Clinical & Experimental Immunology
The Journal of Translational Immunology
Clinical and Experimental Immunology
Guest Editor: Marina Botto

Translational Mini-Review Series on B Cell-Directed Therapies: Recent advances in B cell-directed biological therapies for autoimmune disorders

OTHER ARTICLES PUBLISHED IN THIS MINI-REVIEW SERIES ON B CELL-DIRECTED THERAPIES
B cell-directed therapy for autoimmune diseases. Clin Exp Immunol 2009; 157: doi:10.1111/j.1365-2249.2009.03977.x
The pathogenic role of B cells in autoantibody-associated autoimmune diseases – lessons from B cell-depletion therapy. Clin Exp Immunol 2009; 157: doi:10.1111/j.1365-2249.2009.03978.x

M. C. Levesque
Department of Medicine, Division of Rheumatology and Immunology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Accepted for publication 8 May 2009
Correspondence: M. C. Levesque, University of Pittsburgh Medical Center, BST S709, 3500 Terrace Street, Pittsburgh, PA 15261, USA.
E-mail: MCL40@pitt.edu

Summary

B cell-directed therapies are promising treatments for autoimmune disorders. Besides targeting CD20, newer B cell-directed therapies are in development that target other B cell surface molecules and differentiation factors. An increasing number of B cell-directed therapies are in development for the treatment of autoimmune disorders. Like rituximab, which is approved as a treatment for rheumatoid arthritis (RA), many of these newer agents deplete B cells or target pathways essential for B cell development and function; however, many questions remain about their optimal use in the clinic and about the role of B cells in disease pathogenesis. Other therapies besides rituximab that target CD20 are the furthest along in development. Besides targeting CD20, the newer B cell-directed therapies target CD22, CD19, CD40–CD40L, B cell activating factor belonging to the TNF family (BAFF) and A proliferation-inducing ligand (APRIL). Rituximab is being tested in an ever-increasing number of autoimmune disorders and clinical studies of rituximab combined with other biological therapies are being pursued for the treatment of rheumatoid arthritis (RA). B cell-directed therapies are being tested in clinical trials for a variety of autoimmune disorders including RA, systemic lupus erythematosus (SLE), Sjögren's syndrome, vasculitis, multiple sclerosis (MS), Graves' disease, idiopathic thrombocytopenia (ITP), the inflammatory myopathies (dermatomyositis and polymyositis) and the blistering skin diseases pemphigus and bullous pemphigoid. Despite the plethora of clinical studies related to B cell-directed therapies and wealth of new information from these trials, much still remains to be discovered about the pathophysiological role of B cells in autoimmune disorders.

Keywords: antigens CD19, antigens CD20, antigens CD22, BAFF, B lymphocyte

Introduction

An increasing number of B cell-directed therapies are in development for the treatment of autoimmune disorders [1–6]. Rituximab (Rituxan™; Genentech, South San Francisco, CA, USA) is the best-studied of the B cell-directed therapies and is approved as a treatment for active rheumatoid arthritis (RA) that is refractory to therapy with an anti-tumour necrosis factor (TNF) drug [7,8]. A generic version of rituximab (Reditux™; Dr Reddy's Laboratories Ltd, Hyderabad, India) is the first biosimilar monoclonal antibody (mAb) and is now available and approved for use in

Table 1. Approved and investigational uses of rituximab for treating autoimmune disorders.

