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B cells in the pathogenesis and treatment of rheumatoid arthritis

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Abstract

Purpose of review—Our understanding of the multiple physiological and pathogenic functions of B cells in rheumatoid arthritis continues to expand. In turn, the availability of effective agents targeting the B cell compartment increases. In this review, we discuss novel insights into the roles of B cells in RA and recent evidence regarding the efficacy of B cell depletion and biomarkers of treatment response.

Recent findings—Recent data has further elucidated the requirements for the generation of ectopic lymphoid structures in the rheumatoid synovium, their frequency, and role in pathogenesis. Additional studies have described the phenotype of infiltrating B cells in the synovium and the unexpected role for B cells in bone homeostasis. In addition to pathogenic roles for B cells, there is also mounting evidence for regulatory B cell subsets that may play a protective role. New data on radiographic progression, efficacy in early disease, the role of re-treatment, and biomarkers of treatment response continue to refine the role of B cell depletion in the treatment armamentarium.

Summary—The past few years have seen new advances in immunology applied to the study of RA with surprising observations and interesting new insights into etiology and pathogenesis.

Keywords

B cells; rheumatoid arthritis; rituximab

Introduction

Rheumatoid arthritis (RA) is a systemic auto-inflammatory disorder manifested by aggressive synovitis that over time causes bone, tendon and cartilage damage. While different cell types play pathogenic roles in RA, a prominent participation of the B cell has long been appreciated since the discovery of rheumatoid factor and has been re-highlighted over the past several years. Thus, rheumatoid factor (RF) and anti-cyclic-citrillunated peptide (anti-CCP) autoantibodies are well-established indicators of disease and disease severity and may precede the onset of disease by many years. Recently elucidated novel roles for autoantibodies in RA include the amplification of tissue injury by antibodies against citrullinated proteins in collagen-induced arthritis in mice [1], the demonstration that arthritogenic antibodies can activate mast cells and induce RA-like disease in K/BxN mice at least in part through the production of TNFα and IL1 [2], and the ability of immune complexes to activate RF-specific B cells by the synergistic engagement of the B cell receptor and toll-like receptors [3].

Although B cells have been considered important as producers of autoantibodies, their antibody independent roles and utility as a major therapeutic target have not been appreciated until more

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recently. In this review we shall discuss the most relevant biological and pathogenic functions of B cells in RA with a focus on new insights over the past year and the therapeutic benefit and mechanisms of B cell depletion.

Novel insights into patho-physiological functions of B cells in RA

The ever-expanding autoantibody independent role for B cells in the disease process, including cytokine secretion, antigen presentation, and the organization of other inflammatory cells, are discussed further below.

Ectopic lymphoneogenesis

B cells may provide a critical link between the development of tertiary lymphoid tissue within the inflamed synovium (ectopic lymphoneogenesis) and the propagation of the autoimmune process. This contention has been supported by the finding of germinal center (GC) like structures within the inflamed RA synovium and the profound effect of B cell borne lymphotoxin (LT) α on lymphoid architecture. A particularly provocative example of the central participation of B cells in the pathological process taking place in tertiary lymphoid tissue is the demonstration that CD4 T cell activation in the rheumatoid synovium is dependent on the presence of B cell follicles and that the depletion of B cells in this model inhibits the T cell production of IFNγ and IL-1 [4]. However, the precise requirements for the generation of these lymphoid structures, their frequency, and role in the pathogenesis of RA have remained unclear. A recent ambitious study led by Baeten, Tak, and colleagues provided surprising evidence that synovial lymphoid neogenesis is a dynamic process related to the degree of inflammation as opposed to the specific autoimmune process in RA [5]. Ectopic lymphoid structures were found in 30% of RA patients but were notably also observed in spondyloarthritis and osteoarthritis, albeit at a lower frequency. In RA, progression to full-blown GC reactions (defined by the presence of follicular dendritic cells) was rare (only 2 of 35 samples), and consistent with this finding the authors were unable to detect antigen-driven clonal expansion and affinity maturation of B cells in a smaller number of RA samples analyzed (n=8). These results are surprising and distinct from previous studies which found a higher frequency of GC-like structures (on the order of 20%) [6]. Moreover, it has previously been shown that B cells in synovial aggregates undergo affinity maturation and somatic hypermutation [7,8], suggesting a role for these ectopic structures at least in the amplification if not initiation of the autoimmune response. Possible explanations for the discrepant findings include the selection of distinct stages of disease and the use of different definitions of GC reactions.

