

## Review

**B cells in rheumatoid synovitis**

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**Abstract**

In rheumatoid arthritis, T cells, B cells, macrophages, and dendritic cells invade the synovial membranes, establishing complex microstructures that promote inflammatory/tissue destructive lesions. B cell involvement has been considered to be limited to autoantibody production. However, recent studies suggest that B cells support rheumatoid disease through other mechanisms. A critical element of rheumatoid synovitis is the process of ectopic lymphoid neogenesis, with highly efficient lymphoid architectures established in a nonlymphoid tissue site. Rheumatoid synovitis recapitulates the pathways of lymph node formation, and B cells play a key role in this process. Furthermore, studies of rheumatoid lesions implanted in immunodeficient mice suggest that T cell activation in synovitis is B cell dependent, indicating the role played by B cells in presenting antigens and providing survival signals.

**Introduction**

The role played by B cells in the pathogenesis of rheumatoid arthritis (RA) has recently come back into focus. The discovery of B cell generated autoantibodies in patients with RA provided the first clue that the disease is immune mediated. This original observation drove therapeutic approaches to RA and generated intense interest in how B cells influence the disease process. By the late 1960s it was clear that other factors play a major role, and since 1980 treatment strategies have focused on the effector mechanisms of macrophages and the cytokines that are produced during inflammatory responses. The recent success of B cell depletion therapy using rituximab [1] – a chimeric mouse/human monoclonal antibody directed against CD20 – has led researchers and clinicians to re-examine the role played by B cells in the etiology of RA.

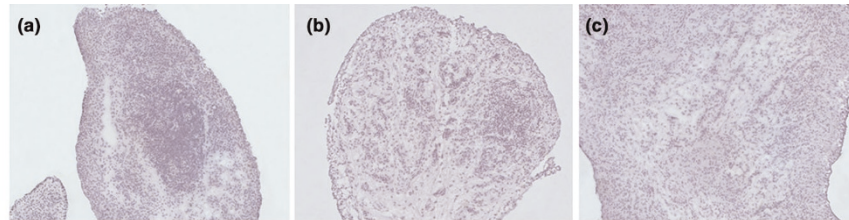
**Activation of CD4<sup>+</sup> T cells by synovial B cells**

CD20<sup>+</sup> B cells, a prominent population in rheumatoid synovial tissue, are present in the majority of RA patients. Immunohistochemical analysis reveals that they can acquire a very distinct follicular organization, which is reminiscent of the

functional unit of secondary lymphoid tissues, the germinal centers. The organization of B cells in ectopic germinal centers [2] raises interesting questions about their role in promoting rheumatoid disease. Is their only contribution the production of autoantibodies, or do they participate in other mechanisms that lead to synovial inflammation?

Takemura and coworkers [3] addressed this question by depleting B cells from the rheumatoid synovium. Severe combined immunodeficient mice were engrafted with synovial tissue taken from RA patients, allowing for subsequent explantation and analysis of morphology, cytokine production, and function of germinal centers. The contribution of engrafted B cells to inflammatory processes in these mice was assessed using antibody mediated cell depletion. A 3-day course of anti-CD20 antibodies caused total dissociation of B cell follicular structures, as well as dramatic reductions in the levels of IL-1 $\beta$  and IFN- $\gamma$ , suggesting that B cells affect the function of the T cells and macrophages that generate these proinflammatory cytokines.

Takemura and coworkers [3] also examined the role played by CD4<sup>+</sup> T cells in inflammatory processes in engrafted synovium by isolating T cell clones from microdissected synovial follicles. These clones were adoptively transferred by tail vein injection into mice engrafted with autologous synovial tissue. Monitoring of cytokine production in the engrafted synovium revealed that transfer of T cell clones caused a threefold to fourfold increase in IFN- $\gamma$  transcripts, and a twofold to threefold increase in the abundance of IL-1 $\beta$  and tumor necrosis factor (TNF)- $\alpha$  transcripts. Notably, transfer of T cell clones isolated from peripheral blood did not stimulate cytokine production. This selective activation of tissue-derived T cells is most consistent with the model in which these cells recognize a local antigen, and suggests that they drive the inflammatory response by producing IFN- $\gamma$  and regulating the function of macrophages and synoviocytes that are the source of IL-1 $\beta$  and TNF- $\alpha$ .

**Figure 1**

B cells in rheumatoid synovium. Tissue biopsies were collected from patients with active synovitis. Infiltrates of B cells and T cells are often arranged in complex microarchitectures. Three patterns can be distinguished. **(a)** T cells and B cells can form typical germinal centers. **(b)** Alternatively, they can be clustered in aggregates that lack follicular dendritic cells and have no germinal center reaction. **(c)** The third pattern is formed by T cells and B cells infiltrating diffusely into the synovial stroma. Reproduced with permission [7].

