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Immunopathologic role of B lymphocytes in rheumatoid arthritis: Rationale of B cell-directed therapy

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Abstract

Although the immunopathogenesis of rheumatoid arthritis (RA) remains unclear, recent advances have paved the way for new therapies, such as anti-cytokine and cell-directed therapies. Here, B cells have re-gained interest concerning the pathogenesis of a number of autoimmune diseases after observing that patients with RA and non-Hodgkin lymphoma, who received anti-CD20 therapy leading to B cell depletion, demonstrated remarkable improvements. The underlying modes of action appear to be related to B cell functions, such as deletion of memory B cells, interruption of immune activation, antigen-presentation and production of inflammatory cytokines. In many RA patients, synovial extrafollicular germinal centers develop, where B cells play an intimate role in local inflammation and the generation of memory B cells and plasma cells. These local processes lead to activation of the immune system and ultimately to joint destruction in RA. Recent data demonstrating the clinical value of B cell depletion in refractory RA patients substantiate the notion that B cells are important players in the pathogenesis of the disease. Future studies should clarify which functions are affected by B cell depletion, providing the promise of new avenues to patient-tailored therapies.

Keywords: B lymphocytes; Rheumatoid arthritis; B cell-directed therapies

Contents

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1. Introduction

Rheumatoid arthritis (RA) is one of the most frequent systemic autoimmune diseases, affecting about 0.5–1% of the adult population. In the last decades, RA has been considered to be preferentially mediated by T cells and macrophages, which are important players in the pathophysiology of RA [1,2]. More recently, increasing evidence of a pivotal role of B cells involved in immune dysregulation in RA was obtained and fueled by clinical improvements in RA patients receiving B cell depleting therapies such as rituximab, an anti-CD20 antibody [3]. It is important to bear in mind the contribution of B cells under healthy conditions to maintain the integrity of the body and the striking clinical examples of humoral immunodeficiency syndromes when B cell functions are impaired [4].

Initial observations for the potential of anti-CD20 therapy in RA came from case reports of patients with lymphoma, who had been treated with rituximab and showed improvement in concomitant RA symptoms [5,6]. In this regard, it is well known that patients with RA with high disease activity are at greater risk of developing non-Hodgkin lymphoma (NHL), clearly emphasizing a central role of B cell activation in the pathology of RA [7]. In a very first case report, successful treatment of arthritis with rituximab was observed in a patient suffering from NHL associated with RA [5], followed by another patient who had chronic lymphocytic leukemia and RA and achieved a complete remission of RA after B cell depletion [6]. These case reports suggested that B cell depletion therapy may be a promising novel treatment for refractory RA.

Table 1
Immune functions of B cells

Roles of B cells in the regulation of immune responses
• As precursors of (auto)antibody-secreting plasma cells
• As antigen-presenting cells
• In the differentiation of follicular dendritic cells in secondary lymphoid organs
• In lymphoid organogenesis
• In the development of effective lymphoid architecture
• In the initiation and regulation of T and B cell responses
• In the differentiation of effector T cells
• In the polarization into cytokine-producing effector B cells
• In the expression of co-stimulatory molecules
• In immune regulation (IL-10 positive B cells)

2. The role and importance of B cells under healthy conditions

B cells are phylogenetically the most recent evolutionary development in the immune system and are far more than just passive precursors of antibody-secreting cells. As recognized in recent years [8–10], they play an essential role in regulating immune responses, and dysregulation of B cell responses may consequently lead to an attenuated immune response and potentially to the development of autoimmune disease. In the healthy immune system, B cells are responsible for maintenance of cellular and humoral memory, fulfilling a number of important functions (Table 1). Following initial exposure to an antigen or pathogen, some B cells become memory cells and other ones differentiate into plasma cells. When the same antigen is encountered again, the cells are primed and the response raised against the pathogen is usually rapid and effective. Evidence also suggests that B cells are crucial in the orchestration of inflammatory pathways and provide important links between innate and adaptive immunity. Like T cells and macrophages, B cells produce pro-inflammatory cytokines. Moreover, they regulate the trafficking of immune cells firstly by producing chemokines and secondly by responding to chemokines through chemokine receptors expressed on their surface. In addition to the ability of activating antigen-presenting cells (APCs), B cells efficiently act as APCs themselves [8]. Furthermore, they are not only involved in T cell activation and expansion, but are also crucial in the organization and development of architecture of lymphoid tissues. Evidence shows that whenever the B cell system is impaired or depleted, lymphoid tissue lacks proper organization, and normal functioning of the immune system is disrupted [11].