Disease	Description	Phase	Status or outcome
Rheumatoid arthritis	Anti-TNF inadequate responders (IR)	Phase II	Effective [30]
	Anti-TNF IR (DANCER)	Phase IIB	Effective [7]
	Anti-TNF IR (REFLEX)	Phase III	Approved [8]
	Early RA	Phase I/II	Ongoing
	MTX IR (RUMBA)	Phase II	Ongoing
	MTX IR, rituximab plus anti-TNF	Phase II	Ongoing
	MTX IR (SCORE, SERENE)	Phase III	Ongoing
	MTX naive	Phase III	Ongoing
	Non-biological DMARD IR (SUNDIAL)	Phase III	Ongoing
SLE	Non-renal disease (EXPLORER)	Phase II	Not effective [36]
	Lupus nephritis (LUNAR)	Phase II	Not effective
	Lupus nephritis	Phase II	Ongoing
ITP	Chronic ITP	Phase II	Effective [39]
	Acute adult ITP	Phase II	Ongoing
	Chronic ITP	Phase II	Ongoing
	Untreated, adult ITP	Phase III	Completed
	Chronic ITP	Phase III	Ongoing
Pemphigus	Refractory; > 30% BSA	Phase II	Effective [40]
	Refractory or steroid-dependent	Phase II/III	Effective [41]
	Severe pemphigus	Phase III	Ongoing
Sjögren's syndrome (SS)	Primary SS (pSS)	Phases I/II	Effective [43]
	pSS	Phase II	Ongoing
	pSS, early, active disease (TEARS)	Phases II/III	Ongoing
Myositis	Refractory polmyositis	Phase II	Ongoing
	Adult dermatomyositis		
	Refractory juvenile dermatomyositis		
Graves' disease	Mild relapsing	Phase II	Effective [55]
	New onset and relapsing disease	Phases I/II	Effective [54]
	Graves' opthalmopathy	Phases II/III	Ongoing
Myasthenia gravis	Refractory myasthenia gravis	Phases I/II	Ongoing
	Refractory myasthenia gravis	Phase II	Ongoing
Multiple sclerosis	Relapsing–remitting MS	Phase II	Effective [60]
	Relapsing–remitting MS	Phase II	Effective [61]
	Primary progressive MS	Phases II/III	Ongoing
ANCA-associated vasculitis	Churg–Strauss syndrome	Phases II/III	Ongoing
	Wegener's granulomatosis	Phases II/III	Ongoing
	Microscopic polyangiitis (RAVE)		
	Maintenance *versus* azathioprine	Phase III	Ongoing

TNF, tumour necrosis factor; RA, rheumatoid arthritis; SLE, systemic lupus erthematosus; MTX, methotrexate; DMARD, disease modifying anti-rheumatic drug; ITP, immune thrombocytopenic purpura; MS, multiple sclerosis; ANCA, anti-neutrophil cytoplasmic antibody.

India. Like rituximab, many of the new biological therapies that target B cells cause B cell depletion; other B cell-directed therapies target pathways essential for B cell development and function [3,9–11]. This review will focus primarily on therapies that result in direct or indirect depletion of some or all B cells, as opposed to therapies that primarily block B cell activation or development such as co-stimulation blockers (abatacept and 7-related protein-1), cytokines (tocilizumab and baminercept) and B cell receptor-targeted therapies (abetimus and edratide). For many of the B cell-targeted therapies that induce B cell depletion, parallel development and treatment trials are in progress for autoimmune disorders [3,9–11] and B cell malignancies, including non-Hodgkin's lymphoma (NHL) [12] and chronic lymphocytic leukaemia (CLL) [13].

Table 1 lists the investigational studies that are under way to test the safety and effectiveness of rituximab in various autoimmune disorders. Table 1 emphasizes the dominant role currently played by rituximab in the area of B cell-targeted therapies. However, rituximab's dominance over other B cell-directed therapies (Table 2) for treatment of autoimmune disorders will probably diminish as other B cell-directed therapies are approved by the Food and Drug Administration (FDA). Table 2 lists other B cell-targeted

Table 2. Investigational B cell-directed biological therapies for treating autoimmune disorders.

