

Mechanisms of allergic diseases

(Supported by an educational grant from Merck & Co., Inc.)

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B cell-directed therapies for autoimmune disease and correlates of disease response and relapse

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Recent advances have led to the development of mAbs that effectively deplete B cells in human beings and target pathways essential for B-cell development. 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 diseases but not all. This finding implies that self-reactive B cells are playing important pathogenic roles in autoimmune disorders beyond the production of autoantibodies. Clinical studies of B cell-directed therapies are beginning to illuminate the effects of B-cell modulation on immune function using a variety of mechanistic approaches, including delineation of B-cell subsets by flow cytometry, measurement of serum autoantibodies and cytokines, and tests of immunocompetence. Recent clinical studies have been performed in patients with rheumatoid arthritis and SLE suggesting the depletion of memory cells accounts at least in part for the clinical efficacy of rituximab therapy, but these findings, although provocative, require further investigation in larger cohorts. Memory B cells are not the only targets of depleting antibodies; therefore, other B-cell populations of therapeutic relevance may be modulated by these interventions. Moreover, pathologic B-cell responses may be favorably influenced by other targeted approaches such as those using anti-B-cell activating factor belonging to the TNF family (BAFF) or anti-CD22 antibodies.

(*J Allergy Clin Immunol* 2008;121:13-21.)

Key words: *B lymphocyte, antigens, CD20, B-lymphocyte activating factor, rituximab*

Recent advances have led to the development of mAbs that effectively deplete B cells in human beings and target pathways essential for B-cell development.^{1,2} Among these agents, rituximab (Rituxan; Genentech, South San Francisco, Calif) has been the most studied in human beings. Rituximab binds to **CD20**, which is almost exclusively expressed on human B cells.^{3,4} Other mAbs that target B cells are in phase III of clinical development and include ocrelizumab (humanized anti-CD20; Genentech), ofatumumab (humanized anti-CD20; Genmab, Copenhagen, Denmark), epratuzumab (humanized anti-**CD22**; Immunomedics, Morris Plains, NJ), and belimumab (humanized anti-B-lymphocyte stimulator; Human Genome Sciences, Rockville, Md).^{1,5,6}

In addition to treatment of hematologic malignancies such as non-Hodgkin lymphoma⁷ and chronic lymphocytic leukemia,⁸ B-cell-directed therapies have an important role in the treatment of autoimmune disorders.^{6,9-11} These therapeutic antibodies have proven to be largely well tolerated except for the frequent occurrence of mild-to-moderate infusion reactions. However, rituximab treatment, which has the most extensive clinical use to date, may rarely be associated with serum sickness, agranulocytosis, fatal infections including progressive multifocal leukoencephalopathy from John Cunningham virus, and death from other causes.¹²⁻¹⁶

AUTOIMMUNE DISORDERS AND THE CLINICAL UTILITY OF B-CELL-DIRECTED THERAPIES

For autoimmune disorders, rituximab treatment has produced a range of clinical and serologic effects depending on the disease. For example, in large clinical trials, rituximab treatment has been shown in combination with methotrexate to reduce disease activity in patients with **rheumatoid arthritis (RA)**.¹⁷⁻¹⁹ Methotrexate is the most commonly prescribed disease-modifying antirheumatic drug for the treatment of RA and is usually the comparator for new therapies in clinical trials. Many of the new biologics, including the B cell-targeted agents, are tested in combination with methotrexate and compared with a placebo plus methotrexate alone using a composite endpoint; number of tender and swollen joints, physician and patient global assessment, patient pain, functional disability, and acute phase reactants levels (erythrocyte sedimentation rate, C-reactive protein). For example, the American College of Rheumatology (ACR) response criteria require 20%, 50%, or 70% improvement in tender and swollen joint counts plus a minimum of 3 of the other 5 measures listed.

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Supported by National Institutes of Health contracts 7U19AI056362 (Autoimmunity Centers of Excellence) and NO1-AI-15416 (Immune Tolerance Network) and a research grant from Genentech, Inc.

Disclosure of potential conflict of interest: M. C. Levesque has consulting arrangements with Genentech; has received research support from the National Institutes of Health, Genentech, and Amgen; has served as an expert witness for Merck; and has served as a member of the Arthritis Foundation. E. W. St. Clair has consulting arrangements with Biogen Idec, Genentech, Bristol Myers Squibb, Novartis, Human Genome Sciences, Medimmune, and Synovex; has received research support from the National Institutes of Health, Genentech, and Amgen; and has been a part of American College of Rheumatology Research and Education Foundation.

Received for publication October 29, 2007; revised November 30, 2007; accepted for publication November 30, 2007.

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0091-6749/\$34.00

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doi:10.1016/j.jaci.2007.11.030

Terms in boldface and italics are defined in the glossary on page 14.