3. Immunopathogenic role of B cells in rheumatoid arthritis

After several years of research on the role of T cells in the pathogenesis of RA including anti-CD4 therapy

[12], recently the influence of B cells regained interest. Similar to healthy conditions as outlined above, also in the case of RA these cells appear to have many more functions than just being precursors of plasma cells producing auto-antibodies [13]. Nevertheless, all facets of B cells in RA pathogenesis have not been completely delineated so far. Some of these major functions will now be discussed in more detail in order to provide a better understanding to what extent B cell depletion or B cell modulation may reduce inflammation in general and in the joints in particular.

3.1. Production of auto-antibodies

Rheumatoid factors (RF) as auto-antibodies against IgG have been known since 1940. They represent one of the two objective classification criteria of RA, detectable in about 80% of RA patients. Recently, the discovery of auto-antibodies to citrullinated peptides, such as anti-CCP [14] has improved the diagnostic specificity for RA to 90–98%. RF, anti-CCP and many other known auto-antibodies in RA (i.e. anti-keratin, anti-filaggrin, etc.) correlate very well with radiologic progression [15], providing indirect evidence for humoral disturbances in RA patients, especially in those with high disease activity.

Auto-antibodies form and enlarge immune complexes leading to activation of B cells and follicular dendritic cells (FDCs) via Fc receptors and complement receptors 1 and 2 (CR1 and CR2, also known as CD21 and CD35) [16,17] expressed on their surfaces, contributing to activation of the immune system. In addition, B cells express a specific B cell receptor (BCR). All these receptor systems, similar to the toll-like receptors (see below), combine the innate and adaptive immune system on the surface of B cells.

Immune complexes can be processed by B and other cells for antigen presentation, thereby enhancing local inflammatory processes. In RA, disease activity usually correlates with increased levels of RF-secreting cells, which also leads to higher auto-antibody levels in the synovial fluid as compared to the serum [18].

3.2. Production of cytokines

One of the central effector mechanisms of activated immune cells is the production of certain cytokines. This is extensively known for T cells and macrophages, and it has also been demonstrated for B cells [19]. Other studies showed that stimulated B cells secrete TNF-α, lymphotoxin and IL-6, which can act not only as autocrine growth and differentiation factors, but can also amplify immune responses [13]. Furthermore, they are able to produce IL-10, which activates FDCs and also stimulates B cell function via a feedback loop, perpetuating chronic inflammation. However, IL-10 as an immune regulatory cytokine can also prevent inflammation and therefore is not only pro-inflammatory [20]. Although cytokine production by B cells in RA synovitis appears to be of pathogenic importance, this has not been studied in greater detail yet.

3.3. Interactions with chemokines and chemokine receptors

Distinct expression patterns of chemokine receptors CXCR3, 4 and 5 in the blood of RA patients have been identified as compared to controls [21], indicating that there are changes in lymphocyte trafficking under disease conditions. A number of chemokines have been identified that contribute to the formation of ectopic lymphoid tissue. CXC chemokine ligand (CXCL) 13 and CXCL21 play a key role in the generation of germinal centers. While CXCL13 is involved in the recruitment of B cells, CXCL21 is responsible for attracting T cells. Both are also very important for the structure of the rheumatoid synovium. Another cytokine, lymphotoxin (LT)-β, which is produced by activated T cells, appears to be essential for the formation of B cell follicles in rheumatoid synovial lesions [11]. Current data suggest that chemokines and their receptors are involved in the organization of extrafollicular structures, whereas the value of blocking these systems as a therapeutic approach remains to be tested.

3.4. Antigen presentation and T cell activation

A number of immune cell types are able to act as APCs. It has been shown that B cells are able to internalize and process antigens into antigenic peptides. These antigenic peptides are then efficiently presented by B cells via major histocompatibility complex (MHC) class II molecules, one of them being HLA-DR4, which has been identified long ago as having an important association with RA [22]. Through antigen presentation by B cells, T cells become activated, leading to further TNF-α production, which subsequently activates macrophages.

