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Pathogenic Roles of B Cells in Human Autoimmunity: Insights from the Clinic

Review

Flavius Martin[1,*] and Andrew C. Chan[1,2,*]
[1]Department of Immunology
[2]Department of Antibody Engineering
Genentech, Inc.
One DNA Way, MS-34
South San Francisco, California 94080

The pathogenic roles of B cells in human autoimmune diseases involve a multitude of mechanistic pathways and include the well-established contributions of autoantibodies and immune complexes that induce local inflammatory reactions and tissue destruction. Recent results using several novel B cell-directed therapies have provided new insights into additional roles of B cells in human autoimmunity. In this review, we will highlight some of these studies and discuss how clinical insights parallel murine models of normal immunity and autoimmunity.

Treatment of autoimmune disorders has begun a transformation from an armamentarium of nonspecific therapies that affect a wide range of cells and signaling pathways to targeted therapies that modulate specific cytokines (e.g., tumor necrosis factor and IL-1), cell types (e.g., $CD3^+$ T cells and $CD20^+$ B cells), or inhibit a unique signaling pathway (Kamradt and Mitchison, 2001; Davidson and Diamond, 2001; Keystone, 2003; Gottlieb, 2003; Shanahan et al., 2003). The advent of targeted therapies in the treatment of human autoimmune diseases has not only significantly improved patients' lives and changed clinical therapeutic paradigms but has also led a renaissance in understanding pathogenesis of human disorders (Anasetti et al., 1992; Weinblatt et al., 1999; Lipsky et al., 2000; Pisetsky, 2000b; Cohen et al., 2002; Kremer et al., 2003; Keystone et al., 2003; Edwards et al., 2004). Our insights into the pathogenic roles of B cell dysfunction in human autoimmune diseases circa 2004 remain limited. While prophylactic or interventional preclinical studies in rodent models of human diseases are enlightening, many of these genetically homogeneous models do not translate well into the heterogeneous nature of human biology and diseases. Hence, a challenge to drug discovery as well as human immunology is to understand how probing B cell biology in human autoimmunity translates back to these preclinical models of human disease and then applying this new knowledge to advance patient care. In this discussion, we will review how therapies in human autoimmune diseases have provided insights into our expanding understanding of B cell functions in human autoimmune disorders (Figure 1).

The scientific routes of B cell modulation began with the elegant surgical approaches in birds and underscored the importance of the Bursa of Fabricius in B cell ontogeny (Cooper et al., 1965). This was followed by pioneering work using anti-immunoglobulin (Ig) reagents in which pre- and perinatal treatment of mice with anti-Ig polyclonal reagents successfully depleted surface positive B lineage cells (Cooper et al., 1980). Such a strategy was not applicable to adult animals that have high levels of circulating Igs that function as a sink for the depleting anti-Ig reagents. Attempts to translate these observations into clinical use for B cell lymphomas focused initially around the idea of anti-idiotype (Id) antibodies passively delivered or induced through active immunization (anti-Id vaccines) (McCarthy et al., 2003). Although this approach seemed ideal from a scientific perspective, the preexisting suppressed immune systems of cancer patients, phenomenon of tumor escape, as well as manufacturing related factors have delayed the translation of these therapies to clinical practice. The alternative approach of using antibodies directed against pan-B cell targets was faster to reach fruition through the development of antibodies targeting the CD20, CD22, and CD52 surface molecules (Treon and Anderson, 2000; Juweid, 2003; Cersosimo, 2003; Robak, 2004). All these approaches rely on the ability of passively infused antibodies to deplete antigen-bearing cells. This depletion is temporary and ceases through the continuous repopulation of the B lineage from precursors residing within primary hematopoietic organs.

In contrast to the B cell malignancies, non-Hodgkin's lymphoma, and chronic lymphocytic leukemia, in which one or more proliferating B cell clones can be identified at a cellular and molecular level, the identity and specificity of the pathogenic B cell clones in autoimmune diseases are rarely known. In a subset of lymphoma and leukemia patients, the malignant B cell clones produce low-affinity paraproteins (antibodies) that manifest autoimmune paraneoplastic syndromes. These paraproteins bind a variety of self-antigens, including those expressed on red blood cells (RBCs) that result in autoimmune hemolytic anemia (e.g., IgM cold agglutinin disease), or C1 esterase complement inhibitor that manifests clinically with angioedema (Silberstein, 1994; Pascual et al., 1997; Ward, 2001). Treatment of the underlying lymphoma or leukemia and eradication of the malignant B cell clone improves the associated clinical autoimmune manifestations. For most autoimmune diseases, however, the mechanistic relationship between pathogenic B cells, their products (e.g., autoantibodies), and clinical manifestations remains elusive. Nonetheless, the emerging findings suggest that B lineage depletion or modulation might be beneficial and provides a basis to further explore the role of B lineage function in human biology.

Autoantibodies—Diagnosis and Pathogenesis

Autoantibodies have served historically as the sine qua non to indicate a role for B cells in autoimmune diseases. They provide diagnostic and prognostic criteria, play a requisite role in disease pathogenesis, and serve as surrogate markers for disease activity.

The first category, while not mutually exclusive with the others, represents a large proportion of autoantibodies and typically is not thought to play a role in disease

*Correspondence: flavius@gene.com (F.M.); acc@gene.com (A.C.C.)

