The Role of B Cells in the Pathogenesis of Rheumatoid Arthritis

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ABSTRACT. The classical paradigm for rheumatoid arthritis (RA) pathogenesis holds that CD4+ T cells mediate joint damage both directly and by driving non-T effector cells to release inflammatory cytokines. By contrast, the new paradigm that is developing centers on an interaction of CD4+ T cells with B cells. Evidence reviewed in this article shows that autoreactive B cells can be driven by the T cells to produce IgG autoantibodies that may be directly involved in joint damage, and B cells are known to be critical in activating CD4+ T cells. As the B cell appears to play an important role in the RA process, it is appropriate to consider how B cell-mediated effects might be reduced or prevented in patients with this disease. As the targeted depletion of B cells with a monoclonal antibody such as rituximab appears to be clinically effective in RA patients, this approach shows great therapeutic potential. (J Rheumatol 2005;32 Suppl 73:14-18)

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INTRODUCTION

The development of rheumatoid arthritis (RA) has a strong genetic basis. This genetic predisposition involves major histocompatibility complex (MHC) class II genes as well as many non-MHC genes; the identities of some of the latter have been suggested by sibling linkage studies[1-4]. In individuals with a genetic predisposition to RA, the appropriate environmental stimulus, for example exposure to infection or even smoking, triggers a series of cellular and molecular events, likely initiated by activities of CD4+ T cells.

The evidence for the critical role played by CD4+ T cells in the pathogenesis of RA is now very substantial[5] and is centered on 2 mechanisms in particular. First, RA has been shown to be associated with particular MHC class II molecules, for example the human leukocyte antigen (HLA)-DRB1 *0401 and *0404 alleles in white populations[6]. These alleles have a common sequence motif in one of the hypervariable regions of the gene (called the shared epitope)[7]; the binding and presentation of peptides and their recognition by T cells are controlled by variations in this region of the gene. Second, there is pronounced infiltration of CD4+ T cells, including a number of activated cells, into the synovial tissue of patients with RA[5].

Other supportive evidence for the importance of CD4+ T cells includes the presence of clonal expansions of T cells in the synovium that indicate a response to stimulating antigens. Moreover, CD4+ T cells themselves are critically involved in the stimulation of non-T effector cells to produce inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin 1 (IL-1)[5]. As evidenced by the effectiveness of recently developed therapies, these cytokines have a critical role in the pathogenesis of RA and its associated joint destruction.

Although the classical paradigm of RA pathogenesis is centered on the role of the CD4+ T cells, new evidence has emerged in the last few years that some parts of the pathogenetic mechanism in RA depend on B cells. Exciting recent work, described in this article, has shown that IgG autoantibodies produced by autoreactive B cells may be directly involved in tissue damage in RA, and that these B cells, and possibly other types of B cells, can mediate synovial CD4+ T cell activation. Indeed, there is now much support for a new paradigm for RA pathogenesis, the most telling contrast with the classical model being the critical role played by B cells, and possibly autoantibodies. The article also reviews new and provocative data for the role in RA of disease-relevant autoantibodies, especially those directed to citrulline-containing proteins. It will also review the way in which B cells might stimulate the infiltration and activation of CD4+ T cells, perhaps in the synovium, and the possible role of B cells in cytokine production.

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EVIDENCE FOR THE INVOLVEMENT OF B CELLS IN RA

The K/BxN transgenic model of arthritis. Some of the most exciting evidence for the role of B cells and autoantibodies in the pathogenesis of RA has come from an animal model of inflammatory arthritis, the K/BxN transgenic model of arthritis. This model was developed by a group of workers who were studying T cell responses to bovine ribonuclease in a K/B transgenic mouse (KRN T cell receptor expressed on a C57BL/6 background). When the K/B mouse was crossed with the non-obese diabetic (NOD) mouse, the resulting hybrid mouse (K/BxN) developed a very severe arthritis with a pathology typical of RA[8]. Subsequently, it was shown that the only inherited factors that were important in causing the disease in these mice were the T cell receptor from the KRN mouse and MHC class II genes from the NOD mouse, referred to as I-A^{g7} [9]. The interaction between the CD4+ T cells and I-A^{g7} caused T cell activation and resulted in the production of autoantibodies that mediated joint damage in the hybrid mouse[10]. The self-antigen to which these autoantibodies were directed was found to be a ubiquitous glycolytic enzyme, glucose-6-phosphate isomerase (GPI)[9].