Abbreviations used

ACR:	American College of Rheumatology
AID:	Activation-induced cytidine deaminase
ANCA:	Antineutrophil cytoplasmic autoantibody
BAFF:	B-cell activating factor belonging to the TNF family
BCR:	B-cell receptor
CCP:	Cyclic citrullinated peptide antibody
Dsg:	Desmoglein
iCOS:	Inducible costimulator
ITP:	Immune thrombocytopenic purpura
RA:	Rheumatoid arthritis
RAG:	Recombination activating gene
RF:	Rheumatoid factor
TdT:	Terminal deoxynucleotidyltransferase
Treg:	T-regulatory

Rituximab has been approved for the treatment of refractory RA. In a phase II trial, 4 weekly infusions of rituximab 375 mg/m² and methotrexate were shown at week 24 to produce superior

clinical responses to methotrexate alone (ACR50 response rates, 43% vs 13%).¹⁷ The patients in this trial received premedication with methylprednisolone before each infusion of rituximab as well as a 2-week course of oral prednisone to improve tolerability. This need for glucocorticoids was further investigated in a 9-arm study in which 2 infusions of 500 or 1000 mg of rituximab were administered 2 weeks apart in combination with 3 different glucocorticoid regimens (placebo, intravenous methylprednisolone premedication, and intravenous methylprednisolone premedication plus oral prednisone for 2 weeks).¹⁸ Both doses of rituximab were effective in reducing disease activity independent of glucocorticoid regimen, whereas 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 randomly allocated to receive 2 placebo or 1000-mg rituximab infusions 2 weeks apart on background methotrexate therapy.¹⁹ The patients received premedication consisting of intravenous methylprednisolone 100 mg plus a 2-week course of oral prednisone. At week 24, the rituximab-treated group (n = 311) showed significantly

GLOSSARY

B-CELL ACTIVATING FACTOR BELONGING TO THE TNF FAMILY (BAFF): B lymphocyte stimulator; required for B-cell development, binds to the BAFF receptor and to transmembrane activator and calcium-modulator and cyclophilin. Mutations in TACI can cause common variable immunodeficiency.

CD5: Associated with B cells that make polyreactive, autoimmune IgM.

CD20: Surface protein involved in Ca⁺⁺ flux, present on mature B cells (normal and malignant) but not present on plasma cells.

CD22: Also known as Siglec-2, involved in B-cell activation and proliferation, expressed on mature B cells.

CD27: A TNF-receptor family member present on activated and memory B cells and plasma cells that mediates costimulatory signals.

CD40L (CD154), CD69, INDUCIBLE COSTIMULATOR (iCOS): Markers of activated T cells that provide B-cell help for immunoglobulin class switch. CD69: expressed on multiple activated leukocyte subsets including T cells, B cells, eosinophils, and neutrophils. iCOS: belongs to the CD28 family of proteins, induces differentiation to memory and plasma B cells. CD40L deficiency causes the primary immunodeficiency X-linked hyper-IgM syndrome; ICOS deficiency can cause common variable immunodeficiency.

GERMINAL CENTER: Develops in secondary lymphoid organs in response to antigen; clonal proliferation, somatic hypermutation, receptor editing, isotype class switch, affinity maturation, apoptosis, selection by antigen, and generation of memory B cells occur in germinal centers.

MARGINAL ZONE: The region between the splenic red and white pulp; where B and T cells likely initially encounter antigen; marginal zone B cells express IgM, low levels of IgD. An absence of marginal zone memory B cells in patients with common variable immunodeficiency may relate to an inability to generate a protective polysaccharide response.

MEMORY B CELL: Generated in the germinal center, differentiates into high-affinity plasma cells; CD27⁺, CD20⁺.

PEMPHIGUS VULGARIS: A blistering skin disease presenting with painful intraepidermal bullae of the mucosal and skin surfaces caused by breakdown of cell adhesion within the epithelium. Associated with an antibody against Dsg 3. The pemphigus group includes pemphigus vulgaris, foliaceus, drug-induced, and paraneoplastic pemphigus.

PHYTOHEMAGGLUTININ: A mitogen that causes polyclonal *in vitro* T-cell proliferation in an antigen-independent manner.

PLASMA CELL: No longer expresses surface Ig but makes large quantities of antibody. B-cell development can be simplified as (1) pro-B:

H-chain rearrangement begins; (2) pre-B: H-chain rearrangement complete, pre-B-cell receptor expressed; (3) immature B: H-chain and L-chain rearrangement complete, surface IgM expressed; (4) mature B: surface IgM and IgD expressed; (5) activated B cells: antigen responsive; (6) plasma cells.

RECOMBINATION ACTIVATING GENE (RAG), TERMINAL DEOXYNUCLEOTIDYLTRANSFERASE (TDT), ACTIVATION-INDUCED CYTIDINE DEAMINASE (AID): Immunoglobulin production depends on VDJ recombination with site-specific cleavage followed by end repair. Antigen affinity maturation occurs through somatic mutation; class switch changes IgM and D to IgG, A, or E. RAG-1 and RAG-2 initiate cleavage; TdT adds nucleotides to the recombination ends; AID is involved in affinity maturation and class switch. RAG deficiency leads to Omenn syndrome, a severe combined immunodeficiency variant; AID deficiency can cause autosomal-recessive hyper-IgM syndrome.

RHEUMATOID ARTHRITIS (RA): Requires 4 of the following for >6 weeks: (1) morning stiffness for >1 hours, (2) arthritis of 3 or more joints (proximal interphalangeal, metacarpophalangeal, wrist, elbow, knee, ankle, metatarsophalangeal), (3) symmetric joint involvement, (4) rheumatoid nodules, (5) positive serum rheumatoid factor, and (6) radiographic changes in or adjacent to affected joints. ACR guidelines are used for instituting disease-modifying antirheumatic drugs and for following clinical/functional response.