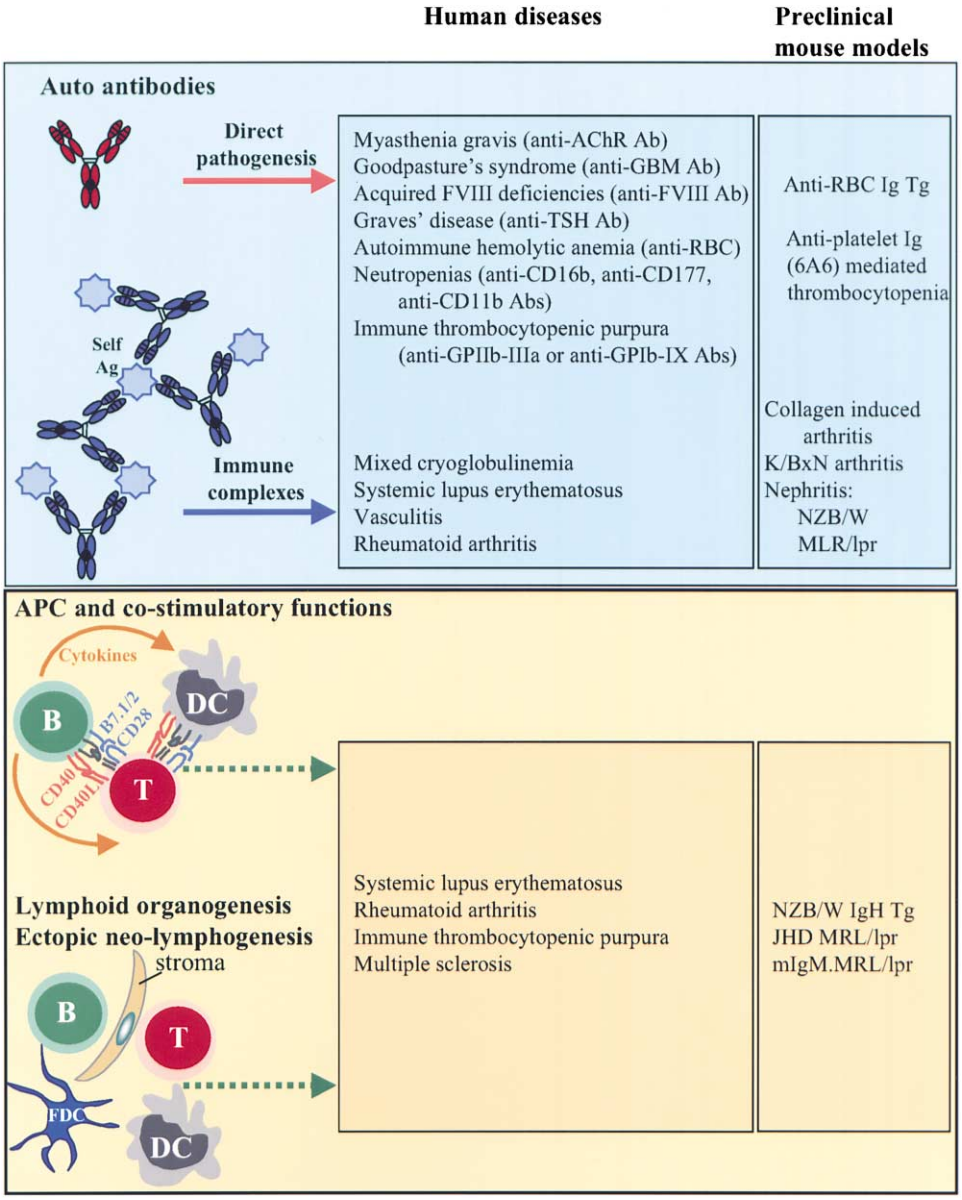

Figure 1. Effector Mechanisms of Human and Mouse B Cell Autoimmunity

"Classic" antibody-dependent mechanisms are responsible either directly or through immune complexes for the pathogenesis of specific autoimmune diseases. Several other mechanisms, including antigen presentation (APC), costimulatory functions (surface molecules and secreted cytokines), and the ability to support de novo lymphoid tissue organization (neolymphogenesis) may explain other parts of B cell involvement in complex autoimmune conditions.

AchR, acetylcholine receptor; GMB, glomerular basement membrane; FVIII, factor VIII; TSH, thyroid-stimulating hormone; RBC, red blood cells; GPIIb-IIIa, glycoprotein IIb-IIIa; GPIb-IX, glycoprotein Ib-IX; Ag, antigen; B, B cell; T, T cell; FDC, follicular dendritic cell; DC, dendritic cell.

pathogenesis. However, the latter may merely reflect our lack of understanding of antigen specificity and its role in disease pathogenesis. Nonetheless, many antibodies provide highly sensitive and/or specific diagnostic criteria for the presence of a specific clinical syndrome. For example, the presence of anti-nuclear antibodies (ANA) serves as an extremely sensitive diagnostic test for systemic lupus erythematosus (SLE). This autoimmune disorder is characterized by T, B, and myeloid cell dysregulation that gives rise to immune complex (IC)-mediated deposition and inflammatory damage in a variety of organs, including skin, lung, kidney, blood vessels, and the nervous system (Pisetsky, 2000a). However, this serologic marker is observed in a variety of other autoimmune disorders, and the level of serum ANAs does not correlate with the severity of clinical disease. Patients can have persistently high ANA titers despite the absence of clinical disease. Conversely, patients experience disease flares without any alterations in ANA levels. Other types of autoantibodies in SLE patients can also be used to aid diagnosis and prognostication of patient subsets—the presence of

anti-Sm antibodies is highly specific for SLE diagnosis, anti-RNP antibodies identify a subset of patients with a different clinical course of mixed connective tissue disease, anti-phospholipid antibodies are associated with an increased risk of thrombotic events, anti-ribosomal P antibodies identify patients with increased risk of central nervous system disease, and anti-Ro (SSA) antibodies are causally linked with an increased risk for the development of complete heart block (CHB) in the newborns of SLE patients (Ruiz-Irastorza et al., 2002; Reichlin, 2003; Buyon and Clancy, 2003). In the case of anti-Ro antibodies, only 50% of women have symptomatic SLE or Sjogren's syndrome at the time their babies are born with CHB. Yet, a large proportion of these asymptomatic women ultimately develop clinical SLE and Sjogren's disease over the ensuing decade (Brucato et al., 2002).

