2. INTRODUCTION

Based on its clinical efficacy and safety profile, rituximab (Rituxan; Genentech, Inc., San Francisco, CA, USA and Biogen-IDEC Pharmaceutical Corporation, San Diego, CA, USA or Mabthera; Roche, Milan, Italy) has become part of the standard of care for certain forms of non-Hodgkins Lymphoma (NHL), and there have also been a number of studies documenting its utility in other B-cell malignancies. From this extensive clinical experience, there has been increasing interest in the use of rituximab for the treatment of autoimmune diseases in which autoantibodies and B lymphocytes are known to play central roles in pathogenic pathways. It has now been two years since I last reviewed this topic (1). At that time, the literature was limited primarily to case reports and a few small open-label clinical trials. Subsequently this area of clinical investigation has rapidly advanced, and there is substantial evidence that an agent that kills B cells through the
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Table 1. Immunobiologic functions of B lymphocytes in health and disease

<table>
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<tr>
<td>1. Provide cognate help for T cells</td>
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<td>2. Produce cytokines (e.g., IL-4 and IL-10) that support other</td>
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<td>3. Antigen-uptake via surface Ig and immune complexes for</td>
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<td>4. Autoantigen-induced production of autoantibodies that are directly</td>
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<td>5. Constitutive production of autoantibodies by plasma cells</td>
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<td>6. Memory cell (semi-dormant) awaiting antigen re-exposure</td>
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<table>
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<th>In disease</th>
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<tr>
<td>1. Provide cognate help for autoreactive T cells</td>
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<td>2. Produce cytokines that support other mononuclear cells</td>
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<td>3. Autoantigen-uptake via surface Ig and immune complexes for</td>
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<td>4. Autoantigen-induced production of autoantibodies that are directly</td>
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<td>5. Constitutive production of autoantibodies by plasma cells</td>
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<td>6. Autoactive memory cell awaiting (sequestered) autoantigen re-</td>
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<td>7. Disease-associated uncontrolled clonal proliferation (or prolonged</td>
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<td>8. Direct infiltration of end organs (e.g., the kidneys in SLE, the</td>
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<td>9. Expression of adhesion and other co-stimulatory molecules that</td>
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<td>10. Synthesis of chemokines that induce leukocyte infiltration</td>
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<td>11. Production of factors that initiate and sustain angiogenesis and</td>
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In different systems, the anti-CD20 antibody rituximab has been shown to delete B cells by reliance on different cellular mechanisms: apoptosis can be induced by hypercross-linking of membrane-associated CD20 molecules; antibody dependent cytotoxicity (ADCC) can be evoked through interaction with FcγR on adjacent mononuclear cells; and recruitment by rituximab of complement-dependent cytotoxicity has also been implicated. But most reported studies have been performed in simplified in vitro systems with cell lines that may not be representative of in vivo responses. There is also evidence that clinical responses of different B-cell malignancies to rituximab vary in their dependence on specific antibody-mediated deletional pathways. These insights also appear relevant when considering how rituximab may induce clinical responses in patients with autoimmune diseases, which also vary widely in underlying pathogenesis. These diseases affect individuals who may have defects in these same afferent pathways, either based on inheritance of the genetic backgrounds that also predispose them to disease, or as a consequence of the inflammatory autoimmune disease process itself.

The following sections provide a review of the current state-of-the-art of our understanding of the efficacy, toxicities, and mechanism(s) of action involved in the use of the anti-CD20 chimeric antibody, rituximab, for the treatment of patients with autoimmune disease.

3. RHEUMATOID ARTHRITIS

Rheumatoid Arthritis (RA) represents a common (0.6-1.7% prevalence) form of chronic symmetric polyarticular inflammatory arthritis that is associated with progressive joint destruction and disability, and systemic immune abnormalities. While B cells and plasma cells have long been known to be common in the cellular infiltrates in rheumatoid synovium, the pioneering rituximab trial of Edwards and coworkers provided direct evidence that B cells play central roles in pathogenesis (2). More recently, the safety and efficacy of rituximab were documented in a multiple arm double-blinded controlled study of classic seropositive (i.e., rheumatoid factor positive) RA with active disease despite treatment with oral methotrexate, the current standard of care (3). From four treatment groups, each with ~30 patients, comparisons were made with a control group that received weekly oral methotrexate. In the other groups, patients received rituximab alone (i.e., monotherapy) at 1000 mg on day 1 and 15, or combinations that included rituximab and methotrexate, or rituximab and cyclophosphamide (750 mg on days 3 and 17).

In general, at week 24, the proportion of patients with 50% improvement in disease symptoms, by the criteria of the American College of Rheumatology (ACR), was significantly greater with the rituximab-methotrexate combination (43%, P = 0.005) and the rituximab-cyclophosphamide combination (41%, p = 0.005) compared with methotrexate alone (13%)(Figure 1). Moreover, a higher proportion of patients treated with rituximab had a 20 percent improvement in disease symptoms, according to the ACR criteria (i.e., ACR20) (65 to 76 percent vs. 38 percent, p ≤ 0.025). Even for this lower threshold of clinical response, co-treatment with cyclophosphamide or methotrexate resulted in benefits for a greater proportion of the patients compared with rituximab alone. Taken together, this study showed that a single course of rituximab, with or without co-treatment, provided significant improvement in disease symptoms at both weeks 24 and 48 (Figure 1), and these responses were comparable to those commonly achievable with anti-TNF-α biologic agents.

