

## The role of B cells in animal models of rheumatoid arthritis

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## 1. ABSTRACT

Rheumatoid arthritis is a chronic systemic inflammatory autoimmune disease that affects approximately 1% of the population. Recent studies demonstrate a significant improvement in clinical symptoms in patients treated with Rituximab, an anti-CD20 monoclonal antibody that depletes pro-B cells and mature B cells but not plasma cells. These findings indicate that B cells are an important contributor to the pathogenesis of RA. In this review we will examine the role of B cells in several different murine models of RA. There are a number of antibody-dependent mechanisms by which B cells support inflammatory processes in the joint. However, there are also antibody-independent mechanisms that involve B cell/T cell collaboration where B cells may modulate autoreactive T cell responses. In addition, B cells may be an important source of cytokines that either stimulate or inhibit autoimmune responses. Understanding the role of B cells in RA will provide new and directed therapeutic approaches to the treatment of disease.

## 2. INTRODUCTION

The discovery that B cells produced autoantibodies in patients with RA was the first indication that RA is an immunological disease (1). However, because autoantibodies, in particular rheumatoid factor (RF), did not always correlate with disease and therapeutic reduction of RF did not yield convincing clinical results, interest in B cells in RA waned (2, 3). Recently, the role of B cells has regained prominence based on several studies in which a monoclonal antibody that depletes B cells (anti-CD20, Rituximab) has significant therapeutic efficacy in RA patients (4, 5). In patients treated with Rituximab the amount of autoantibody did not necessarily correlate with clinical responses; suggesting other B cell activities may be involved in disease. What can animal models tell us about the function of B cells in RA?

Animal models combined with the use of transgenic and gene deletion techniques have opened new avenues to define the molecular and cellular components

that contribute to the pathogenesis of human disease. Among the most widely used mouse models of RA that involve B cells are collagen-induced arthritis (CIA), antigen-induced arthritis (AIA), immune-complex-mediated arthritis (ICA), K/BxN, and proteoglycan (PG)-induced arthritis (PGIA) (6-9). In these models, antibodies and immune complexes (IC) are required for the development of arthritis. In animal models requiring de novo production of autoantibodies, T cell helper activity is required. However, antibodies specific for type II collagen (CII in the CIA model) and for glucose- 6-phosphate isomerase (GPI in the K/BxN model) are able to transfer arthritis (10-12). This review will focus on the function of B cells in murine models of RA and will opine on their roles in RA.

### 3. HOW DO B CELLS BECOME AUTOREACTIVE?

B cells express a diverse repertoire of receptors that are specific for both non-self and self-antigens (13). Silencing of self-reactive B cells is essential for preventing autoimmune disease. Mechanisms that silence self-reactive B cells in the bone marrow include deletion, anergy and receptor editing (14, 15), however, this system is leaky and the presence of self-reactive B cells can readily be detected (16-19). In the periphery, Fas-dependent T cell interaction can result in B cell elimination (20). Self-reactive B cells that are not deleted are maintained in an inactive state (anergy) by the continuous occupancy and signaling by self-antigen in the absence of T helper signals (21). Additional tolerance mechanisms that regulate autoreactive B cells were recently demonstrated to occur within germinal centers (22). William et al. demonstrated that autoreactive B cells are specifically activated and form germinal centers but have few antibody forming cells. In addition, a separate mechanism regulates clonal expansion and selection of B cell mutants limiting the generation of high-affinity self-reactive antibodies (22). Regulatory T cells are also involved in controlling autoreactive B cells activation by inhibiting B cell proliferation and the maturation of autoantibody responses (23, 24). With all these mechanisms in place how do self-reactive B cells break tolerance to become autoreactive? Anergic B cells may be rescued by transient loss of self-antigen (21) or defective self-antigen signaling. Also, T helper signals induced by either self or cross-reactive antigens on infectious organisms have the potential to stimulate self-reactive B cells (25-27). Inflammatory conditions, where cytokines and costimulatory molecules are upregulated, could lower the threshold for self-reactive B cell activation. For example, over expression of B cell activating factor belonging to the TNF family (BAFF) sustains B cell activity triggering severe autoimmune disease (28, 29). Thus, there may be intrinsic defects in the B cell that prevent the maintenance of self-tolerance or extrinsic signals that trigger autoreactive B cell activation.

