Immunopathologic Aspects of Rheumatoid Arthritis: Who Is the Conductor and Who Plays the Immunologic Instrument?

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ABSTRACT. The inflammatory process of rheumatoid arthritis (RA) resembles a symphony orchestra playing a piece of music—not a song that anyone wants to hear, but a song nevertheless. Each cellular player has a distinct role, and all must coordinate in order to play their discordant "music" successfully. Rheumatoid synovitis consists of resident cells and invading immune cells that together arrange the inflammatory process in RA. There are 3 major types of synovitis that RA can comprise: germinal center synovitis, aggregate synovitis, and diffuse synovitis. Germinal centers are highly organized complex structures that are functionally competent. Aggregates are B cells and T cells arranged in defined follicles, yet they lack germinal center reactions. Diffuse synovitis is the least organized but can still cause significant damage. For each of these types of synovitis, the cellular players and their molecular instruments vary significantly. Differences in lymphoid microorganizations draw attention to the process of lymphoid organogenesis as a fundamental pathway of rheumatoid synovitis, a process that lends stability and sustainability to dysfunctional immune responses. This article will address how tissue-resident and invading cells, in particular T cells, B cells, dendritic cells, and synoviocytes, are brought together in different "symphonic" arrangements and how this process of lymphoid organization affects disease outcome and therapeutic options in RA. (J Rheumatol 2007;34 Suppl 79:9-14)

Key Indexing Terms:
RHEUMATOID SYNOVITIS T CELLS B CELLS DENDRITIC CELLS SYNOVIOCYTES

INTRODUCTION

Rheumatoid arthritis (RA) is a complex autoimmune syndrome with an increasing number of cell types, cellular mediators, and signaling pathways implicated in the inflammatory networks of the disease[1]. The immunoinflammatory process resembles an orchestra at work, with RA as the resulting song. Key components in rheumatoid synovitis are detailed in Figure 1, and each cell type, molecule, and pathway has a different role depending on the type of synovitis in a particular patient. The research summarized here will define roles for some of the "players" and pathways in the pathogenesis of RA.

Lymphoid neogenesis

A close relationship exists between the assembly of highly complex lymphoid structures and inflammation. Lymphoid neogenesis is a process whereby complex lymphoid formations that optimize antigen storage, recognition, and cell-cell communication emerge in nonlymphoid tissues, where they support chronic persistent inflammatory reactions. Such newly formed lymphoid organs are durable structures that enable the generation of immunologic memory and allow for longterm immune responses. This process may be one of the critical steps in turning an acute immune response into a chronic inflammatory disease[2].

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There are distinct types of lymphoid microstructures generated in synovial lesions of patients with RA[3]. Germinal centers in the synovium are highly organized, complex lymphoid microstructures that include T cells, B cells, and follicular dendritic cells, which are structurally and functionally indistinguishable from germinal centers formed in lymph nodes[2]. Aggregates are B cells and T cells arranged in defined follicular clusters, but they lack follicular dendritic cells and thus cannot generate germinal center reactions. Diffuse synovitis is the least organized assembly, but can still elicit significant damage in patients with RA[2]. Patients normally commit to one type of lymphoid structuring in the synovium, with persistence and stability of the pattern over time[2].

One important question is whether the "music" produced by each of these different types of lymphoid microstructures varies from patient to patient or whether the same music is played despite the different players and instruments. In a study examining the levels of immunoglobulins (IgG) produced by different types of synovitis, we quantified IgG-specific transcripts in synovial biopsies representing the different categories of synovitis. Additionally, we measured human antibodies in mouse blood by implanting synovial biopsies into immunodeficient mice (the human synovium-SCID chimera model). In other cases, we found that tissue containing the germinal centers produces the highest levels of IgG, that aggregates produce intermediate levels, and that tissues with diffuse synovitis have the lowest concentrations of IgG transcripts[4]. These results suggest that there are significant differences in the inflammatory potential among the 3 types of synovitis.

