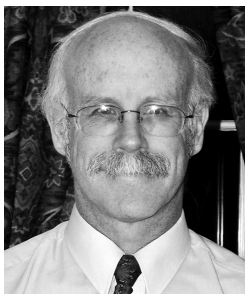
Editorial

Discovering That B Cells Are Important in Rheumatoid Arthritis



The past half-century has seen each new advance in immunology and molecular biology applied to the study of rheumatoid arthritis (RA), with surprising observations and interesting new insights on etiology and pathogenesis (Table 1). Although these theories are often debated as if they were mutually exclusive, there is a growing appreciation that RA is a complex disease and that different etiologic factors and pathogenic mechanisms may pertain not only within a population but even within the same patient at different times during the course of the disease. The evolution of concepts of the role of B cells in the pathogenesis of RA has been particularly interesting. Autoantibodies and hence B cells dominated research on RA in the 1960s and 1970s, but were eclipsed in the 1980s and 1990s by other areas of research including work on T cells, fibroblast-like synoviocytes, and cytokines. Nevertheless, work on autoantibodies and B cells never stopped, and there were notable discoveries, such as the role of citrullinated peptides as one of the targets for autoantibodies in RA. Recently, B cell depletion has been tested in a randomized, double-blind, placebo controlled clinical trial for RA; the successes of this trial have renewed enthusiasm for the role of B cells in this disease[1]. These trials have also raised a host of new questions for both basic and clinical researchers.

The discovery of rheumatoid factor (RF) as a factor in the serum of patients with RA that agglutinated IgG-coated erythrocytes, and the characterization of RF as an autoantibody recognizing the Fc portion of IgG provided the first evidence that RA might be an autoimmune disease. While RF has proven to be a useful diagnostic and prognostic test, whether RF is important in the pathogenesis of RA or is merely an epiphenomenon has been uncertain and hotly debated for over 50 years. Nathan Zvaifler's 1973 review, "Immunopathology of joint inflammation in rheumatoid arthritis," was a superb exposition on how RF might be important in the joint inflammation of RA[2]. Major pieces of evidence include the following:

1. In rheumatoid synovitis, immune complexes consisting of rheumatoid factor, IgG, and complement were abundant and formed large inclusions in polymorphonuclear leukocytes (PMN) and in type A synoviocytes (macrophages).
2. Ingestion of similar complexes was shown to induce cellular activation and to release lysosomal enzymes by PMN.
3. Small immune complexes that did not form precipitants, fix complement, or induce activation of leukocytes developed these proinflammatory capabilities when incubated with IgM RF.
4. In established RA (but not in early RA), synovial tissue was found to contain plasma cells synthesizing both IgM and IgG RF and forming self-aggregating, complement-fixing complexes in the absence of any exogenous antigen.
5. IgG fractions from the serum of patients with RA were shown to induce joint inflammation when injected into the same patient's asymptomatic knee joint, while similar IgG preparations from healthy individuals injected into the other asymptomatic knee joint had no effect.

From these and other observations it was proposed that RA could be viewed as an immune complex disease. In early disease, RF and other autoantibodies capable of forming immune complexes must be derived from blood, since plasma cells are absent in the synovium. In established disease, plasma cells are present in synovium, such that RF and other autoantibodies could be synthesized and form immune complexes *in situ*.

The biggest advance in the treatment of RA in the past 15 years has been the introduction of anti-tumor necrosis factor-α (TNF-α) therapy. Synovial macrophages appear to be the major source of TNF-α, but the driving force for TNF-α production by these synovial macrophages is not certain. One hypothesis to explain macrophage TNF-α production is that immune complexes ingested by synovial macrophages are responsible. If this is true, then RF-producing B cells might be an attractive therapeutic target. Based on this hypothesis, Edwards and colleagues proposed that B cell depletion (BCD) therapy might treat RA[3-5].

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Table 1. Evolving concepts for the pathogenesis of RA.