### 4. ANTIBODY-DEPENDENT INDUCTION OF ARTHRITIS

In RA, once self-tolerance is broken and self-reactive T cells and B cells are activated, autoantibodies are

one of the main contributors to events that lead to cartilage destruction and bone erosion. Several different mechanisms are responsible for the inflammatory effects of autoantibodies including immobilization of autoantibodies to cartilage surfaces, activation of the complement cascade, and cross-linking of Fc gamma receptors (FcγR) on macrophages and neutrophils. These events cause cell activation and the release of proinflammatory cytokines and matrix metalloproteinases ultimately resulting in cartilage degradation (30).

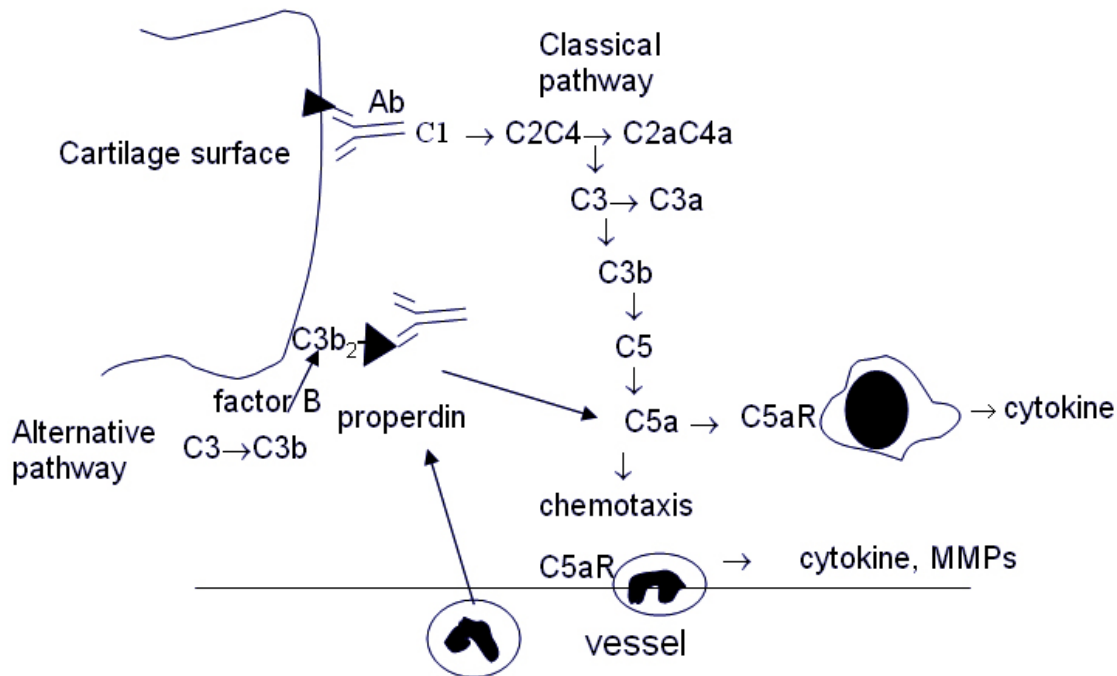
#### 4.1. Autoantibody is directly pathogenic for autoantigen

In several arthritis models autoantibodies are specific for autoantigens expressed in the joint. Cartilage is composed primarily of proteoglycans (PG) and collagens. As a normal process of cartilage metabolism PG and collagen are released from the cartilage matrix. In the K/BxN model, where the autoantigen GPI is an intracellular protein, GPI is associated with extracellular structures along the articular cartilage surface (31). These proteins and carbohydrates may serve as targets for autoantibodies directly binding to cartilage surfaces. In PGIA, binding of autoantibodies to articular cartilage is associated with the depletion of cartilage PG (32). PG-specific autoantibodies are cytotoxic to mouse and human chondrocytes in the presence of complement (32, 33). In CIA, monoclonal antibodies to CII bind to chondrocytes causing disorganization of CII fibrils and increased matrix synthesis (34, 35). Degraded cartilage could release fragments of PG and collagen, providing a target for pathogenic autoantibody. Damaged cartilage may also expose neoepitopes that have not been previously encountered by the immune system. Release of cartilage components and neoepitopes may contribute to the progression of arthritis by a positive feedback loop that recruits autoantibodies and immune complexes to the cartilage surface in a process known as epitope spreading. Damage to the cartilage could instigate subsequent inflammatory events that contribute to chronic and perhaps progressive nature of RA as new autoantibodies may be directed to previously undamaged cartilage components.

