The B Cell in the Pathogenesis of Rheumatoid Arthritis

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Classically, B-cells have been considered to play a secondary role in the pathogenesis of rheumatoid arthritis, restricted to the production of autoantibodies.

Nevertheless, the unexpected good clinical response that the systemic depletion of B-cells has shown in a well-controlled clinical trial in patients with rheumatoid arthritis has revitalized the interest in this cell type in the pathogenesis of this autoimmune disease. Several evidences suggest that B-cells can regulate the course of the immune response through antibody production independent mechanisms.

These mechanisms include antigen presentation and the release of soluble factors such as proinflammatory cytokines, metalloproteinases, and chemokines.

This article reviews experimental data supporting that the participation of B-cells in the pathogenesis of rheumatoid arthritis occurs through multiple mechanisms.

Key words: B-lymphocytes. Rheumatoid arthritis. Pathogenesis.

La célula B en la patogenia de la artritis reumatoide

Hasta hace poco tiempo se consideraba que las células B tenían un papel secundario en la patogenia de la artritis reumatoide, limitado a la producción de autoanticuerpos. Sin embargo, la sorprendente buena respuesta clínica que la depleción sistémica de las células B ha demostrado en ensayos clínicos controlados y aleatorizados con pacientes con artritis reumatoide ha revitalizado el interés por el papel del linfocito B en la patogenia de esta enfermedad autoinmunitaria. Diversas evidencias indican que las células B pueden regular el curso de la respuesta inmunitaria mediante mecanismos alternativos no dependientes de la producción de anticuerpos. Estos mecanismos incluyen la presentación de antígenos y la liberación de factores solubles como citocinas proinflamatorias, metaloproteasas y quimiocinas. En esta revisión se resumen los datos experimentales que indican que la célula B participa en la patogenia de la artritis reumatoide mediante un mecanismo multifactorial.

Palabras clave: Linfocito B. Artritis reumatoide. Patogenia.

Introduction

Rheumatoid arthritis (RA) is an chronic inflammatory disease that causes the progressive destruction of diarthrodial joints and that leads to loss of function and shortens the life expectancy of patients by interfering with vital organ function.1,2 Although the pathogenesis of RA is complex, in general terms its basic mechanisms are well established: a) the proliferation of endotelial and synovial cells, especially fibroblast-like synoviocytes (FLS); b) the recruitment and activation of proinflammatory circulating cells: T- and B-lymphocytes; and monocytes, and c) the secretion of cytokines and chemokines; mainly by monocytes/macrophages and FLS.3 Multiple lines of evidence point to the CD4+ T-lymphocyte as the initiator of synovitis in RA, through the recognition of a still unknown arthritogenic antigen.4,5 The monocyte/macrophage seems to play an important role in the perpetuation of inflammation through the secretion of soluble proinflammatory mediators, among which tumor necrosis factor alpha (TNFα) stands out predominantly. FLS are the effector cells that carry out the joint destruction by invading joint tissue and secreting metalloproteinases (MMP) in response to the inflammatory synovial environment. With respect to B-cells, their role in the pathogenesis of RA has largely been considered as secondary and limited to the production of autoantibodies, without any apparent pathogenic role. Nonetheless, results from a recent controlled and randomized clinical trial have shown that the systemic depletion of B-cells is useful for the management of the
symptoms and signs of RA. This unexpected result has shifted a part of the research effort in rheumatology toward the study of the implication of B-cells in the pathogenesis of RA.

This review attempts to summarize the point in which we currently stand in our knowledge of the role that B-cells play in the pathogenesis of RA. In light of the current studies, it seems evident that B-cells participate in RA synovitis by more than one mechanism, including the activation of T-lymphocytes, the production of autoantibodies, and the secretion of diverse soluble factors with both proinflammatory and effector activity.

The question of which of these B lymphocyte mediated actions is most relevant in RA or if other, still unknown actions exist remains still unanswered.

Pathogenesis of Rheumatoid Arthritis

RA is an autoimmune disease in which the unleashing event is assumed to be an exaggerated immune response to an environmental antigen that affects a genetically susceptible individual. The first pathogenic event in RA is probable mediated by the activation of antigen-dependent T-cells. We currently do not know what the exogenous element, one or several, that causes this alteration of the immune response is, or whether it is the same for all affected individuals or, on the contrary, differs among the affected subjects. In the past few years there has been an intense effort to try and identify the T cell clones that lead to the first events in joint destruction in RA, because this could help define the nature and the structure of the initial antigen. Unfortunately this objective still has not been reached, making it probable that several antigens are probable involved in the initiation of the inflammatory process in a predisposed host.

