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To B or Not to B the Conductor of Rheumatoid Arthritis Orchestra

Rita A. Moura · Luis Graca · João E. Fonseca

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Abstract Rheumatoid arthritis (RA) is a chronic, systemic immune-mediated inflammatory disorder that mainly targets the joints. Several lines of evidence have pointed to B cell function as a critical factor in the development of RA. B cells play several roles in the pathogenesis of RA, such as autoantibody production, antigen presentation and T cell activation, cytokine release, and ectopic lymphoid organogenesis. The success of B cell depletion therapy in RA further supports the relevance of these cells in RA progression. In addition, recent studies have also highlighted the B cell role in the first weeks of RA onset. The present article is a review focused in the immunopathogenic B cell-dependent mechanisms associated with RA development and chronicity and the importance of the recent discoveries documented in untreated very early RA patients with less than 6 weeks of disease duration.

Keywords Rheumatoid arthritis · B cells · Autoantibodies · Antigen presentation · Synovium · Rituximab

R. A. Moura · J. E. Fonseca (✉)
Rheumatology Research Unit, Instituto de Medicina Molecular,
Edifício Egas Moniz,
Faculdade de Medicina da Universidade de Lisboa,
Av. Professor Egas Moniz,
1649-028 Lisbon, Portugal
e-mail: jecfonseca@gmail.com

L. Graca
Cellular Immunology Unit, Instituto de Medicina Molecular,
Edifício Egas Moniz,
Faculdade de Medicina da Universidade de Lisboa,
Av. Professor Egas Moniz,
1649-028 Lisbon, Portugal

J. E. Fonseca
Rheumatology Department, Centro Hospitalar Lisboa Norte, EPE,
Hospital de Santa Maria,
Av. Professor Egas Moniz,
1649-028 Lisbon, Portugal

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that preferentially affects the joints. RA has a prevalence of around 1 % worldwide in the adult population. Disease onset usually occurs between 30 and 50 years of age and it is more frequent in women. RA is characterized by chronic pain and progressive joint damage with high risk of functional disability and consequent diminished quality of life and premature mortality [1]. Although the cause that triggers RA autoimmune process remains unknown, it has been demonstrated that several types of cells from both innate and adaptive immune system actively participate and form complex networks of cell–cell interactions that contribute to the development and chronicity of rheumatoid synovitis and systemic inflammation (Fig. 1). Several lines of evidence have pointed to B cell function as a critical factor in the development of RA (Fig. 2). B cells are responsible for the production of autoantibodies, such as rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA), which form immune complexes that deposit in the joints, activate complement, and cause inflammation. Furthermore, B cells are very efficient antigen presenting cells (APC) that activate T cells through the expression of costimulatory molecules. B cells also function as cytokine- and chemokine-producing cells that promote leukocyte infiltration in the joints and formation of ectopic lymphoid structures, thus aggravating angiogenesis and synovial hyperplasia. Importantly, recent studies have also documented a B cell intervention since the first weeks of RA onset. Indeed, it has been demonstrated that very early RA patients, with less than 6 weeks of disease duration, have disturbances in circulating memory B cells [2] and increased levels of cytokines related with B cell activation and survival [3, 4]. Moreover, the recent results observed in RA patients after B cell depletion therapy suggest that the re-establishment of active disease might be associated with alterations in B cell activating factor (BAFF)-binding receptors and an increase in