In accord with the latter, another recent paper found that 50% of RA synovial samples had functional sites of ectopic lymphoneogenesis based on quantitative PCR identification of activation-induced cytidine deaminase (AID), the enzyme required for somatic hypermutation and class-switch recombination, and the detection of abundant anti-CCP antibody producing plasma cells [9]. Overall, the combined data suggests that ongoing GC reactions may actually be considerably more common than has previously been appreciated in RA, although not necessarily specific to the autoimmune process.

The factors controlling the generation of ectopic lymphoid structures have been the subject of much study [6,10]. Indeed, the study by Baeten and Tak found abundant synovial expression of CXCL13 (a major B cell attractant), as well as CCL19 (a T cell attractant), and BAFF (B cell activation factor), with higher expression in RA than other arthritides. CXCL13 can be produced by synoviocytes and follicular dendritic cells in the RA synovium and was recently also found to be secreted by RA synovial T cells, indicating a direct role for both the inflamed synovial stroma and synovial T cells in the recruitment and organization of B cells [11]. A gene expression analysis study of RA synovial tissues confirmed prior reports that lymphoid

aggregates are associated with elevated expression of CXCL13, CCL21, CCR7, and LTα and LTβ. Additionally, they observed enhanced expression of CXCL12 and CCL19 and the associated receptors CXCR4 and CXCR5, chemokine-chemokine receptor pairs important for the attraction of B cells, T cells, and dendritic cells [12]. Activation of the IL7 pathway was also seen, a notable finding given that IL7 receptor signaling is essential for inducing LTα1β2 on lymphoid inducer cells. This stimulates the LTβ receptor on lymphoid organizer stromal cells, leading to the production of critical chemokines such as CCL19, CCL21, and CXCL12.

Evidence for altered B cell activity in RA

Other recent studies have characterized B cell phenotypes in both the peripheral blood and synovium of RA in more detail. The results have not been entirely consistent, perhaps because of variability in disease phenotypes and therapy. For example, we have reported that, in contrast to systemic lupus, a disease that is characterized by profound alterations in peripheral blood B cells, RA patients tend to have similar peripheral blood B cell profiles to healthy controls. Interestingly, we did find that RA patients on anti-TNF (etanercept: TNF receptor-Ig p75 decoy that binds both TNF and LTα) display a paucity of follicular dendritic cell networks and germinal center (GC) structures in lymphoid tissue accompanied by a peripheral blood memory B cell lymphopenia. This suggests for the first time in humans that anti-TNF treatment may disrupt GC reactions at least in part via effects on follicular dendritic cells. Moreover, the efficacy of these drugs could be in part mediated by their anti-B cell effects [13]. Treatment with either etanercept or anti-TNF monoclonal antibodies (adalimumab or infliximab) has been associated with a decrease in ectopic lymphoid structures in the synovium that correlated with good clinical response [14].

Souto-Carneiro and colleagues recently described an increased frequency of post-switch CD27 +IgD- peripheral blood memory B cells in RA patients with longer disease duration compared to shorter disease duration or normal controls [15]. They also noted an increase in the frequency of pre-switch memory B cells in the peripheral blood of RA patients after anti-TNF. Interestingly, the majority of synovial B cells in RA expressed CD27, prompting the authors to suggest that trafficking of memory B cells into inflamed tissue is regulated by TNF, although changes in memory B cell generation or maintenance are alternative possibilities.