Aside from rheumatologic disorders, examples of pathogenic autoantibodies include the anti-acetylcholine receptor (AChR) and anti-glomerular basement membrane (GBM) antibodies in myasthenia gravis and Goodpasture's syndrome, respectively. Anti-AChR antibodies alter AChR organization on the postsynaptic neuronal plate and functionally block acetylcholine neurotransmission to induce muscle paralysis, in particular impairment of diaphragmatic function, with progression to respiratory failure and death (Vincent, 2002). The histological demonstration of these complement fixing antibodies on myelinated fibers from affected patients and ability of plasma exchange and intravenous immunoglobulin (IVIG) to improve clinical course together support a pathogenic role for anti-AChR antibodies in MG (Richman and Agius, 2003). Binding of anti-GBM antibodies to the α3 chain of type IV collagen in the lung and kidneys results in pulmonary hemorrhage and glomerulonephritis, respectively, the latter of which can progress to renal failure. Suppression and removal of these pathogenic antibodies with immunosuppressive agents and plasma exchange guide the therapeutic principles of anti-GBM disease (Hudson et al., 2003). Hence, both of these pathogenic antibodies support a definitive role for B lineage cells in human disease.

Immune Complexes, Complement, and Fc Receptors in Disease Pathogenesis

The contributions of antibodies to disease are not solely dependent upon their direct binding to end-organ tissue antigens but also through indirect mechanisms, including IC formation, IC deposition, complement activation, and FcR activation. These complement-activating ICs are found in many autoimmune disorders and deposit in a variety of tissues (e.g., kidney, lung, and blood vessels) to cause end-organ damage (e.g., kidney failure, pulmonary hemorrhage, and vasculitis, respectively). A classical example of an IC-mediated disease is the Hepatitis C-associated syndrome of mixed cryoglobulinemia, in which complement activating IgG and IgM ICs (containing Hepatitis C antigen) are deposited in the skin, kidney, and peripheral nerves to induce cutaneous purpuric lesions, glomerulonephritis, and mononeuritis multiplex, respectively (Trendelenburg and Schifferli, 2003). Treatment is dependent upon the extent of involvement but traditionally geared toward decreasing cryoglobulin production through the use of immunosuppressive therapies and, in severe cases, removal of the pathogenic complexes through plasma exchange.

A role for ICs in autoimmune disorders is also supported by the genetic association of Fc receptor (FcR) polymorphisms and human disease. FcRs for IgG include the high-affinity FcγRI (CD64) receptors and the low-affinity FcγRII (CD32) and FcγRIII (CD16) receptors. Genetic polymorphisms for both low-affinity IgG receptors have been described (Ravetch and Bolland, 2001; Binstadt et al., 2003). The major FcγRIIA polymorphism resides at amino acid 131. FcγRIIA(His131) confers higher affinity for IC binding than FcγRIIA(Arg131). His131/His131 phagocytes are more efficient in IC phagocytosis and clearance than cells bearing the Arg131/Arg131 low-affinity receptor allelic genotype. The major FcγRIIIA polymorphism resides at amino acid 158. Val158 confers high-affinity binding while Phe158 confers low-affinity binding. Patients expressing two copies of FcγRIIA(Arg131/Arg131) or IIIA (Phe158/Phe158) receptors have been reported in some studies but not in others to exhibit increased association with a number of autoimmune diseases, including immune-mediated thrombocytopenia (ITP), SLE, and multiple sclerosis (MS). These polymorphic differences may result not only in prolonged IC circulation, deposition, and tissue damage but may enhance inflammatory and proliferative responses of FcR-bearing cells in disease.

The ability of ICs to activate the complement cascade and liberate proinflammatory complement degradation components that enhance IC clearance, augment phagocyte activation, and modulate cellular and humoral immunity provide additional effector mechanisms by which B cell products can contribute to disease (Holers, 2003; Walport, 2001a, 2001b). In preclinical models, inhibition of complement activation, using soluble forms of complement receptors or antibodies that bind complement degradation products, have demonstrated efficacy in preclinical models of ischemia-induced injury, lupus, and collagen-induced arthritis (Weisman et al., 1990; Couser et al., 1995; Wang et al., 1995, 1996). Translation of some of these principles to the clinics are in progress and will provide us with a greater understanding of these effector pathways in autoimmune disease. A phase II study of the anti-C5 inhibitor antibody eculizumab (5G1.1, Alexion Pharmaceuticals, Cheshire, CT) that prevents the generation of the C5a anaphylatoxin has demonstrated short-term safety and tolerability in patients with rheumatoid arthritis (RA) and clinical benefit compared to control patients (Tesser et al., 2001).