Experiments have shown that serum from the K/BxN mouse could immunoprecipitate recombinant GPI and was able to bind strongly to GPI in an ELISA. Using T cells derived from hybridomas and lymphomas from the K/BxN mouse, it was shown that the T cells recognize a GPI peptide when it is presented by I-Ag^7-expressing presenting cells, and react by stimulating the B cells to produce the anti-GPI autoantibody[9]. Together, these studies confirmed that GPI was the protein being recognized in the K/B x N mouse.

Crucially, it has also been shown that the anti-GPI autoantibody produced in this model was pathogenic. When serum from the K/B x N mice was injected into normal mice, the recipient mice developed marked arthritis. When the serum was passed over an affinity column containing GPI, the bound proteins (once eluted) produced arthritis in the recipient animals but the flow-through fraction, despite containing a large amount of IgG, did not. When the serum was passed over a control column (without GPI), the bound proteins eluted from it produced no arthritis in the recipient mice, showing that the arthritogenic component in the serum was anti-GPI antibodies[9].

Other recent studies in the K/BxN mouse model have shed more light on the way in which the autoimmune response to GPI produces arthritis. It has been shown that the GPI molecule itself appears to be present on the cartilage surface, and that anti-GPI autoantibodies can therefore bind to the articular surface and form immune complexes[11]. Effective mechanisms of inflammation are then set in place, some of which involve Fc receptor-bearing effector cells and, importantly, the activation of the alternative complement pathway[12]. One hypothesis that has been suggested is that because the cartilage surface lacks certain inhibitors of the complement pathway, complement activation is greater than it would be in other tissues; this may explain why the autoantibodies appear to produce immunopathology only in the joints of these animals[11].

In addition, the involvement of neutrophils in the pathogenesis of arthritis in the K/BxN mouse has been reported[13], and there is substantial evidence that mast cell pathways are also critical[14,15]. It is also interesting that both IL-1 and TNF- are prominently involved in the K/BxN model of arthritis[16]; these cytokines are centrally implicated in the pathogenesis of RA, as noted above.

As described, IgG autoantibodies from this K/BxN model of arthritis can initiate a severe and destructive arthritis, in this case mediated by anti-GPI antibodies or complexes that bind to the cartilage. The autoantibodies are not directed to a joint-specific antigen such as type II collagen or human cartilage glycoprotein 39, yet the only disease the animals have is arthritis. This raises the interesting question of whether systemic versus joint-specific antigens may be key to the pathogenesis of RA.

Pathogenic autoantibodies have been well documented in a number of different autoimmune diseases. Examples of autoantibody-mediated diseases include autoimmune thrombocytopenia, myasthenia gravis, and systemic lupus erythematosus (SLE). In diseases such as type 1 diabetes, where T cells are thought to mediate the actual destructive process, autoantibodies are markers of the underlying CD4+ T cell recognition. Even if autoantibodies are not instrumental in the pathogenesis of RA in humans, they still may be important disease markers, because they reveal which antigens are being recognized by disease-relevant CD4+ T cells.

Autoantibodies in RA. The second crucial finding in relation to the function of B cells and production of autoantibodies in RA relates to new insight into the autoantigen targets. The 2 most important antigen groups that are recognized by autoantibodies in RA are rheumatoid factor (RF), which is directed to the Fc region of IgG, and citrullinated proteins. Although relatively little new work has been done on the role of RF, the possible role of antibodies directed against citrulline-containing proteins is rapidly gaining attention.

