Crossroads of B Cell Activation in Autoimmunity: Rationale of Targeting B Cells

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ABSTRACT. B cells have historically been considered as not preferentially involved in the immunopathogenesis of inflammatory joint diseases, in particular rheumatoid arthritis (RA), despite the notion that autoantibodies and immune complexes were involved in pathogenesis and served as diagnostic and classification markers. Following initial reports that patients with non-Hodgkin's lymphoma (NHL) and coexisting RA showed improvement in signs and symptoms of RA after anti-CD20 therapy, the role of B cells in autoimmune diseases was reexamined. Potential mechanisms can be inferred from what is known about the role of B cell functions, in particular antigen-experienced memory B cells. Activation of these cells can be dependent on T lymphocytes or independent of them. Once activated, the cells can efficiently act as antigen-presenting cells, can produce inflammatory cytokines, and may alternatively differentiate into antibody-producing plasma cells. These processes contribute to the activation of other immune cells and ultimately to joint destruction in RA. The development and maintenance of RA may be related to both direct and indirect involvement of these B cell-dependent processes. In systemic lupus erythematosus (SLE), the central pathogenic importance of autoimmune B cells is well recognized, based on early recognition of numerous autoantibodies and clinically important immune complexes. Based on the evidence supporting B cell involvement in the pathophysiology of autoimmune diseases, investigations are evaluating the clinical impact of B cell targeted therapies. B cell targeted therapies in human trials include an anti-B lymphocyte stimulator protein agent, belimumab; an anti-CD20 agent, rituximab; and an anti-CD22 antibody, epratuzumab. (J Rheumatol 2006;33 Suppl 77:3-11)

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INTRODUCTION

The traditional model of rheumatoid arthritis (RA) describes a central pathogenetic role for CD4+ T cells and macrophages[1]. There is a growing recognition that RA is a more complex disease, with an enhanced activation of most locally present immune cells, including B cells. In this context, new molecular targets have been identified, leading to clinical development of novel therapeutic approaches. One avenue currently being more intensely explored is modulation of B cell activity, after early observations in the treatment of difficult autoimmune thrombocytopenia and autoimmune hemolytic anemia indicated a potential therapeutic role for B cell depletion in refractory patients[2].

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Accumulating evidence suggests that B cells play an important part in the pathology of RA. The usefulness of B cell depletion for treating RA demonstrates 2 major ideas: (1) RA does not seem to be solely dependent on T cells; and (2) B cells play a pathogenic role in RA that is more central than simply that of the precursor of producing autoantibodies. This review describes the role of B cells in the pathogenesis of rheumatic diseases, with a particular focus on RA, a disease not completely dependent on B cells but dependent on an important immunopathogenic role of these lymphocytes. Since the use of anti-CD4 therapy in RA permitted a more detailed insight into the role of T lymphocytes[3], application of B cell directed therapies will contribute to our understanding of RA immunopathogenesis; but we must bear in mind that the cause of this disease has not been identified.

IMPORTANCE OF B CELLS

B cells are an important component of normal immune response and are responsible for the maintenance of cellular and humoral protective memory[4,5]. B cells are directly and indirectly involved in the regulation of certain parts of immune activation and can ultimately differentiate into antibody-producing cells. Additionally, they are involved in the orchestration of inflammatory processes, and most importantly, they link innate, inborn, and acquired adaptive immunity by using specific receptors expressed on B cells.

During the last 5 to 10 years, it has become increasingly apparent that B cells are not simply precursors of antibody-producing or autoantibody-producing cells. One of the most important functions of memory B cells is antigen presentation. B cells are also involved in the regulation of T cell activation, anergy, and differentiation, and the expansion of T cells. Moreover, B cells regulate follicular dendritic cell differentiation and the organization of the lymphatic architecture[6]. B cells also produce proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6), and immunoregulatory cytokines such as IL-10. It will be important to discover the extent to which cytokines produced by B cells contribute to certain steps in immune activation. In this regard, B cells may also provide specific regulatory functions by producing certain cytokines, such as IL-10, that have a protective capacity[7]. B cells are also intimately involved in lymphoid organization, as demonstrated by specific mice that lack B cells and subsequently do not develop M cells in the gut[4-6] (Figure 1).