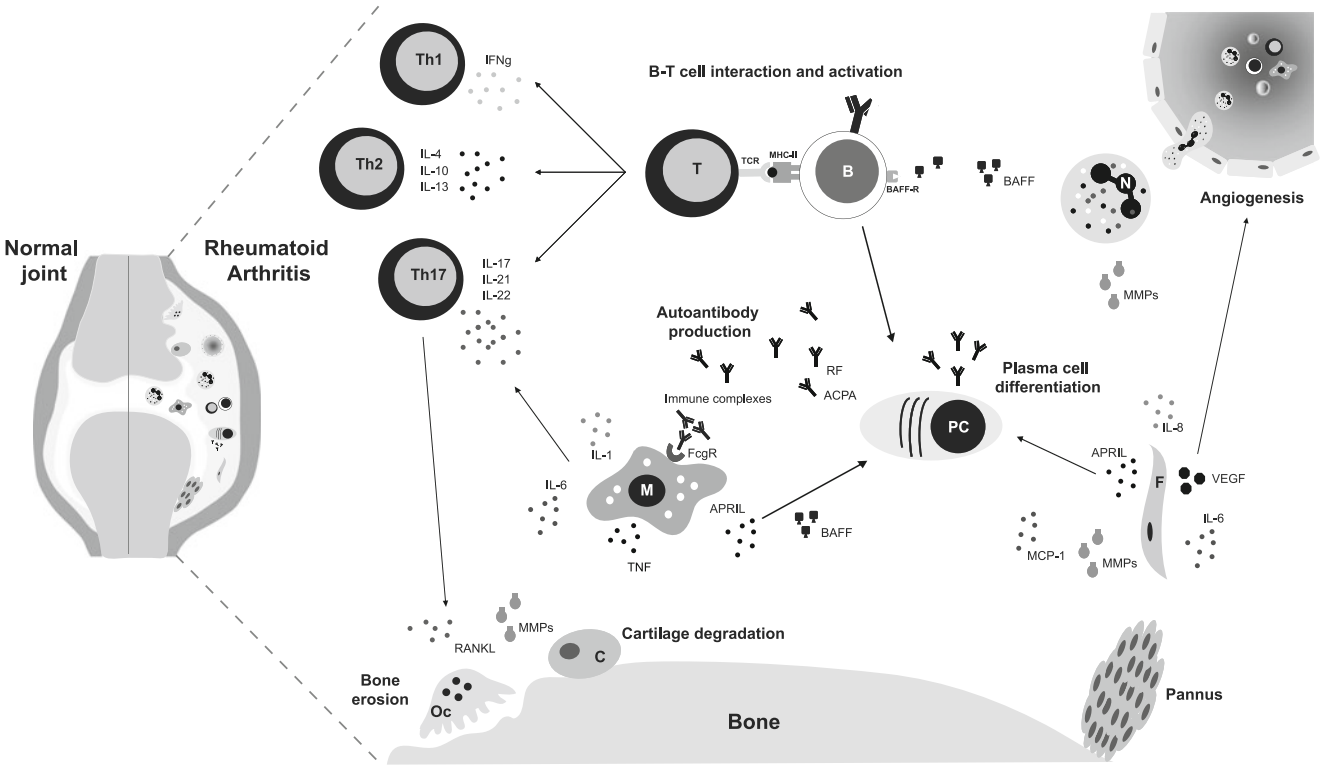

Fig. 1 Cellular interactions and cytokine networks in rheumatoid arthritis (RA). In RA, the inflammatory process leads to a cellular infiltration of the synovial membrane, with simultaneous angiogenesis, pannus formation, swelling, and pain. B–T cell interactions result in the activation and differentiation of plasma cells, responsible for the production of autoantibodies. These autoantibodies form immune complexes that activate Fc gamma receptors expressed by monocytes. Activated monocytes, neutrophils, and fibroblasts can release high levels of cytokines that further activate not only B and T cells, but also chondrocytes and osteoclasts, leading to cartilage and bone destruction. *APRIL* A proliferation-inducing ligand, *ACPA* anti-cyclic citrullinated protein antibody, *B* B cell, *BAFF* B cell activating factor, *BAFF-R* BAFF receptor, *C* chondrocyte, *F* fibroblast, *FcgR* Fc gamma receptor, *IL* interleukin, *IFNg* interferon gamma, *M* monocyte, *MCP-1* monocyte chemoattractive protein, *MHC-II* major histocompatibility complex class II, *MMPs* matrix metalloproteinases, *N* neutrophil, *Oc* osteoclast, *PC* plasma cell, *RANKL* receptor activator of nuclear factor kappa-B ligand, *RF* rheumatoid factor, *T* T cell, *Th* T helper, *TCR* T cell receptor, *TNF* tumor necrosis factor, *VEGF* vascular endothelial growth factor

class-switch recombination process, affecting the memory B cell pool [5]. These observations suggest that an earlier introduction of B cell-directed therapies, such as B cell depletion or indirect B cell targeted therapies affecting B cell receptors or its ligands might be of beneficial clinical use to induce early remission in RA patients.