#### 4.2. Immune-complexes activate complement

Antibodies and immune complexes (IC) immobilized on cartilage surfaces initiate inflammation through activation of the complement cascade. In RA patients, complement components are co-localized with immune complexes in joints and are decreased in synovial fluid. These observations suggest a role for complement in the inflammatory process (36).

Three different pathways activate the complement cascade; classical, mannose-binding lectin, and alternative pathways. The first step in the classical pathway is the formation of C1qrs complex. C1q is pro-inflammatory stimulating the release of inflammatory chemokines/cytokines (37). Active C1s is capable of degrading types I, II, and IV collagens, decorin, and activating matrix metalloproteinase-9 (38-41). Activated C1s activates the classical pathway by cleaving C2 and C4,



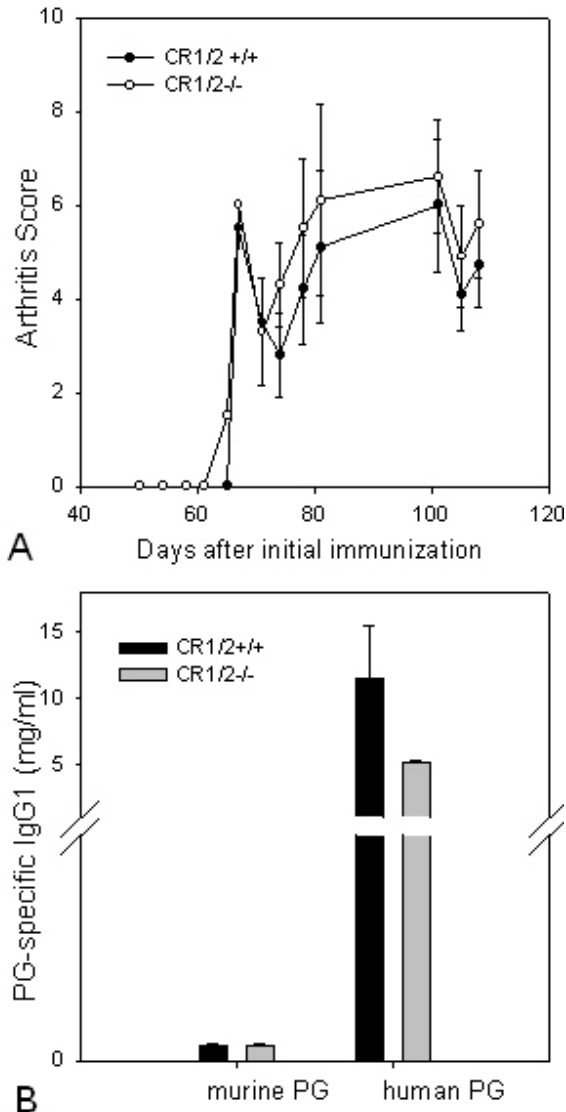
**Figure 1.** The classical and the alternative pathways of complement activation are involved in the development of arthritis. In the classical pathway immobilization of antibodies on cartilage surfaces or immune complexes in the synovial joint initiates C1qrs formation resulting in the C3a and C5a activation. Activation of the alternative complement pathway may occur through ICs binding of C3b, which can then bind to cell surfaces stabilizing the activation of C3a and C5a. The contribution of neutrophil properdin to the IgG-C3b<sub>2</sub> complex enhances activation of the alternative pathway. In both pathways activation of C5a, which is a strong chemoattractant for neutrophils and stimulates proinflammatory cytokine production, is crucial for the development of arthritis.

forming a C4bC2a complex also designated as C3 convertase. This convertase cleaves C3 into C3a and C3b. C3a also displays pro-inflammatory properties (42). A deficiency in C3 in K/BxN serum transfer model and in CIA prevents arthritis (43, 44). C3b forms a complex with C4b and C2a, creating a C5 convertase capable of cleaving C5 into C5a. C5a is a strong chemoattractant for neutrophils and is also pro-inflammatory, stimulating IL-1, IL-6, IL-8, and TNF- $\alpha$  expression (45-50). In CIA and K/BxN targeted deletions of C5 and C5aR, or neutralization of C5, are associated with resistance to arthritis (43, 50-52).