Rituximab therapy was also generally well tolerated in these patients. The majority (85 to 90%) of adverse events were associated with rituximab infusions, and these were mild or moderate, and each of the treatment groups had a similar incidence of adverse events. Overall, a total of 16 serious adverse events were reported in 14
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Figure 1. American College of Rheumatology clinical responses of rituximab treated RA patients at weeks 24 and 48. In these data, ACR 20 denotes at least a 20 percent improvement in disease symptoms according to the American College of Rheumatology (ACR) core set of outcome measures, ACR 50 is a 50 percent improvement, and ACR 70 is a 70 percent improvement. P values are for comparisons with the methotrexate-monotherapy (control) group. Reproduced with permission from the New England Journal of Medicine (3).

patients, with the highest incidence among patients receiving rituximab plus cyclophosphamide. Serious infections occurred in one patient (2.5%) in the control group and in a total of four patients (3.3%) in the rituximab groups (two patients in the rituximab-monotherapy group and two in the rituximab–cyclophosphamide group).

As rituximab is a chimeric antibody that contains murine variable regions, there has been concern that patients might develop specific antibody responses, which lead to impaired efficacy and/or potential allergic reactions. Notably, human anti-chimeric antibodies (HACA) developed in only 5 of 117 patients (4.3%) in the rituximab-treated groups. While this may seem paradoxical that a specific antibody response can be induced in a patient with massive B-cell depletion, it is compatible with evidence that not all B-lineage cells are depleted (reviewed in (4)). In any event, no specific clinical manifestations were observed in those patients, and the relevance of such induced anti-rituximab antibodies may only become clear after such patients later receive retreatment.

In all of these RA patients, despite evidence of effective depletion of peripheral blood B cells (Figure 2A), levels of total IgG, IgM, and IgA immunoglobulins remained within normal ranges, and there was no effect on anti-tetanus antibody titers from prior immunizations. In contrast, rituximab treatment was associated with rapid and large decreases in rheumatoid factor (RF) autoantibody levels (Figure 2B) that persisted through week 24. In contrast, patients receiving methotrexate alone had only modest transient decreases in RF levels, which were deemed likely due to corticosteroids. The responsiveness of the RF levels to rituximab treatment is potentially important, as RA is believed to involve an immune complex-mediated process (reviewed in (5)), and high titer RF are associated with more aggressive articular disease, extra-articular manifestations, and increased mortality (6). The greatest induced decreases were in IgG RF, which may be the most important contributor to in vivo pathogenesis (7). Significant decreases were also induced in levels of autoantibodies to cyclic citrullinated peptides (CCP) (3), which are becoming accepted as a specific diagnostic marker for RA, as these autoantibodies may arise in about half of patients even before the development of clinically detectable disease (8). In contrast, levels of anti-microbial Abs did not fall, suggesting that these protective responses may be the products of different B-cell subsets.

More recent blinded control studies have supported the desirable safety and efficacy profile for Rituximab in RA patients (9;10). Significantly, Rituximab infusions with weekly oral methotrexate have demonstrated attractive clinical response rates in patients who were inadequate responders to one or more TNF blocking agents (10). Rituximab treatments have also been shown to provide clinical benefits for patients with seronegative RA (9). Taken together, these recent studies suggest that B-cell deletion therapy can affect underlying pathways of disease in RA (discussed further below). As a consequence, a regimen of rituximab and methotrexate was approved by the US Food and Drug Administration in early 2006 for RA patients who had inadequate response to TNF blockade. More recently, this novel B-cell deleting agent was also approved for RA treatment in Canada and the European Union.
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Figure 2. Median levels of peripheral CD19+ B cells and median changes in the levels of total RF autoantibody in rituximab treated RA patients during the 24-week study period. Panel A shows the levels of peripheral CD19+ B cells during the study period, and Panel B shows the median change in total RF activity. Reproduced with permission from the New England Journal of Medicine (3).

4. AUTOANTIBODY MEDIATED THROMBOCYTOPENIA

Chronic idiopathic thrombocytopenic purpura (ITP) in adults (female predominance with an incidence of about 5 per 100,000) is an autoimmune disease in which circulating pathogenic IgG autoantibodies are believed to be responsible for the destruction of mature platelets, leading to potentially life threatening bleeding diatheses (11). In part because of this relatively simple pathogenesis, ITP has become an attractive system to evaluate responses to immune-based therapeutic approaches. Moreover, clinical responses can easily be assessed. The levels of circulating platelets can directly reflect the efficacy of therapeutic interventions—low platelet count indicates continued activity of the disease, while the normalization of platelet count is a direct indicator of response and/or remission.