The activation of T-lymphocytes leads to multiple effects such as the proliferation of synovial and endothelial cells, the recruitment of other proinflammatory cells present in the circulation such as macrophages/monocytes and B-cells, the secretion of cytokines and proteases by macrophages and fibroblasts and, lastly, to the production of autoantibodies. T-lymphocytes constitute 50% of the cells present in the rheumatoid synovium, most of them CD4+, while B-lymphocytes and plasmatic cells constitute less than 5% of total. The role of B-lymphocytes in the pathogenesis of RA has recently gained notoriety. The recent success with the use of rituximab (chimeric monoclonal anti-CD20 antibody) has led researchers and clinicians to reconsider the role of B-lymphocytes in the pathogenesis of RA.

In a large group of patients with RA, synovial membrane infiltrating cells are organized in a way that is similar to germinal centers of secondary lymphoid organs, forming a microstructure constituted by T-cells that envelop B-cells and surrounded by a layer of dendritic cells. In this heterogeneous microstructure interactions are carried out, both physical and through soluble factors, mainly cytokines, generating signals that are capable of modulating the function of FLS. All of these changes lead to hyperplasia of the synovial membrane, which is transformed into an exceedingly vascular granulating tissue (pannus) that invades and destroys joint tissue through the action of MMP produced mainly by FLS.

B-Lymphocytes

Lymphocytes are cells that are in charge of specific or adaptive immune responses. Lymphocytes that mature in the bone marrow are called B-lymphocytes (bone marrow, bursa) and are responsible for the synthesis of antibodies when faced with an antigenic challenge; those that mature in the thymus are called T-lymphocytes, originating cell-mediated immune responses. Once maturity is attained, lymphocytes are distributed through different organs and lymphoid tissue. From an immunological standpoint these organs are divided into primary lymphoid organs, those in which lymphocyte development takes place (lymphopoiesis) from an antigen-independent process (fetal liver during pregnancy and bone marrow in adults), and peripheral (spleen, lymph nodes, mucosal associated lymphoid tissue, Peyer’s patches). B-lymphocytes migrate to the latter organs once they have matured to develop a response to antigen.

B-cells develop from bone marrow stem cells where, through a process of maturation and activation (Figure 1), they acquire unique antibody specificity (variable Fc region of immunoglobulin). After migrating via the circulation, they nest in perifolicular areas, germinal centers and the memory compartment of lymph nodes as well as in the spleen, to finally return to the blood marrow, where they remain in the form of plasma cells. Maturity and survival of B-cells in these different stages depends on the liberation of trophic and survival signals through surface receptors such as vascular cell adhesion molecule (VCAM)-1 and soluble factors such as BAFF or BLYS (B cell activating factor). Mature B-lymphocytes have a short lifespan unless they undergo activation after coming into contact with an antigen, dendritic cells, or T-lymphocytes. Activated B-lymphocytes undergo clonal expansion while those that are not are condemned to programmed cell death in a matter of days.

B-Lymphocytes and Rheumatoid Arthritis

In a large group of patients, synovial membrane infiltrating cells are organized similar to the germinal centers of the
secondary lymphoid organs (follicular synovitis). These structures contain both T- and B-cells and are surrounded by dendritic cells. In other patients, B and T cell cumulus lacking dendritic cells are formed, but without the typical germinal reaction.

Less frequently, some rheumatoid synovial present inflammatory infiltrates that are organized in a diffuse form without an organized lymphoid organ structure. It has been pointed out that the presence of these 3 histological patterns in patients with RA is stable over time and is repeated in all of the affected patients’ joints. Factors involved in the formation of these patterns and their clinical consequences are still clarified and detailed. However, there seems to be a correlation between the histologic patterns and B cell activity markers, indirectly indicating that these cells are determinant to the synovial infiltration pattern in RA. Synovial B-lymphocytes contained in the germinal centers express larger quantities of RNAm for IgG than those present in synovial cell aggregates, while patients with a diffuse pattern of synovial B cell infiltration contain the lowest IgG RNAm titers of the 3. It is known that the organization of lymphoid cells is orchestrated by chemokines (low molecular weight cytokines involved in chemotaxis). Two chemokines denominated CXCL13 (B cell chemotactic chemokine 1 or BCA-1) and CCL21 (secondary lymphoid organ chemokine or SLC) seem to be determinant in the formation of synovial infiltrates in RA. The origin of these chemokines and what would result from a therapeutic blockage of these molecules or their receptors for the management of RA is still undetermined.