SJÖGREN SYNDROME: Four or more of the following: ocular symptoms (dry eyes >3 months or tear substitute >3 times a day); oral symptoms (dry mouth for 3 months, recurrent/persistent swollen glands, frequent drinking of liquids); ocular signs of positive Schirmer test, Rose Bengal score; histopathology of aggregates of >50 mononuclear cells/mm² on salivary biopsy; positive test result on salivary flow tests; autoantibodies: anti-Ro (SS-A), anti-La (SS-B), or rheumatoid factor.

SLE: Four of the following: malar or discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal disease, seizures/psychosis, hematologic abnormality (leukopenia/lymphopenia/thrombocytopenia), autoantibodies (anti-dsDNA, anti-Smith, and/or antiphospholipid), positive antinuclear antibody.

T-REGULATORY (Treg) CELLS: Regulatory CD4⁺ or CD8⁺ cells, can express the transcription factor FoxP3 and/or the cytokine TGF-β. Congenital absence of Foxp3 Treg cells causes immune dysregulation, polyendocrinopathy, enteropathy, X-linked, an immunodeficiency associated with polyorgan autoimmunity. Therapies that successfully treat autoimmunity or allergy can be associated with elevated numbers of Treg cells.

greater improvement than the placebo-treated group ($n = 209$), with higher ACR20, ACR50, and ACR70 response rates compared with 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.

Rituximab has been widely investigated in *SLE* because of the potentially serious toxicities of immunosuppressive agents such as glucocorticoids in moderate or high doses, azathioprine, mycophenolate mofetil, and cyclophosphamide that are currently in use for the treatment of this disease. A major barrier to the study of new therapies for *SLE* has been the heterogeneity of disease and the difficulties with developing valid outcome measures across multiple organ systems. Regardless, several small trials in adults and children with *SLE* have shown that intravenous rituximab at various doses, often in combination with other immunosuppressive agents, may improve myriad manifestations of *SLE*, including skin rash, alopecia, arthritis, nephritis, hemolytic anemia, and thrombocytopenia.²⁰⁻²³ Proof of efficacy and safety of rituximab therapy for *SLE* await the results of the phase III randomized, placebo-controlled trials of rituximab therapy for lupus nephritis (a phase III, randomized, double-blind, placebo-controlled, multi-center study to evaluate the efficacy and safety of rituximab in subjects with ISN/RPS class III or IV lupus nephritis [LUNAR]) and moderate-to-severe *SLE* without active nephritis (a randomized, double-blind, placebo-controlled, multi-center phase III/III study to evaluate the efficacy and safety of rituximab in subjects with moderate to severe systemic lupus erythematosus [EXPLORER]).

Rituximab therapy has also been used in a variety of other autoimmune settings. Some success has been reported using rituximab treatment for idiopathic thrombocytopenic purpura (ITP), an acquired hemorrhagic condition associated with accelerated platelet consumption and antiplatelet autoantibodies that mainly target glycoprotein IIb/IIIa on the surface of platelets.²⁴ Although the majority of patients with ITP can be treated successfully with prednisone therapy, a minority requires the use of other immunosuppressive agents to achieve a significant platelet response. The efficacy and safety of rituximab has been systematically reviewed for adults with ITP.¹⁶ Among the 19 eligible studies in this review ($n = 313$ potentially evaluable patients), rituximab treatment produced a complete response in 46.3% of patients (platelet count $> 150 \times 10^9$ cells/L) and a partial response in 24.0% of patients (platelet count $50-150 \times 10^9$ 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 9 patients died. Despite the widespread use of rituximab therapy for ITP, no controlled trials have formally tested the efficacy and safety of this agent for this condition.

CD20 B-cell depletion using rituximab has also been tried in patients with refractory *pemphigus vulgaris* and pemphigus foliaceus. These severe blistering skin diseases are associated with autoantibodies directed against desmogleins, desmosomal proteins responsible for keratinocyte adhesion. Several lines of evidence implicate antibodies to desmoglein (Dsg)-1 in the pathogenesis of pemphigus foliaceus, whereas antibodies to Dsg 3 with or without antibodies to Dsg 1 are believed to be causal in pemphigus vulgaris. These conditions are usually treated with prednisone, methotrexate, azathioprine, mycophenolate mofetil,

and other immunosuppressive drugs. In an open, prospective trial of 11 patients with refractory pemphigus vulgaris, 9 patients treated with a combination of 10 infusions of rituximab and 6 infusions of intravenous immune globulin over a 6-month period had resolution of skin lesions and sustained remissions of 22 to 37 months.²⁵ Impressively, all other immunosuppressive therapy was discontinued in these responders before the end of the rituximab treatment period. Results from another open, prospective trial of steroid-refractory pemphigus vulgaris or foliaceus showed that 4 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 ± 7.9 months in 9 of these responders.²⁶ Although serum levels of IgG and IgG₄ antibodies strongly correlated in these studies with clinical response to rituximab therapy, exceptions were described in which persistently high serum levels of anti-Dsg 1 and anti-Dsg 3 antibodies or increases in their levels were detected in 5 of the 18 patients from the latter study who had achieved a durable clinical remission. Case reports indicate that paraneoplastic pemphigus, a malignancy-driven autoimmune disorder, may be sensitive or resistant to rituximab therapy.²⁷