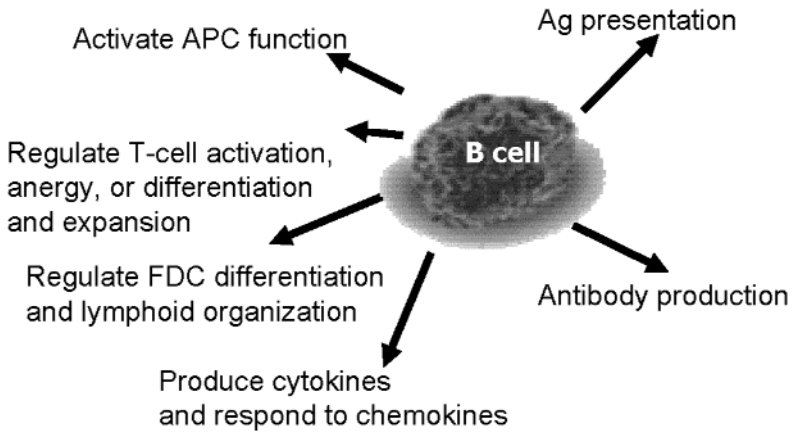

Figure 1. The various functions of B cells. APC: antigen-presenting cell; FDC: follicular dendritic cell. Adapted from Lipsky. *Nat Immunol* 2001;2:764-6[4], with permission.

HUMORAL IMMUNITY AND IMMUNOLOGIC MEMORY

Humoral immunity appears to be based on immunologic memory provided by memory plasma cells, which secrete protective antibodies, and by memory T cells and B cells, with the latter reacting to antigen challenge by differentiating into plasma cells. How the 2 differentiation pathways of B cells memory or plasma cells relate to each other, how cells are selected into these memory populations, and how these populations are maintained remain enigmatic, although recent studies have shown that the expression of B lymphocyte-induced maturation protein-1 (Blimp-1) is restricted to plasma cell lineage[8].

Recent clinical trials using B cell depletion in RA support the concept that humoral immunity, as evidenced early by the production of rheumatoid factor (RF) and anti-citrullinated peptide (anti-CCP) antibodies, plays a significant role in RA. Another end product of B cell differentiation may be the formation of immunocomplexes that maintain rheumatoid synovitis.

Fresh insights into humoral autoimmunity suggest that B cell activation via Toll-like receptors (TLR) can occur in the absence of the cognate antigen of the B cell receptor[9,10]. Despite limited knowledge of the B cell memory compartment, clinical experiences show that autoantibody-producing plasma cells have different biologic properties, since those differ in their correlation to disease activity and respond differently to conventional therapies. The distinction between short-lived plasma cells and long-lived plasma cells may therefore also define response to therapy, as recently shown in a lupus model[11]. What is not well understood is the mechanism by which B cells differentiate from naive into memory or plasma cells under the influence of antigen and antigen-presenting cells and with the help of T cells. Recent evidence in the mouse suggests that memory B cells also have different lifespans[12].

T CELL ACTIVATION IN RHEUMATOID SYNOVIUM IS B CELL-DEPENDENT

An important series of mouse studies by Takemura and colleagues has demonstrated that T cell activation in RA is B cell-dependent[13,14]. T cell-driven inflammation in the synovial membrane of RA is frequently associated with the formation of tertiary lymphoid structures (lymphoid follicles with germinal centers, GC). Data suggest that recruitment or *in situ* maturation of follicular dendritic cells, which are exclusively present in GC, may be a critical factor for synovial membrane GC formation[14]. Primary follicles are absent, emphasizing the role of anti-

gen recognition in the development of inflammation-associated lymphoid neogenesis that is consistent with molecular data of local B cell differentiation[15]. A striking dependence of T cell activation on B cells was confirmed in B cell depletion studies in a preclinical model[13]. Treatment with anti-CD20 antibodies that deplete B cells resulted in the destruction of these extrafollicular GC and in a complete loss of the dendritic follicular cell networks. The other interesting result was the disruption of T cell activation. These data support the idea that B cells have a central role in synovial inflammation when GC are involved in the pathogenesis.