Although there no doubt exists heterogeneity in the specificities of autoantibody responses between affected individuals, and diverse autoantigen targets may be recognized even within a single individual, the most common target of these IgG autoantibodies in ITP is the platelet membrane glycoprotein IIb/IIIa complex (12). Patients with ITP also have circulating CD4+ T cells reactive with gpIIb/IIIa, which promote the production of anti-gpIIb/IIIa antibodies that aid the pathologic accelerated clearance of platelets (13).

During the development of chronic ITP, the production of anti-platelet antibodies is commonly believed to begin in the spleen. Moreover, if the levels of circulating platelets cannot be maintained with modest doses of corticosteroids and/or other immunosuppressive agents, patients often undergo splenectomy, although this intervention has significant lasting immunologic consequences (14). Those who do not respond to splenectomy are believed to have an autoimmune process that has disseminated beyond the spleen. This presumably explains why patients with autoimmune thrombocytopenia differ from those with thrombocytopenia secondary to systemic lupus erythematosus, which is a more generalized disease of autoimmune hyperactivity, as lupus patients are believed to less commonly derive lasting clinical benefits from splenectomy (15). In fact, in a recent literature review only 66% of ITP patients had a complete response after splenectomy (14), and the frequency of relapse seems to increase further with longer duration of followup (16), indicating that better therapeutic approaches are needed. It is also an unavoidable fact that, at the present time, trials of new biologic therapies include a majority of patients who have failed other approaches, including splenectomy.

Extending observations from earlier case reports and small series of pediatric and adult patients with ITP (17), Cooper et al have recently reported an open label study of the use of rituximab in 57 adult patients with chronic ITP (18). In this landmark study, all patients had platelet counts of less than 30 x 10^9/L and had previously received two or more immunosuppressive agents. Patients received standard rituximab regimens (i.e., rituximab 375 mg/m² intravenously once a week for four consecutive weeks). While most patients required continued daily oral corticosteroids, about a third received only minimal corticosteroid doses limited to the first and second infusions but none subsequently.

In this study, rituximab was shown to be efficacious, as 31 of 57 ITP patients (54%) achieved clinical response with increases of platelet counts to at least 50 x 10^9/L and 18/57 (32%) patients had complete responses, representing normalization to platelet counts of more than 150 x 10^9/L. However, due to very low platelet counts, 7 patients needed additional therapy, and 5 of these patients received IVIG within the first few weeks of therapy.

Splenectomy did not affect rituximab responder status. These patients also did not differ in the characteristics of their responses, as defined by time to achieve response or duration of response. Significantly, in this study 31 of the 57 patients had previously undergone splenectomy, which included eight of the 18 patients who achieved a complete response. Prior splenectomy also did not influence the duration of clinical and laboratory responses, which was found to also directly correlate with
the magnitude of the improvement in platelet count, such that 16 of the 18 patients with complete remissions displayed normal platelet counts over a median of 72.5 weeks (range 24 to 165 weeks) from the initial infusion. However, patients with longer histories of ITP (i.e., > 15 yr) were less likely to respond.

Akin to other rituximab studies, most infusion associated adverse events were mild, with only a single more serious event, bronchospasm, which did not interfere with the completion of the treatment. There were also no overall changes in immunoglobulin levels or infectious complications (17). As there is currently no commonly accepted assay with proven clinical utility for the diagnosis or monitoring of these patients (19), determinations of relevant autoantibody levels could not determined in these patients.

Among the 18 patients who achieved a complete response, a broad range in the kinetics of their responses was demonstrated, and three distinct patterns of platelet level responses were identified (18). In seven patients, there was an early platelet increase with normalization even before the completion of the fourth infusion, which may suggest a high degree of rituximab responsiveness of the B-lineage cells that may be the source of pathogenic autoantibody. In an additional five patients, normal platelet levels were not achieved until week seven to eleven. For the remaining six patients, there was a slow steady increase detected with a median of eight weeks, so that normal counts were not achieved until between weeks 13 and 31. In explanation, one may speculate that these differences may in part reflect the turnover of involved B-lineage cells at different stages of differentiation, that are involved or contribute to pathogenesis. Direct studies on the impact of therapy on B-cell subsets at sites of disease are currently limited, but suggest that deletion may be efficient in some patients, but not in others (20).

5. SYSTEMIC LUPUS ERYTHEMATOSUS

SLE represents a generalized autoimmune process (occurring in about 15-50 per 100,000 population with a strong female predominance) affecting multiple organs, often with nephritis and cytopenias, primarily due to pathogenic IgG autoantibodies (21). For progressive disease, several immunosuppressive regimens are now commonly employed, and the best validated involves monthly cyclophosphamide infusions for the treatment of renal disease (reviewed in (22)). In general, investigations have been hindered by the fact that patients vary greatly in the severity and clinical manifestations of their disease (i.e., specific organ system involvement), which makes the design of clinical trials and comparisons of patient responsiveness especially challenging (23). However, hyperactive B cells and autoantibody production appear to be invariant features.