Unexpectedly, a genetic deletion of factor B, a component of the alternative complement pathway, renders mice resistant to CIA and K/BxN serum transfer (43, 44). The participation of the classical pathway was excluded in the K/BxN serum transfer model based on the evidence that C1q and C4-deficient mice are susceptible to arthritis (43) whereas in CIA, both classical and alternative pathways may play a role (44). The alternative pathway may be activated by IC bound C3b which is constitutively cleaved from C3 in the serum (53). The interaction of IC-C3b with complement proteins properdin, factor B and factor H functions as an amplification loop for the alternative pathway (54). The importance of the alternative pathway correlates with neutrophils as the predominant cell population recruited to inflamed joints. Neutrophils are a major source of properdin and C5a is a potent inducer of

properdin as well as a neutrophil chemotactic and degradative agent (55). This scenario is supported by the findings that either blocking neutrophil recruitment by C5a or depletion of neutrophils reverses arthritis (51, 56). Figure 1 depicts the classical and the alternative pathways of complement activation in synovial joints in arthritis.

Complement C3 and C4 cleavage products bind to complement receptors, CR1 (CD35), CR2 (CD21), and CR3 (CD11b/CD18). The C3 cleavage product C3d binds to CR2 on B cells and follicular dendritic cells. Antigen bound C3d co-ligates CR2 and the B cell antigen receptor increasing B cell receptor signaling thereby enhancing antibody responses to low concentrations of antigen (57, 58). Disruption of the *Cr2* gene results in the reduction of B cell responses to T-dependent antigens (59). CR1 and CR2 are also important for the processing and presentation of complement tagged antigens (60). In the mouse, the *Cr2* gene codes for both CR1 and CR2. Del Nagro et al. showed that CR1/2 -deficient mice are resistant to arthritis and that C3d conjugated to type II collagen is sufficient to induce CIA in the absence of Freund's complete adjuvant (61). To address the importance of CR1/2 in PGIA, we immunized female wild type (WT) and CR1/2-deficient mice with human PG in CFA with two boosters in IFA at 3-week intervals. Arthritis developed in WT mice 3 weeks after the last injection with PG. Contrary to the above results for CIA, the PG-specific antibody response and the development of arthritis were similar in WT and CR1/2-



**Figure 2.** CR1/2<sup>-/-</sup> mice are susceptible to PGIA and PG-specific antibody levels are similar to WT. (A) WT (n=10) and CR1/2<sup>-/-</sup> (n=10) mice > 3 month of age, were immunized intraperitoneally (i.p.) with 150 µg of human PG measured as protein, emulsified in CFA. Mice received booster immunization at week 3 and 6 with 100 µg of PG in IFA. Mice were monitored biweekly for signs of arthritis subsequent to the final immunization, and scored blindly for clinical signs of arthritis on a scale from 1 to 4 as follows: 0, normal; 1, mild swelling affecting more than one digit; 2, moderate erythema and swelling of the paw; 3, more intense erythema, swelling and redness affecting a greater proportion of the paw; 4, severe erythema, swelling and redness affecting the entire paw. Each animal receives a cumulative score ranging from 0 to 16, based on individual paw scores of 0 to 4. (B) PG-specific IgG1 was evaluated by ELISA from serum of immunized mice.

deficient mice (Figure 2A and Figure 2B). We were unable to identify a requirement for CR1/2 in autoantibody production possibly because of the multiple injections of

PG and/or administration of adjuvant was able to overcome the requirements for CR1/2 in B cell antibody production.

#### 4.3. Immune complex cross-linked FcγR activates inflammation.

Cross-linking FcγRs triggers various cellular functions including antibody production, antibody-dependent cellular cytotoxicity (ADCC), phagocytosis, and the release of inflammatory cytokines and chemokines (62-64). FcγRI and FcγRIII are dual-chain receptors capable of transducing signals through the shared γ-chain, which contains an immuno-tyrosine based activation motif (ITAM)(65). In animal models of arthritis including CIA, AIA, K/BxN serum transfer, anti-type II collagen transfer, and PGIA, targeted deletion of the FcR shared γ-chain prevents disease, thereby demonstrating a critical role for FcγR in arthritis development (43, 66-70).