Citrullination is the process by which the arginine residues in a peptide chain are converted to citrulline by deimination after the protein has been synthesized. Citrullination is catalyzed by the enzyme peptidyl arginine deiminase (PAD) (reviewed in van Venrooij and Pruijn[17]). Autoantibodies have been found in RA patients that do not bind certain arginine-containing peptides but do bind to the same peptides containing citrulline[18]. The ELISA that is used to determine levels of these antibodies features a cyclic peptide containing citrulline (cyclic citrullinated peptide; CCP) that is derived from filaggrin, the prototypic citrullinated protein antigen identified as a target in RA.

Once anti-CCP antibodies are produced, they can recognize not only filaggrin but a number of other citrulline-containing proteins, including fibrin and fibrinogen, both of which can be expressed prominently in the joint[17]. Anti-CCP antibodies may be present in over 70% of RA patients but in fewer than 5% of healthy individuals[18-20]. Anti-CCP autoantibodies may therefore be more specific than any other known autoantibodies in RA, including RF. In epidemiologic and longitudinal studies, anti-CCP antibodies have been shown to predate the development of RA by a number of years and can predict the development of RA[21,22]. Unfortunately, the specificity of the CD4+ T cell that drives B cells to make these anti-CCP antibodies has not yet been identified. Although the autoantibody binds to specific peptides of filaggrin, for instance, the peptide initially recognized by the T cell could be any of a number that are derived from this or other proteins. Notably, as these citrullinated peptides and proteins are created in the joint space itself, they may represent new types of self-antigen to which the T cell has not previously been exposed or tolerized.

PAD, the enzyme responsible for citrullination, exists in 4 forms in mammals. Expression of mRNA for these enzyme forms has been measured in mouse synovium using a reverse transcriptase-polymerase chain reaction with isotype-specific primers[23]. Although PAD2 was expressed in the synovium, the level of expression was no different during the induction of experimental arthritis from that in control tissue; however, the expression of PAD4 by neutrophils was seen only in the presence of inflammation, and expression appeared to correlate with the degree of inflammation[23]. A case-control association study has shown that polymorphisms of the gene that encodes the PAD4 isoenzyme, called *PADI4*, is associated with RA in Japanese populations[24]. Previous studies had suggested that a chromosome 1 locus, which encompasses PADI genes, is associated with RA. The level of association of various PADI genes with RA was investigated by looking at a number of single nucleotide polymorphisms (SNP) in this region. Certain SNP in the region encompassing exons (coding sequences) 3, 4, and 5 of *PADI4* are strongly associated with disease[24]. From these data, one haplotype of *PADI4* was identified to be associated with disease susceptibility to RA. This haplotype affected the stability of transcripts and was associated with serum levels of anti-CCP antibody in RA patients.

If *PADI4* alleles are confirmed to be associated with the development of RA, this has far-reaching implications for the pathogenesis of disease, as the autoimmune response in question would not be just a marker of the process. If citrullination of proteins is a critical genetic predisposition, the immune response to these altered proteins and likely anti-CCP antibodies must be critically involved in the development of the disease. If this can be confirmed in other human populations, it will be one of the most exciting findings of recent years and should offer tremendous insight into the pathogenesis of RA.

Interaction of T cells and B cells. Another potential mechanism for B cell involvement in RA has emerged from work conducted with another animal model of the disease[25] in which rheumatoid synovial tissue from patients with RA is implanted into non-obese diabetic-severe combined immunodeficient (NOD-SCID) mice. Tissues that lacked B cells did not support the infiltration and activation of adoptively transferred CD4+ T cell clones. Dependence of T cell activation on B cells was further demonstrated in studies in which mice were treated with a monoclonal antibody to CD20 (rituximab) to deplete their B cells. Subsequent examination of the grafted synovial tissue showed that the antibody treatment reduced infiltration by T cells. In addition, the ability of the tissues to produce the inflammatory cytokines interferon-γ and IL-1 was inhibited in a dose-dependent manner in the B cell-depleted mice (Figure 1[25]). Thus, the ability of synovial T cells to drive a Th1-type inflammatory process appears to be governed by the presence of B cells.