At the present time, the outcome of several small open trials of rituximab treatment of lupus patients has been reported. In a very recently published Phase I/II dose escalation study from the University of Rochester (24), the responses of 17 SLE patients, including 7 with nephritis, were evaluated when rituximab was given either as a single infusion of 100 mg/m² (low dose), a single infusion of 375 mg/m² (intermediate dose), or as 4 infusions (1 week apart) of 375 mg/m² (high dose). Cyclophosphamide and bolus corticosteroids were not allowed. In general, rituximab was well tolerated with only 3 serious adverse events, which were thought to be unrelated to rituximab administration. Notably, blood B-cell depletion was highly variable, even in patients who received high dose treatments, and only 11 of 17 had marked B-cell depletion (to < 5 CD19+ B cells/ul). Patients with this level of B-cell depletion exhibited significant clinical improvement at 2 and 3 months (P = 0.0016 and P = 0.0022, respectively, by paired t-test), and this improvement persisted for 12 months. The best responses were for rash, mucositis, alopecia and arthritis. While there were no consistent responses for patients with nephritis, none of these patients received the high dose regimen. Significantly, there were no significant changes in anti-native DNA antibody or complement levels in these rituximab-treated patients. However, six patients did develop human antichimeric antibodies (HACAs), which were more common if a patient received low dose treatment and had limited B-cell depletion. Treatment associated low IgM levels were demonstrated in many of these patients, but only minor decreases were documented in total IgG levels, and no decreases in anti-tetanus and anti-pneumococcal antibody responses from prior vaccinations were detected.

In a report of a British open trial, the responses of six female patients with active SLE to a combination regimen were described, in which three patients had renal disease, one had CNS disease, also with Raynaud’s phenomena, arthritis, lymphopenia, anemia and cutaneous vasculitis. These patients, who were resistant to standard immunosuppressive therapy, received two infusions of 500 mg of rituximab in combination with two 750 mg infusions of cyclophosphamide and high dose oral corticosteroids (25). After treatment, marked blood B-lymphocyte depletion was documented in all patients, which persisted for months. During the follow-up of 6-18 months, most patients demonstrated significant clinical improvement of fatigue, arthralgia/arthritis, serositis, and skin vasculitis, while 2/3 patients with renal involvement displayed improvement.

Consistent with active autoantibody-immune complex mediated disease, before treatment all patients in the British trial had depressed levels of complement and elevated levels of anti-native DNA antibodies. Following treatment, in most patients C3 levels normalized at least transiently, and some patients also had decreases in anti-native DNA antibody levels. Levels of other autoantibodies were not reported. While modest decreases in serum Ig were common, these levels remained within normal range. For some patients, treatment appeared to provide benefits that extended beyond the period of B lymphocyte depletion, enabling decreases in maintenance daily doses of corticosteroids. In general, treatment was safe and well tolerated, with only minor infections reported. Significantly, disease activity subsequently flared in most
patients, suggesting that such patients may anticipate the need for re-treatment.

In a recent open trial from Greece, the responses of 10 patients with active lupus nephritis were evaluated (26). This report was important as the greater homogeneity of clinical involvement simplified efficacy measures, and because the standard of care for patients with this common clinical presentation often requires cytotoxic agents that are associated with potential for serious toxicity and even ovarian failure. These 10 SLE patients with active proliferative (4 focal, 6 diffuse) nephritis were treated with rituximab (4 weekly infusions of 375 mg/m²) and oral corticosteroids for 10 weeks (26) and other agents were discontinued. Significantly, this regimen induced 5 complete remissions and an additional 3 partial remissions, which were sustained in many cases at 12 months. Akin to other trials, if a patient had only limited or only transient peripheral blood B-cell depletion there was little chance for a clinical response. Only one patient had a significant adverse event, which was a hypersensitivity reaction with rash and fever but without hemodynamic instability. Nonetheless, this reaction prevented completion of the infusions.

In all patients significant decreases in autoantibodies to native DNA were noted, although levels generally remained detectable. Levels of circulating immune complexes also decreased, while complement levels normalized. Of interest, many of these patients also displayed significant decreases in serum IgM levels (discussed further below). A key finding was that the levels of the activation/co-stimulation molecule, CD40L, on blood CD4⁺ T cells also significantly decreased following treatment. There were also concurrent decreases in the T-cell activation markers, CD69 and HLA-DR, with greatest improvements in the patients with complete remissions. These findings suggested that treatment resulted in a decrease in the activation level in the T-cell compartment, presumably due to removal of B lymphocyte co-stimulation.

The impressive results in this trial raise the question of whether these lupus patients were somehow different than those in other trials. It is possible that inherited genetic variations or acquired immune abnormalities in these ethnic Greek SLE patients are less likely to interfere with clinical responses than have been described in other recent SLE studies (27). Alternatively, it may be relevant that most of these patients had previously received potent immunosuppressive regimens, including cyclophosphamide and mycophenolate mofetil. Hence, it is intriguing to speculate that even when treatments are not concurrent, greater clinical responses could in part reflect the impact of rituximab on lymphoid tissues that have previously been treated with other potent immunosuppressives, possibly through residual effects on myeloid cell precursor populations.

