li SJ, Schultz BE, Sprengeler PA, Burrill LC, Mendonca RV, Sweeney MD, Scott KC, Grothaus PG, et al. Discovery of selective irreversible inhibitors for Bruton's tyrosine kinase. *ChemMedChem*. 2007; 2:58–61. [PubMed: 17154430]
84. Honigberg LA, Smith AM, Sirisawad M, Verner E, Loury D, Chang B, Li S, Pan Z, Thamm DH, Miller RA, et al. The Bruton tyrosine kinase inhibitor PCI-32765 blocks B-cell activation and is efficacious in models of autoimmune disease and B-cell malignancy. *Proc Natl Acad Sci U S A*. 2010; 107:13075–13080. [PubMed: 20615965]
85. Brown JR. Ibrutinib (PCI-32765), the first BTK (Bruton's tyrosine kinase) inhibitor in clinical trials. *Curr Hematol Malig Rep*. 2013; 8:1–6. [PubMed: 23296407]
86. Di Paolo JA, Huang T, Balazs M, Barbosa J, Barck KH, Bravo BJ, Carano RA, Darrow J, Davies DR, DeForge LE, et al. Specific Btk inhibition suppresses B cell- and myeloid cell-mediated arthritis. *Nat Chem Biol*. 2011; 7:41–50. [PubMed: 21113169]
87. Ng YS, Wardemann H, Chelnis J, Cunningham-Rundles C, Meffre E. Bruton's tyrosine kinase is essential for human B cell tolerance. *J Exp Med*. 2004; 200:927–934. [PubMed: 15466623]
88. Leung WH, Tarasenko T, Biesova Z, Kole H, Walsh ER, Bolland S. Aberrant antibody affinity selection in SHIP-deficient B cells. *Eur J Immunol*. 2013; 43:371–381. [PubMed: 23135975]
89. Shojaaee S, Chan LN, Buchner M, Cazzaniga V, Cosgun KN, Geng H, Qiu YH, von Minden MD, Ernst T, Hochhaus A, et al. PTEN opposes negative selection and enables oncogenic transformation of pre-B cells. *Nat Med*. 2016; 22:379–387. [PubMed: 26974310] Authors show acute inhibition of PTEN, as well as hyperactivating the PI3K pathway can lead to increased central tolerance.

Figure 1.
Proposed Mechanisms/Targets of B cell Therapies in Autoimmunity.

Table 1
Properties of B cell targeted therapeutics

Target	Name	Format	Mechanism of action	Implications
CD20	Rituximab	Chimeric human IgG1 mAb	B cell depletion via CDC	Non-Hodgkin's Lymphoma, CLL, RA, GPA and MPA, MS
	Veltuzumab	Human IgG1 mAb	Depletes peripheral and tissue B cells	ITP, PV
	Ofatumumab	Human IgG1 mAb	B cell depletion via ADCC and CDC	CLL, Phase II RRMS, Phase III RA
	Ocrelizumab	Human IgG1 mAb	B cell depletion via CDC	'Breakthrough Therapy' Designation for RRMS, Phase II RRMS, Phase III PPMS
	Obinutuzumab	Human IgG1 mAb	B cell depletion via increased ADCC	Rituximab-Resistant CLL, Phase II Lupus Nephritis
CD19	MEDI-551	Human IgG1, Glycoengineered mAb	Improved FcγRIIIa effector potency and depletion of peripheral and tissue B cells	Phase I RRMS, Phase II/III Neuromyelitis Optica
	Blinatumomab	Bispecific × CD3	B Cell-mediated cytotoxicity due to proximity to T cells	R/R-ALL
	XmAb5574	Human IgG1, Fc Engineered mAb	Inhibition of BCR signaling via enhanced and selective affinity for FcγRIIB	Phase I/II SLE
	XmAb5871	Human IgG1, Fc Engineered mAb	Interacts with CD19 and binds FcγRIIB with increased affinity	Phase II RA, Phase II SLE, Phase II IgG4 Related Disease
CD79	MGD010	Bivalent DART × FcγRIIB	Bivalent human antibody targeting both CD79b and FcγRIIB	Phase I in Healthy Subjects
CD22	Epratuzumab	Human IgG1 mAb	Induction of inhibitory signaling in B cells	Phase III SLE
sBAFF	Belimumab	Human IgG1 mAb	Prevents interaction of BAFF with BAFF-R, TACI and BCMA	Autoantibody Positive SLE, Phase III Active Lupus Nephritis
sBAFF and sAPRIL	Atacicept	Fusion protein of TACI-R and human IgG1 Fc	Prevents interaction of BAFF with BAFF-R, TACI and BCMA and APRIL with TACI and BCMA	Phase II/III SLE
PI3Kδ	Idelalisib	Small-molecule inhibitor	Inhibits PI3K signaling pathway	Small Lymphocytic Lymphoma, CLL, Non-Hodgkin's Lymphoma, Phase I Allergic Rhinitis
	TGR 1202	Oral small-molecule inhibitor	Inhibits PI3K signaling pathway	Phase II Relapsed or Refractory Hematologic Malignancies
Btk	Ibrutinib	Irreversible, small-molecule inhibitor	Stabilizes Btk in an inactive conformation	Mantle Cell Lymphoma, CLL, and Waldenström's Macroglobulinemia
	CGI 1746	Reversible, small-molecule inhibitor	Stabilizes Btk in an inactive conformation	Phase I RA