An unexpected role for B cells in bone homeostasis

Interestingly, in RA ectopic lymphoid follicles can develop not only in the synovium but also in other locations that can be affected by the disease process such as the lung [16]. Moreover, there is an evolving literature that lymphoid neogenesis may occur in bone marrow [17–19]. In particular, lymphoid aggregates have been described in subchondral bone marrow (BM) adjacent to the joint in RA, with evidence of high endothelial venules (HEVs), follicular dendritic cell networks, and CXCL13+ cells. Bugatti and colleague described this local inflammatory process associated with bone erosions and osteoclast recruitment, implicating the bone compartment in ongoing joint damage [17].

Schett and colleagues have further demonstrated that B cells dominate these BM aggregates to a dramatic extent even compared to synovium, with molecules involved in B cell chemotaxis, homing and activation up-regulated (including CXCL13, CCL21, BAFF) and evidence of increased osteoblasts (bone-forming cells) and osteoclasts (bone-resorbing cells) [18,19]. However, in contrast to Bugatti et al., they suggest a putative protective role for these B cell infiltrates due in part to a shift in the balance of bone homeostasis toward osteoblasts. Along these lines, B lymphocytes in these lesions express bone morphogenetic protein (BMP)-6 and -7, which are important stimulators of new bone formation. In a recent paper, this same group explored the effect of deletion of Btk, an essential molecule for B cell development, on arthritis

in TNF-transgenic mice. TNF transgenic mice deficient for Btk display low B cell counts and serum immunoglobulin but still develop synovial inflammation [20]. Interestingly, bone marrow infiltrates were significantly diminished in these mice, leading to impaired bone formation and an increased invasion of synovial inflammatory cells into the bone marrow, again suggesting a protective role for these B cells.

Overall, these provocative studies suggest a previously unappreciated role for B cells in regulating bone homeostasis. Whether B cells contribute to joint damage versus protection from erosions is unclear but may depend on the developmental stage and activation state of the predominant B cell subset present. Certain subsets of memory B cells have recently been described to express RANKL [21], a key cytokine that regulates bone homeostasis and the balance between osteoblasts and osteoclasts towards osteoclastogenesis. On the other hand, B cell precursor populations can be a prime producer of osteoprotegerin (OPG)- a soluble decoy receptor of RANKL and a potent physiological inhibitor of osteoclastogenesis [22], as well as BMPs.

Cytokine production

The ability of B cells to produce RANKL versus OPG under different conditions highlights the critical role of B cells as secretors of cytokines, potentially in a polarized fashion. Thus, B cells are the main producers of LTα and an important producer of TNF, both cytokines that as previously discussed play central roles in lymphoid development and organization. A novel function of B cells in promoting lymphangiogenesis and lymph node expansion in response to immunization has also recently been described via the expression of VEGF-A (vascular endothelial growth factor-A) [23]. This is intriguing given the long appreciated presence of angiogenesis in the RA synovium. B cells can also secrete multiple other cytokines [24], including IL-1, IL-4, IL-6, IL-8, IL-7, G-CSF, GM-CSF, IL-10, IL-12 and TGFβ [24–26], and may do so in a polarized fashion similar to Th1/Th2 cells [27,28]. For example, Bar-Or and colleagues have found that naive human B cells tend to produce pro-inflammatory cytokines (IL-6, TNF) and LT in response to BCR/CD40 and anti-inflammatory cytokines (IL-10) in response to CD40 alone [29]. In contrast, memory B cells have a greater tendency to produce pro-inflammatory cytokines. It has been proposed that TNF secreting CD27+ memory B cells accumulate in the salivary glands in primary Sjogren's syndrome and also may contribute to multiple sclerosis (MS) [29]. Given the accumulation of memory B cells in the RA synovium, it is interesting to speculate a similar cytokine secreting function in this disease.