Overall, these open trials point out that not only are patients with SLE highly heterogeneous with regard to organ involvement, disease severity and in vivo autoantibody profiles, but that there may also be great heterogeneity relating to the underlying immunobiology of the B lymphocytes and B-cell dependent influences on pathogenesis.

6. CAN CURRENT ANTI-CD20 REGIMENS REESTABLISH “SELF” IMMUNE TOLERANCE?

Preliminary reports suggest that after rituximab treatment the composition of the residual blood B-cell pool in SLE patients is highly variable and not all types of B-lineage cells are depleted from the bloodstream with equivalent efficiency (28). Based on surface phenotype detected in flow cytometric studies, it was found that memory B cells (i.e., CD19⁺ CD27⁺) may predominate among the few residual B cells. This was unexpected as in normal adults the majority (70-80%) of circulating B cells have the phenotype of naïve B cells (i.e., CD19⁺ CD27⁻). These findings therefore raise the concern that naïve B cells may be more efficiently deleted than the memory B cells that preserve the pathologic autoreactivity, and that this may lead to later reemergence of disease.

In another preliminary report, single cell PCR studies were used to characterize the antibody genes expressed in the blood B cells of rituximab-treated RA patients (29). At 17 months after treatment, at the time that overall blood B-cell levels were recovering, there was a disproportionate overexpression of hypermutated antibody genes, a marker of post-germinal center B cells (i.e., not naïve). Hence, in this study reconstitution did not appear to solely reflect replenishment in the periphery with naïve new B-cell emigrants from the central compartment (i.e., bone marrow). In contrast, these findings suggest that when the depleting effect of rituximab wears off, there may be expansions of persistent memory B cells. Therefore, these findings may predict that therapeutic interventions with current regimens may not have the capacity to completely restore immunologic tolerance, as lymphocytes with the capacity for pathologic autoimmunity remain. In more recent studies, the period of recovery of peripheral blood B cells was shown to primarily reflect a predominant blood B-lineage population that is CD38⁺ IsgD⁺ CD10⁺ CD24⁺, and which do not display the CD27 memory B-cell marker, and their antibody gene rearrangements are without extensive somatic hypermutation. These “transitional B cells” (30;31) are believed to be antigenically naïve recent bone marrow emigrants that derived from immature B cells. These limited studies further highlight the importance of direct evaluations of the effect of treatment on mononuclear populations in lymphoid tissues, and especially at sites of disease (discussed in (32)).

7. CD20 EXPRESSION BY B-LINEAGE CELLS AND RITUXIMAB SUSCEPTIBILITY

To understand the clinical implications of rituximab treatments, we need to better understand the effect of these treatments on specific B-cell subsets. In general, CD20 is not substantially shed by B cells, and in vitro interactions do not appear to directly down-regulate membrane expression of CD20 (33), which might enable
escape from anti-CD20 antibody interactions. However, B-lineage cells at early and late stages of differentiation that do not express CD20 can still be recruited into the pathogenesis of an autoimmune disease (discussed further below).

There is also evidence that surface CD20 expression can be modulated, as in vitro encounter of B cells with CD40L was shown to induce down-regulation of CD20 in a ligand-dependent manner (34). Hence, germinal center B cells affected by B-T interactions may be less susceptible or even resistant to rituximab treatment, which could lead to clonal escape. Studies of lymphoma cell lines have also demonstrated that a B cell can display surface CD20 but still be resistant to apoptosis induced by in vitro incubation with an anti-CD20 antibody. If the B cell is susceptible, the antibody was shown to cross-link membrane-associated CD20 that was then drawn into specialized lipid rafts capable of activation signaling that led to apoptotic death (35). In contrast, changes in the membrane-associated CD20 domains of B-cell lines that are impervious to rituximab were shown to interfere with these lipid raft interactions (36). While it is currently unknown whether this mechanism can affect the responsiveness of B cells in patients with autoimmune disease, the greater relevance of such pathways are highlighted by reports of an analogous defect in the capacity of some allelic variants of FcR to enter and interact in lipid rafts (37).

Of great importance for the treatment of autoimmune diseases, fully differentiated plasma cells, the antibody factories of the body, do not display CD20, and hence are not directly affected by anti-CD20 antibody therapy. In health, immunization with exogenous protein antigens recruit follicular B cells (also termed B-2) into germinal center reactions, which can yield Ig-secreting cells in lymph nodes and/or spleen and also generate the semi-dormant B cells that retain antigenic memory (i.e., memory B cells), and both can lack CD20. These cells migrate to the bone marrow, where some plasma cells survive months or even years without proliferating. Memory B cells may have similar long life-spans, and persist for decades. In contrast, non-protein antigens (i.e., carbohydrates and lipids) recruit what have been termed innate-like B cells (i.e., marginal zone B cells and B-1 cells) into extra-follicular responses, to rapidly generate large numbers of plasmablasts that locally secrete antibodies, primarily in the spleen, but which may turnover rapidly due to their survival for only a few days.