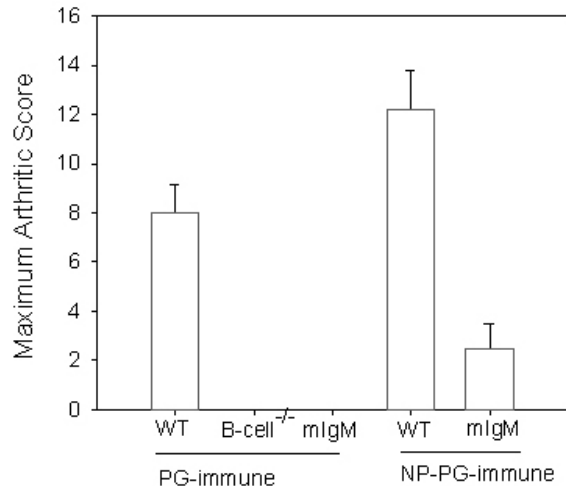
Although these models have demonstrated the importance of FcR γ-chain expression, there is a clear difference in the critical role FcγRI and FcγRIII play in animal models. In antigen-induced arthritis (AIA), neither FcγRI nor FcγRIII are involved in joint swelling and leukocyte infiltration but cartilage damage is substantially reduced in FcγRI<sup>-/-</sup> mice (71, 72). In ICA, FcγRI and FcγRIII are involved in cartilage destruction (73). FcγRIII is the principal activating FcγR mediating joint inflammation in ICA, K/BxN serum transfers, CIA, and PGIA models (43, 73-75). This disparity in the requirement for FcγRs suggests that different mechanisms may be involved in the development of disease. Recently, an important role for the human FcγRIIIa activating receptor was shown in a transgenic mouse model (76). FcγRIIIa-transgenic mice developed disease in both CIA and passive transfer of anti-CII antibody more rapidly than control mice. Also, these mice developed spontaneous autoimmunity (76). These results suggest the human FcγRIIIa may contribute to the severity of RA.

A third classification of FcγRs in mice is the FcγRIIb single chain receptor that contains an immuno-tyrosine based inhibitory motif (ITIM) in its cytoplasmic tail. Cross-linked FcγRIIb with the FcγRIII or the B cell receptor inhibits IC-triggered inflammation and antibody production respectively (77-79). Thus, this is likely the reason deletion of FcγRIIb enhances antibody production and accelerates the development of CIA, PGIA, and ICA (66, 70, 73, 80).

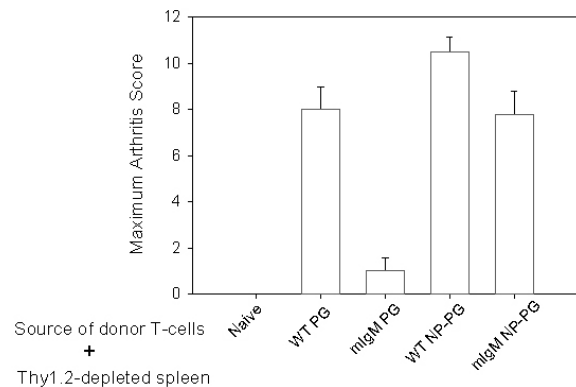
#### 5. ANTIBODY-INDEPENDENT INDUCTION OF ARTHRITIS

##### 5.1. B cells function as antigen-presenting cells

In addition to the role of autoreactive B cells in autoantibody production, B cells have the capacity to promote T cell activation. The question of whether B cells activate T cells *in vivo* has been studied using B cell depletion by anti-IgM treatment or B cell deficient mice. These studies have concluded that B cells are required for priming T cells to some antigens and not others (81-85). In CIA and PGIA, depletion of B cells prevents the



**Figure 3.** Targeting PG to the NP-specific B cell receptor with NP-PG in mIgM mice induces modest arthritis in the absence of antibody production. WT, B cell<sup>-/-</sup>, and mIgM mice were immunized with PG and WT and mIgM were immunized with NP-PG as described in Figure 2. Development of arthritis was monitored for onset and severity.