The Immunopathogenic Role of B Cells in Rheumatoid Arthritis

B Cells Produce Autoantibodies

Autoantibodies are the serological hallmark of autoimmune diseases and serve as indicators of the break in self-tolerance. RA is characterized by the presence of several autoantibodies in serum and synovial fluid. The first autoantibody to be documented in RA, RF, was described by Waaler in 1940 [6], and it was later found to be directed to the constant (Fc) region of IgG. There are several autoantigens recognized by the autoantibodies present in RA that include cartilage components (for instance, anti-type II collagen, anti-human cartilage glycoprotein 39) [7], enzymes (anti-glucose-6 phosphate isomerase (anti-GPI), anti-enolase) [8], nuclear proteins (anti-RA33) [9], stress proteins, and ACPA [10]. The production of autoantibodies is nevertheless supported by autoreactive T cells that closely interact with B cells and also contribute for RA development and progression. Indeed, it has been demonstrated in mice models of arthritis that antigen-specific T cells homing to lymphoid organs and T cells that migrate to the joints provide help to B cells for systemic autoantibody production [11]. The clinical significance and pathogenic roles of these autoantibodies are, however, largely unknown, except for RF and ACPA, whose clinical usefulness, as diagnostic and prognostic factors, has been clearly acknowledged.

Rheumatoid Factor

RF are autoantibodies that directly bind to the Fc portion of the normal human IgG and are locally produced in RA by B

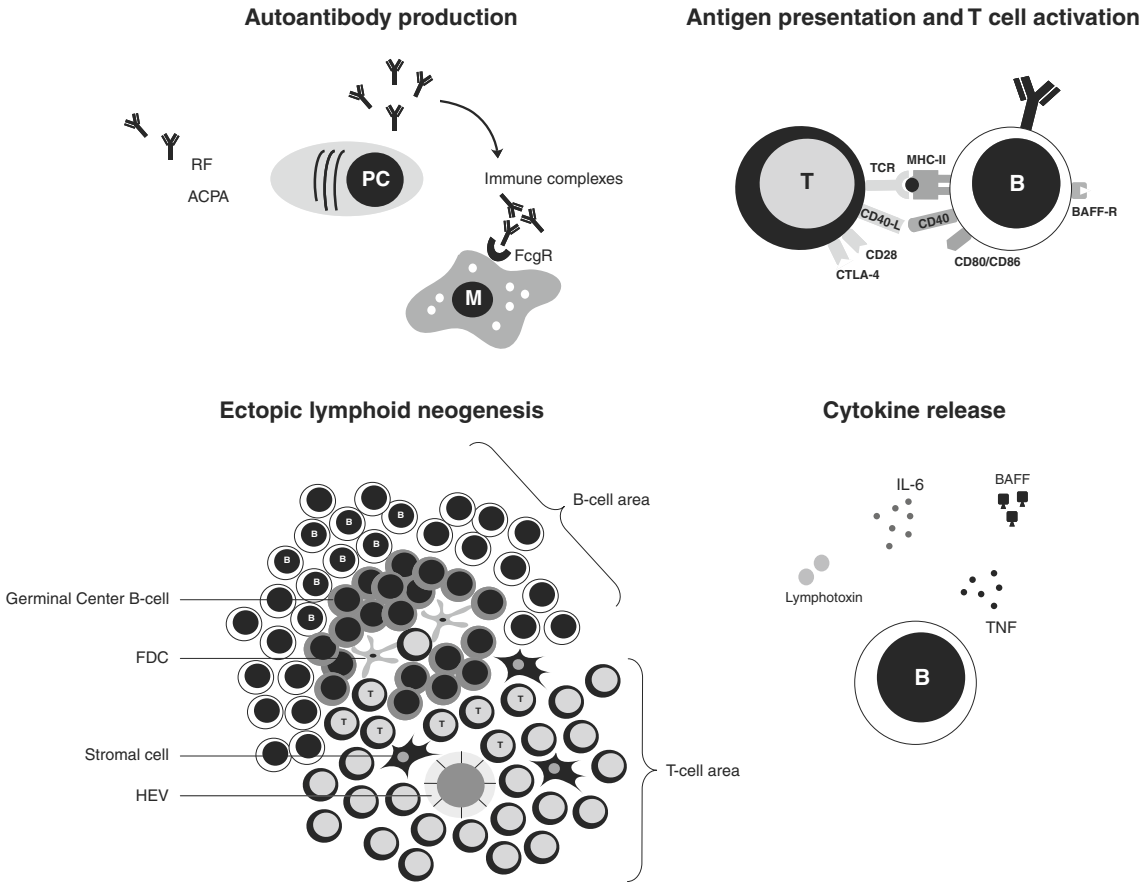

Fig. 2 An overview on the roles of B cells in rheumatoid arthritis (RA) pathogenesis. B cells play several important roles in RA development and progression. B cells produce autoantibodies that form immune complexes and activate Fc gamma receptors (*FcgR*), inducing proinflammatory cytokine production; they function as antigen presenting cells and activate T cells, release cytokines upon activation, and participate in ectopic lymphoid neogenesis. *ACPA* anti-cyclic citrullinated protein antibody, *B* B cell, *BAFF* B cell activating factor, *BAFF-R* BAFF receptor, *CD* Cluster of Differentiation, *CTLA-4* cytotoxic T-lymphocyte antigen 4, *FDC* follicular dendritic cell, *HEV* high endothelial venule, *IL* interleukin, *M* monocyte, *MHC-II* major histocompatibility complex class II, *PC* plasma cell, *T* T cell, *TCR* T cell receptor, *TNF* tumor necrosis factor