Takemura et al. [23] found that the development of RA germinal centers is critically dependent on MHC class II positive cells and on the presence of B cells. When CD4+ T cell clones were transferred into SCID mice, the animals developed arthritis, but only in the presence of B cells. When B cells were depleted, no disease occurred, suggesting a critical dependence of the

arthritis pathophysiology on the presence of viable B cells, since all other compartments of the immune system remained intact [23]. These authors also showed that the production of pro-inflammatory cytokines by T cells can be disrupted by B cell depletion. Thus, follicular CD4 T cells failed to function in the absence of B cells in studies using human synovium-SCID mouse chimeras.

3.5. T cell-dependent and -independent B cell activation

In the classical view, B cell activation is dependent on T cells, since affinity maturation of B cell receptors (BCRs) by somatic hypermutation and class switching takes place in germinal centers after B cells encounter antigen and receive appropriate T cell help by CD40/CD154 interaction. Recent data provide evidence that B cell activation can also occur in the absence of direct T cell help by using FcγRIIb, Toll-like receptors (TLRs) and transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI) dependent activation in addition to BCR-ligation [4,16,24].

3.6. Ectopic lymphoid organogenesis

The rheumatoid synovial lesion is a highly vascularized tissue infiltrated by T and B lymphocytes and macrophages. In a substantial number of RA patients, aggregates of B lymphocytes surrounded by T cells are arranged in follicle-like structure around a network of FDCs [25,26]. These structures appear to be functional ectopic germinal centers. Since they represent the anatomical structures of affinity maturation, differentiation and proliferation of B cells, the local immune response is currently considered as the site of the autoimmune process. However, a major problem here is that the potential (auto)antigen in RA synovium has not been identified so far. Further evidence of enhanced local B cell activation has been reported by Ohata et al. [27], who demonstrated that B cell activating factor (BAFF or BlyS) is produced by fibroblast-like synoviocytes in RA synovium and contributes to enhanced B cell survival. Of note, the production of BAFF was shown to be enhanced under the influence of TNF-α or IFN-γ, indicating a close collaboration of different cell types in the inflamed synovium.

3.7. Relationship between innate and adaptive immunity in RA

In a reversal of conventional immunology in which the innate immune system activates the adaptive one, it is believed that in RA and likely in other entities this relationship can operate in the opposite direction, activating macrophages and leading to inflammatory damage induced by an activated adaptive immune system [1,2]. Experiments in animal models have demonstrated that activation of B cells via TLRs plays a significant role in the development of antibody-mediated, collagen-induced and adjuvant-induced arthritis [28–30]. Current data suggest that TLR-dependent B cell activation allows an immune response to be initiated and/or propagated independently of T cells. The relative impact of these distinct pathways of B cell activation under conditions of health and disease needs to be explored in further studies. However, it is likely that TLRs have a similar role in RA to that observed in the healthy immune response.

4. Clinical perspectives

Taken together, with increasing understanding of the mechanisms in which B cells are involved, there is strong evidence arising showing that B cells play a central role in regulatory and effector immune functions in the pathogenesis of RA, making them an attractive target for the treatment of RA and other autoimmune diseases. New insights into the various functions of B cells may help us to further reveal the secrets of RA and other systemic autoimmune diseases, such as SLE, Sjögren's syndrome, etc., where B cells have long been considered to be of pivotal importance for the pathogenesis. This knowledge brings new possibilities for novel therapeutic approaches.

Of particular importance, data currently available about the use of anti-CD20 (rituximab) in RA do not indicate a greater susceptibility to infection, i.e. a similar rate of serious infections was observed in the rituximab and control groups at weeks 24 and 48 in a phase II trial [3]. Similarly, reports of open studies using anti-CD20 (rituximab), anti-CD22 (epratuzumab) or anti-BLyS (belimumab) in RA and a variety of diseases do not show a specific tendency to develop infections, although we do not have long-term safety data in patients since studies were initiated very recently.