Agent	Pharmaceutical company	Target	Form	Diseases	
				Phases I/II	Phase III
Anti-CD20 therapies					
Ocrelizumab	Genentech/Roche/Biogen-Idec	CD20	Humanized mAb	RA	RA
				MS (RR)	SLE (lupus nephritis)
				RA	
Ofatumumab	GSK/Genmab	CD20	Human mAb	RA	RA
				MS	
Veltuzumab	Nycomed/Immunomedics	CD20	Humanized mAb	ITP	
SBI-087	Wyeth/Trubion	CD20	SMIP	RA	
Tru-015				SLE	
Anti-CD22 therapies					
Epratuzumab	UCB/Immunomedics	CD22	Humanized mAb		SLE
Anti-CD19 therapies					
MDX-1342	Medarex	CD19	Human mAb	RA	
BAFF and APRIL Blockers					
Belimumab	GSK/Human Genome Sciences	BlyS (BAFF)	Human mAb	RA	SLE
Atacicept	EMD Serono	BAFF April	TACI-Ig receptor	RA[a]	SLE
				MS	
A-623	Anthera Pharmaceuticals	BAFF	Peptide fusion protein	SLE	
CD40 – CD40L blockers					
Ruplizumab (hu5c8; BG9588)	Biogen-IDEC	CD40L (CD154)	Humanized mAb	SLE	terminated (thrombotic events)
				ITP	
Toralizumab (IDEC-131)	Biogen-IDEC	CD40L (CD154)	Humanized mAb	SLE	terminated (thrombotic events)
				ITP	

[a]Alone and in combination after rituximab therapy. ITP, immune thrombocytopenic purpura; SLE, systemic lupus erythematosus; MS, multiple sclerosis; MS (RR), MS relapsing–remitting; RA, rheumatoid arthritis; mAb, monoclonal antibody; BAFF, B cell activating factor belonging to the TNF family.

therapies that are in clinical development and highlights the stage of development for each drug and the autoimmune diseases that are being targeted with these agents. The role of B cells in autoimmune diseases and the effects of B cell depletion therapies are the topic of a complementary review in this series [14].

Rituximab

Rituximab binds to CD20, which is expressed on human B cells and is expressed at a low level on a small subset of T cells [15–19]. After binding to CD20, rituximab induces B cell depletion by complement- and antibody-mediated cytotoxicity, although there is some evidence to suggest that some non-circulating tissue B cells bind rituximab but are not depleted [11,20]. Rituximab has proved to be well tolerated, except for the frequent occurrence of mild-to-moderate infusion reactions that may be dependent on complement fixation via the Fc portion of the antibody [21–24]. In addition, rituximab treatment may rarely be associated with serum sickness, agranulocytosis, fatal infections, including progressive multifocal leucoencephalopathy (PML) and death from other causes [25–29].

For autoimmune disorders, rituximab treatment has produced various clinical effects depending on the disease. In large clinical trials of patients with RA, rituximab treatment has been shown in combination with the disease-modifying anti-rheumatic drug (DMARD) methotrexate (MTX) to reduce disease activity [7,8,30]. Many of the new biologicals, including the B cell-targeted agents, are tested in combination with MTX and compared to a placebo plus MTX alone. For these studies, a composite end-point is used that includes the number of tender and swollen joints, physician and patient global assessment, patient pain, functional disability and acute phase reactant levels (erythrocyte sedimentation rate, C-reactive protein). The most commonly used composite end-point is the American College of Rheumatology (ACR) response criteria which requires 20, 50 or 70% improvements in tender and swollen joint counts plus improvements in a minimum of three of the other five measures listed above.

Rituximab has been approved for the treatment of refractory RA patients who have had inadequate responses to anti-TNF therapy. In phase II trials, different dosing regimens of rituximab (four weekly infusions of rituximab 375 mg/m^2, 500 or 1000 mg of rituximab administered 2 weeks apart) were tested in combination with MTX and were shown to have similar efficacy and to produce superior clinical responses to MTX alone [7,30]. In these RA trials, the effectiveness of rituximab in reducing disease activity was independent of a glucocorticoid regimen, although intravenous methylprednisolone improved tolerability during the first rituximab infusion. In a large phase III trial, patients with an inadequate response to anti-TNF agents (etanercept, adalimumab and infliximab) were randomized to receive MTX therapy and either two placebo or 1000-mg rituximab infusions 2 weeks apart [8]. At week 24, the rituximab-treated group ($n = 311$) showed significantly greater improvement than the placebo-treated group ($n = 209$), with higher ACR20, ACR50 and ACR70 response rates compared to placebo of 51% *versus* 18%, 27% *versus* 5%, and 12% *versus* 1%, respectively. The rate of serious infections was slightly higher in the rituximab group (5·2 per 100 patient-years) than the placebo group (3·7 per 100 patient-years), but tuberculosis or other opportunistic infections were not reported during the 24 weeks of the study. A recent meta-analysis suggests that treatment of RA with rituximab is not associated with an increased incidence of serious infections [31].