cells present in lymphoid follicles and germinal center (GC)-like structures that develop in inflamed synovium [12]. RF has been hypothesized to be a pathogenic autoantibody with a key role in the physiopathology of RA [13]. In fact, RF can be detected in serum and synovial fluid samples from patients and its presence is associated with more aggressive articular disease, a higher prevalence of extra-articular manifestations, and increased morbidity and mortality [14]. RF induces the formation of immune complexes at the sites of synovial inflammation, the activation of complement and leukocyte infiltration by means of the downstream products of complement activation [15]. RFs are found in multiple immunoglobulin isotypes (IgM, IgG, and IgA), although IgM-RF is the one usually measured in clinical assays, being detected in 60–80 % of RA patients [16]. RF has been observed in many other autoimmune diseases, including systemic lupus erythematous, as well as in non-autoimmune conditions, such as chronic infections, and also in healthy individuals. Nevertheless, RF from healthy subjects are of the IgM class, polyreactive and with low affinity [17], whereas RF from RA patients belong to all classes and exhibit affinity maturation [18]. High titers of IgM and IgA-RF isotypes have higher diagnostic specificity to RA [19]. Accordingly, high titers of RF have been associated with worse prognosis. Indeed, it has been shown that a high titer of IgA-RF is associated with radiologic erosion, extra-articular manifestations, and worse outcomes [14, 19]. Moreover, high concentrations of IgG-RF within the confines of the joint are associated with the formation of larger, complement-fixing complexes which, together with IgM-RF-based complexes, are likely to amplify the inflammatory process [20]. The association between high titers of RF and worse prognosis suggests that RF may have an important role in the pathogenesis of RA. RF has proven to be, together with ACPA, the most useful diagnostic marker of RA, having been included in the 1987 ACR classification criteria for RA [21] and in the new ACR/EULAR classification criteria [22]. Furthermore, it is thought that B cells

with RF specificity migrate into the synovium of RA patients, therefore presenting a variety of antigens to T cells. This leads to the perpetuation of local inflammatory responses and amplification of RF production in the synovium. Thus, RF may prolong B cell survival and hence maintain its own production.

Anti-Citrullinated Protein Antibodies

ACPA are found in 70–90 % of RA patients and have high disease specificity (90–95 %) [23]. They are rarely present in other diseases or in healthy individuals. ACPA were first detected in RA in 1998 [10], a result later confirmed by other groups [24, 25]. These autoantibodies recognize peptides or proteins containing citrulline, a non-standard amino acid generated by the post-translational modification of arginine by peptidylarginine deiminase enzymes [26], in a process known as citrullination. Of interest, citrullination occurs during a variety of biologic processes, including inflammation, apoptosis, or keratinization. ACPA are locally produced in RA joints, where proteins are citrullinated during the inflammatory process [25]. In fact, several citrullinated proteins can be found in RA synovium, such as filaggrin, fibrin, vimentin, nuclear, and stress proteins [27]. However, the major citrullinated protein in RA joint was found to be fibrin [28]. Immune complex formation between ACPA and citrullinated proteins with subsequent complement fixation and joint deposition occurs in RA synovium, which is thought to perpetuate RA synovial inflammation, causing a vicious cycle [29]. ACPA-positive disease is associated with increased joint damage and low remission rates [30]. Evidence from animal models and in vivo data suggest that ACPA are pathogenic on the basis of induction of arthritis in rodent models and because immunological responses are present in ACPA-positive patients in a citrulline-specific manner [31]. Interestingly, the majority of ACPA-positive patients is also positive for RF [32]. Histologically, ACPA-positive patients have more infiltrating lymphocytes in synovial tissue, whereas ACPA negative have more fibrosis and increased thickness of the synovial lining layer [33]. Importantly, ACPA autoantibodies not only have a better diagnostic value than RF in terms of sensitivity and specificity but also seem to be better predictors of poor prognostic features such as progressive joint destruction [32]. Moreover, they also help to predict disease outcome in patients with undifferentiated arthritis [34]. In 2007, ACPA were formally included in the EULAR guidelines for the diagnosis of early RA [22].