EARLY ANTIBODY FORMATION IN RA

Antibodies against immunoglobulin G (IgG), IgM-rheumatoid factor (IgM-RF), and antibodies against CCP are the autoantibodies most frequently found in patients with RA. Since these autoantibodies represent a breakdown of immune tolerance, they indicate a common mechanism leading to the development of autoreactive plasma cells on the basis of precursor autoreactive B cells.

RF as an IgM molecule is an efficiently complement-activating antibody, contributing to synovitis and to loss and destruction of cartilage. RF testing is commonly used in clinical practice as an indicator of humoral imprinting, reflecting the breakdown of immune tolerance of B cells; however, the specificity of RF testing is low. The introduction of tests for anti-CCP antibodies that have a higher specificity for RA especially in combination with RF (as high as 98%) has expanded diagnostic options[16]. Anti-CCP testing is useful for diagnosing RA in patients with early synovitis, as well as for differentiating RA from other inflammatory arthritides and other connective tissue diseases, such as SLE. The presence of RF and/or anti-CCP was observed in patients tested years prior to the onset of the disease, indicating that the breakdown of immune tolerance in the B cell system is a very early step in the disease pathogenesis. IgM-RF and anti-CCP tests have proven to be very helpful in the early detection of RA[17]. However, the apparent discrepancy is that these humoral disturbances are currently insufficient for clinical decisions prior to the onset of disease; therefore, further studies are needed to identify a more comprehensive profile of patients at risk[18].

POTENTIAL ROLES OF B CELLS IN THE IMMUNOPATHOGENESIS OF RA

B cells appear to be important in the immunopathogenic activity seen in patients with RA. At least 4 different processes or specific roles can be identified as to how B cells are involved in the pathogenesis of RA[13,19-23] (Figure 2). These 4 mechanisms may contribute to different degrees in distinct diseases where B cells are involved in the pathogenesis, although this has not been proven.

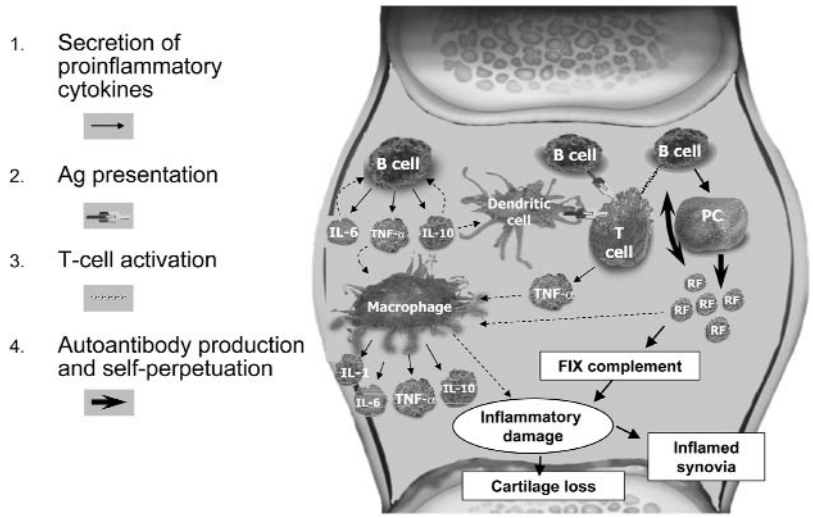

Figure 2. Potential roles of B cells in the immunopathogenesis of RA. Their precise contribution has yet to be elucidated; however, a number of researchers have proposed various mechanisms in which B cell involvement is implicated[1-4]. From Shaw, et al. *Ann Rheum Dis* 2003;62 Suppl 2:ii55-ii59, with permission.

B cells produce proinflammatory cytokines, including TNF-α and IL-6. These cytokines may then activate macrophages, amplifying the proinflammatory signal and resulting in enhanced levels of IL-1, IL-6, and additional TNF-α. IL-6 itself is an important cytokine for B cell growth and may act in an autocrine fashion. The differentiation of B cells into plasma cells allows subsequent production of RF and anti-CCP antibodies and local production of immune complexes. All the above mechanisms appear to contribute to inflammatory damage. The other important cytokine produced by B cells is IL-10. IL-10 activates dendritic cells by enhancing antigen presentation, and with the help of T cells, further allows the differentiation of B cells into plasma cells. B cells are also important in T cell activation and vice versa. This is supported by recent studies in patients with autoimmune disease that demonstrated a deactivation of T cells after selective B cell depletion[24,25]. Another important aspect that has been neglected is antigen presentation by B cells. These pathogenic roles of B cells in the development of RA may also apply to other autoimmune diseases, including SLE.