Rituximab has been investigated as a therapy in systemic lupus erythematosus (SLE) due to the potentially serious toxicities of other immunosuppressive agents used to treat this disease, and because more efficacious therapies are needed for many SLE manifestations. Several small trials in adults and children with SLE have shown that rituximab, often in combination with other immunosuppressive agents, may improve diverse manifestations of SLE, including skin rash, alopecia, arthritis, nephritis, haemolytic anaemia and thrombocytopenia [32–35]. Proof of efficacy and safety of rituximab therapy for SLE await the final results of two phase III randomized, placebo-controlled trials of rituximab therapy; one is a study of lupus nephritis (LUNAR) and the other is a study of moderate-to-severe SLE without active nephritis (EXPLORER). An abstract published at the 2008 American College of Rheumatology (ACR) meeting indicated that SLE subjects receiving rituximab in the EXPLORER trial did not have major or partial clinical responses that were different than subjects treated with placebo [36]. However, a subgroup analysis of African American and Hispanic SLE subjects in the EXPLORER trial indicated significant responses to rituximab compared to placebo. A recent press release from Genentech regarding the LUNAR study (http://www.gene.com/gene/news/press-releases/display.do?method=detail&id=11947) indicated that the trial failed to achieve its primary end-point, although details about the trial's results have not been published.

Several reports suggest success using rituximab treatment for idiopathic thrombocytopenic purpura (ITP), which is an acquired haemorrhagic condition associated with accelerated platelet consumption and anti-platelet autoantibodies that bind mainly glycoprotein IIb/IIIa on the surface of platelets [37,38]. The chronic form of ITP typically affects adults and the acute form often affects children. Although the majority of patients with ITP can be managed successfully with prednisone therapy, some patients require the use of other immunosuppressive therapies to achieve a significant platelet response [37]. The efficacy and safety of rituximab has been reviewed systematically for adults with ITP [29]. Among the 19 eligible studies in this review ($n = 313$ potentially evaluable patients), rituximab treatment pro-

duced a complete response in 46·3% of patients (platelet count > 150×10^9 cells/l) and a partial response in 24·0% of patients (platelet count 50–$150 \times 150 \times 10^9$ cells/l), with a median time to response of 5·5 weeks from the first dose of rituximab and a median response duration of 10·5 months. Ten of the patients in this group had a severe or life-threatening event, and nine patients died. A phase II study of rituximab therapy for patients with chronic ITP has now been published [39]. Forty per cent of subjects had good responses and the remainder failed to respond; many of the latter underwent splenectomy. A *post hoc* analysis of the study results indicated that responders were significantly younger than non-responders and that no other factors could be identified that differentiated the responders and non-responders. Another phase II trial of rituximab for refractory, relapsing or chronic ITP is ongoing and a phase III trial is currently under way to test the efficacy and safety of rituximab for chronic ITP. A phase II study of acute ITP is nearing completion.

B cell depletion using rituximab has also been tried in patients with refractory pemphigus vulgaris and pemphigus foliaceous. These severe blistering skin diseases are associated with autoantibodies directed against desmogleins, which are desmosomal proteins responsible for keratinocyte adhesion. Several lines of evidence implicate antibodies to desmoglein 1 (Dsg 1) in the pathogenesis of pemphigus foliaceous, while antibodies to desmoglein 3 (Dsg 3) with or without antibodies to Dsg 1 are believed to cause disease in pemphigus vulgaris. Pemphigus vulgaris and pemphigus foliaceous are treated usually with prednisone, MTX, azathioprine, mycophenolate mofetil and other immunosuppressive drugs. In an open, prospective trial of 11 patients with refractory pemphigus vulgaris, nine patients treated with a combination of 10 infusions of rituximab and six infusions of intravenous immune globulin over a 6-month period had resolution of skin lesions and sustained remissions of 22–37 months [40]. Impressively, all the other immunosuppressive therapies were discontinued in these responders before the end of the rituximab treatment period. Results from another open, prospective trial of steroid-refractory pemphigus vulgaris or pemphigus foliaceous showed that four weekly infusions of rituximab 375 mg/m^2 produced a complete remission in 18 (86%) of 21 patients, although the disease relapsed after a mean of 18·9 $\pm$ 7·9 months in nine of these responders [41]. While serum levels of immunoglobulin (Ig)G and IgG4 anti-Dsg 1 and anti-Dsg 3 antibodies decreased typically with clinical responses to rituximab therapy in these open label studies, exceptions were described in which persistently high serum levels of these autoantibodies or increases in their levels were detected in five of the 18 patients from the latter study, despite the fact that these subjects had achieved a durable clinical remission [40,41].