Another aspect that implicates B-lymphocytes in the generation of an infiltrative pattern in RA is provided by 2 members of the TNF superfamily, concretely lymphotoxins LT-α and LT-β, which are also differentially expressed in RA synovitis. IL-10, an immunoregulatory cytokine, is overexpressed in synovial tissue and inhibits the production of pro-inflammatory cytokines, such as TNF-α and IL-6, and promotes anti-inflammatory cytokines, such as IL-10. This suggests a role of IL-10 in the modulation of the inflammatory response in RA. However, the role of IL-10 in B cell differentiation and function is complex and may differ depending on the context.

The interaction between B cells and other immune cells, such as T cells and dendritic cells, is crucial for the development of the immune response. B cells can be activated by antigens presented by dendritic cells, leading to the production of autoreactive antibodies that can contribute to the pathogenesis of RA. The therapeutic targeting of B cells, such as with rituximab, an anti-CD20 monoclonal antibody, has shown promise in clinical trials for the management of RA.

Figure 1. Development of B-cells. Represented here are the stages of maturation of B-cells, their anatomical localization, and the expression of cell surface markers, including CD20, the target molecule for rituximab. IL indicates interleukin; MMP, metaloproteinase; TNF, tumor necrosis factor.
expressed according to the type of inflammatory infiltrate. LT-β is known to be synthesized by the B cell and its RNAm present in synovial tissue is strongly associated with the production of IgG. Immunohistochemical studies have determined that LT-β is produced by a specific B lymphocyte subtype (LT-β+) that are arranged in the follicular mantle zone of the lymphocyte aggregate and not anywhere else, indicating its key role in the disposition of ectopic lymphoid organ generation that takes place in the rheumatoid synovium.

Based on diverse experimental data, 3 hypotheses have been formulated on the potential role of B-cells in RA (Figure 2): a) B-cells regulate the function of T-cells, fundamentally CD4+, by acting as their main antigen presenting cells and inducing their activation as well as the production of proinflammatory factors; b) generation of autoantibodies by T-cells favors the formation of immune complexes, that through their binding with Fc receptors and the activation of complement activate the production of proinflammatory factors by macrophages; and c) infiltrate B-cells directly produce soluble factors, some with a proinflammatory capacity (cytokines) and others with the ability to directly remodel the extracellular matrix, as is the case with MMP.

It has not been determined which of these hypothesis is more relevant or if other, still unknown mechanisms, are involved.

**B-Lymphocytes as Antigen Presenting Cells**

B-cells have the capacity to present antigens to lymphocytes T in a very efficient manner. There is solid evidence that has been obtained in animal models that attributes B-lymphocytes with the capacity to regulate the activity of T-cells in the rheumatoid synovial. In very elegant experimental studies, synovial tissue from patients with active RA and follicular synovitis were implanted in animals with severe combined immunodeficiency; when these animals underwent the injection of CD4+ T-lymphocytes extracted from the synovial follicles of RA patients, production of proinflammatory cytokines such as TNF-α, IL-1β (IL-1β) and interpheron gamma (IFN-γ) in the implanted synovium was 2 to 3 times higher than when the animals received CD4+ clones from peripheral blood or saline solution. However, when animals were implanted with synovial tissue that has scarce or null quantities of CD20+ B-cells (patients with diffuse synovitis), the

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**Figure 2. Schematic representation of the possible roles of B-cells in RA.**
transference of the same T cell clones did not elevate noticeably the levels of cytokines in the synovial implants, in spite of the presence in both synovial implants of CD83+ dendritic cells capable of presenting antigens. Also, the administration of human anti-CD20 monoclonal antibodies (rituximab) to the mice produced a delayed reduction dependent of IFN-γ and IL-1β in the implanted synovium.\textsuperscript{17}

These observations indicate that the activation of T-cells in the rheumatoid synovial depends on the presence of B-cells in the synovial microenvironment. The nature of T/B cell interaction, apparently essential to synovial swelling, is unknown; nonetheless 2 possibilities seem equally plausible: the B cell acts as an antigen presenting cell to autoreactive T-cells, inducing their activation, or the presence of B-cells in the rheumatoid synovial, through the liberation of soluble factors, generates survival signals for T-cells.\textsuperscript{15}

Production of Autoantibodies in RA

In the 1970s, a series of immunological findings established RA as a B cell mediated disease. These findings included: the frequent detection of autoantibodies, especially rheumatoid factor (RF), the presence of immune complexes, the scarcity of complement in the rheumatic joint, and the detection of immunoglobulin and complement deposits in the cytoplasmic inclusions of macrophages. All of this led to the conclusion that a local immune response directed against joint structures produced arthritogenic antibodies.\textsuperscript{24} The presence of plasma cells and B-lymphocytes in the rheumatoid synovial organized as lymphoid follicles gave a cellular basis to this pathogenic hypothesis.