MECHANISMS OF T CELL-DEPENDENT ACTIVATION OF B CELLS

The involvement of T cells in RA supports the notion that B cells are activated by T cells and vice versa. In the affected organs (i.e., the synovium), it was demonstrated that B cell activation and post-recombination processes of the B cell receptor contribute to the development of affinity and maturation of the B cell receptor[15,26,27]. Most of the post-recombination processes, such as somatic hypermutation and Ig class switching, have been considered to be dependent on the interaction with T cells using CD40-CD40L ligation[28]. In this context, conglomerates or aggregates of CD20+ B cells can be identified in a substantial number of patients with RA[15]. These B cells are surrounded by T cells and form GC-like structures in RA. The degree and architecture of infiltration of the synovial membrane can be differentiated based on the intensity of inflammation or infiltration. In some patients, there is significant infiltration and lymphoid follicular structures are present. In others, there is only moderate infiltration or even slight infiltration of these cells[29]. This is observed not only in patients with RA but also in the kidneys of patients with SLE[30]. It has also been described in patients with Sjögren's syndrome and in patients with myasthenia gravis, and in the central nervous system of patients with multiple sclerosis[31-33]. The presence of extrafollicular centers suggests a similarity among these different autoimmune diseases, indicating that B cell differentiation into memory and plasma cells occurs in the inflamed organs.

MECHANISMS OF T CELL-INDEPENDENT ACTIVATION OF B CELLS

Recent research in B lymphocyte stimulator (BLyS), also known as B cell-activating factor (BAFF), has added to the understanding of T cell-independent activation of B cells. Human and *in vitro* studies of severe immunodeficiency have demonstrated dependency on transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) and its ligand BAFF for Ig class switching, a classical mechanism considered to be under strict T cell dependency[34-36]. This indicates that post-recombination can occur independently of direct involvement of T cells. Since BAFF levels in autoimmune diseases, including RA, are often remarkably increased and these patients tended to have enhanced B cell activity including hyperimmunoglobulinemia, there is a possibility that some of the disturbances of B cells occurring in autoimmunity may be T cell-independent[34,35] (Figure 3). As a consequence, targeting B cells would elaborate on a common denominator of both pathways.

Activation of B cells can also occur in the absence of T cells by crosslinking immune complexes to B cell receptors. The important issue with regard to BAFF and apoptosis-inducing ligands, which belong to the TNF-receptor ligand family, is that when they are expressed they lead to the survival of plasma cells. This may lead to a cycle of continuous antibody-mediated inflammation and tissue destruction. There is also Ig class switching in the absence of T cells[37]. Further along in the differentiation process, the BAFF receptor 3 is also involved in promoting B cell survival by allowing alternative B cells to emerge under enhanced levels of BAFF. Fibroblast-like synoviocytes of mesenchymal origin express functional BAFF of the TNF family in response to proinflammatory cytokines in the synovium of patients with RA[38].

This is important because BLyS has been found to be elevated in patients with RA, SLE, and Sjögren's syndrome[23] (Figure 4). There is a higher level of BLyS in the synovial fluid than in plasma, indicating that B cell differentiation is taking place locally and that BLyS appears to be one of the driving forces. Most recently, fibroblast-like synoviocytes have been shown to produce BLyS in the RA synovium, in contrast to synovium from controls[38]. The same study showed that BAFF production in these cells can be enhanced by the proinflammatory cytokines TNF-α and interferon gamma (IFN-γ) and increased B cell survival. This clearly substantiates the close interrelation of different cells, mechanisms, and certain signals in the orchestration of synovial autoimmunity.