B Cells Participate in Ectopic Lymphoid Organogenesis

Rheumatoid synovium is a highly vascularized tissue infiltrated by cells from both the innate (macrophages and neutrophils) and adaptive (B and T cells) immune systems (Fig. 1). B cells have an important role in lymphoid organogenesis by contributing for the activation and regulation of effector T cells. Nonetheless, follicular helper T cells are critical for humoral immunity, not only for the generation of long-lived and high-affinity plasma and memory B cells but also as providers of survival and differentiation signals to B cells [35]. The presence of lymphoid microstructures in rheumatoid synovial tissue can enhance the sensitivity of antigen recognition, optimize the collaboration of immunoregulatory and effector cells, and support the development of aberrant immune responses. It is possible to distinguish at least three different patterns of synovial infiltration in RA patients: a diffuse rearrangement of B and T cells, aggregates of B and T cells without a follicular microstructure, and synovial lymphocytes arranged in B cell follicular-like structures with B and T cells arranged around a network of follicular dendritic cells that resemble functional ectopic GCs [36, 37]. Interestingly, the patterns of lymphoid infiltrates correlate with clinical disease activity [38] and severity [39]. Some chemokines have been identified to contribute to the formation of these ectopic lymphoid structures, namely CXCL13 [40] and CXCL12 [41], which play a key role in the generation of GC as responsible for the recruitment of B and T cells, respectively. Additionally, another cytokine, lymphotoxin (LT)-β, produced by activated T and B cells, also appears to be essential for the formation of primary B cell follicles in rheumatoid synovium [42]. BAFF, an important B cell survival cytokine, has also been detected at high levels in rheumatoid synovial fluid [43], suggesting that the local production of BAFF in RA joints might favor the activation of autoreactive B cells with simultaneous ectopic lymphoid tissue formation [44]. Other cytokines are known to have an important role in the recruitment of B cells towards RA synovium and hence contribute to the development and organization of lymphoid structures. In fact, previous studies have documented an increase in RA joints of interleukin (IL)-6 [4, 45], a major B cell chemoattractant [46], and IL-21, a cytokine that directly affects plasma cell differentiation and supports autoantibody production [3, 47]. Of interest, both these cytokines reflect the polarization and activation of Th17 and follicular helper T cells, respectively, which importantly contribute for B cell activation [48, 49]. In fact, recent studies have identified a cytokine profile supporting neutrophils and Th17 cells activation not only in serum from untreated very early RA patients but also in synovial fluid from established RA patients [4], thus reinforcing the important cellular interactions that contribute for RA pathogenesis and influence B cell physiopathology in this autoimmune disease.

B Cells Can Function as Antigen Presenting Cells

B cells can function as APC and have a central role in autoimmunity. A B cell is classified autoreactive when its

B cell receptor (BCR) targets a self-antigen. This contrasts with other APC, whose activation depends on the recognition of molecular patterns from the antigen as foreign. In many cases, autoimmunity arises when antigens that are sequestered from the immune system become accessible. B cells have the ability to present antigen efficiently, since they can find T cells in secondary lymphoid organs shortly after antigen entrance, and BCR-mediated endocytosis allows them to concentrate small amounts of specific antigen [50, 51]. In RA, autoreactive B cells can function as APC and present processed self-antigens to T cells, thus allowing the development of an autoreactive immune response [52, 53]. RF^+ B cells are believed to play an important role in antigen presentation. In fact, it has been demonstrated that they can take up antigen–antibody immune complexes through their membrane Ig receptors, which have RF specificity [52]. These autoreactive B cells then process and present peptides from the antigen to T cells, inducing T cell activation and help. Furthermore, in animal models of RA, genetic manipulation to generate mice with B cells that are unable to secrete antibodies demonstrated that antigen presentation by B cells is critical for disease development, as targeting of the autoantigen to specific B cells resulted in T cell activation and joint inflammation in the absence of autoantibodies [54].