From an autoimmunity standpoint, cytokine production by B cells may either stimulate or inhibit pathogenic responses. Thus, B cells are able to suppress autoimmunity in different animal models either through the production of IL-10 or TGFβ as well as by cytokine independent functions. Collectively, these observation are starting to coalesce into the important concept of regulatory B cells (Breg) [30]. Mauri and colleagues have demonstrated that a transitional-marginal zone precursor B cell subset is protective against murine inflammatory arthritis via the production of IL-10 [31]. In a murine autoimmune diabetes model it has recently been shown that B cell depletion therapy may lead to prolonged remission through the expansion of both T regulatory and B regulatory cells [32]. In human disease, Bar-Or and colleagues have found that IL-10 production was impaired in peripheral B cells of patients with active MS, a defect that was reversed after B cell depletion therapy [33]. Whether this is due to a shift in the predominant B cell subset present or a functional change in the cytokine secreting capacity of the B cell compartment is unclear. Moreover, the relevance of these findings to human RA remains to be delineated.

New insights into B cell depletion in RA

Initially based on the idea that RF-producing B cells could perpetuate themselves and induce production of TNF, Edwards and Cambridge first hypothesized that B cell depletion could have a beneficial impact in patients with RA. Three large randomized trials subsequently paved the way for approval of rituximab in TNF refractory disease [34] [35] [36]. These cohorts have now been followed in open-label extension studies, and as these and other trial and registry data accumulate, the role of rituximab in the armamentarium of the rheumatologist continues to be refined. In particular, recent data has demonstrated that rituximab reduces radiographic progression and has efficacy even in early disease. Our understanding of long-term safety, retreatment, and use along with other biologic agents also continues to be elucidated. Finally, critical insights regarding biomarkers of responsive patient groups and treatment response are beginning to emerge.

Clinical usage

Rituximab has recently been shown to benefit many patients with early disease as well as long-standing disease or that resistant to other biologic agents. Results from the IMAGE study, which included 748 patients without prior use of either biologics or methotrexate, were recently reported. In this group, high risk patients who had baseline high DAS28 scores or high CRP were found to have greater DAS28 improvement at week 52 if they received rituximab in addition to methotrexate [37].

Like other effective treatments for RA, rituximab therapy also reduces radiographic progression. REFLEX protocol patients, who had established disease resistant to other therapies, were followed for at least 2 years, and radiographs of wrists and feet were compared at week 56 to those from baseline. Patients in the original intention-to-treat rituximab group had significantly less radiographic progression at week 56 [38]. To evaluate patients earlier in the disease course, the IMAGE study included patients naive to both rituximab and methotrexate, and those on the combination had less radiographic progression at week 52 than those on methotrexate alone [37]. Radiographic changes several years into therapy have yet to be determined, and no direct comparisons with other biologic therapies have been made.

Currently, rituximab is generally used clinically after failure of at least one TNF antagonist as well as traditional DMARDs. In a prospective cohort study of over 300 patients who switched from an anti-TNF agent to either rituximab or a different TNF antagonist, those who switched due to lack of effect of the first agent tended to do better on rituximab than an alternate TNF agent [39]. Another question of clinical interest is whether anti-TNF agents may be safely and effectively used after failure of rituximab, a potential concern given the long-lasting duration of B cell depletion. Of 2578 patients who were treated with rituximab as part of one of several clinical trials, 158 subsequently received another biologic therapy, largely TNF inhibitors (81%). Of these, 88.6% had peripheral B cell depletion (CD19+ $\leq$ 80 cells/μl) when they received the next biologic therapy. The overall rate of serious infections prior to (median interval 7 months: 6.63 events per 100 pt years) and after receipt (mean follow-up of 11 months: 4.93 events/100 pt yrs) of the subsequent TNF inhibitor was similar to prior trials of patients on rituximab without another biologic agent (4.31 events per 100 patient-years) [40]. While this is an observational study only, it does suggest that serious infections may not be significantly more common in patients who take a biologic agent after B cell depletion.