Another mechanism by which B cells can be activated in the absence of T cells is by TLR. They are also known as "pathogen-associated molecular pattern receptors" or

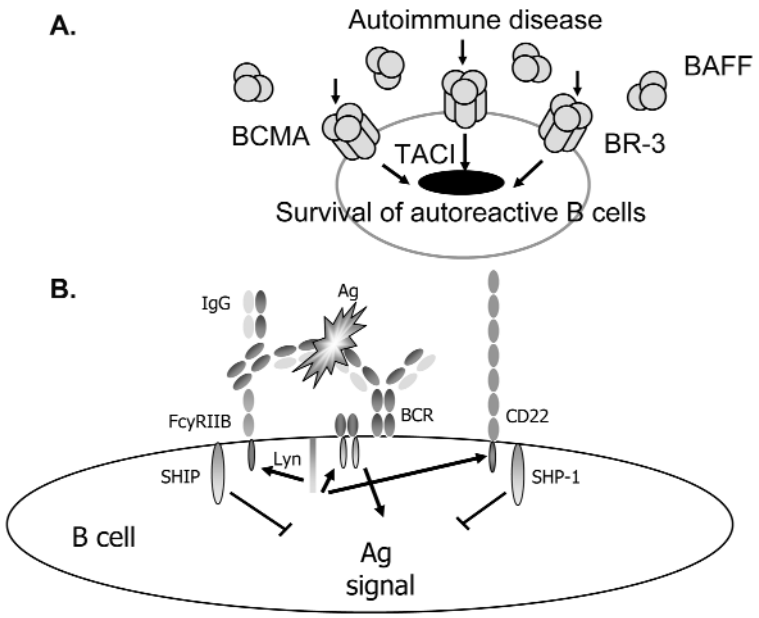

Figure 3. T cell-independent B cell activation by unique receptors on B cells. A. Activation by BAFF and its 3 receptors. B. Activation by cross-linking BCR and FCγIIB receptor. Ag: antigen; BCR: B cell receptor; SHP-1: SH2-containing inositol phosphatase; SHIP: Src homology-2 domain-containing inositol polyphosphate 5'-phosphatase. From Ohashi, et al. *Curr Opin Immunol* 2002;14:744-59, with permission.

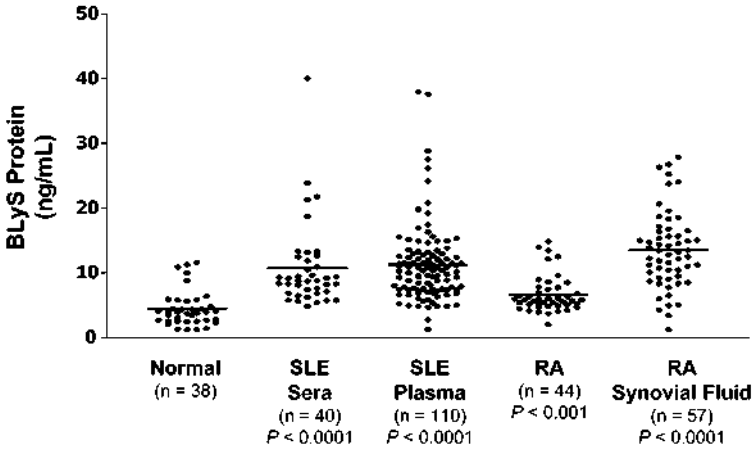

Figure 4. BLyS is elevated in patients with autoimmune disease. BLyS: B-lymphocyte stimulating protein; SLE: systemic lupus erythematosus; RA: rheumatoid arthritis. From Zhang, et al. *J Immunol* 2001;166:6-10[23], with permission.

"pattern recognition receptors" and are expressed by nearly every cell in the body. TLR-7, TLR-8, and TLR-9 are the most important of these with respect to B cells. Bacterial DNA is the natural ligand of TLR-9, and single-stranded RNA is the ligand of TLR-7 and TLR-8. All 3 receptor-ligand interactions lead to activation of B cells in a nuclear factor-κB-dependent activation pathway[9,10]. As a result, B cells can differentiate into (auto) antibody-producing plasma cells or produce proinflammatory cytokines and chemokines[10].