B–T Cell Interactions

Previous studies by Takemura and colleagues have demonstrated that T cell response in RA synovitis and ectopic lymphoid organization is B cell dependent [55]. When $CD4^+$ T cells were transferred into severe combined immunodeficiency mice, the animals developed arthritis in the presence of B cells, but when B cells where depleted, no disease occurred. Moreover, the production of proinflammatory cytokines by T cells could be disrupted by B cell depletion. In fact, it has been demonstrated that B cell depletion enhances T regulatory cell activity, essential for the suppression of arthritis [56]. Nevertheless, recent studies in mouse models of arthritis support the importance of T cell intervention in RA pathogenesis, irrespective of B cell relevance for disease progression. Indeed, it has been shown that non-depleting pro-tolerogenic anti-CD4 monoclonal antibodies prevented the onset of chronic autoimmune arthritis in SKG mice. Furthermore, the antibody treatment also inhibited disease progression in arthritic mice, although without leading to remission. Interestingly, protection from arthritis was associated with an increased ratio of Foxp3 and decreased IL-17-producing T cells in the synovia [57]. The discovery in mice of a new lineage of $CD4^+$ effector T helper cells that selectively produce IL-17, termed Th17 cells, has provided new insights into the immune regulation and pathogenesis of some autoimmune diseases [58]. The role of Th17 cells in the inflammatory process that occurs in collagen-induced arthritis (CIA) mice served as a trigger to investigate the possible involvement of this T cell subset in human RA progression [59]. Of interest, Th17 cells have indeed proven to be associated with RA pathogenesis [60]. Recent findings document that signaling pathways regulated by IL-17 contribute for the development of spontaneous GCs in autoimmune BXD2 mice [61] through activation of autoreactive B cells by NF-κB signaling [62]. Moreover, studies performed in CIA mouse model have shown that local BAFF gene targeting inhibited proinflammatory cytokine expression, suppressed generation of plasma cells and Th17 cells expansion, thus ameliorating joint pathology [63]. Importantly, recent in vitro studies have shown that Th17 cells are also effective B cell helpers due to their ability to stimulate B cell differentiation and class-switch recombination [64]. Indeed, Th17 cells express B cell chemoattractant CXCL13, which can have important consequences not only in autoimmunity but also in protective immunity against pathogens [65]. Of interest, a B cell- and Th17-derived cytokine patterns have been recently identified in circulation of untreated very early RA patients with less than 6 weeks of disease duration [3, 4], thus reinforcing the notion of an interaction between B and Th17 cells since early RA development. Taken together, these observations imply that the reciprocal activation of autoreactive B and T cells is fundamental for RA progression.

Lessons from Animal Models of Arthritis

Animal models have been used extensively in studies of RA pathogenesis. In particular, the discovery of K/BxN mouse model in 1996 [66] brought new attention to the idea that antibodies to systemic autoantigens might be a principal effector mechanism in initiating the inflammatory process in the joint. Similarly to human RA, this model presents lymphocyte infiltration of the synovium, synoviocyte proliferation, pannus formation, cartilage and bone erosion, polyclonal B cell activation, hypergammaglobulinemia, and autoantibody production. Despite RF not being produced, the disease in this mouse model was shown to be antibody-mediated. The K/BxN mice were originally generated by crossing a T cell receptor (TCR) transgenic strain (KRN-C57BL/6) with non-obese diabetic mice. Unexpectedly, the transgenic TCR from F1 offspring recognized a peptide derived from a ubiquitously expressed protein, glucose-6-phosphate isomerase (GPI). The T cell-dependent B cell activation by GPI, together with a costimulatory signal via CD40L-CD40 interaction, leads to the production of arthritogenic anti-GPI autoantibodies. Furthermore, serum transfer to wild-type, B cell-deficient- or lymphocyte-deficient mice led to rapid onset of arthritis,

although transient, unlike the persistent arthritis in the K/BxN mouse [67]. Importantly, K/BxN mice devoid of B cells did not develop arthritis [66]. The identification of the target of the pathogenic antibody as GPI demonstrated that antibody to an ubiquitous antigen could lead to joint-specific disease and, therefore, renewed attention for the role of antibodies and immune complexes as a cause of RA. Consequently, there was a revived interest in B cells as important effector cells in RA, as has been assumed in earlier years following the discovery of RF. Although the reasons that support B cells and antibodies in particular as main disease drivers in the K/BxN arthritis mouse model are clearly corroborated by the literature, recent work developed with this same mouse model has brought new attention for the role of T cells in arthritis susceptibility. It was reported that K/BxN mice housed in different institutions can develop a gut microbiota with distinct characteristics. It was shown that some microorganisms from the gut promote Th17-biased immune responses thus leading to increase susceptibility to arthritis [68]. In addition, it has been demonstrated that the use of monoclonal anti-CD8 therapy in K/BxN mice can lead to disease improvement, while the levels of the disease-related anti-GPI antibodies did not change [69]. These results support the notion that not only B cells but also T cells contribute to the chronic polyarthritis in K/BxN mice. Accordingly, in other animal models of arthritis, such as CIA or SKG mice, the physiopathology of arthritis has been associated to T cell-dependent mechanisms [59, 70, 71]. Nevertheless, B cells have also proven to be crucial for disease progression [54, 72, 73].