To determine whether the activation of CD4<sup>+</sup> T cells in these experiments was restricted by human leukocyte antigen (HLA) class II molecules, T cell clones were transferred to mice engrafted with HLA-DRB1 mismatched synovium. All T cell clones that were shown to function in autologous tissue also had proinflammatory activity in heterologous tissue that shared one common HLA-DRB1 allele. Thus, all components required to trigger the activity of the CD4<sup>+</sup> T cell clones are present in heterologous synovial tissue, and antigens driving inflammation are shared by different patients. However, adoptive transfer of T cells to mice carrying tissue mismatched for both alleles did not stimulate cytokine production, documenting the need for HLA-restricted recognition events.

Finally, in order to assess directly the role played by B cells in CD4<sup>+</sup> T cell activation, Takemura and coworkers [3] engrafted mice with rheumatoid synovial tissue containing few or no B cells. Such experiments were possible because not all patients form germinal centers in synovial infiltrates. Rather, heterogeneity exists in the organization and the composition of tissue invading T cells and B cells. In some patients, B cells are absent from the synovial lesions. Alternatively, B-cell free synovial tissues can be generated by using depleting anti-CD20 antibody. Adoptive transfer of HLA-DRB1-matched T cell clones into mice engrafted with synovial tissue lacking B cells failed to stimulate cytokine production, indicating that B cells are critical in supporting *in situ* activation of T cells and in maintaining the continuous production of proinflammatory cytokines by macrophages and synoviocytes.

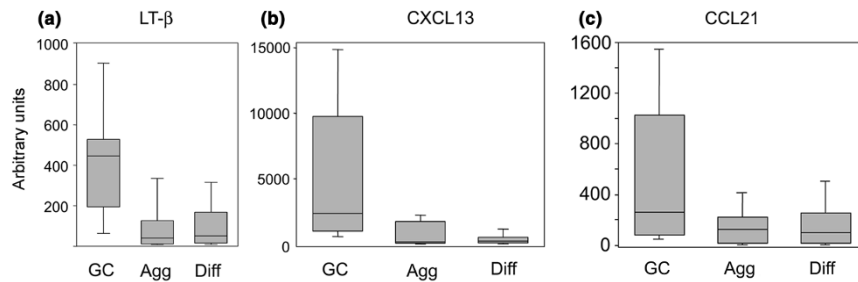
### **B cells and ectopic lymphoid organogenesis**

The data reported by Takemura and coworkers [3] suggest that B cells are absolutely required to support the activation and effector function of synovial CD4<sup>+</sup> T cells, extending their functional contribution to the disease process far beyond autoantibody production [4]. The nature of this supportive interaction is as yet unclear, although two possibilities seem likely: B cells present antigens to autoreactive T cells, driving their activation; or the presence of B cells in synovial tissue generates survival signals for T cells.

In order to appreciate how B cells function in rheumatoid synovitis, it is important to understand their role in the histological architecture of synovial lesions. The joints of some RA patients exhibit what appear to be functional ectopic germinal centers (Fig. 1a), with T cells and B cells arranged around a network of dendritic cells. Lesions in other patients do not form follicular microstructures, but rather contain aggregates of T cells and B cells (Fig. 1b), whereas still other lesions have a diffuse arrangement of T cells and B cells (Fig. 1c). These patterns of synovial histology are stable over time and consistent within patients, with tissues from distinct joints exhibiting the same type of inflammatory lesion.

Interestingly, these patterns can be correlated with biomarkers for B cell activity. The relationship between B cells and the establishment of complex lymphoid architectures has been studied in a large cohort of synovial biopsies, all collected from patients with active rheumatoid synovitis (authors' unpublished data). As a first step, B cell activity in the tissues was determined by quantifying immunoglobulin transcription. Interestingly, rheumatoid synovium can display a wide range of IgG production, varying over several orders of magnitude. Tissues containing germinal centers tend to have the highest levels of IgG mRNA, tissues containing B cell aggregates have intermediate levels, and tissues with a diffuse arrangement of T cells and B cells tend to have the lowest levels.

Clearly, the functional significance of these patterns makes it critical to understand how lymphoid organogenesis occurs in synovial tissue. Organization of cells in lymphoid organs and in inflammatory lesions is orchestrated by chemokines – soluble messengers that stimulate chemotactic behavior. Two such molecules, namely CC chemokine ligand (CCL)2 and CCL18, were previously proposed to play a role in recruitment of lymphocytes and macrophages to rheumatoid lesions. Analysis of the levels of these molecules in synovial tissue, however, reveals no significant correlation between patterns of B cell organization and levels of CCL2 or CCL18 mRNA production, suggesting that other chemokines are