In small studies, rituximab treatment has demonstrated limited evidence of clinical efficacy in other autoimmune disorders,^{2,17,28} such as primary *Sjögren syndrome*,^{29,30} dermatomyositis and polymyositis,³¹⁻³⁴ Graves disease,^{35,36} and myasthenia gravis.³⁷⁻³⁹ Results have been especially promising in studies of rituximab therapy for Wegener granulomatosis and antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis.⁴⁰⁻⁴² A large, multicenter trial is currently underway investigating the effects of rituximab therapy for ANCA-associated vasculitis that will shed more light on its efficacy for this indication. Beneficial effects of rituximab treatment have also been reported in a phase II trial in multiple sclerosis, which is not usually considered an autoantibody-mediated disease.⁴³ In a similar way, Simon et al⁴⁴ have reported successful treatment of atopic eczema in an open-label trial of rituximab treatment despite the absence of treatment-associated declines in allergen-specific IgE antibodies. Therefore, B-cell-directed interventions may favorably influence the clinical features of autoimmune disorders, opening new opportunities for improving the care of patients with these conditions. However, at this stage, large, randomized, placebo-controlled trials are lacking that provide convincing evidence of clinical efficacy and safety except in the case of RA.

Most autoimmune disorders are characterized by the presence of autoantibodies and abnormalities of B-cell function.^{6,11} Autoantibodies may form immune complexes that engage other immune cells and can activate complement, triggering an inflammatory response. B cells may also participate in immune responses by activating T cells, secreting cytokines, and influencing lymphoid structure.⁴⁵⁻⁵⁰ In some organ-specific diseases such as pemphigus vulgaris and pemphigus foliaceus, ITP, and Graves disease, rituximab therapy typically reduces serum levels of autoantibodies in accordance with improvements in disease activity.^{6,11,16,25,26,36,51,52} Autoantibodies associated with these organ-specific diseases bind to antigens predominantly expressed in the target tissue; their levels often correlate with disease activity and in many cases have been shown to play a significant role in disease pathogenesis.

In contrast, the relationships among rituximab-induced B-cell depletion, changes in autoantibody levels, and reductions in disease activity have been far less consistent in the cases of RA

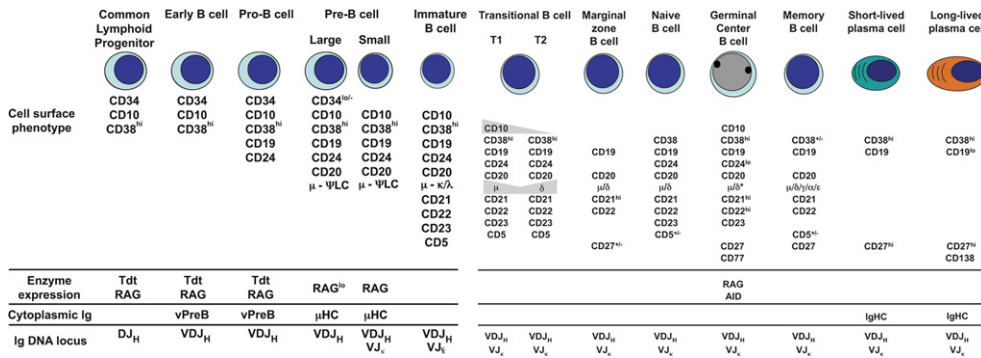


FIG 1. Human B-cell development. The developmental steps of human B cells are outlined. Initial development of B cells in adults occurs in the bone marrow and includes development of progenitor cells into pro-B-cell and pre-B-cell populations. The bone marrow stages of human B-cell development are characterized by the expression of CD19 by pro-B cells, the subsequent expression of CD20 by pre-B cells, the rearrangement of the heavy-chain and light-chain immunoglobulin loci, and the expression of cell surface IgM. Self-reactive B cells undergo either deletion or immuno-

globulin receptor editing to eliminate autoreactive B cells. After exiting the bone marrow, transitional B cells undergo further development in peripheral lymphoid organs and circulate in the blood as naive B cells. Naive B cells encounter antigen and are stimulated via T-dependent and independent pathways in germinal centers and in the marginal zones of lymphoid organs to form memory B cells, plasmablasts, and long-lived plasma cells. The introduction of AID-mediated somatic mutations in germinal center B cells and the subsequent expression of CD27 mark the transition from naive B cells to memory and plasma cell B-cell populations. The *asterisk* for μ/δ at the germinal center stage indicates that μ and δ immunoglobulin heavy-chains are expressed during the pregerminal stage with subsequent replacement by γ , α , or ϵ or less frequently by continued μ or δ expression at later stages of germinal center development and at the memory B-cell stage. Most terminally differentiated plasma cells are characterized by the absence of CD20 expression and low or negative expression of CD19 and surface immunoglobulin.