The therapeutic efficacy of IVIG in a limited subset of autoimmune diseases, including ITP and MG, also suggests roles for the contribution of antibodies, complement, and FcRs in B cell-mediated autoimmunity. ITP is a clinical syndrome manifested by immune-mediated destruction of platelets (Beardsley, 2002). These patients demonstrate high levels of autoantibodies directed against platelet surface glycoproteins that are associated with accelerated platelet destruction (McMillan et al., 2001; McMillan, 2003). IVIG is manufactured from pooled human plasma, and its therapeutic effects in ITP involve a multitude of mechanisms that include Fc blockade to decrease clearance of antibody-laden platelets, inhibition of complement deposition, and more

recently evidence for engagement of inhibitory FcγRIIB receptors. In a murine model of autoantibody-induced ITP, the therapeutic effect of IVIG was dependent upon the induced expression of the inhibitory FcγRIIB receptor on splenic macrophages that, in turn, inhibits Fc-mediated opsonization of antibody-bound platelets (Samuelsson et al., 2001). Similarly, the protective effects of IVIG in the K/BxN arthritis model are dependent on the ability of CSF-1-dependent macrophages to upregulate FcγRIIb expression on CSF-1-independent "effector" macrophages (Bruhns et al., 2003). Hence, IVIG may function, in part, by inhibiting effector responses through this inhibitory receptor. Independent of mechanism, the limited therapeutic benefits of IVIG in specific autoimmune disorders support a pathogenic role for antibodies and ICs in these diseases.

The therapeutic application of plasma exchange has provided additional support for the role of antibodies and ICs to implicate B lineage cells in disease (Saydain et al., 2002). Therapeutic apheresis is a technique by which plasma and cellular components are separated and autoantibodies, ICs, or toxins are removed from the plasma. This technique has been modified to include protein A immunoadsorption to specifically extract pathogenic autoantibodies and ICs. While plasma exchange has been used in a wide number of autoimmune diseases, its therapeutic application is accepted as standard of care in only a handful of autoimmune diseases, including anti-GBM disease and MG, and adjunctive therapy in cryoglobulinemia and ITP. Nonetheless, modulation of circulating ICs and pathogenic antibodies by simple removal has proved effective in some of these B lineage-mediated diseases.

B Cell Depletion

The development of therapeutics that specifically target B cells during the past decade has provided an entrée to better translate preclinical observations into human biology and disease. These therapeutics have been led by the use of rituximab (Rituxan®, Genentech, Inc., South San Francisco, CA and Biogen-IDEC, Cambridge, MA; Mabthera®, F.Hoffman-LaRoche, Ltd., Basel, Switzerland), a chimeric monoclonal antibody directed against the CD20 molecule. CD20 is a transmembrane glycoprotein expressed exclusively on normal and malignant B cells. Its expression is regulated during B cell development emerging in late pre-B cells and extinguished on plasma cells. Given the prominent historical role of autoantibodies, the absence of CD20 on plasma cells that presumably are responsible for producing autoantibodies raised fundamental skepticism in applying this therapy to autoimmune diseases. Yet, the efficacious use of Rituxan in a number of autoimmune disorders has brought into question some of the preconceptions of the biology of B lineage cells in autoimmune disorders. Ideally, one would design a B cell-depleting therapy for autoimmunity to meet several criteria: (1) deplete all or most pathogenic B lineage cells or their precursors (assuming that pathogenic cells and their products have a reasonably short lifespan), (2) pathogenic B cell repopulation should not occur or should be significantly delayed upon repletion from early bone marrow progenitors, (3) interrupt or influence non-B cell effector pathogenic pathways that are dependent or downstream of B cell-initiated processes. To date, clinical results exist to support the first two criteria while the third awaits more formal scientific validation.

Clinical data for Rituxan in autoimmune diseases is emerging from a variety of double-blinded placebo-controlled trials though a large amount of data has already emerged from smaller Phase I/II studies and case series reports (Edwards et al., 2002; Silverman and Weisman, 2003). The use of Rituxan in ITP led its entrée into the realm of autoimmune diseases. In four case series reports, 33%–54% of ITP patients, who had failed standard of care corticosteroid and splenectomy therapies, experienced partial or complete responses to Rituxan, in most cases in the absence of concomitant corticosteroids (Saleh et al., 2000; Stasi et al., 2001; Zaja et al., 2003; Cooper et al., 2004). Interestingly, two patterns of clinical responses emerged. Early responders experienced immediate increases in platelet counts after the first or second antibody infusion that were followed by a continued rise until a peak count was achieved between weeks 6 and 10. In contrast, late responders had minimal immediate effects. Rather, they had a delayed effect in which peak counts were achieved within the 6 to 8 week period. These distinguishing patterns suggest that Rituxan may operate through at least two distinct mechanisms in ITP. In the first, the effects of Rituxan are too rapid to be accounted for by depletion of circulating antiplatelet antibodies but potentially could be expanded through Fc receptor-mediated functions. In the latter phase, the sustained effects may function through alterations in anti-platelet antibodies and/or interruption of T-B cell cooperation or other B cell-dependent events in disease pathogenesis. To date, these small clinical studies reveal no direct correlation of anti-platelet antibody levels with clinical responses and raise the likelihood of additional mechanisms by which B lineage cell depletion modulates ITP (see below).