Rituximab treatment has also demonstrated limited evidence of clinical efficacy in other autoimmune disorders [2,30,42], including primary Sjögren's syndrome [43–48], dermatomyositis and polymyositis [49–52], Graves' disease [53–55], myasthenia gravis [56–59], multiple sclerosis [60,61], Wegener's granulomatosis and anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis [62–65]. There are ongoing phases II and III trials of rituximab for each of these autoimmune disorders (Table 1). In addition, there is an ongoing phase I trial of rituximab for treating psoriasis, but there have been case reports that suggest rituximab therapy may induce psoriasis [66,67].

In small, early-phase studies, Sjögren's syndrome patients treated with rituximab have shown increased salivary secretion and significant improvements in fatigue compared to patients treated with placebo [46,47]. The salivary secretion improvements have been most pronounced in subjects with early disease. However, there continues to be some concern about an increased incidence of serum sickness in Sjögren's syndrome patients treated with rituximab [43,47]. Beneficial effects of rituximab treatment have also been reported in phases I and II trials in relapsing–remitting multiple sclerosis (MS) [60,61]. In these MS studies, the authors reported reduced numbers of gadolinium-enhancing lesions on magnetic resonance imaging (MRI) and reduced numbers of relapses following rituximab treatment. Results have also been especially promising in studies of rituximab therapy for Wegener's granulomatosis and ANCA-associated vasculitis [62–65]. A large, multicentre trial (RAVE) is currently under way investigating the effects of rituximab therapy for ANCA-associated vasculitis. Therefore, B cell-directed interventions may influence favourably the clinical features of autoimmune disorders, opening new opportunities for improving the care of patients with these conditions. However, for many autoimmune disorders besides RA, large, randomized, placebo-controlled trials are lacking at this stage that provide convincing evidence of clinical efficacy and safety.

Other anti-CD20-directed therapies

Other monoclonal antibodies that target CD20 are in phases II and III of clinical development and include ocrelizumab (humanized anti-CD20; Genentech, South San Francisco, CA, USA), ofatumumab (humanized anti-CD20; Genmab, Copenhagen, Denmark), veltuzumab (humanized anti-CD20; Immunomedics, Morris Plains, NJ, USA) and the related agents TRU-015 and SBI-087 (humanized anti-CD20 SMIP; Trubion, Seattle, WA, USA). Two other anti-CD20 mAbs, tositumomab (Bexxar; GlaxoSmithKline, Brentford, UK) [68] and ibritumomab (Zevalin; Biogen IDEC, Cambridge, MA, USA) [69] have been approved as treatments for B cell malignancies and are each conjugated to radioisotopes to potentiate their killing action. These latter agents have not been studied in autoimmune disorders.

Rituximab is a chimeric antibody and this may account for some of the infusion reactions observed with the drug

[21,23]. In contrast, all the newer anti-CD20 biologicals being studied in autoimmune disorders are human or humanized monoclonal antibodies. The side-effect profiles and clinical activity of newer anti-CD20 drugs appear to differ somewhat from rituximab [70–73]. Whether this will translate into significant clinical differences and whether these newer anti-CD20 drugs supplant rituximab for treatment of RA and haematological malignancies remains an open question.