**Figure 4.** Targeting PG to the B cell receptor with NP-PG primes arthritogenic T cells. Nylon wool purified T cells ( $1 \times 10^7$ ) from naïve WT, PG-or NP-PG-immunized WT or mIgM mice, plus Thy1.2 (CD90)-microbead (Miltenyi Biotec, Auburn, CA) depleted spleen cells ( $2 \times 10^7$ ) from arthritic WT mice, and 100  $\mu$ g antigen (PG or NP-PG) were mixed in saline and adoptively transferred i.p. into SCID mice. SCID mice were examined for arthritis onset and severity every three days following cell transfer.

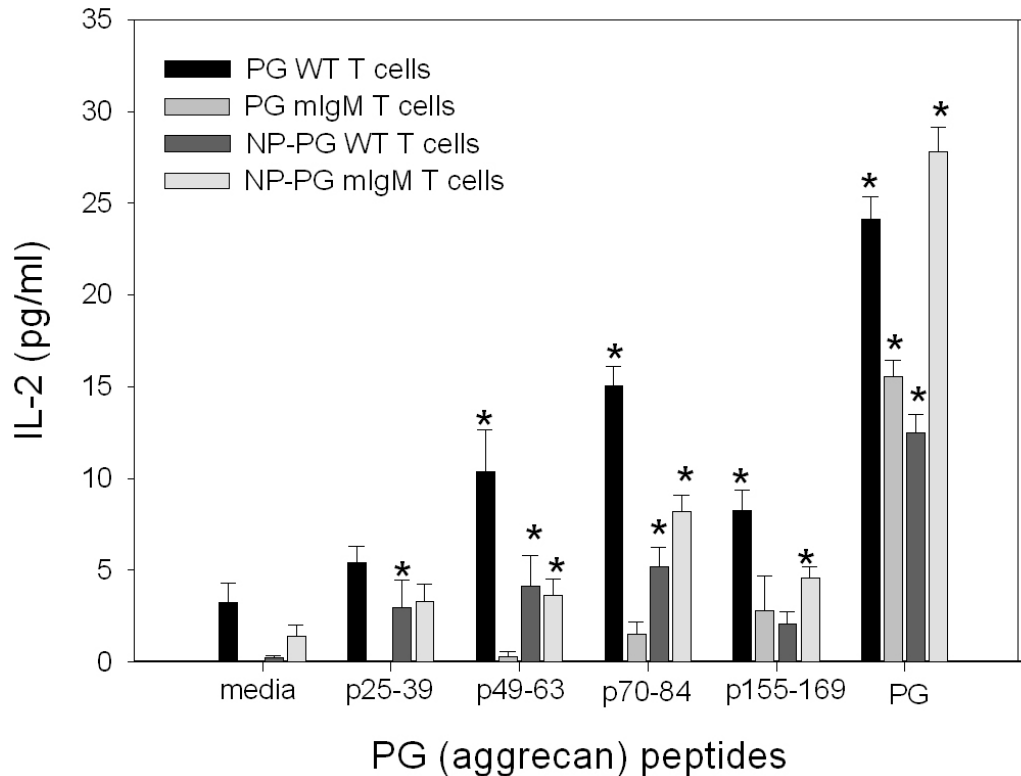
development of arthritis (86, 87) and T cell activation is substantially reduced in PGIA (87)

Antigen-specific B cells are able to process and present antigen  $10^3$ - $10^4$  fold more efficiently than other antigen-presenting cells (APCs) suggesting that they are more likely to induce T cell priming to small doses of antigen (88-91). In PGIA, B cells isolated from PG-immunized mice present PG peptides to T cells  $10^4$  fold more effectively than peritoneal macrophages (92). This interaction between B cells and T cells is dependent on the

expression of MHC class II, ICAM-1, and CD86 (92). Similarly, B cells isolated from CII-immunized mice present collagen peptides to T cells very efficiently (93).