The use of Rituxan in autoimmune disorders is most advanced in the treatment of patients with rheumatoid arthritis (RA). This disease involves a multicellular inflammatory response involving infiltration of lymphocytes and granulocytes, proliferation of synovial fibroblast and macrophages, and neovascularization of the lining surrounding joints. This proliferative process not only induces swelling, erythema, and pain of multiple joints but progress to destruction and loss of bone density and architecture. The diagnosis of RA is aided typically by the presence of low-affinity IgM rheumatoid factor (RF) autoantibodies directed against the Fc portion of IgG. In a multicenter randomized double blind controlled study of 161 patients, those treated with Rituxan and a short course of corticosteroids in conjunction with methotrexate or cyclophosphamide achieved greater clinical responses than patients treated with corticosteroids and methotrexate alone (Shaw et al., 2003; Edwards et al., 2004). Analysis of serologic changes in a smaller series of 22 RA patients demonstrated a 60% decrease in IgM RF and 75% decrease in anti-cyclic citrullinated peptide (CCP) antibodies in Rituxan responders (Cambridge et al., 2003). Despite the longer half-life of IgG, when compared to IgM, IgG RF decreased more rapidly and to a greater extent (80%) than IgM RFs (60%) following Rituxan therapy. In contrast, no

change in anti-pneumococcal capsular polysaccharide IgG antibodies and a 23% decrease in anti-tetanus toxoid IgG antibodies were detected 3 months following treatment. Similar serologic effects have been observed in two additional studies with Rituxan treatment (De Vita et al., 2002; Tuscano, 2002). This preferential decrease in autoantibodies suggests that autoreactive B cells and their corresponding plasma cells may have a shorter lifespan while non-self-reactive plasma cells are relatively more resistant to Rituxan and have a longer lifespan.

Since RF is not thought to play a direct pathogenic role in rheumatoid arthritis, as transfer of RF-positive serum in the *skg* model of RA is not sufficient to confer disease (Sakaguchi et al., 2003), the therapeutic efficacy of Rituxan reported in these studies identifies B lineage cells as playing a key, but undefined, pathogenic role in RA. B cells exist as lymphoid aggregates within the synovium of RA patients and may function as APCs to provide important costimulatory signals in promoting effector T cell expansion (Weyand and Goronzy, 2003). Addition of antibodies that block T and B cell cooperation results in loss of these T-B cell aggregates (Takemura et al., 2001). Hence, B cell depletion may remove a critical component of T-B cell cooperation required for disease. While RF alone is not sufficient to induce disease, RF-containing ICs may promote synoviocytes to induce local release of inflammatory factors and exacerbate the inflammatory cascade. Conversely, decreases in RF and other autoantibodies may attenuate these local inflammatory ICs and result in clinical improvement. Additional clinical studies of synovial B cells prior to and following Ritxuan therapy will be revealing in dissecting the specific mechanistic roles of systemic and local (e.g., synovial) B cells in RA and the effects of Rituxan on RA pathogenesis.

Longer term follow-up analysis of a handful of Rituxan-treated RA patients have been reported and reveal that most undergo clinical relapse. In the study reported by Cambridge and colleagues, 13 of 15 responders relapsed within 17 months following B cell repletion. In all but one patient, return of autoantibodies preceded relapse. Return of autoreactive antibodies, through their corresponding plasma cells and precursors, may occur through a stochastic recapitulation of pathogenic events involving a new set of naive B cells freshly generated from the bone marrow. Supporting this scenario are data on subset composition for repopulating blood cells as well as their VH gene repertoire assayed by single cell PCR (Rouziere et al., 2003). Peripheral B cells from patients with moderate to severe RA have a large component of isotype-switched memory cells with heavily somatically mutated VH genes. During and immediately following B cell repletion, the repertoire is dominated by phenotypically naive cells with germline VH regions. Preceding reemergence of autoantibodies and clinical relapse, the peripheral B cell repertoire begins reaccumulating mutations and slowly converges to resemble the predepletion repertoire. Together, these early studies suggest that pathogenic B cell clones and their progeny can be modulated by Rituxan therapy and that autoreactive and allogeneic responses likely have distinct kinetics of longevity and biology.

Recent studies of four patients with circulatory inhibitors against Factor VIII, an important component of the coagulation cascade, also demonstrate differences between self and non-self antibodies. These patients have circulating FVIII inhibitors (anti-FVIII autoantibodies), despite immunosuppression with corticosteroids and chemotherapeutic agents, which functionally compromise their coagulation cascade, that result in bleeding complications requiring RBC transfusions. Treatment with Rituxan resulted in a rapid decrease in their acquired FVIII inhibitor and return of their endogenous FVIII levels (Wiestner et al., 2002). Interestingly, one of these four patients had mild hemophilia A and received recombinant human FVIII (rFVIII) perioperatively, which appears to have triggered an increase in FVIII inhibitor activity and further compromised his already low FVIIIc level. Administration of Rituxan in this patient resulted in rapid resolution of the autoantibody (against endogenous FVIII) whereas the alloantibody (anti-rFVIII) response persisted for months. Hence, similar to the fate of serum RF, the biologic effects of these autoreactive B cells appear to be far shorter lived than non-self-reactive B cells.