1.	Autoimmune disease mediated by immune complexes
	Discovery and characterization of rheumatoid factor (RF)
	RF-containing immune complexes in synovium and blood
	RF production by plasma cells in synovium
	RF as a risk factor for developing RA
2.	T cell responses to arthritogenic autoantigens
	Linkage to DR
	Collagen-induced arthritis and other animal models of autoimmune inflammatory polyarthritis
	T cell responses and autoantibodies to a variety of autoantigens found in joints
	Role of TH1, TH2, regulatory T cells, and interleukin 17-secreting T cells in animal models
	Linkage of RA to allele of PTPn22
3.	Abnormalities in cytokine network
	Predominance of macrophage and fibroblast cytokines in synovium
	Clinical success of treatment with TNF inhibitors and interleukin 1 receptor antagonist
4.	Abnormal phenotype of fibroblast-like synoviocytes (FLS)
	Anchorage-independent growth of FLS
	Invasion and destruction of cartilage when FLS were transplanted into SCID mice
	Mutations in p53 in FLS
5.	Citrullinated peptides as the target autoantigens in RA
	Autoantibodies to citrullinated peptides in RA
	Presence of citrullinated proteins in inflammatory synovitis
	Autoantibodies to citrullinated peptides as a risk factor for developing RA
	Linkage of RA to allele for arginine deimidase
6.	Autoantibody-mediated arthritis
	Passive transfer of erosive arthritis with anti-glucose-6-isomerase IgG in animal models
7.	Abnormal T cell phenotype in RA
	Increased expression of NKG2D, a stimulator coreceptor, by peripheral blood and synovial T cells, and increased expression of MICA/B, for one of the ligands for NKG2D, in RA synovium
	Accelerated aging of T cells (and myeloid cells) in patients with RA and in normals who are HLA-DR-0401
8.	Central role of B cells
	See item 1
	Animal experiments demonstrate an important role for B cells in autoimmunity even when B cells are engineered so they cannot secrete immunoglobulin
	B cell-dependent T cell activation in an animal model where RA synovial explants are transplanted into SCID mice
	Clinical success of B cell depletion therapy
9.	RA as a complex disease
	See items 1 to 8
	Heterogeneity in genetic predisposition and autoantibodies
	Heterogeneity in course
	Heterogeneity in response to therapy (including failure of 50% of patients to have a good response even with our best therapies)
	Changes in pathophysiology over the course of the disease due to epitope-spreading or accumulation of damage

A problem with testing this B cell depletion hypothesis was that conventional therapies affecting B cells, e.g., glucocorticoids and cyclophosphamide, are nonspecific. The development of rituximab as a therapy for B cell non-Hodgkin's lymphoma in the 1990s provided a B cell-specific reagent and thus an opportunity to test BCD therapy. Rituximab is a chimeric monoclonal antibody, with mouse variable regions and human constant regions, directed against human CD20, a cell-surface antigen only present on B cells. Treatment with rituximab leads to a profound and prolonged depletion of immature, naive, and memory peripheral blood B cells, but does not affect plasma cells, which are CD20-negative[6,7]. Thus, serum levels of protective antibodies such as those directed against tetanus toxoid and pneumococcal polysaccharides are not affected[8]. This lack of effect on plasma cells reduces the potential for toxicity, but raises the possibility that rituximab would also not affect the production of autoantibodies unless they were produced by short-lived plasma cells.