In general, Ig-secreting cells do not appear to be intrinsically long lived, as these cells die rapidly in simple culture conditions. In vivo survival has been shown to be highly dependent on one or more signals from soluble factors and cognate (i.e., membrane associated) from adjacent cells that provide “niches” for cell survival. In health there is believed to be a limited number of these niches, and defining the factors responsible for the lifespan of these B-lineage cells is a topic of active investigation. In certain autoimmune diseases, the availability of these niches may be greatly expanded, presumably due to enhanced production of these pro-survival factors. Cells producing pathogenic autoantibodies may also be prominent in ectopic lymphoid infiltrates in end organs, such as affected synovium in RA, and kidneys and other tissues in SLE (discussed below).

8. DO PRO-SURVIVAL INFLUENCES OPPOSE ANTI-CD20 MEDIATED DELETION?

Although not yet directly investigated, it is highly likely that in autoimmune diseases, and perhaps other conditions, the susceptibility of CD20-bearing B-lineage cells to deletion may also be affected by local pro-survival influences. For plasma cell longevity, these signals are reported to include IL-5, IL-6, stromal cell-derived factor-1 alpha (SDF-1, CXCl12), TNFα, and ligands for CD44, an adhesion receptor for hyaluronic acid (38). Pathogenesis is also affected by the factors responsible for the migration and subsequent localization of affected B-lineage cells. Plasma cells in the bone marrow express CXCR4, CXCR6, CCR10, and CCR3 (39), and these Ig-producing cells have been shown to migrate towards gradients of SDF-1 (40), CXCL9 (a monokine induced by IFN-γ), CXCL10 (IFN-γ-inducible protein 10 and CXCL11 (IFN-inducible T cell alpha chemottractant) (40). The adhesion receptor, CD44, and its major ligand, hyaluronic acid, are also involved in homing to the bone marrow (41).

In studies of lupus-prone mice, lymphoid infiltrates associated with a local inflammatory autoimmune response in the kidneys of lupus-prone (NZB × NZW) F1 mice were shown to support the survival of post-immunization antigen-specific plasma cells (42). These intra-renal plasma cells may produce autoantibodies directed at renal/glomerular autoantigens (43). In addition, in another well characterized murine lupus system, a defect in chemotaxis to CXCL12/SDF-1 was believed to contribute to the pathologic accumulation of plasma cells in the spleen (44).

Patients with certain autoimmune diseases are reported to have similar abnormalities. In human lupus, circulating levels of plasma cells correlate with overall disease activity (45), although the prevalence of plasma cell infiltrates in lupus kidneys is currently a controversial topic. In RA, it was shown that at 4-6 days after tetanus toxoid immunization boost, IgG anti-tetanus toxoid antibodies were produced by both cultured peripheral blood lymphocytes and synovial cells obtained surgically from RA patients (46). These findings suggest that rheumatoid synovium is well suited to support the survival of Ig-secreting cells, even when generated at other sites. More recently, rheumatoid synovial tissues were shown to be rich sources of CXCL12/SDF-1 (47). In fact, SDF-1 produced by fibroblast-like synoviocytes can contribute to the resistance of B cells to apoptosis, which supports the hypothesis that specialized synovial “nurse-like cells” mediate homing and survival of B cells (48;49).

Of special clinical importance, the TNF family member, BAFF (also known as BlyS, TALL-1, THANK,
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and zTNF4), has potent B-cell specific pro-survival effects that contribute to physiologic clonal selection and the survival of mature B cells. While it is not required by B-lineage precursor cells in the bone marrow, the clonal survival of B cells after peripheral encounters with BCR ligands appears to require BAFF, which may increase intracellular levels of anti-apoptotic Bcl-2 family members (50). BAFF has also been shown to be involved in both T-dependent and T-independent immune responses, and recently human follicular dendritic cells (FDC), which play central roles in germinal center reactions, have been shown to be important sources of BAFF (51). In addition, overexpression of BAFF has been implicated in the pathogenesis of RA, SLE and other autoimmune diseases (52). In particular, BAFF is increased in rheumatoid synovial fluid (53), but this may just be a reflection of the local recruitment of myeloid cells. A related TNF family member, APRIL, is often produced under the same conditions as BAFF, and it shares receptors and pro-survival biologic activities (reviewed in (54)). The expression and availability of these factors may be greatly expanded during the pathogenesis of an autoimmune disease, especially at sites that have developed ectopic lymphoid tissue. However, it is currently unknown how any of these factors may affect B cell responsiveness to anti-CD20 treatment.

Other membrane associated mechanisms may also enhance B-cell survival, as studies of B-cell lines have shown that signaling via α4 β1 integrin, as occurs for marginal zone B cells (55), can also promote resistance to apoptotic stimuli through effects on p53 expression and up-regulation of Bcl-xL (56;57). Potentially relevant to the development of future therapeutic regimens, patients with rituximab treatment-resistant NHL, which is associated with Bcl-2 overexpression, were more responsive to co-treatment with rituximab and a standard regimen of cytotoxic chemotherapy agents that was ineffective alone (58). Preliminary studies suggest that such resistant NHL patients may also benefit from co-treatment with rituximab and a factor that inactivates transcripts for Bcl-2 (59). The mechanisms by which co-treatment regimens may provide enhanced benefits for autoimmune patients remain to be determined, but these pathways are attractive candidates for investigation (discussed below).