Role of CD4+ T cells and B cells in rheumatoid synovitis

To examine the cellular characteristics of synovitis, Takemura and colleagues[5] utilized the human synovium-SCID mouse model[6] to explore the role of follicular CD4-positive (CD4+) T cells in rheumatoid synovitis. They adoptively transferred follicular CD4+ T cell clones into autologous grafts and subsequently measured the production of inflammatory cytokines[5]. The researchers discovered that CD4+ T cells are the fundamental drivers in the production of interferon-γ (IFN-γ), interleukin 1ß (IL-1ß), and tumor necrosis factor-α (TNF-α), all critical cytokines in the pathophysiology of rheumatoid synovitis. To identify what role B cells played in the generation of T cell-mediated inflammatory cytokines, the group then adoptively transferred follicular CD4+ T cell clones into mice in which B cell-deficient synovial tissues had previously been implanted. CD4+ T cells transferred into such "B cell-less" chimeras failed to boost the production of IFN-γ, IL-1ß, and TNF-α in the synovial lesions[5]. These experiments were the first to suggest that T cell function in rheumatoid synovitis is critically dependent upon B cells, and assigned a key function to T cell/B cell interaction in the disease process.

To further examine the role of B cells in the pathogenesis of RA, Takemura and colleagues again used the SCID mouse model and injected the chimeras with increasing amounts of anti-CD20 antibody[5]. CD20 is a cell-surface marker expressed on the majority of B cell subsets. During the differentiation process, B cells express a combination of different cell-surface markers, which enables researchers to distinguish the stage of B cell ontogeny. CD20 is present from the pre-B cell stage to the mature B cell stage and absent from both the pro-B cell stage and plasma cells[7]. The results of this experiment are illustrated in Figure 2[5].

Depletion of CD20+ B cells from the synovial implants markedly reduced the production of IFN-γ and IL-1ß, which are T cell- and macrophage-derived cytokines, respectively. As noted, these cytokines play prominent roles in the inflammatory "orchestra" of synovitis. Targeted therapies that muffle the effector functions of particular players, such as B cells and T cells, can dramatically alter the subsequent release of destructive cytokines. The interdependence of the different players required to produce a piece of music thus provides a novel therapeutic strategy in RA. If we can quiet one of the players, we will critically affect the work of the entire group. This principle should be especially effective if we succeed in targeting the vitally important players, allowing us to dampen the inflammation and eventually prevent the disastrous effects of RA-associated synovitis.

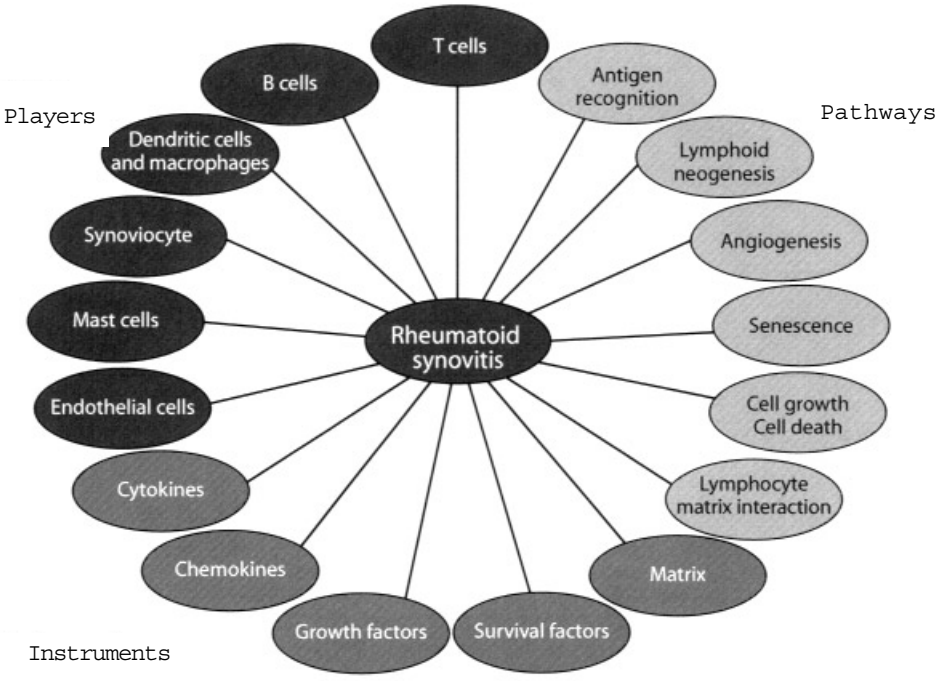

Figure 1. Players, instruments, and pathways of RA.