Edwards and colleagues initiated an open trial of BCD therapy and in 2001 presented data on 5 patients with refractory RA treated with this therapy at the American College of Rheumatology (ACR) meeting in Philadelphia[9]. BCD therapy consisted of high-dose steroids (100 mg intravenously at time of rituximab infusion plus 60 mg for the first week and 30 mg for the second week), cyclophosphamide (750 mg twice, 2 weeks apart), and rituximab (1 g twice, 2 weeks apart). The results were spectacular — all patients achieved an ACR50 response, and an ACR70 response was achieved in 4 of 5. Responses were long-lived, much longer than would be expected based on the short-term therapy with prednisone and cyclophosphamide. There was considerable excitement about these results, including headlines in the lay press about a cure for RA. Understandably, success in this very small open study was greeted with considerable skepticism. However, since then the results of several additional open studies, including Higashida, *et al* in this issue of *The Journal*[10], have supported clinical efficacy. Even more important, a randomized, double-blind, placebo controlled study has now been published providing strong evidence that B cell depletion therapy is effective for treatment of RA[1]. A second controlled trial has confirmed the efficacy of rituximab added to methotrexate and has found that neither the steroids nor cyclophosphamide are necessary[11,12]. With BCD therapy both RF and total IgM decreased significantly[13]. In contrast, IgG anticitrullinated peptide antibodies in serum decreased without a significant change in total IgG or protective antibodies, indicating that IgG autoantibodies can be selectively affected[13].

What does the success of rituximab in RA mean? It certainly suggests that B cells play an important and ongoing role in synovial inflammation, but just how they play this role is debatable. There are several mechanisms that could explain the success of rituximab; the 2 most likely are: (1) As hypothesized by Edwards, *et al*[1], BCD therapy may decrease levels of immune complexes in synovial tissue by eliminating memory B cells needed to regenerate short-lived autoantibody-secreting plasma cells, thence decreasing TNF-α production by macrophages. (2) An alternative

hypothesis is that rituximab may work by depleting the B cells that serve as antigen-presenting cells, thus decreasing T cell activation and T cell-dependent synovial inflammation. In support of this second mechanism are 2 brilliant studies: The first used mice engineered so B cells could express immunoglobulin on their surface but could not secrete immunoglobulin. This study established unequivocally in a mouse model of systemic lupus erythematosus that B cells play a role in the development of autoimmune disease beyond the production of autoantibodies, i.e., T cell activation was dependent on B cells, even when the B cells could not secrete autoantibodies[14]. The second study used human synovial tissue transplanted into SCID mice to show that elimination of synovial B cells by rituximab led to a profound and rapid decrease in the production of interferon-γ (a T cell derived cytokine) as well as TNF-α[15].

The success of rituximab in RA in these short-term studies suggests that we may have a new therapy with a distinct mechanism of action, but substantial questions remain to be answered. Anti-CD20 therapy appears to be well tolerated. However, there are very limited data on the longterm effects of rituximab in this population, and even less data on the longterm effects of repeated treatments. In addition, data on radiographic progression of disease have not yet been reported, and will be critical for assessing the effect of rituximab on damage. Until we know that anti-CD20 therapy halts erosions and loss of cartilage its use will probably be limited. On the other hand, a particularly tantalizing aspect of rituximab therapy for autoimmune disease is that for some patients the beneficial effects appear to be long-lived even without retreatment; these longterm responders raise the possibility that eliminating autoimmune memory B cells may reset the autoimmune response. Further studies on the immunologic changes associated with longterm responses will be of great interest. In addition, it is interesting that, as with other remittive agents, response to rituximab is heterogeneous. A substantial proportion of patients have a good response, i.e., ACR50, but many patients have a weak response or no response. What determines who responds and whether biomarkers predicting response to specific agents can be discovered are certain to be major areas of research.

It must be noted that besides rituximab other anti-CD20 monoclonal antibodies are being developed, and there are also agents that target B cells through different molecules, e.g., anti-CD22 and inhibitors of BLyS/BAFF. Trials with these additional agents are not as far along as with rituximab, but will be extremely interesting to follow.

The success of rituximab as a treatment for RA is a remarkable new twist in the continuing studies of this fascinating disease. For some it will seem that B cells are just now being discovered as important agents in the pathogenesis of RA; others will remember the importance of B cells recognized long ago and will be cheered by their long overdue rediscovery.

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Dr. Looney has conducted an open clinical trial of rituximab in SLE that was partially supported by Genentech and IDEC; he has also been paid to advise Genentech, IDEC, and Roche on the development of rituximab for SLE.

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