9. DISTINCT SETS OF B-LINEAGE CELLS, DIRECT AND INDIRECT EFFECTS ON IG-SECRETING CELLS

Although clinical experience is still relatively limited, review of the literature suggests that, even when anti-CD20 results in a marked blood B-cell depletion and subsequent clinical improvement, antibody titers may not be affected. Based especially on the British experience, it also appears that when rituximab is used with a second potent agent, decreases in disease-associated autoantibody levels are more common, and there may also be marked reductions of total IgM, but without concurrent effects on levels of total IgG or titers of antibodies to bacterial antigen vaccines (e.g., tetanus toxoid or Pneumovax TM). It is possible that differential effects on types of antibody responses are in part due to the circulating half-lives of different Ig isotypes, and IgM is known to have a much shorter half life than most IgG. In addition, these antibody responses likely come from different B-cell compartments.

To understand the potential efficacy of CD20 targeted therapy we need to better characterize the in vivo immunobiology of the differentiation and trafficking of different types of B cells and Ig-secreting cells. Although human immunology may differ, studies of mice have shown that there are three tiers of mature B lymphocytes: B-1 cells; marginal zone B cells; and the re-circulating follicular B cells (B-2 cells) that take part in germinal center reactions. These B cells reside at different anatomic sites, with different local milieus, and each is involved in immune responses to different types of antigens that require different signals and intracellular interactions for activation, clonal selection and subsequent differentiation to Ig-secreting cells. Different factors are also likely to be required for their survival and trafficking.

In the spleen, most Ig-secreting cells are relatively short lived and these may derive from different cellular sources than the Ig-secreting cells which predominate in the bone marrow. In the mouse, more than 80% of circulating IgM comes from B-1 cells, which traffic from the peritoneal cavity to the spleen to secrete IgM, where most of these immunoglobulin-secreting cells are relatively short-lived (60).

The outcome of therapeutic intervention may therefore reflect the cellular lifespan of the Ig-producing cells, the rates of turnover or replenishment from their precursors, and the variable availability of supportive niches that enable their subsequent survival. Notably, pediatric populations treated with rituximab, even as monotherapy, seem to be much more susceptible to depletion of total IgM levels, which could be due to developmental differences in IgM-secreting cells. For instance, in early development IgM-secreting cells may express higher levels of CD20. Alternatively, IgM-secreting cells may turnover more quickly in these younger patients. In this case, if the lifespan of these Ig-secreting cells is limited and/or if cell turnover can be accelerated, the level of pathogenic autoantibody deriving from a B-lineage cell that does not express CD20 may still be indirectly affected by rituximab.

Another possible reason that certain IgM autoantibodies are relatively more susceptible to rituximab treatment (e.g., IgM RF in RA)(61) could be that there are differences in the susceptibility of B cells that reside in ectopic lymphoid infiltrates at sites of disease, compared to those in peripheral lymphoid tissue. We may therefore speculate that antibodies arising from prior vaccinations are much less affected because these are produced by long-lived plasma cells in the bone marrow, which is more protected from the effects of anti-CD20 antibody treatment.

These concepts may help to explain the different kinetic patterns for normalization of platelet counts observed after anti-CD20 depletion therapy in adult ITP
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patients (18). Speculatively, in the more rapid responders the pathogenic autoantibody-secreting cells may have expressed CD20 at high density, enabling efficient and fast depletion. In contrast, in the patients with the delayed responses, the pathogenic autoantibodies could be from B-lineage cells that were protected from Rituximab due to either diminished surface CD20 expression and/or local pro-survival effects. After many weeks of continued production these non-susceptible cells completed their life span, but were not subsequently replaced by autoreactive precursors.

10. SLE – FACTORS THAT MAY BOTH PREDISEPOSE AND INTERFERE WITH CLINICAL RESPONSE

While many patients with autoimmune diseases appear to benefit from anti-CD20 therapy, certain individuals do not respond. Rituximab has been shown to deplete B cells through a range of mechanisms (reviewed in (62)), and the pathway(s) responsible for clinical responses remain controversial. In part, this is because most observations were made in vitro on cell lines, and different mechanisms may predominate in vivo for different types of normal and neoplastic B-lineage cells.

Of special importance, B cells coated with anti-CD20 antibodies may be cleared in part by interactions with cellular receptors for the IgG1 constant regions (i.e., Fc receptors), especially Fc RIIfa that is expressed on a variety of cells, including phagocytic cells (63). However, the inefficient B-cell depletion seen on a subset of SLE treated with rituximab correlates with the inheritance of a genotype of Fc RIIfa associated with lower affinity binding interactions (27). A similar correlation was previously reported for the responses of NHL patients (64), although not found for CLL (65). In fact, Fc R dysfunction may be an integral part of the genetic factors and functional immunologic defects that predispose some patients to SLE, and inheritance of this same Fc RIIfa (F 176) low affinity allele is a strong risk factor for the development of SLE and nephritis across ethnic populations (66-68). Although controversial, other types of Fc R may also be involved in SLE predisposition (69). A correlation of the Fc RIIfa (V176) high affinity allele has also been reported for chronic ITP in pediatric populations (70), which may in part predict the responders in trials.