Over the course of the following years, the relevance of RF started to be questioned. A group of patients were negative for RF and the infusion of RF into healthy individuals did not produce signs and symptoms of the disease.\textsuperscript{25} In the past few years, the potential pathogenic role of autoantibodies in RA has once again been examined based on animal model findings and the above commented response that has been observed in clinical trials using anti-B cell therapy. Both of the groups of antigens that are recognized by autoantibodies in RA and that recently have become the focus of greater attention are citrullinated proteins and glucose-6-phosphate isomerase. Many patients with RA have antibodies against diverse proteins.\textsuperscript{24} Of special interest are antibodies directed against citrullinated proteins. Citrullination represents a posttranslational modification due to enzymatic deamination of arginine residues to residues of citruline on defined proteins. It is known that citruline is the specific antigen against which these antibodies are directed. It is believed that citrullination is a product of an abnormal protein metabolism and that it is related to abnormal B cell apoptosis due to an aberrant clonal stimulation.\textsuperscript{25,26} In spite of what was initially said,\textsuperscript{27} protein citrulination is not specific to RA.\textsuperscript{28} There is recent evidence that anticitrulline antibodies are directly implicated in the generation of arthritis in a collagen-induced arthritis (CIA) murine model.\textsuperscript{29} However, the pathogenic relevance of these antibodies in human RA is still to be determined. In general, anticitrulline antibodies are considered less sensitive but more specific that RF and appear earlier in the course of the disease.\textsuperscript{26,30,31}

The discovery that antibodies directed against glucose-6-phosphate isomerase (GPI) produced by B-lymphocytes can induce arthritis in healthy mice\textsuperscript{32} has led to the study of the implication of these autoantibodies in patients affected by RA. There is 64% of positivity for anti-GPI antibodies in RA patients, especially in those with extraarticular manifestations,\textsuperscript{33} but studies done afterward have not detected such an elevated presence,\textsuperscript{26} and these antibodies have been described in other inflammatory joint diseases,\textsuperscript{34} cancer, and autoimmune processes.\textsuperscript{35,36,37} GPI is a very ubiquitous cytoplasmic protein, also known as autocrine motility factor (AMF) or neuroleucin, playing a relevant role in the energy cycle that intervenes in glucose metabolism. GPI also has other functions and acts as an extracellular molecule with signaling capacity. The reason for which such a widely distributed protein is capable of generating an eminently articulate disease in mice seems to reside in its non-specific capacity to bind to circulating GPI.\textsuperscript{38} Still, it is unknown if antibodies against it have the capacity to induce selective inflammation in human joints.

Production of Soluble Factors by B-Cells

Finally, it seems that B-cells may play a relevant role in the final effector mechanisms that cause structural damage in RA joints. B-cells release proinflammatory cytokines such as TNFα, IL-1β, and lymphotoxin.\textsuperscript{39,40} It is well established that TNFα and IL-1β are potent inducers for the liberation of MMP, which are final pathway effector substances in joint disease by FLS.\textsuperscript{41,42} Braun et al\textsuperscript{43} has demonstrated that LT-α1β2 secreted by B-lymphocytes have functions beyond the conditioning of the tridimensional disposition of the inflammatory network, leading to profound changes in the function of FLS by increasing the synthesis of IL-1β, MMP, and chemokines such as CCL2, CCL5, and CCL8. B-cells have been shown to be capable of producing a liberating MMP-9 (gelatinase) in response to cytokines such as IL-1, or after activation of phorbol esthers.\textsuperscript{20} The true role that proteolytic enzymes liberated by B-cells in the degradation of the cartilage and bone matrix in RA has not been studied. Recently, several members of the chemokine family have been implicated in tissue destruction.
mechanisms after inducing the secretion of MMP (collagenases and gelatinases) in FLS of patients with RA. But until now very little is known about the pattern of cytokine production by peripheral blood B-cells and practically nothing is known on the type, quantity and functional capacity of chemokines produced by B-cells by synovial tissue infiltrating B-cells in patients with RA. In light of current knowledge, it seems evident that B-lymphocytes are important in the pathogenesis of RA and participate in synovial inflammation through several different mechanisms. In addition to their classic role producing autoantibodies, B-cells present self-antigens to CD4+ T-cells in the rheumatoid synovial, participate in the synovial follicular structures and liberation of diverse soluble proinflammatory factors and have the capacity to remodel the extracellular matrix. The relative importance of these B cell functions in synovial inflammation is still currently unknown and it is very probable that their study will lead to more specific and effective anti-B cell therapy.

References