In addition to ITP, RA, and FVIII deficiency, therapeutic effects of Rituxan have been reported in small case series of patients with pemfigus vulgaris (anti-desmoglein-3 antibodies) and pemfigus foliaceus (anti-desmoglein-1 antibodies) (Salopek et al., 2002; Goebeler et al., 2003; Dupuy et al., 2004). In addition to clinical benefit, pathogenic antibody titers decrease, but do not disappear, following Rituxan administration and return at relapse. In at least one patient, clinical relapse and autoantibody increase was documented in the complete absence of peripheral B cells, suggesting a complex relationship between B cells, autoantibodies, and clinical effect. Patients with MG have similarly demonstrated decreases in anti-AChR antibodies, which is accompanied by a slow clinical improvement (Wylam et al., 2003). Finally, patients with IgM-associated polyneuropathies with anti-MAG (myelin-associated glycoprotein) autoantibodies have also been reported to have decreased anti-MAG titers and clinical improvement following Rituxan administration (Levine and Pestronk, 1999; Pestronk et al., 2003). In contrast, two patients with antiganglioside IgG antibodies did not improve even up to 1 year following depletion (Rojas-Garcia et al., 2003). This dichotomy of responsiveness does not simply reflect differences between IgM and IgG autoantibodies since other IgG autoantibodies are modulated by Rituxan therapy.

The effects of Rituxan on autoreactive and non-self-reactive antibodies have only recently been studied in SLE patients. Three open label studies have been reported (Leandro et al., 2002; Anolik et al., 2003; Eisenberg, 2003). While the overall efficacy is unknown at this time, the immunologic parameters in these studies demonstrate a further disconnect between B cell depletion and reduction of anti-dsDNA antibodies. Interestingly, these patients have a large percentage of circulating plasmablasts ($CD27^{++}CD20^{-}$), memory ($CD27^{+}CD20^{+}$), and/or germinal center ($CD38^{+}CD20^{+}$) B cells. Analysis of the effects of Rituxan on the survival of these B cell subsets will likely provide greater insights into the half-life and survival factors that regulate these germinal center (GC)-derived cells.

B cell differentiation into Ig-secreting plasma cells has been elegantly explored in humans through the investigation of the CD40/CD40L axis (Arpin et al., 1995). GCs represents a major site to permit crosstalk of antigen-activated T, B, and follicular dendritic cells to promote T and B cell expansion and effector functions. In particular, the CD40/CD40L axis is critical within these GC processes for appropriate generation of memory B cells and long-lived $CD38^{++}CD27^{++}$ antibody-forming cells (plasmacytes). Blockade of the CD40/CD40L axis inhibits activation-induced cytidine deaminase-mediated somatic hypermutation, Ig class switch, GC formation, and maintenance and the generation of long-term B cell memory. Patients with inherited mutations of CD40L are unable to undergo Ig class switch and develop elevated levels of IgM but reduced levels of serum IgG and IgA.

Selective patients with active SLE have significantly elevated numbers of circulating $CD38^{+}IgD^{+}$ preswitch GC cells, $CD38^{+}IgD^{-}$ postswitch GC cells, $CD38^{-}CD27^{+}$ memory cells, and $CD38^{++}$ plasma cells. Some of these cells have variable levels of inappropriate CD40L expression and in vitro can undergo spontaneous proliferation and Ig secretion that is blocked with an anti-CD40L mAb (Grammer et al., 2003; Huang et al., 2002). Treatment of SLE patients with an anti-CD40L antibody (BG9588, 5c8, Biogen-IDEC, Inc.) resulted in substantial decreases in anti-ds DNA antibody, proteinuria, and improved clinical SLEDAI score (a conglomerate measure of clinical and laboratory parameters relevant to the severity of SLE disease). Correspondingly, decreases in $CD38^{+}IgD^{+}$ preswitch GC cells and $CD38^{++}$ plasmacytes with less dramatic effects on $CD38^{+}IgD^{-}$ postswitch GC cells were observed in treated patients. Hence, CD40/CD40L blockade may operate at multiple levels, including inhibiting GC initiation, differentiation of GC cells to $CD38^{++}$ plasmacytes, and interfere with GC maintenance in autoantibody generation.

Together, the body of emerging clinical data involving B cell depletion and modulation in a number of human diseases all indicate a clear pathogenic role for B lineage cells, effector functions, and products in disease pathogenesis. Independent of the direct and indirect roles for antibodies in disease, these data also demonstrate that self-reactive B cells and their products likely exhibit distinct properties as compared to non-self-reactive B cells. Additionally, studies of the CD40/CD40L axis further indicate that targeting of specific subsets of downstream B cell effector function is feasible and will be of benefit in certain human autoimmune disorders.

Mechanisms of B Cell Pathogenicity—Humans and Mice

In this final section, we will link observations on B cell effector functions in human disease with those in murine models of disease and discuss how these parallels might aid in understanding the mechanisms of action for B cell depleting therapies in human autoimmunity. We will begin by continuing the theme of potential differences in the precursors of self- and non-self-reactive antibodies, then discuss non-antibody-dependent contribution of B cells as antigen-presenting cells, and finally, review the role of B cells in lymphogenesis and ectopic neolymphogenesis in disease pathogenesis.

Plasma Cells

B cell pathogenesis in autoimmune diseases has been historically attributed to autoantibodies that, in either soluble or IC forms, are thought to initiate local inflammatory cascades. The plasma cell source of these autoantibodies is much less well characterized in humans with respect to exact phenotype, location, or longevity (Arce et al., 2002; Hauser et al., 2003). Populations of long-lived plasma cells have been well characterized in response against foreign antigens (Manz et al., 1997; Traggiai et al., 2003). These cells, in both mice and humans, are enriched in the bone marrow environment. The existence of long-lived autoimmune plasma cells has been demonstrated in murine models of SLE and suggested in humans. Analysis of these cells in humans, however, has proven difficult due to the extreme low frequency and poor understanding of their precursors and intermediates. Although long- and short-lived terminally differentiated plasma cells downregulate surface expression of CD20, and hence would not be depleted by anti-CD20 therapies, very little is known about their immediate precursors in autoimmune diseases. The minimal decreases observed in total serum Ig and more drastic reductions in autoantibody levels following Rituxan treatment suggest either different precursor sensitivities to depletion or, alternatively, that autoantibody-producing plasma cells are enriched in shorter-lived subsets.