Complement has also been implicated in rituximab-induced B-cell depletion (reviewed in Cartron et al. (62)). In vitro studies have shown that the ability of anti-CD20 antibody to induce complement-mediated lysis correlated with the capacity to translocate CD20 into lipid rafts (71), and this mechanism may be important for responsiveness of CLL patients (72). Significantly, C1q deficient mice displayed an impairment of rituximab-induced depletion of human CD20 transduced syngenic lymphoma cells (73). Importantly, inherited complement deficiency is a strong risk factor for human and murine SLE. In fact, human C1q deficiency is reported to have near 100% penetrance for lupus disease, and acquired complement deficient states may be even more common in SLE patients (74). Hence, the same pathways that contribute to autoimmunity and dysregulated autoantibody production and function may also interfere with the efficiency of rituximab mediated B-cell deletion. Patients with other types of autoimmune disease, for example RA, are not known to have these same immunologic abnormalities.

11. PERSPECTIVE AND CONCLUDING REMARKS

Although the evidence is still limited, there is now convincing support for the use of anti-CD20 antibody based therapy in the treatment of patients with certain autoimmune diseases. While best documented in RA, interim trial results have also been impressive for the less common diseases of ITP and SLE, and potentially others will also be explored in the near future. Although such an exercise may be premature, I believe that several important lessons can be gleaned from available reports.

11.1.B cell depletion

In patients with autoimmune diseases, it appears that effective depletion of peripheral blood B cells generally requires doses of rituximab that are comparable to those used in NHL. However, depletion in many SLE patients in particular is often inadequate (discussed below), and in most cases clinical responses do not occur if effective blood B-cell depletion does not occur. Moreover, the duration of depletion can be highly variable, lasting from weeks to more than a year. It is also possible that some patients, especially with SLE, may require even higher doses to achieve responses. While the current full dose regimes often results in 3-9 months of depletion of B cells in the bloodstream, the effects at other anatomic sites, including sites directly involved in disease, need to be better characterized.

11.2.Safety profile

Based on available reports, rituximab treatments are generally well tolerated in patients with autoimmune diseases. While first infusion reactions are still common, these are generally mild and it is uncommon that these prevent completion of therapy. There are no reported cases of cytokine storm responses akin to the tumor lysis syndromes that infrequently have been associated with the treatment of NHL patients. However, some SLE patients may have a greater tendency toward developing antibody responses to rituximab (HACA) and serum sickness (75), suggesting that these patients may be at somewhat greater risk for these adverse effects.

11.3.Immune defects may diminish efficacy

Recent reports support the notion that the distinct pathways of immune pathogenesis associated with autoimmune diseases can adversely affect the patient’s responsiveness to therapy. Although still poorly understood, impaired efficacy could derive from differences in the efficiency of host capacity for antibody effector mediated functions, such as induction of complement and FcR mediated host responses. These postulated differences are especially important as the same pathways may also contribute to autoimmune disease predisposition, severity and clinical involvement.
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11.4. Therapeutic intervention as an investigative tool

The greatest potential importance of this new therapeutic approach is that it enables the induction of highly focused immune deficits that can provide investigative opportunities to better dissect and define the central pathogenesis pathways at work (76). In the past, most molecular and cellular observations in these patients have been characterizations of stable ongoing processes. Interventions with rituximab appear to be an effective tool to perturb the immune systems of patients with autoimmune disease in a specific fashion, and then assess the outcome. An example maybe the case of seronegative RA, which limited experience indicates is not responsive to anti-CD20 therapy (61), suggesting the fundamental pathogenetic pathways may be very different than in patients with seropositive RA disease.

Available studies have also provided evidence that in certain diseases combination regimens may be more effective, but the pathways responsible for these benefits are currently unknown. Exploration of known B-cell survival pathways should be highly informative. It is obvious that studies restricted to the blood, the body’s internal highway/transit system, will therefore be inadequate.

We need to now extend our investigations to sample the full range of lymphoid organs and sites of disease, where immune cells work together in physiologic/protective and pathophysiologic/destructive responses. Consideration of the full range and potential functions of B-lineage cells will be essential. Herein may be the greatest opportunities to understand the basis for disease, and to develop, refine and monitor safe and effective biologic therapies for the future.

12. REFERENCES

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44. L.D. Erickson, L.L. Lin, B. Duan, L. Morel and R.J. Noelle: A genetic lesion that arrests plasma cell homing to the bone marrow. *Proc Natl Acad Sci* (USA) 100(22), 12905-10 (2003)


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**Abbreviations:** ACR, American College of Rheumatology; BCR, B-cell receptor; CCP, cyclic citrullinated peptide; gpIIb/IIIa, Glycoprotein IIb/IIIa; HACA, human anti-chimeric antibody; ITP, idiopathic thrombocytopenic purpura; NHL, Non-Hodgkins Lymphoma; slg, surface Ig; RA, rheumatoid arthritis; RF, rheumatoid factor; SLE, systemic lupus erythematosus.

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