The findings of the SCID mouse study suggesting that B cells are instrumental in the activation of synovial CD4+ T cells are consistent with the specific antigen-presenting role of B cells. After taking up antigen through the immunoglobulin receptor, B cells process the antigen and express peptides bound to MHC class II molecules, which are recognized by the CD4+ T cell. Because of this internalization of antigen, B cells are estimated to be 100 to 1000 times more potent as stimulators of T cell activation to a specific antigen compared with other types of antigen- presenting cells, such as macrophages or dendritic cells[26]. B cells that are specific for RF may also take up immune complexes and present a number of different autoantigens. Such B cells act as highly efficient antigen-presenting cells for low concentrations of immune-complexed antigen[27]. B cells (especially when activated) express increased surface levels of molecules important in the costimulation of T cells, such as B7 and CD40[28].

Cytokine production by B cells. Finally, it appears that B cells may play a critical part in the effector mechanism of tissue damage in RA. New evidence suggests that B cells may release proinflammatory cytokines, such as TNF-α, IL-1, and lymphotoxin[29,30], and that they are capable of producing IL-6 and IL-10, which further stimulate B cell function via a feedback loop, thus perpetuating chronic inflammation. B cells may also be involved in another aspect of the inflammatory cascade by releasing cytokines that have important effects on follicular dendritic cells and the development of germinal centers[29,31].

B cell depletion in the clinical setting. The ability of B cells to produce certain autoantibodies, efficiently present antigen to activated CD4+ T cells, and enhance inflammatory responses are mechanisms by which they may influence the pathogenesis of RA. A critical role for B cells in RA is supported by recently presented evidence

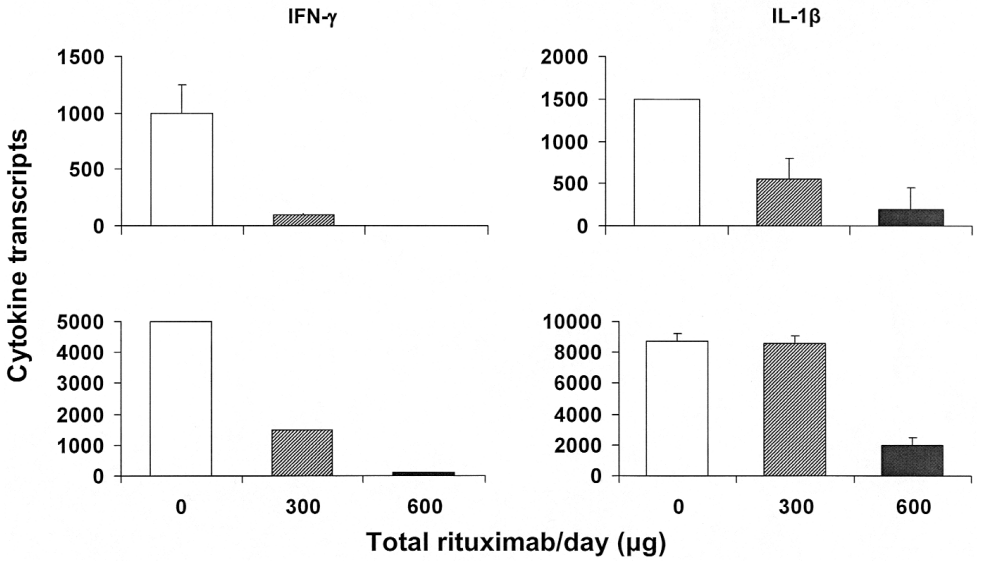

Figure 1. Effect of B cell depletion on synovial interferon-γ (IFN-γ) and interleukin 1β (IL-1β) in human synovium-SCID mouse chimeras 6 days after treatment with rituximab[25]. From: Takemura S, *et al.* T cell activation in rheumatoid synovium is B cell dependent. J Immunol 2001;167:4710-8. With permission of The American Association of Immunologists, Inc.