The anti-CD20 drug, ocrelizumab, enhances antibody-dependent cell-mediated cytotoxicity (ADCC) and has reduced complement-dependent cytotoxicity (CDCC) compared with rituximab [72]. These characteristics may improve ocrelizumab's tolerability in autoimmune disorders while making it less desirable for haematological malignancies. The Fc portion of ocrelizumab was modified to reduce complement-dependent cytotoxicity, because complement activation may lead to some of the side effects associated with rituximab [22]. Ocrelizumab binds to a different, but overlapping, epitope of the extracellular domain of CD20 compared with rituximab. A published phases I/II trial of ocrelizumab in RA patients on MTX and with active disease demonstrated that ocrelizumab infusions were associated with minimal infusion reactions despite the absence of concomitant corticosteroids during the infusions [72].

The anti-CD20 agent, ofatumumab, is in the late stages of development for several disorders, including CLL, NHL, MS and RA. In studies of CLL patients, ofatumumab was associated with significant B cell depletion [74]. Ofatumumab binds to a more proximal portion of CD20, closer to the B cell membrane. Preclincal studies suggest that ofatumumab has a slower rate of dissociation from CD20 than rituximab and this results in greater CDCC and lysis of rituximab refractory B cell lines [71,75]. In preclinical studies, ofatumumab has demonstrated higher potency and longer duration of efficacy than rituximab [70]. However, the period of B cell depletion after ofatumumab therapy is similar to that of rituximab, with the gradual recovery of normal B cells approximately 6 months after therapy [74]. In addition, infusion reactions appear similar after ofatumumab administration compared to rituximab in patients treated for CLL [74]. Head-to-head clinical studies will need to be performed to assess whether there are significant differences between ofatumumab and rituximab in the treatment of autoimmune disorders and B cell malignancies.

The anti-CD20 mAb, veltuzumab, is in phase II studies for treatment of ITP with plans for further trials in other autoimmune disorders through a licensing agreement with Nycomed. An early study suggested that veltuzumab had similar binding characteristics as rituximab [76], but a more recent report indicates that veltuzumab has reduced off-rates during CD20 binding and is more effective *in vitro* at lysing tumour cells and more effective *in vivo* than rituximab in three lymphoma models [73]. The difference in CD20 binding off rate is due to a single amino acid difference in the CDR antigen-binding region of veltuzumab compared to rituximab [73].

The small modular immunopharmaceutical (SMIP™), SBI-087, is a humanized version of Trubion's TRU-015. Both compounds consist of single-chain variable regions (V_L and V_H) that bind CD20, and which are fused by means of a modified human IgG1 hinge domain to engineered constant regions that encode human IgG1 constant heavy domains (CH2 and CH3). The small size of SMIPs may enhance tissue penetration, and their unique formulation may lower infusion-related reactions due to less CDCC [77]. SBI-087 is in phase I studies for RA and SLE and TRU-015 is in phase II studies for RA.

Anti-CD22-directed therapies

Epratuzumab (Immunomedics) is a humanized anti-CD22 that induces preferential depletion of naive and transitional B cells and reduces total B cells by about 35% [78,79]. Epratuzumab also blocks activation and proliferation of anti-immunoglobulin-stimulated B cells from SLE patients after co-incubation with CD40L or CpG; this suggests that epratuzumab not only depletes B cells, but also regulates their function. Interestingly, epratuzumab inhibited the proliferation of B cells from patients with SLE but not normal B cells under various culture conditions [79]. A phase II study of epratuzumab treatment in SLE patients reported promising results with British Isles Lupus Activity Group Index (BILAG) scores decreased by greater than 50% in all 14 patients at some point during the study [78]. There were few infusion reactions during the phase II trial. Epratuzumab is in phase III trials for SLE and haematological malignancies. For treatment of haematological malignancies the effects of epratuzumab appear to be augmented by concomitant treatment with anti-CD20 therapy.

Another anti-CD22 therapy, CAT8015 (MedImmune, Gaithersburg, MD, USA) is being developed for use as a treatment for haematological malignancies. The role of CAT8015 in autoimmune disorders is not clear.