**Figure 2**

B cells support ectopic lymphoid organogenesis in rheumatoid synovitis. cDNA was generated from synovial biopsies of patients with rheumatoid arthritis, and production of CXC chemokine ligand (CXCL)13, CC chemokine ligand (CCL)21, and lymphotoxin (LT)- $\beta$  mRNA was quantified by real-time polymerase chain reaction. Tissues were categorized as having germinal centers (GCs), aggregates, or diffuse infiltrates as described in Fig. 1. Results are shown as box plots with medians, 25th and 75th percentiles as boxes, and 10th and 90th percentiles as whiskers. **(a)** Tissues with GCs produced significantly higher levels of the tumor necrosis factor-like ligand LT- $\beta$  – a molecule that is involved in lymphoid organogenesis. **(b)** These tissues also expressed the highest concentrations of the B cell recruiting chemokine CXCL13. **(c)** Similar to lymph nodes, GC-forming synovial tissues contained high levels of the T-cell attracting chemokine CCL21. Reproduced with permission [5]. Copyright 2001, The American Association of Immunologists, Inc.

more important in directing how B cells are recruited to the synovial membrane and determining how they arrive at a distinct site in the synovial microenvironment.

Indeed, analysis of other chemokines reveals an intriguing relationship between lesion architecture and chemokine levels. Takemura and coworkers [5] found that two chemokines, namely CXC chemokine ligand (CXCL)13 and CCL21, are major determinants of germinal center formation. CXCL13 has been implicated in the recruitment of B cells, whereas CCL21 has been shown to attract T cells. Again, both of these chemokines are found at high levels in synovial tissues that are capable of forming germinal centers and at low levels in other rheumatoid lesions (Fig. 2b,c). Thus, CXCL13 and CCL21 are not only involved in regulating the positioning of T cells and B cells in structured lymphoid organs; they also serve a critical role in supporting the infrastructure of the rheumatoid lesion. The end result is a process of ectopic lymphoid neogenesis, providing the disease lesion with robust structural support. It is important to recognize that this process is directly related to the functioning of B cells and relies on their contributions far beyond the release of autoantibodies.

Takemura and coworkers [5] provided a clue to the process by which B cells may regulate ectopic germinal center formation. They observed that two members of the TNF superfamily, lymphotoxin (LT)- $\alpha$  and LT- $\beta$ , were differentially expressed in tissues with distinct B cell organization. These proteins form  $\alpha 1\beta 2$  heterotrimers and, along with CXCL13 and CCL21, have been implicated in lymph node formation in mouse knockout models. There is now evidence that in rheumatoid synovitis LT- $\beta$  is supplied by B cells. Analysis of LT- $\beta$  mRNA production in synovial tissue reveals a strong correlation with levels of IgG production, two markers closely

related to synovial architecture (Fig. 2a). The relationship between LT- $\beta$  production and IgG production was the first hint that B cells are the cellular source of the TNF-like ligand. Indeed, immunohistochemical analysis has identified expression of LT- $\beta$  on a subset of B cells. Specifically, LT- $\beta^+$  B lymphocytes are positioned in the follicular mantle zone, assigning a critical contribution of that structure to ectopic lymphoid organogenesis.

Braun and coworkers [6] demonstrated recently that LT- $\alpha 1\beta 2$  has functions beyond regulating how B cells are embedded into a three-dimensional network. LT- $\alpha 1\beta 2$  stimulation also causes profound changes in the function of synovial fibroblasts. In particular, LT- $\alpha 1\beta 2$  stimulation led to the induction of a number of factors in fibroblast-like synoviocytes that contribute to inflammation and T cell recruitment. These include IL-1 $\beta$ ; matrix metalloproteinases; the T cell attracting chemokines CCL2, CCL5, and CCL8; and cell adhesion molecules required for efficient attachment of lymphocytes. These data support a critical role for LT- $\beta$  producing B cells in sustaining principal disease pathways in rheumatoid synovitis. Such disease pathways include the process of lymphoid organogenesis as well as sustaining the activation of tissue resident fibroblasts.

## Conclusion

It is now clear that B cells play multiple roles in the rheumatoid disease process. In addition to their classical role as producers of autoantibodies, B cells are central to the activation of CD4<sup>+</sup> T cells in synovial tissue, and depletion of B cells disrupts the production of proinflammatory cytokines by T cells and macrophages. B cells have also been implicated in the process of ectopic lymphoid organogenesis. In normal secondary lymphoid tissue, germinal center formation requires the expression of numerous cytokines and

chemokines. The observation that synovial follicular structures express high levels of several of these molecules suggests that rheumatoid synovitis utilizes similar building blocks and construction principles as lymph nodes. The end results are highly effective and complex three-dimensional arrangements of lymphocytes and fibroblasts that provide ideal conditions for continuous immune stimulation. B lymphocytes supply LT- $\beta$ , a key factor in ectopic lymphoid neogenesis, and thus hold a central position in shaping the synovial microenvironment and turning it into a disease permissive site.

### Competing interests

This manuscript is based on a transcript of a talk given by CMW at a symposium held at the ACR meeting sponsored by Genentech. CMW received an honorarium from Genentech for this talk. CMW does not have a consulting contract with Genentech but has participated at an Advisory Board Meeting on one occasion.

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