Regardless of whether autoantibodies originate from long- or short-lived precursors, it is widely accepted that most pathogenic autoantibodies increase their affinity through somatic mutations. For normal immune responses, this process occurs within the specialized GC environment. Recent data from a murine model of RF autoantibody generation, however, suggests that there are situations in which high-rate somatic mutations can be generated outside of the GC (William et al., 2002). Microdissection of dividing cell foci demonstrate that mutations are readily detected at the T cell zone—red pulp interface in immunoglobulin transgenic MLR/lpr mice, where the normal Fas/FasL mechanisms for maintaining tolerance are compromised. Paralleling these observations, patients with Fas/FasL pathway defects (human autoimmune lymphoproliferative syndrome), with defects in B and T cell apoptosis, develop in both cellular and humoral autoimmunity.

The exact cellular source of autoantibodies in autoimmune diseases becomes important when one tries to explain the large variability seen in autoantibody reduction in patients. Treatment of nonhuman primates with clinically relevant doses of anti-CD20 antibodies demonstrates significantly different rates for depletion of circulating and lymphoid-laden B cells (Reff et al., 1994; Schroder et al., 2003). Additionally, there appears to be differential sensitivities to depletion of various B cell subsets (Vugmeyster et al., 2003). It is conceivable that the variable effects on autoantibodies may result from the differential depletion of these self- and non-self-reactive plasma cell precursors.

Antibody-Independent Effector Functions

The antibody-independent contributions of B cells in human autoimmunity have also been paralleled in mu-

rine models of disease. The ability of B cells to alter T cell activation and expansion was suggested by the generation of an IgH Tg NZB/W SLE-prone mouse strain expressing a germline immunoglobulin (Ig) transgene (Wellmann et al., 2001). Despite a restricted B cell repertoire, these Ig transgenic mice developed high titers of anti-dsDNA autoantibodies but not IC deposits or glomerulonephritis. They also had improved mortality, when compared to their NZB/W littermates, and displayed reduced T cell activation. A similar finding of B cell-mediated disease has also been demonstrated in the New Zealand obese (NZO) diabetes-prone mouse (Haskell et al., 2002). Hence, autoimmune B cells appear capable of driving T cells to mediate certain subsets of autoimmune manifestations. These distinct contributions are consistent with the identification of genetic elements in the NZM2140 SLE-prone mouse model in which clinical and serologic manifestations of disease can be linked, in part, to T (the *sle3* locus), B (*sle2*), and B-T components (*sle1*) (Nguyen et al., 2002).

The contributions of antibody-independent mechanisms to B cell function is further illustrated in MLR/lpr mice made deficient in their ability to secrete Ig but with intact membrane Ig B cells and B cell effector functions—mIgM MRL/lpr (Chan et al., 1999). Despite the absence of secreted Ig and anti-ds DNA antibodies, these mice still develop interstitial nephritis, vasculitis, and less, but still significant, glomerulonephritis and mortality when compared with secretory sufficient MLR/lpr. In contrast, JHD MLR/lpr mice rendered completely B cell-deficient exhibit minimal disease and mortality (Shlomchik et al., 1994). Hence, effector B cell functions, independent of secreted antibody, are also of paramount importance in disease development. A favored hypothesis to explain these antibody-independent effects is the ability of B cells to present antigen to T cells as part of the pathogenic process. Such a possibility clearly exists, though it should be pointed out that these experimental systems utilize immunoglobulin heavy chain transgenes that can limit the autoreactive B cell receptor repertoire.

Direct inhibition of B/T collaboration, through CD40/CD40L blockade, has been demonstrated to be beneficial in several mouse models of lupus. Anti-CD40L treatment blocks both Ig class switching and somatic mutation, reduces anti-dsDNA IgG antibodies, and induces a variable period of general B and T cell unresponsiveness (Kalled et al., 2001; Wang et al., 2003). In addition, CD40 is required for the development of CD8 memory T cell responses (Bourgeois et al., 2002). Together, these data demonstrate multiple mechanisms by which B/T interaction, presumably through antigen presentation or various cosignals and cytokines, may promote T cell effector functions in disease pathogenesis. Additionally, in MLR/lpr mice with B cells lacking β2-microglobulin, and hence MHC class I expression, the contribution of B cells to CD8 T cell activation can also occur independent of antigen crosspresentation (self-antigen presented on MHC class I to CD8 T cells) (Chan and Shlomchik, 2000). Together, these data open the possibility of APC-dependent and APC-independent mechanisms for B cells in autoimmune mouse models of lupus and potentially in human disease.

Lymphogenesis and Neolymphogenesis

The newly recognized role of B cells in lymphogenesis and ectopic neolymphogenesis may also contribute to autoimmunity and serve as a therapeutic mechanism by which B cell depletion or modulation improves human autoimmunity. Dissection of attraction, migration, and retention signals have demonstrated that lymphoid organs and structures are highly dependent on networks of TNF family molecules, integrins, and chemokines (Fu et al., 1997; Cyster, 1999; Ansel and Cyster, 2001). In generating appropriate lymphoid architecture, B cells play an early requisite role through their interactions with organ stroma and provide a milieu permissive for secondary seeding by T cells and dendritic cells. This sequence of events for neolymphogenesis was elegantly demonstrated by the ectopic introduction of a B cell chemoattractant in the pancreas that initiates a cascade of cellular and developmental events leading to the creation of a new lymphoid structure (Luther et al., 2000). A similar dependence on B cells has also been demonstrated in splenic organogenesis as well as normal M cell development in the gut (Ngo et al., 2001; Golovkina et al., 1999). The complementary scenario has been attempted by pancreatic expression of a T cell chemoattractant. However, in this parallel experimental system, lymphoid organogenesis did not progress to the same stage as with B cells, demonstrating a cellular hierarchy in lymphogenesis (Luther et al., 2002).