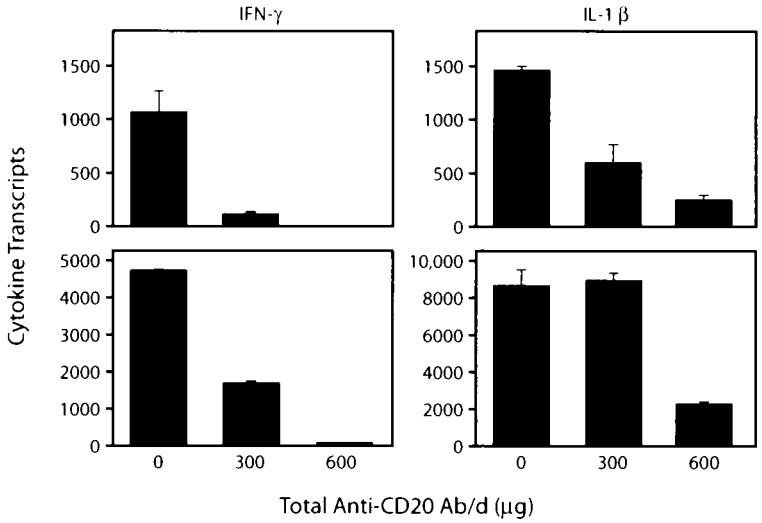

Figure 2. Production of T cell and macrophage-derived cytokines after depletion of CD20+ B cells. From J Immunol 2001;167:4710-8, American Association of Immunologists, Inc., with permission.

Roles of CXCL13, CCL21, and lymphotoxin-ß in rheumatoid synovitis

In addition to IFN-γ and IL-1ß, CXCL13 and CCL21 are important chemokines that assist in the formation of germinal center arrangements. These chemokines are highly expressed in tissues that form germinal centers but are low in aggregate and diffuse lymphoid structures[8]. The cellular origin of CXCL13, a chemokine intimately involved in guiding B cells into the tissue, has been assigned to synovial fibroblasts. CCL21 predominantly derives from dendritic cell populations. Other essential players in the process of lymphoid neogenesis include lymphotoxin-α and lymphotoxin-β. In a study comparing tissue cytokine patterns in a large cohort of synovial biopsies, these 2 cytokines emerged as critical elements in germinal center and aggregate synovitis[8]. To identify important components in the generation of synovial germinal centers, Takemura and colleagues collected synovial tissue biopsies from 64 different patients and used a multivariate logistic regression analysis to determine which variables predicted formation of germinal centers. The model revealed that CXCL13, CCL21, and lymphotoxin-ß were important predictors for lymphoid neogenesis in the rheumatoid synovium, whereas lymphotoxin-α lacked predictability[8]. This study concluded that there are architectural cues for lymphoid neogenesis that are required for the generation of these structures. These cues derive from interactions between the resident synovial cells that produce CXCL13 and the tissue-invading cells that produce lymphotoxin-ß, which ultimately act in concert to generate synovitis in RA.