How to Target B Cells?

B Cell Depletion Therapy: the Case of Rituximab

The identification of a surface antigen that is both exclusively and highly expressed on B cells is essential if B cells are to be targeted effectively. CD20 expression is limited to B cells, begins in humans at the early pre-B cell stage, and persists until the B cells undergo terminal plasma cell differentiation. This specificity is vital when selecting a viable therapeutic target, thus making CD20 an attractive, if not ideal, target. Rituximab (RTX) is a genetically engineered mouse–human chimeric monoclonal antibody specifically targeting CD20. RTX depletes B cells by inducing cell lysis, which can be mediated by complement-dependent cytotoxicity, antibody-dependent cell-mediated cytotoxicity, or apoptosis [74]. RTX was first approved as a treatment of relapsed or refractory $CD20^+$ B cell non-Hodgkin's lymphoma (NHL) in 1997 [75]. In this context, RTX has been shown to diminish the number of B cells in the blood and bone marrow of NHL patients for 9–12 months after just one treatment [76]. Importantly, RTX was generally well tolerated, with a very low incidence of infections. In 1998, Edwards and Cambridge published a seminal paper proposing, for the first time, the use of RTX treatment in RA patients [13], which was highly controversial and revolutionary at the time. The results observed in a small open-label study conducted by these authors provided the first indication of the therapeutic potential of RTX in RA [77]. In fact, in this study, patients who were non-responsive to DMARD therapy had a clear-cut improvement in disease symptoms that was evident even beyond 6 months after a single RTX treatment. Indeed, circulating B cells were depleted to near undetectable levels and no major adverse events attributable to the treatment were observed. Notably, the work developed by Edwards and Cambridge [77] together with further studies [78, 79] provided robust evidence that B cells play a key role in RA and that selective B cell depletion using RTX could be beneficial. Importantly, the first randomized, double-blind, placebo-controlled trial using RTX in any autoimmune disease was the phase IIa trial in RA reported by Edwards et al. in 2004 [79]. These results were also reinforced by a phase IIb clinical trial in 2006 [80]. Despite the overall clinical efficacy of TNF antagonists in RA, a substantial fraction of RA patients do not respond to anti-TNF therapies [81–83]. Of note, the clinical efficacy of RTX was also confirmed in a large study of RA patients refractory to TNF-antagonist therapy [84]. Despite some controversy, there is evidence supporting the higher efficacy of RTX in patients with ACPA and/or RF in the serum [80, 84–88]. Although RTX treatment has proven to be efficient in RA patients for a variable period of time, clinical relapses eventually occur [89], which means that re-treatment is routinely necessary. B cell return into the circulation generally antedates clinical relapse among RTX-treated RA patients. In some patients, relapse is virtually coincident with B cell return, whereas in others, relapse occurs months or even years after B cell repopulation. Importantly, disease reappearance is temporally more closely associated with a rise in titers of circulating autoantibodies (RF, for instance) related to putative pathogenic autoreactive B cells than with global B cell return per se [89]. The reasons that might account for clinical relapse can include: (a) incomplete B cell depletion in secondary lymphoid organs or bone marrow with rescuing and the survival of some pathogenic B cells that could restore the disease [90, 91]; (b) activity of long-lived plasma cells residing in the bone marrow or in the synovial tissue, which are not targeted by RTX [79]; (c) persistence of autoreactive T cells in RTX-treated patients that could lead to the activation of newly generated pathogenic B cell clones; and (d) increased BAFF circulating levels observed during B cell depletion therapy with RTX, which could promote a support for surviving or re-emerging pathogenic B cells

[92]. Therefore, the continuous research for new B cell targeting agents that directly act on other B cell antigens (for instance, CD19, CD22, or other CD20 epitopes) may possibly reveal even more promising results.