and SLE. Autoantibodies in RA and SLE may be associated with disease severity or selected clinical phenotypes, but their levels are not strong correlates of disease activity, except for perhaps anti-double-stranded DNA levels in lupus nephritis.^{51,53-58} Rituximab therapy produces variable decreases in both serum rheumatoid factor (RF) and anti-CCP (cyclic citrullinated peptide antibody) levels, irrespective of improvements in clinical disease activity.^{19,59} The case appears to be similar for SLE, in which the hallmark autoantibodies react with double-stranded DNA and multiple ribonuclear proteins.⁶⁰ Although preliminary studies suggest SLE improves with rituximab treatment, observed reductions in disease activity are not consistently correlated with decreases in serum autoantibody levels.^{61,62} This finding implies that self-reactive B cells are playing important pathogenic roles in RA and SLE beyond the production of autoantibodies, and that rituximab-induced B-cell depletion acts through these other mechanisms.

Moreover, multiple sclerosis is not typically considered autoantibody-mediated yet may improve after rituximab-induced B-cell depletion therapy.^{43,63-65} In multiple sclerosis, rituximab therapy is associated with significant reductions in central nervous system B cells but essentially unchanged levels of cerebrospinal fluid and serum total immunoglobulins, cerebrospinal fluid oligoclonal bands, and serum myelin oligodendrocyte glycoprotein and serum myelin basic protein autoantibody levels.⁶³⁻⁶⁵ Because animal models suggest that immunoglobulin production is not essential to autoimmune disease development,⁶⁶ rituximab-induced improvements may be related to effects on other B-cell functions such as antigen presentation,⁴⁵⁻⁴⁷ cytokine production,^{48,49} and cell:cell interactions with T cells.^{47,50}

B-CELL DEVELOPMENT

An overview of human B-cell development is important to understand the potential mechanisms of B cell-directed therapies and their effect on autoantibody production and the other functions of B cells. These therapies bind to cell surface molecules on B cells such as CD20, CD22, and CD19 and may therefore differentially affect subsets of B cells depending on the expression of these therapeutic targets during B-cell development. Fig 1 provides a developmental scheme that highlights

important steps and unique functions associated with B-cell development in the bone marrow and in peripheral lymphoid organs such as the spleen and lymph nodes. The expression of cell surface immunoglobulin and the secretion of immunoglobulin are fundamental to B-cell maturation and the acquisition of functions unique to B cells.^{67,68} The classification of B-cell subsets is typically based on cell surface markers and the cell surface expression of different immunoglobulin isotypes.^{67,69-72} Fig 1 describes phenotypic cell surface characteristics that are associated with each stage of B-cell development.

The earliest stages of B-cell development are characterized by cell surface expression of CD34 and CD10 and expression of *recombination activating gene (RAG)* and *terminal deoxynucleotidyltransferase (Tdt)* and rearrangement of the D and J regions at the immunoglobulin locus. The next stages of human B-cell development are characterized by the expression of CD19 by pro-B cells and the later expression of CD20 by pre-B cells. During the pro-B-cell stage, RAG-mediated V region, D region, and J region rearrangement of the immunoglobulin heavy-chain locus is completed, and the pre-B-cell stage in human B-cell development is characterized by cell surface expression of the pre-B-cell receptor (BCR), which consists of the immunoglobulin μ heavy-chain and the surrogate light-chain composed of the vPreB and $\lambda 5$ proteins. A functioning pre-BCR is essential for further B-cell development⁷³ and the subsequent rearrangement of the immunoglobulin light-chain and its expression on the cell surface with the immunoglobulin μ heavy-chain as the BCR.

Autoreactive B cells are deleted or undergo a second round of immunoglobulin light-chain rearrangement (receptor editing) at the pre-B-cell to B-cell transition.^{69,70} B cells with functioning, nonautoreactive BCRs develop further in the bone marrow or in the periphery as immature or transitional B cells, which are characterized by increasing cell surface IgD expression, high CD38 expression, *CD5* expression, and continued but diminished expression of CD10 and CD24 (Fig 2). In human beings, mature naive B cells do not express CD10 but express CD23, IgD, and IgM and lower levels of CD38 than immature transitional B cells (Fig 2). Naive B cells are also characterized by the absence of *activation-induced cytidine deaminase (AID)*-mediated somatic mutations in their immunoglobulin genes.

The activation of naive B cells and their subsequent development in *germinal centers* within secondary lymphoid tissue are characterized by the acquisition of AID-mediated somatic mutations in their immunoglobulin genes, the expression of *CD27* as a marker for memory B-cell populations, downregulation of CD38 expression, and downregulation of IgD expression (Fig 2).⁷⁴⁻⁷⁷ Plasmablasts and long-lived *plasma cells* develop directly within germinal centers or from *memory B cells*.⁶⁸ A key feature of most circulating plasmablasts and long-lived plasma cells in the bone marrow is the absence of CD20 expression,⁷⁸ although CD20 appears to be expressed on a subset of human tonsillar plasma cells.⁷⁹