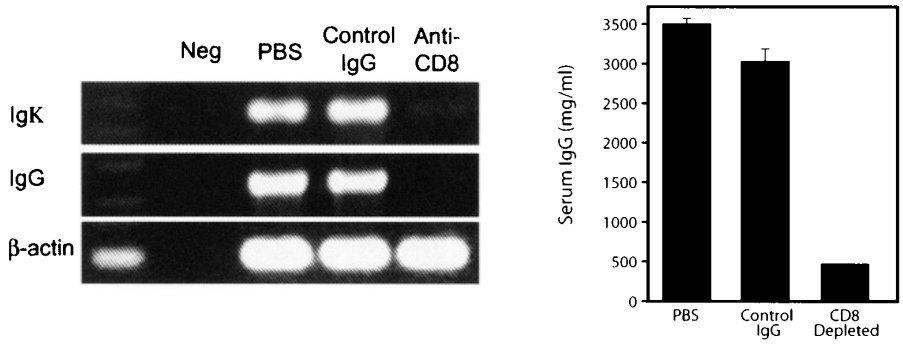

Figure 3. Depletion of synovial CD8+ T cells: effects on IgG production. PBS: phosphate buffered saline. From the J Exp Med 2002;195:1325-36, Rockefeller University Press, with permission.

Role of CD8+ T cells in rheumatoid synovitis

CD8-positive T cells are noteworthy players in the rheumatoid orchestra although they are not always in a lead position. One may compare them to the trumpets in an orchestral arrangement. If present, they cannot be ignored; if absent, the orchestra is still functional. Kang and colleagues examined the role of CD8+ T cells using the SCID mouse model. The group engrafted synovial tissues with germinal centers into experimental mice and then added anti-CD8 antibodies to deplete CD8+ T cells from the grafts[9]. They found that depletion of CD8+ T cells from synovial tissue markedly reduced both IFN-γ and IL-1ß production. This suggests that CD8+ T cells play a substantial role in the inflammatory process by modulating the expression of inflammatory cytokines in RA synovium[9]. Subsequently, Kang and colleagues examined the role that the depletion of CD8+ T cells had on the IgG production in synovial germinal centers. Depletion of CD8+ T cells essentially abrogated the ability of synovial germinal centers to build antibodies (Figure 3)[9].

Kang and colleagues proceeded to examine the effect of CD8+ T cell depletion on the expression of lymphotoxin-ß in the implanted synovial tissues. As described, the cytokine lymphotoxin-ß can be used as a specific marker for germinal centers as it seems to have a key role in the formation of these structures. The results of the experiments showed that removing CD8+ T cells significantly decreased expression of lymphotoxin-ß and thus removed an essential element in the building process of germinal centers[9]. Kang and colleagues went on to more clearly define the subset of CD8+ T cells with functional relevance in rheumatoid synovitis. Specifically, these T cells have the phenotype CD8+CD40L+ and, unusually for CD8 T cells, produce IFN-γ but lack perforin. A caveat to this research is that not all patients and not all rheumatoid tissues have high levels of CD8+ T cells, but for those that do, it is likely that CD8+ T cells play a consequential role in the inflammatory process[9].

Role of the B cell survival factors APRIL and BAFF in rheumatoid synovitis

The players of the rheumatoid orchestra have to be able to survive and thrive in the joint microenvironment. Their instruments need to stay functional and tuned. Thus, they depend on a constant supply of growth and survival factors. B cells are known to receive growth, survival, and differentiation signals from a number of mediators, but 2 factors, APRIL (a proliferation-inducing ligand) and a B cell-activating factor called BAFF (also termed B-lymphocyte stimulator, BLyS), seem to be particularly important[10].

Seyler and colleagues examined the expression levels of APRIL and BAFF in 72 synovial biopsies and compared levels of these 2 survival factors in tissues of patients with germinal center, aggregate, and diffuse synovitis[10]. They discovered that the synovial tissues with germinal centers were highly efficient in the production of APRIL and outperformed both aggregate and diffuse synovitis tissues. In contrast, there was no difference in the expression of BAFF/BLyS among the different types of synovial microstructures. In an effort to identify the cellular source of these growth and survival factors, the researchers identified APRIL- and BAFF-releasing cells in synovial infiltrates. APRIL was predominantly supplied by synovial dendritic cells, and BAFF originated from synovial macrophages[10].