There is a medical need on the one hand in RA patients refractory to conventional therapy as well as to TNF/IL-1 blockade, and on the other hand in patients with other systemic autoimmune diseases where other therapies have failed. B cell-directed therapies either by depletion or modulation have the potential to provide better treatment options by different mechanisms of actions for a number of patients, as discussed elsewhere in this issue (see also Fig. 1). It will be the challenge of future studies to identify patients not only early but also

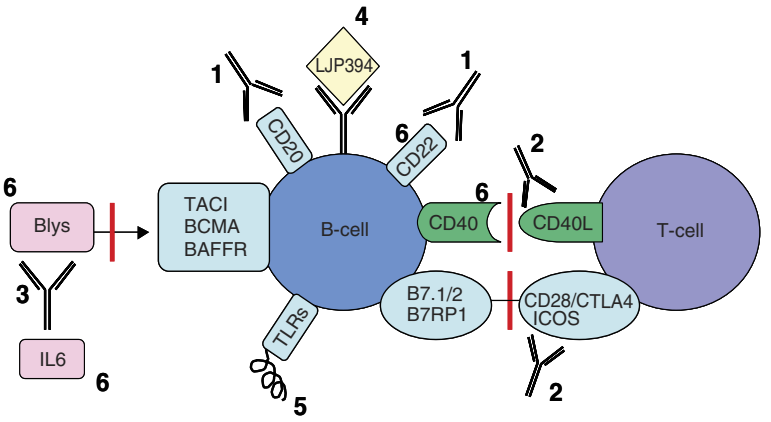

Fig. 1. Possible mechanisms and strategies of B cell directed therapies in autoimmune diseases (modified according to Looney et al. [31]): (1) Use of antibodies against surface antigens. (2) Use of antibodies for blockade of co-stimulatory molecules. (3) Use of antibodies for inhibition of cytokines. (4) Use of tolerogens for induction of cell inactivation. (5) Use of inhibitory and activating oligonucleotides for modulation of receptors. (6) Inhibition and/or modulation of survival factors.

correctly for certain treatment regimens–given the heterogeneity of patients and of new therapies arising–in order to achieve a rapid and effective remission utilizing a patient-tailored therapy.

Take-home messages

- In RA, synovial B cells undergo differentiation and proliferation within extrafollicular germinal centers in a significant number of patients.
- Some pathogenic features of B cells are: production of (auto)antibodies (as plasma cells) and of pro-inflammatory cytokines, interaction with chemokines, antigen presentation and activation of T cells and dendritic cells.
- B cells link innate and adaptive immunity by complement activation and through complement receptors, Toll-like receptors and their ligands, and Ig/Fc receptors.
- B cell-directed therapies, such as B cell depletion, provide evidence that inflammatory circuits dependent on the presence of B lymphocytes can be interrupted. Pro-inflammatory B cell functions in distinct entities appear to be heterogeneous, but reflect the potential of selective therapeutic options.

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The role of aspirin versus aspirin and heparin in cases of recurrent abortions with raised anticardiolipin

The present study was undertaken to compare the role of aspirin versus aspirin plus heparin combination in pregnant women with poor obstetric history and raised anticardiolipin antibodies IgG (IgG(aCL)). Goel N, et al. (Med Scie Monitor 2006; 12: CR132-6), conducted a study on 550 pregnant women, 450 with a history of two or more spontaneous abortions forming the study group, while 100 women with one or more live births and no history of abortion were controls. Their blood was tested to assess the level of IgG aCL by ELISA. The test was strongly positive in 72 (16%) patients of the study group, who were randomized to receive either low-dose aspirin (80mg/day) or a combination of low-dose aspirin (80mg/day) and 5000IU of unfractionated heparin subcutaneously 12 hourly under hospital surveillance. The pregnancy outcome was statistically compared. Of the 39 patients treated with low-dose aspirin, 24 (61.5%) gave birth to live issues compared with 28 (84.8%) of the 33 women given a combination of aspirin and heparin ($p < 0.05$), an overall success rate of 72.2%. Mean birth weight of the babies given treatment with heparin and aspirin was 3.21 ± 0.33 kg compared with 2.7 ± 0.14 kg achieved with aspirin alone ($p < 0.001$). Both treatments were well tolerated. The study provides evidence that in case of recurrent abortions with raised IgG aCL, treatment with a combination of aspirin and heparin showed better outcome than treatment with aspirin alone.