Regulatory B Cells

B cells are traditionally known for their capacity to produce antigen-specific antibodies, thereby contributing to humoral immunity and clearance of pathogens. In autoimmunity, B cells are usually defined as pathogenic due to their capacity to secrete autoantibodies. Nevertheless, reports focusing on B cell-mediated immune suppression have been published over the last three decades, although the regulatory functions of B cells have only been rigorously examined in the last 10 years. The term "regulatory B cells" (Breg cells) to designate B cells with inhibitory properties was used for the first time by Mizoguchi et al. [93, 94]. Suppressor/regulatory B cell populations have predominantly been identified using diverse mouse models of autoimmune diseases [93, 95, 96], suggesting that autoimmunity itself promotes the expansion of these cells as a compensatory mechanism to limit self-directed inflammation. IL-10-producing B cells were the first regulatory B cells to be recognized and were termed "B10" cells [97]. TGF-β-producing B cells are regarded as another regulatory subset involved in tolerance to allergens [98]. Several reports have suggested that absence, or loss, of regulatory B cells can exacerbate manifestations of both allergic and autoimmune diseases [93, 95, 97, 99, 100]. Contrarily to mice models, the knowledge regarding the existence of regulatory B cells in humans is still scarce. In fact, studies suggesting an intervention of these cells in humans have only recently been published [101–103]. Importantly, it has been suggested that patients suffering from autoimmune diseases have an impaired regulatory B cell function [103, 104]. In RA patients, not only the frequency of IL-10^+ B cells is increased after stimulation with CpG and CD40L but also IL-10 production by blood B cells has been reported to be higher in comparison with healthy individuals [104]. As such, it is of therapeutic interest to study the conditions in which these specific B cells can be induced. Once they have been clearly identified, manipulation of regulatory B cells in humans might provide subtle approaches as a treatment for autoimmune conditions.

The Importance of BAFF and Other B Cell-Related Cytokines

BAFF, also known as B lymphocyte stimulator, is a protein member of the TNF family that has an important role in B cell maturation, homeostasis, and survival [105]. Evidence supports that BAFF is implicated in the development of autoimmunity. Indeed, BAFF transgenic mice have B cell hyperplasia, increased levels of immunoglobulins, and prolonged BAFF overexpression that induces autoantibody production [105, 106]. In humans, several autoimmune diseases might be partially triggered by deficient B cell tolerance associated with the overproduction of BAFF that might lead to inappropriate survival of autoreactive B cells [43]. In RA patients, BAFF levels were found to be higher in the synovium than in corresponding serum, suggesting that local production of BAFF in the synovium drives the maturation of autoreactive B cells, exacerbating the inflammatory process [107]. In fact, generalized inflammation and high levels of BAFF may drive continued production of plasma cells-producing pathogenic autoantibodies [108, 109]. A-proliferating inducing ligand (APRIL) is also a key cytokine for B cell activation and maturation. APRIL not only affects class-switch recombination process [110] but also plasma cell differentiation and survival [111]. Of interest, high levels of both BAFF and APRIL, along with their receptors, are observed in the rheumatoid synovium [112], which could explain the maintenance of autoreactive B cells in joints [113]. Furthermore, recent studies have also documented an increase in BAFF and APRIL serum levels in the very first weeks of RA onset, supporting an early B cell activation in RA development [3]. Therefore, targeting BAFF, APRIL, or its receptors may provide a novel therapeutic approach to autoimmunity. Interestingly, in animal models of autoimmune disease, BAFF and/or its receptors' antagonists reduce disease severity and delay disease progression [114, 115]. Clinical trials of a selective antibody to BAFF (belimumab) and with the BAFF/APRIL inhibitor TACI-Ig (atacicept) are currently in progress [116, 117]. In addition, IL-6, important for the development of antibody-producing plasma cells [118] and B cell chemotaxis [46], can also be considered as an indirect B cell target for therapy. In fact, tocilizumab, an IL-6 receptor inhibiting agent that was recently approved for RA treatment, is highly effective in established RA [119, 120] and inhibits B cell hyperreactivity in RA patients [119]. Interestingly, it has been demonstrated that pre-switch (IgD^+CD27^+) and post-switch (IgD^-CD27^+) memory B cells are particularly susceptible to the effects of tocilizumab in vivo [121].

Conclusions

The importance of B cells in RA pathogenesis has been traditionally associated with autoantibody production, namely RF and ACPA, which form immune complexes that further deposit in the joints and exacerbate the inflammatory response. Nevertheless, B cells are more than just autoantibody-producing cells, being able to act in several immune processes involved in the triggering and establishment of RA pathogenesis. Although B cells are clearly

relevant for RA development, important and necessary cellular interactions with other immune players, namely T cells, cannot be dismissed. Indeed, recent observations in untreated very early RA patients reinforce the relevance of not only B cells [2, 3] but also neutrophils and Th17 cells [4] in RA pathogenesis from the very first weeks of disease onset. Therefore, we suggest that new specific therapeutic approaches individually targeting intervenient cells that contribute for RA progression should be reconsidered and carefully analyzed earlier in disease course.

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