Taking this concept a step further, there have now been two trials of concurrent use of rituximab with TNF antagonists. In one small study, eighteen patients received standard rituximab therapy after inadequate response to traditional DMARDs and anti-TNF, and six of these who did not initially respond adequately to the first cycle of rituximab after 2 months were started on etanercept in addition. After another two months, patients treated with combination therapy

demonstrated improved clinical and serological parameters, and after a mean of 18.5 months, there were no serious infections in these patients [41]. A second study, presented in abstract form, also examined combinations of either etanercept or adalimumab and rituximab therapy. Fifty-one patients with active disease despite stable doses of anti-TNF agents and methotrexate were randomized to receive either 500 mg of rituximab (33 patients) or placebo (18 patients) on days 1 and 15. Non-serious infections were comparable between the two groups. At week 24, patients treated with combination therapy were more likely to have an ACR 20 response (30% vs 17%) or ACR 50 response (12% vs. 6%) than those on anti-TNF and methotrexate therapy alone [42]. While neither of these were large studies and long-term safety issues still need to be explored, the potential for increased efficacy using two biologic agents with differing mechanisms of action is appealing.

Biomarkers of response to B cell depletion

Biomarkers to assess the need for re-treatment and even stratify patients into those likely to respond to B cell depletion in the first place are sorely needed. Seropositive patients were found to do better on rituximab in early studies, and recent data continues to support this finding. Of 424 patients in the German RABBIT registry who were observed for at least 6 months after the start of rituximab treatment, there were significantly better outcomes in patients who were RF positive, and a non-significant benefit in those who were anti-CCP positive [43]. Similarly, data from the CERRERA registry, which contains information from 10 European registries including data from 1372 patients treated with rituximab, demonstrated that RF positive patients were more likely to have a better DAS28 improvement at 3 and 6 months [44]. Seropositivity for rheumatoid factor or IgG anti-CCP antibodies, along with a CRP >2.9 mg/dL, was also associated with better ACR50 response in both the REFLEX and SERENE cohorts [45]. Novel predictors of improved responses to rituximab have recently been reported, including lower levels of type I interferons [46], lower serum BAFF levels [47], and a favorable FcγRIII genotype [48].

A question that frequently arises is whether it is clinically helpful to monitor B cell counts in the setting of rituximab therapy. In large series of RA patients, there has previously not been a clear temporal relationship between peripheral B cell return and loss of response making such a recommendation difficult to substantiate. On the other hand, a number of recent publications have found that the detection of residual peripheral blood B cells using high sensitivity flow and the return of B cells, especially with higher fractions of memory B cells, increases the risk of inadequate response and/or relapse [49] [50,51]. The relationship between clinical response and long term depletion of memory B cells in RA bone marrow has also been recently suggested [52] [53]. We have also found that B cell depletion may alter lymphoid architecture by eliminating LTα bearing and TNF secreting B cells, resulting in a prolonged delay in memory B cell reconstitution that correlates with clinical response in SLE [54]. Such mechanisms may explain the delayed acquisition of mutations in the memory B cell compartment after rituximab [55,56].

Although anti-CD20 is usually effective in depleting B cells from peripheral blood, success in depleting B cells from other sites such as lymph nodes or tertiary lymphoid tissues may be highly variable. Failure to deplete in these tissue sites may lead to non-response or early relapse. Thus, Kavanaugh and colleagues found that synovial B cells were decreased but not eliminated by rituximab therapy [57], a result confirmed in other studies [58]. In both these studies higher levels of clinical response correlated with more consistent synovial B cell depletion. Other studies have found that baseline positivity for circulating anti-CCP auto-antibodies, particularly IgM, along with a high infiltration of CD20+ and CD79a+ CD20- B cells in RA synovium (the latter presumably plasmablasts) were predictors of incomplete response after B cell depletion [59]. This is somewhat counter-intuitive as one would expect these biomarkers

to predict a more B cell driven disease. Rituximab did significantly reduce serum autoantibodies (both anti-CCP and RF, particularly IgM isotype) as well as CD20+ B cells in the synovium, but did not affect infiltrating plasmablasts or CD138+ plasma cells at 12 weeks. On the other hand, Thurlings and colleagues [58] found a reduction in synovial plasma cells at 16 weeks that was followed kinetically by reductions in serum autoantibodies and predicted clinical response at 24 weeks.