To examine how B cells or, more directly, how APRIL and BAFF contribute to the disease process of RA, Seyler and colleagues used the SCID mouse model to study the disease process after therapeutically targeting the survival factors[10]. They introduced the transmembrane activator and calcium-modulating and cyclophilin ligand interactor (TACI)-Ig antibody, a soluble decoy receptor that binds to and blocks APRIL and BAFF so that the factors cannot bind to their cognate receptors. Upon blockage of APRIL and BAFF by the decoy receptor construct, expression levels of T cell-produced IFN-γ and TNF-α in germinal center, aggregate, and diffuse synovitis were analyzed. In synovitis tissue with germinal centers, blocking APRIL and BAFF significantly decreased the levels of both IFN-γ and TNF-α (Figure 4A). For aggregate synovitis, knocking down the biologic effect of APRIL and BAFF resulted in enhanced production of the proinflammatory cytokine IFN-γ (Figure 4B). TNF-α levels were diminished, which suggests a complex role for APRIL and BAFF in promoting inflammation. The unexpected proinflammatory effect of APRIL/BAFF blockade was confirmed in tissues with diffuse synovitis; levels of both IFN-γ and TNF-α (Figure 4C) were higher after injection of TACI-Ig compared to sham-treated controls.

This study demonstrated for the first time that APRIL and BAFF regulate both B cell and T cell function in rheumatoid synovitis. Their role in the disease process is complex; these survival factors provide both positive and negative inflammatory signals. In support of the concept that different lymphoid microstructures in the synovium represent fundamentally distinct disease processes, the therapeutic targeting of APRIL/BAFF provided clear evidence that different songs are being played and that taking out one or several players could be beneficial as well as deleterious for the patient[10].

Conclusions

By virtue of its physiologic role, the joint is exposed to immense stress and needs to have rapid and efficient repair mechanisms. Acute inflammatory reactions in the