HUMAN STUDIES OF B CELL-DIRECTED THERAPIES IN AUTOIMMUNITY AND CORRELATES OF DISEASE RESPONSE AND RELAPSE

Clinical studies of B cell-directed therapies are beginning to illuminate the effects of these specific interventions on immune function through a variety of mechanistic approaches, including delineation of B-cell subsets by flow cytometry, measurement of serum autoantibodies and cytokines, and tests of immunocompetence. These studies are shedding light on the role of B cells in the pathophysiology of each autoimmune disorder and may ultimately identify biomarkers of clinical response and permit the design of specific therapies that target only disease-relevant B-cell populations. Most of the work has been done in RA, in which rituximab therapy in combination with methotrexate has been shown in large-scale clinical trials to produce a beneficial response in 50% to 70% of cases; only rarely does it lead to long-term clinical remissions.¹⁷⁻¹⁹

Much of the available information about the mechanisms of B-cell depletion has come from these rituximab trials, with some additional insights from results of smaller controlled and uncontrolled studies involving the use of rituximab and other B-cell-directed agents in patients with SLE and primary Sjögren syndrome. In human beings, administration of two 1000-mg doses of intravenous rituximab 2 weeks apart results in peak serum rituximab concentrations of 400 to 500 ug/mL.⁴ In patients with RA, the mean distribution time for rituximab is 2.4 days, and the half-life is approximately 20 days.^{4,80} After two 1000-mg doses, measurable serum concentrations of rituximab (1-10 ug/mL) can be detected even after 6 months.⁴ In non-Hodgkin lymphoma, the tumor burden and associated saturation of tumor CD20 with rituximab do not appear to influence significantly the pharmacokinetics of rituximab.⁸¹ However, there is an association between blood concentrations of rituximab and lymphoma response rates.⁸² There is a similar relationship between serum rituximab concentrations and the degree of B-cell depletion in patients with SLE.²⁰ In the latter study, there was a significant reduction in disease activity scores in subjects with SLE who depleted B cells to <5 cells/ μ L, whereas subjects who did not deplete B cells to <5 cells/ μ L did not experience significant reductions in SLE disease activity.

In mouse models, there is preferential depletion of peripheral blood naive B-cell populations after rituximab therapy and relatively less reduction in memory B cells and tissue B-cell populations such as *marginal zone* and peritoneal B1 cells.^{11,83-85} The preferential depletion of mouse naive B cells occurs despite equal saturation of all B cells with rituximab, including tissue B cells.^{11,84} In human beings, after administration of rituximab, there is a similar pattern of peripheral B-cell depletion. For

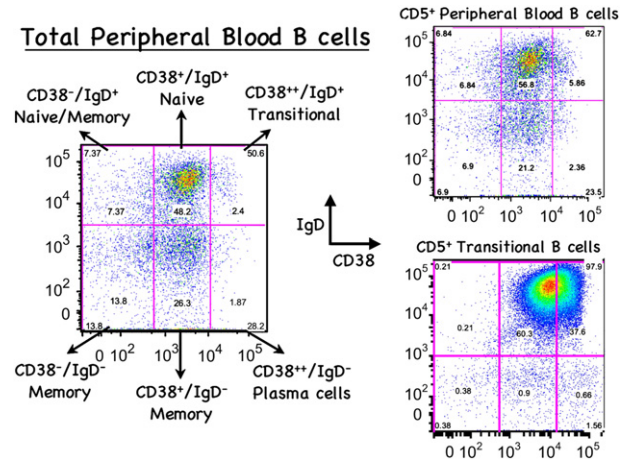


FIG 2. Peripheral blood B-cell subsets on the basis of cell surface expression of CD38 and IgD. Flow-cytometric analysis of CD19⁺ peripheral blood B cells on the basis of expression of CD38 (x-axis) and IgD (y-axis). The upper right flow cytometry plot displays peripheral blood CD5⁺ CD19⁺ B cells on the basis of expression of CD38 and IgD. CD5⁺ peripheral blood B cells from adults have a similar pattern of CD38 and IgD expression compared with CD5⁻ B cells, although CD5⁺ B cells have somewhat higher percentages of naive CD38^{mod}IgD⁺ B cells. For comparison, a plot of CD5⁺ transitional B cells (CD38^{hi}, IgM⁺, IgD⁺, CD10⁺) from a cord blood sample is shown in the lower right flow cytometry plot.

example, in patients with SLE and RA treated with rituximab, during the period of profound B-cell depletion (<5 B cells/ μ L), the highest percentage of the few remaining peripheral blood B cells have a phenotype (CD27^{+/++} and CD38^{+/- or hi}) consistent with memory B cells and plasmablasts.^{86,87} Studies of rearranged immunoglobulin genes from B cells during this phase of depletion are consistent with these findings and reveal high frequencies of highly mutated immunoglobulin sequences likely from memory B cells and/or plasmablasts.⁸⁸