An enhanced frequency of peripheral plasmablasts has been observed in adults and children with active SLE; however, a relative predominance of CD27+ memory B cells has only been identified in adults[39,40]. This represents a characteristic abnormality in SLE and provides a reliable marker of disease activity independent of the presence of anti-DNA antibodies[41].

PROPER SELECTION OF B CELLS AFTER DIFFERENTIATION AND AFFINITY MATURATION

Under normal conditions, the processes of recombination and post-recombination to enhance the affinity of the B cell receptors are under tight control in the bone marrow and subsequently in several lymphoid organs, which protects the body from autoimmunity. Several processes can delete or anergize lymphocytes with self-reactive receptors on their surfaces[42]. Thus, there is receptor revision or simple deletion of these cells. Moreover, intrinsic and extrinsic regulatory pathways can be activated that inhibit the expansion or emergence of these cells. In terms of autoimmunity, as long as no convincing data are available, it is possible that extrafollicular GC, as compared to classical lymphoid organs, may not provide sufficient strict selection of emerging B cells in these structures; and alternatively, T cell-dependent and T cell-independent B cell activation pathways may differ in the organization of B cell receptor selection.

Abnormalities in the composition of B cell subsets in the peripheral blood of patients with RA have also been observed, including enhanced frequency of peripheral blood memory B cells[43,44]. Such an expansion of memory cells, despite the more characteristic enhanced CD27 high-plasma cells, also occurs in patients with SLE[39,40,45].

EVOLVING THERAPEUTIC TARGETS

The introduction of anti-TNF therapies has set new standards for the treatment of RA, in particular for patients with RA with an inadequate response to methotrexate or other disease-modifying antirheumatic drugs. The initial breakthrough of biologic immunotherapies in RA has stimulated a broad interest in additional distinct treatment approaches. All these new approaches in RA treatment will need to provide a comparison of conventional and anti-TNF therapy, including the slowdown of radiologic progression or the need to offer a substantial improvement for inadequate responders.

Investigative treatment strategies now focus on inhibiting the inflammatory and destructive aspects of the disease. Identification of new molecular targets, including cell subsets, receptors/ligands, chemokines, and cytokines that contribute to inflammation and damage, has opened up other intervention possibilities in addition to antibodies against TNF-α and IL-1[46] (Figure 5).

Blockade of costimulatory molecules through blocking CD40/CD40L, ICOS-ICOS-L interactions, or CD28 has been explored at different developmental stages. CD40 binding to CD154 (CD40 ligand) is perhaps the most important costimulatory signal on B cells, which induces activation, proliferation, and Ig class switching. Studies in patients with SLE were hampered by the observation of vascular complications or failure to achieve the primary endpoint[47,48]. It is unlikely that this avenue will be explored further.

Inhibition of cytokines through BAFF blockade is another possible target for intervention. As discussed, BAFF has been important for the survival of human plasmablasts. A fully human IgG1λ monoclonal antibody that neutralizes human BLyS is in early-phase investigations for SLE as well as RA.

IL-1 is a proinflammatory cytokine abundantly found in the synovium of patients with RA. Its proinflammatory effects include induction of IL-6 and cyclooxygenase 2. Its deleterious actions include stimulation of bone and cartilage resorption and inhibition of proteoglycan and articular collagen. IL-6 is another potential target because it regulates acute-phase proteins and activates osteoclast-induced bone resorption. An IL-6 receptor antagonist (MRA) blocking the effect of soluble and membrane-bound IL-6 is being investigated currently in clinical trials for RA and SLE.

By exploring specific surface molecules, antibodies against inflammatory cells have also been investigated. Antibodies to T cells have shown equivocal results in early clinical settings and need further development[49]. Costimulatory modulation of T cells by CTLA4-Ig, a molecule that blocks the interaction between the B7 receptor on antigen-presenting cells and CD28, shows promise in the treatment of inadequate responders to anti-TNF.