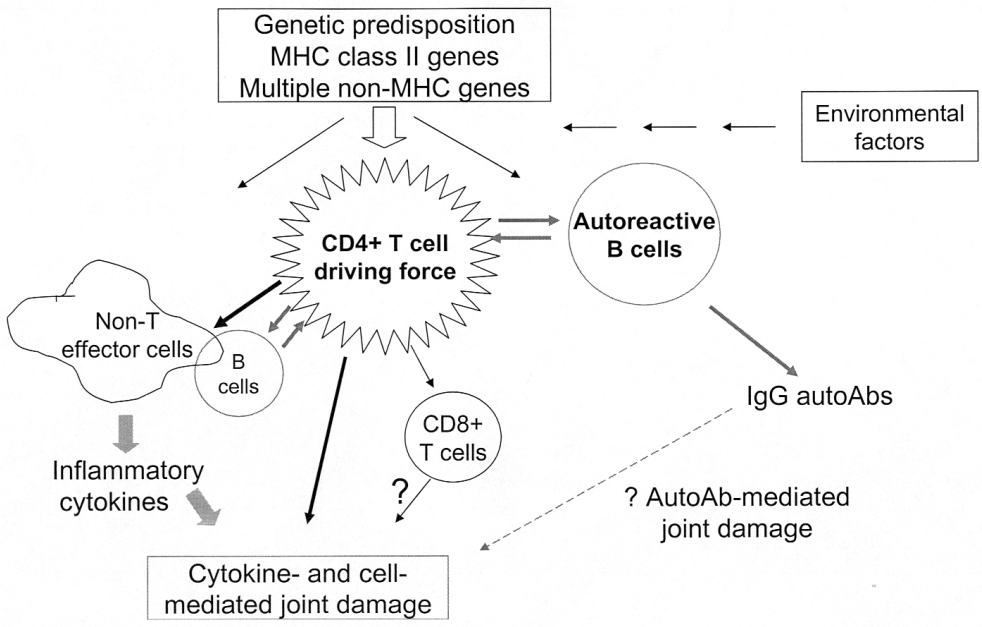

Figure 2. Proposed new paradigm for the pathogenesis of RA, emphasizing the role of B cells (including autoreactive cells). MHC: major histocompatibility complex; Ab: antibody.

that rituximab therapy in RA patients is clinically efficacious. The results of this clinical study are described elsewhere in this supplement[32].

CONCLUSIONS

The classical paradigm for RA pathogenesis holds that CD4+ T cells mediate joint damage both directly and by driving non-T effector cells to release inflammatory cytokines. By contrast, the new paradigm that is developing centers on an interaction of CD4+ T cells with B cells. Evidence reviewed in this article shows that autoreactive B cells can be driven by the T cells to produce IgG autoantibodies that may be directly involved in joint damage, and B cells are known to be critical in activating CD4+ T cells (Figure 2).

As the B cell appears to play an important role in the

RA process, it is appropriate to consider how B cell-mediated effects might be reduced or prevented in patients with this disease. As described in this article, the depletion of B cells with a monoclonal antibody such as rituximab appears to be clinically effective in RA patients. However, B cells can be therapeutically targeted by other mechanisms. Because B lymphocyte stimulator (BLyS), also known as BAFF (B cell-activating factor from the TNF family), is critical for B cell development, inhibiting the production or function of BLyS can greatly interfere with B cell-dependent processes[33]. This might be achievable with anti-BLyS monoclonal antibodies, or through soluble receptor Ig antagonists, which are in development for the treatment of autoantibody-mediated diseases such as SLE. The effects of inhibiting B cells in these ways may also be useful approaches to the clinical treatment of RA.

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