Overall, these results do suggest that the failure to adequately deplete pathogenic B cells in tissue sites is an important predictor of incomplete response to B cell depletion therapy. It is also likely that the therapeutic effect of B cell depletion does not lie solely in the depletion of B cells since not all of the effects of B cells promote autoimmunity. In particular, the fact that a higher fraction of memory B cells and lower fraction of immature transitional B cells during reconstitution correlates with earlier relapse of disease [50,54,60] suggests that the outcome of B cell depletion depends on the balance between protective and pathogenic B cell populations. This hypothesis is in keeping with the previously noted findings of increased B cell production of IL10 after B cell depletion in human MS [33] and the emergence of regulatory B cell populations after B cell depletion in murine diabetes [32].

In RA, transient B cell depletion with rituximab can ameliorate disease for a prolonged period but typically not indefinitely. Some patients do not adequately respond to rituximab treatment, and there is evidence that these patients are unlikely to respond to a second cycle, although this study was limited by the lack of assessment of peripheral blood or synovial B cell depletion [61]. This suggests that some non-responders represent a different pathogenetic subset of RA. There is emerging evidence that repeated courses of B cell depletion in responders are associated with sustained clinical response [62] [61]. However, the optimal timing of repeated B cell depletion remains unclear. In a study of 22 patients followed for at least 5 years, the average duration of clinical benefit was 15 months, with an average of 20 months to retreatment [63]. A small non-randomized study of 48 patients compared fixed retreatment at 24 weeks to on-demand disease activity guided re-treatment. After one year, clinical outcomes and safety profiles were similar in the two groups [64], although in recently reported two year follow-up data presented at the 2009 ACR meeting fixed retreatment resulted in somewhat higher EULAR responses but also increased SAEs and more doses of rituximab. The authors suggest that in practice these factors must be weighed against any potential clinical benefit. Given this and the observation that a small subset of patients may have very prolonged responses after a single course of B cell depletion, our approach remains to tailor the treatment to the patient and consider repeat B cell depletion when disease begins to relapse.

Conclusion

Accumulating data in RA indicates that B cells contribute to disease through multiple mechanisms that include both antibody-dependent and antibody-independent functions. The latter include antigen presentation, T cell activation and polarization, organization of other inflammatory cells, and dendritic cell modulation and are likely critically mediated by the ability of B cells to produce cytokines. Accumulating data indicates that B cells display considerable phenotypic diversity [65], and it is important to recognize that not all of the effects of B cells promote autoimmunity. Thus, we postulate that the therapeutic benefit of B cell depletion depends on the balance of pathogenic and protective B cell functions. If this is so, long-term B cell depletion may not be necessary or advisable given that regulatory B cells are not distinguished from pathogenic B cells with current approaches.

The fact that B cell depletion impacts both the physiological and pathological functions of B cells raises ongoing concern regarding adverse effects on protective immunity. However, a recent meta-analysis supports prior literature regarding the safety of B cell depletion in that

treatment of RA with rituximab was not associated with an increased incidence of serious infections [66], even after repeated courses [62], despite the higher incidence of hypogammaglobulinemia after repeated treatment [67]. The caveat here is that the database remains relatively small. Moreover, there have been reports of fulminant hepatitis B reactivation and rare cases of PML. Thus, patients should be screened for hepatitis B prior to the use of rituximab and viral prophylaxis considered in high-risk patients. Unfortunately, there are no screening methods to identify patients at risk for development of PML although the frequency of PML in RA patients receiving rituximab appears to be quite rare (3/100,000), but not zero [68].

Despite significant progress in this area, important areas of future study include the identification of additional biomarkers, such as unique B cell subsets and autoantibody profiles, to better define responsive patient groups, determine the optimal timing of treatment and role of combination therapy, and the place of B cell depletion in the therapeutic hierarchy. The long-term impact of B cell depletion on both protective immunity and autoimmunity merits further study. Additionally, novel agents to deplete and modulate B cell numbers or function continue to be developed.

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