Anti-CD19-directed therapies

CD19 is expressed by all B cells except at the earliest stage of B cell development; in contrast to CD20, CD19 is expressed at low levels on antibody secreting plasma cells [17]. The anti-CD19 therapy, MDX-1342 (human anti-CD19; Medarex, Princeton, NJ, USA) results in B cell depletion and elimination. MDX-1342 is in phase 1 trials for RA. The results of these studies are awaited eagerly, as it is currently unclear whether anti-CD19 therapy will result in more profound B cell depletion than anti-CD20 therapy and whether these differences will result in better therapeutic responses and/or more side effects.

BAFF and A proliferation-inducing ligand (APRIL) blockers

BAFF regulates B cell development and is necessary for development of transitional T2 and marginal zone B cells in mice and appears to be important for most stages of peripheral B cell development in mice and humans [80–82]. Like BAFF, APRIL also regulates B cell development [83]. BAFF mediates its effects via binding to the BAFF receptor, BCMA and tumour necrosis factor receptor superfamily, member 13B (TNFRSF13B) (TACI), whereas APRIL binds only to BCMA and TACI [84]. Transgenic mice that over-express BAFF develop autoimmune disorders with evidence of circulating rheumatoid factors and lupus-like renal disease [85,86]; these autoimmune disorders are accompanied by lymphocytic disorders with increased B cell numbers, increased immunoglobulin levels, increased germinal centre activity and increased numbers of plasma cells. In a similar fashion, patients with RA, SLE and Sjögren's syndrome have elevated levels of BAFF and APRIL [86–89].

Serum BAFF levels have been measured in patients with autoimmune diseases before and after rituximab therapy. Following rituximab therapy, serum BAFF levels increase significantly during periods of B cell depletion and BAFF levels return to baseline when B cell numbers return to normal values [44,90–92]. The augmented BAFF levels following B cell depletion may contribute to the return of self-reactive B cells, as excessive BAFF has been shown to rescue self-reactive B cells from apoptosis [80,93,94]. Studies are being considered in which B cell depleting antibodies such as rituximab would be combined with a BAFF antagonist to produce more sustained B cell depletion and block return of self-reactive B cells.

In SLE patients, belimumab (human anti-BLyS™ (BAFF); Human Genome Sciences, Rockville, MD, USA) reduces total peripheral B cell numbers; subjects responding to therapy had significantly greater reductions in activated $CD69^+$ B cell numbers than subjects without clinical responses [95]. In a similar way, cynamologous monkeys administered belimumab every 2 weeks had reduced total B cell numbers and decreased numbers and size of splenic lymphoid follicles [96]. In the cynamologous monkeys, chronic administration of belimumab did not lead to changes in total serum IgG and IgM concentrations [96]. Phase III trials of belimumab are in progress to determine the potential clinical efficacy of this agent in SLE. A phase II study of belimumab in SLE did not meet the primary endpoint for the study, but in the extension phase of the study the authors found that among all patients who were seropositive at baseline, 46% had significant improvement at week 52 (difference from placebo 29%, $P < 0{\cdot}05$) and the percentage of subjects who improved increased over time [97,98]. In this analysis, the authors defined seropositive as an ANA level >1:80 or an anti-double-stranded-DNA antibody level >30 IU. There was a significant reduction of flares in the belimumab-treated group after 3 years of treatment and belimumab therapy was associated with sustained improvement in SLE disease activity as defined by a reduction in the frequency of lupus flares [99]. In SLE patients treated with belimumab, there was a four-point reduction from baseline in Safety of Estrogen in Lupus Erythematosus National Assessment–SLE Disease Activity Index (SELENA–SLEDAI) scores, no worsening in Physician Global Assessments and no new BILAG A organ involvement. Belimumab therapy was also associated with increased complement levels. Immunoglobulin levels decreased with belimumab therapy and the percentage of subjects with significant anti-dsDNA reduction was higher in clinical responders compared to non-responders [99].