Studies in human beings of the effects of rituximab on tissue B-cell populations have included studies of synovium, salivary gland, and tonsils. In subjects with RA, comparisons of synovial tissue before and 1 month after treatment with rituximab indicated that about half of the B cells in synovial tissue are depleted by rituximab treatment.⁸⁹ These same authors found that there was a significant reduction in the numbers of plasma cells in synovial tissue in patients who responded to rituximab as opposed to non-responders.⁹⁰ In 2 studies of patients with primary Sjögren syndrome, salivary gland biopsies before and 3 months after rituximab administration revealed nearly complete depletion of tissue B cells; plasma cells were not depleted.^{30,91} In another study involving patients with primary Sjögren syndrome, Pers et al⁹² also found that salivary gland B cells are depleted after rituximab administration. In this study, salivary gland B-cell repopulation began at approximately 12 months, and salivary gland B-cell numbers returned to pretreatment levels after approximately 24 months. The B cells that repopulated the salivary glands after rituximab treatment were CD5⁺ (approximately 1/3 of salivary gland B cells), and many were CD27⁺ memory B cells.⁹² In another study, tonsil specimens from patients with SLE showed long-term (5 years) alterations in B-cell populations after rituximab therapy that paralleled the changes in peripheral blood B-cell populations.⁹³

Most of the observed changes in peripheral blood have focused on depletion and reconstitution of B cells and their subsets.

However, a small population of CD20-expressing T cells is depleted after rituximab treatment,⁸⁷ although total T-cell numbers and the major CD4 and CD8 subsets change little after rituximab treatment.^{62,87,94} The identity of this CD20⁺ T-cell population has not been further clarified. After rituximab treatment, T-cell proliferation does not decline in response to *phytohemagglutinin* or allogeneic stimulation,⁹⁵ and CD8⁺ EBV-specific T-cell immunity is maintained.⁹⁶ However, 1 study has found increased numbers of peripheral *T-regulatory (Treg) cells* in patients with SLE who improve after rituximab therapy.⁹⁴ This finding is of interest because peripheral Treg numbers have been shown in some studies to be low in patients with SLE with active disease.⁹⁴ In this study, the increases in peripheral blood Treg cells were associated with lower overall levels of CD40L⁺CD4⁺ T cells. These results are consistent with a report from another group that showed decreased expression of CD40L, CD69, and *inducible costimulator (iCOS)* on T cells from patients with SLE 1 to 3 months after rituximab therapy.⁹⁷ More recent work indicates that rituximab therapy is associated with increased expression of peripheral blood forkhead box P3 (FoxP3) and CD25 mRNA expression and decreased expression of peripheral blood CD40L mRNA expression.⁹⁸ On the basis of these data, it has been suggested that Treg numbers are increased after rituximab treatment, but their activation state is low. Consistent with this line of reasoning is the finding that TGF- β mRNA is increased in PBMCs from patients with SLE after rituximab treatment.⁹⁸ In contrast with the studies of Treg numbers in patients with SLE, no differences in T-cell or Treg numbers have been observed in patients with RA after rituximab therapy.⁸⁷

Several studies have shown that rituximab therapy does not significantly reduce serum concentrations of total IgG, IgM, or IgA in patients with RA and SLE.^{17,18,59,62,94} However, repeated rituximab treatments of patients with RA may result in reductions in total IgG and IgM levels below the normal range,⁹⁹ although more studies are needed to determine the incidence of hypogammaglobulinemia after repeated cycles of B-cell depletion. Serum immunoglobulin levels and infection rates were recently summarized in a study of patients with RA who had been treated with rituximab in clinical trials and had entered open-label extensions and received further courses of rituximab. The incidence of serious infections remained stable after 4 courses of rituximab therapy, with rates after courses 1, 2, 3, and 4 of 5.36, 4.60, 6.34, and 5.41 events per 100 patient-years, respectively.¹⁰⁰ Among the 1053 patients in this analysis, 761 (72%) consistently had normal IgM and IgG levels. There was at least 1 low serum IgM and IgG level in 261 (25%) and 67 (6%) of the patients, respectively. By 24 weeks after courses 1 to 4 of rituximab, 1.4%, 3.5%, 4.0%, and 4.3% had at least 1 IgG level below the lower limit of normal. In the group with at least 1 IgG level below the lower limit of normal, the rate of serious infections was 6.8 events per 100 patient-years (CI, 4.03-11.49), which compared with an overall rate of 4.9 events per 100 patient-years (CI, 3.93-6.06). Although a trend of a higher rate of serious infection was observed in the group with at least 1 IgG level below the lower limit of normal, it was not statistically significant because of the limited exposure and wide CIs. No reports have described the use of intravenous immune globulin in this population. There are also no significant changes in the serum concentrations of antibodies to common recall antigens such as tetanus toxoid.^{59,61,62} However, antibody responses to new antigens (neoantigens) such as bacteriophage

phiX174 may be diminished during the period of B-cell depletion.^{101,102} In addition, baboons pretreated with rituximab have shown a significant decrease in responsiveness to primary and secondary immunizations with keyhole limpet hemocyanin and fail to switch from IgM to IgG production of antikeyhole limpet hemocyanin antibodies.¹⁰² There are currently no specific recommendations regarding monitoring of immunoglobulin levels in patients treated with repeated courses of rituximab.