Since B cell activation in the pathogenesis of various autoimmune disorders is important, it is not surprising that another target for therapeutic intervention involves B cell depletion or inactivation. One possibility is to target B cells using antibodies to CD20. CD20 is expressed by almost all B cells, with 2 major exceptions (the pro-B cell and the plasma cell). B cell receptor modulation or regulation of B cell activation may also be achieved through ligation of TLR using certain activating or

inhibitory oligonucleotides that need further evaluation. A humanized antibody directed against CD22 (epratuzumab) has been explored in patients with SLE in a Phase IIa trial that provided a favorable safety profile and an early indication for efficacy (Dörner, unpublished observation) and that resulted in ongoing Phase III lupus trials.

EARLY OBSERVATION OF EFFECTIVE ANTI-CD20 THERAPY IN RA

The earliest observations of the potential for anti-CD20 therapy in RA came from case reports of patients with lymphoma who were treated with rituximab and who anecdotally had improvement in concomitant RA symptoms[50,51]. It is well known that patients with RA with high disease activity are at greater risk of NHL, indicating the important role of activated B cells in the pathology of RA[52]. As reviewed elsewhere in this supplement[53], rituximab is being formally evaluated for its role in the treatment of RA.

Recent data show that the repletion of B cells after anti-CD20 therapy is characterized by modulation of B cell subset composition, with a preferential recurrence of naive B cells and a delay in the recurrence of memory B cells[54]. Thus, it might be possible to "reset" the immune system in those patients who positively responded to B cell depletion. Similar observations of recurring naive lymphocytes in relation to successful treatment have only been seen so far after drastic autologous stem cell transplantation in SLE[55].

One concern about B cell depletion has been the consequence of depletion on protective antibody titers. Emery and colleagues have shown that, with rituximab therapy, tetanus antibody titers remained stable, even as RF decreased along with a decline in IgM[56]. This finding of RF decline was more apparent in patients treated with the combination of rituximab and methotrexate.

SUMMARY AND PERSPECTIVE

B lymphocytes have been shown to be precursors of antibody-producing plasma cells and an important component of the immune system involved in antigen presentation, T cell activation, and cytokine production. Extrafollicular germinal center-like structures and disturbances of peripheral B cell homeostasis appear to be a characteristic in patients with RA and SLE. GC-like structures of B cell differentiation often reside in the affected/inflamed organs and this results in generation of memory B cells and (autoreactive) plasma cells. Immune activation of B cells, in particular memory B cells, includes T cell-dependent and T cell-independent B cell activation pathways that play a central role.

B cell directed therapy allows targeting of T cell-dependent and T cell-independent B cell immune activation and represents an important therapeutic approach for patients with RA, especially in those with inadequate response to anti-TNF therapy who otherwise will be subjected to very limited and often experimental therapies. The major challenge of the future will be to determine the most powerful and appropriate place of B cell depletion in RA treatment, in particular at which stages of the disease this strategy offers the most benefit to our patients.

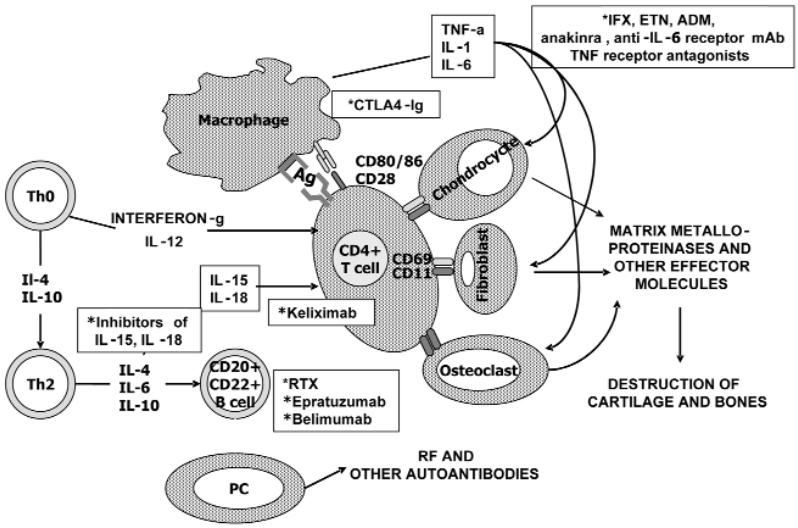

Figure 5. Major cell types and pathways of potential therapeutic targets. Adapted from Taylor. *Curr Opin Pharmacol* 2003;3:323-8[46], with permission.

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