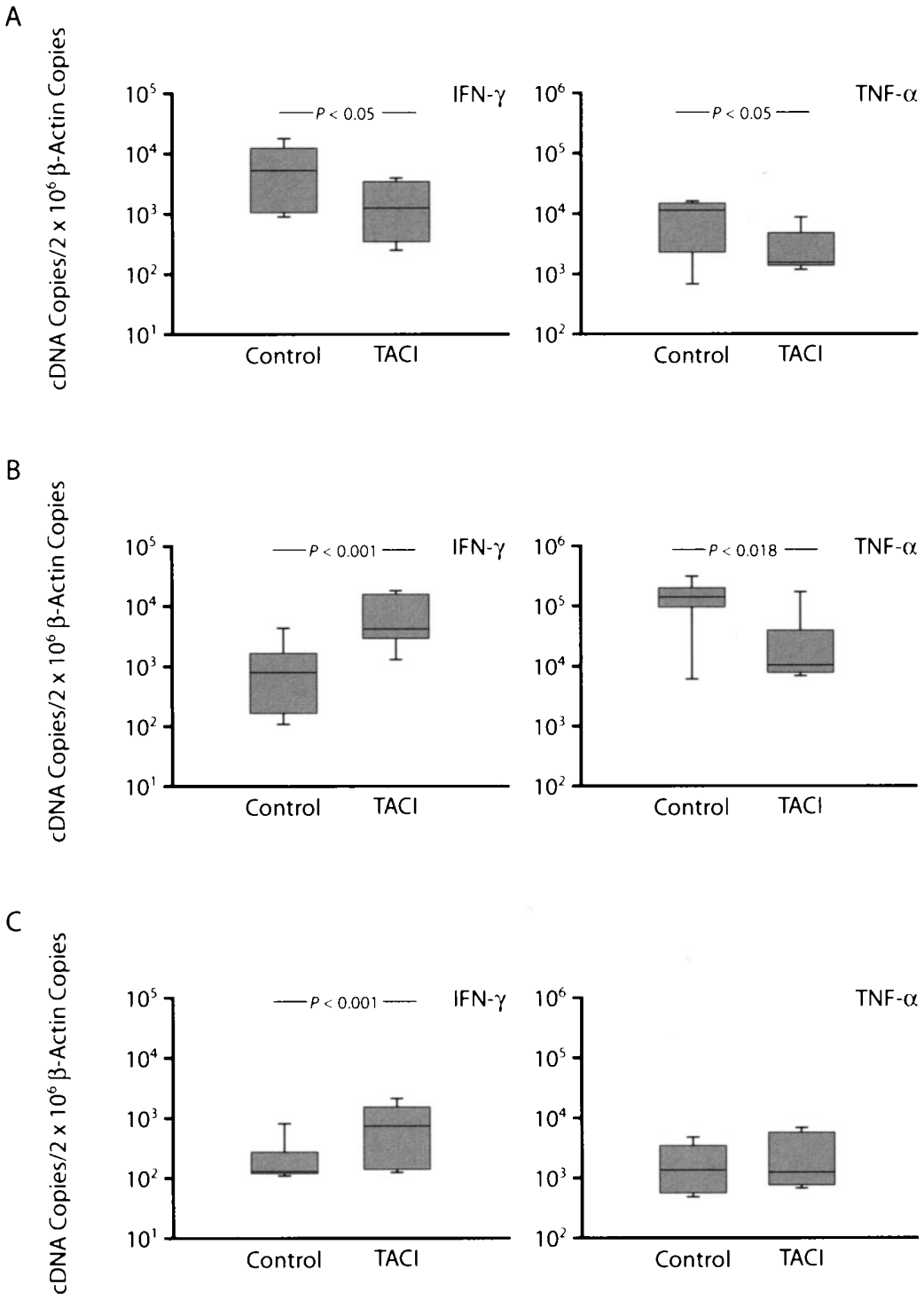

Figure 4. Blocking APRIL/BAFF *in vivo*. Interfering with the biologic action of APRIL and BAFF in rheumatoid synovitis leads to divergent outcomes. Depending on the nature of the synovitic lesions, blockade of APRIL/BAFF is either strongly antiinflammatory (panel A) or causes increased production of proinflammatory cytokines (panels B and C). A. Germinal center synovitis; B. aggregate synovitis; C. diffuse synovitis[10]. TACI: transmembrane activator and calcium-modulating and cyclophilin ligand interactor. From Seyler T.M., et al. J Clin Invest 2005;115:3083-92; American Society of Clinical Investigation, with permission.

Table 1. Role of cellular players in synovitis.

Synovitis	CD4+ T Cell	CD8+ T Cell	B Cells	CXCL13 Cells	CCL21	Lymphotoxin-β	APRIL	BAFF
Germinal center	1	1	1	1	1	1	1	1
Aggregate	1	—	1	—	—	—	**1**	1
Diffuse	1	—	**1**	—	—	—	—	1

1: definite pathogenic role, **1**: possible pathogenic role, — : no known pathogenic role.

joint lining are almost always terminated promptly. In patients with RA, transient inflammation turns into sustained inflammation. The result is a durable disease lesion that has powerful tissue-damaging potential. A look at the disease lesion identifies it as a highly complex, well organized structure. Like the players in an orchestra, a multitude of cellular specialists come together and bring their special talents (Table 1). The tunes are played in concert with strong interdependence among the orchestra members. Removing an instrument group (e.g., by therapeutically targeting one cell type) will have immediate consequences for the entire orchestra.

A preferred therapeutic strategy would paralyze a critical pathway that sustains synovitis. Lymphoid neogenesis, the means of bringing together different cell types and arranging them in sophisticated 3-dimensional structures, emerges as a disease-critical process that should provide a highly effective therapeutic target. If we succeed in disrupting the assembly of the orchestra, then the orchestra will cease playing and the conductor has no chance of continuing his/her work.

Essential elements of the rheumatoid orchestra are CD4 T cells, B cells, CD8 T cells, dendritic cells, and macrophages[11]. Their instruments include cytokines (such as TNF-α, IL-1β, IL-6, leukotriene-α, and leukotriene-β, chemokines (such as CXCL13 and CCL21), and survival factors (such as APRIL and BAFF). As we progress in developing new treatments for RA, we will have to match the sophistication of the disease that presents itself with different sets of immunologic players. Ultimately, we will have to identify the conductor that keeps the orchestra playing.

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