For some diseases, studies of autoantibody levels before and after rituximab therapy have shown correlations between autoantibody levels and disease improvement and relapse. For example, as described, serum levels of antibodies that bind desmoglein in rituximab-treated patients with pemphigus correlate with treatment response.^{25,26} There are similar correlations between ANCA titers and disease remission and relapse in proteinase 3-associated vasculitis.¹⁰³ However, the correlations between autoantibody levels and disease activity are not as clear in disorders such as RA and SLE. For example, although declines in RF levels are variably associated with symptom improvement in patients with RA treated with rituximab, anti-CCP antibody levels change only modestly after rituximab therapy.^{17,19,59,61} The pathogenic role of RF and anti-CCP remains unclear because serum levels of these autoantibodies do not often correlate with disease activity.^{104,105} In patients with SLE, treatment with rituximab has been associated with declines in anti-dsDNA antibody levels in most^{21,22,61,62,86,94,106} but not all studies.^{20,23,107} However, rituximab therapy has not produced any changes in other antinuclear autoantibody levels such as anti-Ro and anti-Sm.^{61,62} Ng et al¹⁰⁸ did observe that patients with SLE with low baseline serum C3 concentrations and detectable anti-Sm, anti-RNP, anti-Ro, and/or anti-La antibodies before rituximab therapy had a greater likelihood of disease flare on reconstitution of peripheral B cells.

Serum *B-cell activating factor belonging to the TNF family (BAFF)* levels have been measured in patients with autoimmune diseases before and after rituximab therapy. 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 human beings.¹⁰⁹⁻¹¹¹ After 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.¹¹²⁻¹¹⁵ The augmented BAFF levels after B-cell depletion may contribute to the return of self-reactive B cells, because excessive BAFF has been shown to rescue self-reactive B cells from apoptosis.^{109,116,117}

Other B cell-targeted therapy may influence B-cell function and lead to clinical improvement of autoimmune disease. In patients with SLE, anti-BAFF (belimumab) therapy 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.¹¹⁸ In a similar way, cyanomolgous monkeys administered belimumab every 2 weeks evidenced reduced total B-cell numbers and decreased numbers and size of splenic lymphoid follicles.¹¹⁹ Chronic administration of belimumab did not lead to changes in total serum IgG and IgM concentrations.¹¹⁹ Further trials of BAFF antagonists are in progress to determine the potential clinical efficacy of this class of agents, and studies are being considered in which B cell-depleting antibodies such as rituximab might be combined with a BAFF antagonist to produce more sustained B-cell depletion and block return of self-reactive B cells.

Several studies have examined the relationships between circulating B-cell subpopulations and disease response and relapse. After rituximab therapy, peripheral blood B cells begin to return after 6 to 9 months. B-cell recovery after rituximab therapy is associated with increasing numbers of CD5⁺ and CD38⁺ B cells that have a transitional B-cell phenotype (IgD⁺, IgM⁺, CD38^{hi}, CD27⁻, CD5⁺, CD10⁺, and CD24⁺)^{62,87,92,120,121} that is associated with an absence of somatic mutations in the rearranged immunoglobulin genes of these transitional B cells.¹²¹ B-cell recovery after rituximab therapy is also associated with increased numbers of B cells with a plasmablast phenotype (CD20⁻, IgD⁻, CD27^{hi}, CD38^{hi}). The absolute numbers of peripheral blood memory B cells typically remain low for at least 1 year after rituximab therapy but gradually recover over time.¹²¹ B-cell repopulation after rituximab therapy for lymphoma is associated with reduced B-cell expression of CD40 and CD80 and reduced numbers of CD27⁺ memory B cells after repopulation.^{97,122} Anolik et al⁸⁶ found that peripheral blood B-cell abnormalities of patients with SLE (naive B-cell lymphopenia, increased memory B cells, and increased plasmablasts) were corrected after rituximab treatment, but it was unclear whether these changes were correlated with clinical parameters.

Leandro et al⁸⁷ reported in rituximab-treated patients with RA that disease relapse was associated with a higher frequency of B cells that had a memory phenotype at the time of B-cell repopulation. In this study, patients with greater than 3×10^6 /L CD27⁺ memory B cells at the time of B-cell repopulation had an earlier relapse than patients with fewer B cells of this phenotype.⁸⁷ These authors also reported that patients with RA treated with rituximab who had a higher proportion of mature B-cell precursors in their bone marrow at 3 months tended to relapse at the time of B-cell repopulation, although interpretation of these results is limited by the small numbers of patients analyzed.¹²³ Similar findings have been reported in long-term studies of patients with SLE treated with rituximab.⁹³ In subjects with the best clinical responses, there were long-term reductions in peripheral blood and tonsil memory B-cell populations and correspondingly high levels of peripheral blood transitional B-cell populations. Treatment responders also had low baseline levels of anti-dsDNA and undetectable levels of autoantibodies to Ro, La, Sm, and RNP.⁹³

CONCLUSION 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 done to understand the mechanism of action of these drugs. Recent studies correlating the clinical effectiveness of rituximab in patients with RA and SLE with depletion of memory B cells^{87,93} need to be confirmed in larger cohorts of patients and need to be studied in other autoimmune disorders. It seems unlikely that memory B cells are the only key target of depleting antibodies; therefore, changes in other B-cell populations such as regulatory B cells may be an important consequence of this treatment approach.

We acknowledge the stimulating exchange of ideas and discussions about B cells and B cell–directed therapies with our colleagues Thomas Tedder, Garnett Kelsoe, Russell Hall, and David Pisetsky at Duke, as well as other members of the Autoimmunity Centers of Excellence.

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