Atacicept (TACI-Ig fusion protein; EMD Serono, Rockland, MA, USA) binds both BAFF and APRIL and has been administered to both SLE and RA patients. In an early-phase trial of SLE patients, atacicept administration was associated with a dose-dependent reduction in immunoglobulin levels, reductions in naive B cells (after an initial brief increase in memory B cells), but no significant changes in anti-tetanus antibody levels [100]. Using a reduction in SELENA–SLEDAI score of greater than 3, the authors observed some effect of therapy on the 12 patients enrolled in the study [100].

In RA patients who were administered atacicept, rheumatoid factor and anti-cyclic citrullinated peptide (CCP) antibodies were reduced with treatment. However, atacicept treatment was not associated with decreases in erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP). On flow cytometry, there were reductions in all subsets of B cells, with the greatest reduction observed in naive B cells; like the studies in SLE patients, after atacicept administration there was a brief initial increase in memory B cells but no effect on T lymphocytes or monocytes. Clinical response data from this study were limited, but subjects who received repeated doses of atacicept evidenced a decrease in mean DAS28 scores from $6{\cdot}4 \pm 1{\cdot}3$ at baseline to $5{\cdot}1 \pm 1{\cdot}4$ at day 85. In this trial of atacicept, no serious infections were noted [101].

Two other BAFF antagonists have been developed and tested in preclinical studies and in early-phase clinical studies. BR3-Fc (BAFF receptor fusion protein; Genentech, South San Francisco, CA, USA) binds BAFF and was tested initially in phase I studies, although its development appears to be on hold. Another BAFF antagonist, A-623 (peptide fusion protein; Anthera Pharmaceuticals, Hayward, CA, USA) has been tested in phase I studies and is being developed for treatment of SLE.

Therapies that block CD40–CD40L interactions

CD40 is expressed by B cells and other antigen-presenting cells and CD40L (CD154) is expressed by T cells [102]. CD40–CD40L interactions are essential for immunoglobulin class-switching, memory B cell development and germinal centre formation [103]. To date, two humanized anti-

CD154 mAbs have been investigated in clinical trials: ruplizumab (hu5c8 or BG9588; Biogen Idec) and toralizumab (IDEC-131; Biogen Idec). Ruplizumab was studied extensively as a therapy for transplant rejection and underwent limited testing for treatment of ITP [104] and SLE [105]. Toralizumab was tested in early-phase studies for treatment of ITP [104] and SLE [106]. There was significant initial excitement related to therapies that blocked CD40–CD40L interactions. Good clinical responses were noted in the trials of ruplizumab for treatment of SLE [105], and ruplizumab treatment reduced circulating plasmablasts in SLE patients [107]. However, enthusiasm for these agents was tempered by thromboembolic events that occurred during the early-phase trials [105] and the observation that activated platelets express CD154 [108]. The Fc portion of ruplizumab is required to aggregate platelets, and a PEG-Fab agent that binds CD154 is being developed that may mitigate some of the thromboembolic side effects of the full-length CD154 mAbs [108]. An anti-CD40 antibody (SGN-40 or dacetuzumab; Seattle Genetics, Bothell, WA, USA) has been developed and is being tested as a treatment for NHL, but has not been studied in autoimmune diseases.

Conclusions and future directions

B cell-directed therapies represent promising treatments for autoimmune disorders, although many questions remain about their optimal use in the clinic. Autoantibody depletion correlates with the clinical effectiveness of these drugs in some, but not all, diseases. This suggests that much work needs to be conducted to understand the mechanism of action of these drugs. To date, only rituximab is currently available for treating patients with autoimmune disorders. However, a number of new B cell-directed therapies are being developed and will probably be available soon for use in the clinic. Of these new agents, two anti-CD20 therapies, ocrelizumab and ofatumumab, appear to be the furthest along in development.

Acknowledgements

The author wishes to acknowledge the stimulating exchange of ideas and discussions about B cells and B cell-directed therapies with his colleagues Bill St Clair, Thomas Tedder, Garnett Kelsoe, Russell Hall and David Pisetsky at Duke and David Lee at Harvard. This study was funded by NIH contracts 7U19A056362 (Autoimmunity Centers of Excellence) and a research grant from Genentech, Inc.

Disclosure

Dr Levesque has received research funding from Genentech for Studies of rituximab.

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