B cells appear not only important for the formation but also maintenance of these structures. These observations on lymphogenesis formation and maintenance may be important to the recent description of lymphoid-like follicles in joints and inflamed glands in patients with RA and Sjogren's syndrome, respectively (Weyand and Goronzy, 2003; Groom et al., 2002). Hence, B cell depletion or CD40/CD40L blockade could lead to dissolution of these organized pathogenic lymphoid infiltrates and interfere with maintenance of long-lasting T helper memory as well as dendritic cell localization and function (Moulin et al., 2000; Ngo et al., 2001). Looking at the B cell effector mechanisms in total, it is possible that interrupting all or a combination of the above-described pathways is required for clinical efficacy. Further dissection of these mechanisms in ongoing clinical trials as well as complex models will be required to fully appreciate the multiple B cell functions in immunity and disease.

Future of B Cell Therapies

A final point of discussion for human clinical efficacy in autoimmune diseases is the potential lack of complete anti-CD20 B cell depletion in secondary lymphoid organs. Current protocols of Rituxan administration result in an excess of drug that should efficiently deplete all $CD20^+$ B cells. Explanations for the lack of complete depletion, as has been observed in nonhuman primate studies, could be diverse and span from intrinsic B cell resistance as well as factors derived from the local microenvironment (Reff et al., 1994; Schroder et al., 2003). While intrinsic B cell resistance has been more difficult to address and manipulate in humans, the local B cell microenvironment with the multitude of cells and survival signals is of prime importance for additional clinical intervention. It is conceivable that additional costimula-

tory signals, such as CD40/CD40L, with effects on B cell differentiation and survival could rescue them from depletion. Such survival signals within these microenvironments would not only protect B cells from death but could provide additional growth signals for the expansion of autoimmune B cells. In addition to CD40/CD40L, blocking additional survival signals, such as BLys/BAFF and Toll-like receptors (TLR), would likely be beneficial for targeting downstream effector functions, particularly for autoimmune diseases in which these signals are deregulated.

Patients with SLE and Sjogren's syndrome have elevated levels of serum BLys/BAFF (Zhang et al., 2001; Cheema et al., 2001; Groom et al., 2002; Stohl et al., 2003; Mariette et al., 2003). As BLys/BAFF induces B cell proliferation in vitro and in vivo, blockade of this pathway results in loss of follicular and marginal zone B cells (Moore et al., 1999; Thompson et al., 2000). Moreover, mice overexpressing BLys/BAFF develop autoantibodies, Sjogren's syndrome, and SLE-like autoimmunity (Gross et al., 2000; Khare et al., 2000; Yan et al., 2000). In turn, blockade of this survival signal in murine models of SLE results in significant delay in autoantibody development, decreased proteinuria, and improved mortality (Yan et al., 2001; Gross et al., 2001). Analysis of immunologic parameters in SLE and RA patients presently treated with BLys/BAFF blockade will provide additional insights into human B cell functions (Baker et al., 2003). Should BLys/BAFF prove pivotal in human autoimmune B cell survival in vivo, combination therapies with anti-CD40L, anti-CD20, and BLys/BAFF blockade may provide improved efficacy by modulating distinct effector mechanisms.

TLR ligands, particularly for TLR9 (CpG oligonucleotides, hypomethylated bacterial, and mammalian DNA), are potent activators of dendritic cells but function as cosignals for long-term serologic memory B cells and self-reactive transgenic B cells (Bernasconi et al., 2002; Leadbetter et al., 2002; Viglianti et al., 2003). In addition, activation of TLR9 in vivo accelerates nephritis in the MLR/lpr lupus mouse model and exacerbates experimental allergic encephalitis (Ichikawa et al., 2002; Anders et al., 2004). Consistent with the notion that bacterial or viral infections may act as triggers for clinical flares of autoimmune disorders, triggering of TLR4 (LPS) in mice prone to the development of myocarditis induces clinical autoimmune myocarditis (Eriksson et al., 2003). Hence, activation of TLRs in dendritic cells and B cells may contribute in disease pathogenesis. Interestingly, ongoing clinical studies are utlizing CPG and CPG-conjugated allergens to rebalance a deregulated autoimmune system in the treatment of asthma and other autoimmune disorders (Krieg, 2003). Whether TLRs are differentially utilized among immune and autoimmune B cells is unclear at this time. These ongoing clinical studies combined with preclinical insights in TLR biology should inform us whether this axis can be harnessed in the treatment of autoimmunity.

The beginning of this first decade of the 21st century has been an exciting time with the emergence of a multitude of therapeutic probes to understand human B cell biology. These studies have not only reinforced the importance of autoantibodies and ICs in disease pathogenesis but also provide additional insights into the biology of self-reactive B lineage cells. The second half of this first decade promises to be even more exciting as the impact of these emerging B cell-directed therapies in disease and their immunologic consequences will likely not only improve patient survival and quality of life but will undoubtedly illuminate the multitude of B cell functions in human immunity and autoimmunity.

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