In addition to the ability of antigen-specific B cells to bind and internalize antigen, antigen may also be internalized through immune-complexes binding Fc $\gamma$ R expressed on dendritic cells and macrophages (94-97). Thus, the question arises is there a role for B cell antigen-presentation independent of antibody production? To test the requirement for B cells independent of antibody production in PGIA, an Ig V<sub>H</sub> transgenic mouse was created with a deletion of the DNA encoding secretion and polyadenylation sequence (98). These transgenic mice, designated mIgM, express membrane IgM but do not secrete Ig. We immunized female WT and mIgM mice with PG in CFA. mIgM mice were resistant to PGIA whereas 100% of WT mice were susceptible (Figure 3). In PGIA both autoantibodies and autoreactive T cells are required for the development of arthritis. Since mIgM mice do not secrete autoantibodies, we needed to distinguish between the role of B cells in autoantibody production and T cell priming. To address this problem, we established a protocol to assess T cell priming in a disease transfer system. In the transfer system, purified T cells from PG-immunized WT or mIgM mice were transferred along with T cell-depleted spleen cells (B cells + APCs) from arthritic WT mice into severe combined immunodeficient (SCID) mice. B cells + APCs provided a source of autoantibody producing cells and antigen-presenting cells. All SCID mice that received T cells from PG-immunized WT mice developed severe arthritis. In contrast, T cells from PG-immunized mIgM developed very mild and transient disease in only 40% of mice (Figure 4). These data demonstrate that T cells from the PG-immunized mIgM were not adequately primed. Thus, the presence of B cells in the mIgM mice was not sufficient to prime a population of autoreactive T cells.

Since antigen binding to the B cell receptor induces cell activation and efficient endocytosis, processing and presentation of peptides to T cells, it is possible that B cells in the mIgM mice need to be specific for PG. In mIgM mice, B cells expressing the V<sub>H</sub>186.2 H chain can pair with any endogenous light chain; however, no B cell receptor specific for PG was detected. When V<sub>H</sub>186.2 pairs with an  $\lambda$  L chain, specificity for the hapten 4 hydroxy-3-nitrophenyl acetyl (NP) is conferred in 2-4% of the B cells (98). We used the high frequency of NP-specific B cells in mIgM mice to directly target PG to the B cell receptor through a hapten-carrier conjugate of NP-PG. WT mice immunized with NP-PG developed severe disease; unexpectedly, mIgM mice immunized with NP-PG developed arthritis although milder than in WT animals (Figure 3). These data suggest that autoreactive T cells activated by antigen-specific B cells can induce mild disease in the absence of autoantibody. To directly test whether antigen-specific B cells in mIgM mice prime autoreactive T cells, we transferred T cells from NP-PG immunized mIgM mice and B cells + APCs from arthritic mice into SCID recipients. T cells efficiently induced arthritis whether derived from mIgM or WT NP-PG



**Figure 5.** T cells from NP-PG immunized, but not PG-immunized mIgM mice respond to peptides of PG that correlate with the development of arthritis. Purified T cells ( $2.5 \times 10^5$ ) from PG or NP-PG immunized WT and mIgM mice were cultured overnight with peptides of repeating sequence of the core protein of human cartilage PG (101) (50  $\mu\text{g/ml}$ ) or glycosaminoglycan-depleted human cartilage PG (12.5  $\mu\text{g/ml}$ ) in the presence of irradiated splenocytes ( $2.5 \times 10^5$ ) from naïve WT mice. Supernatants were collected and examined for the concentration of IL-2 by ELISA. \*Data are statistically significantly different ( $p < 0.05$ ) between unstimulated T cells (media) and T cells stimulated with PG peptides or cartilage PG.

immunized mice (Figure 4). Furthermore, T cells from NP-PG immunized mIgM could synergize with arthritic serum (autoantibodies) for the induction of severe arthritis (data not shown). Thus, autoreactive T cells and autoantibody are required to cause severe arthritis, indicating that both B cell-mediated effector pathways contribute synergistically to autoimmune disease.

Since antigen-specific B cells were required as APCs to activate arthritogenic T cells, it is conceivable that antigen-specific B cells efficiently present certain peptides of PG in the context of MHC class II alleles and the appropriate costimulatory molecules. Therefore, we assessed whether antigen-specific B cells activate a subset of PG-specific T cells that correlate with arthritis development. Peptides of PG (aggrecan core protein-specific synthetic peptides: p25-39, p49-63, p70-84, p155-169) have previously been shown to activate T cells from arthritic BALB/c mice, but not arthritis resistant PG-immunized BALB/c mice (99-101). T cells were isolated from PG- and NP-PG-immunized WT and mIgM mice were re-stimulated with PG peptides (p25-39, p49-63, p70-84, p155-169) or with PG in the presence of irradiated WT splenocytes. T cells from WT and mIgM mice immunized with PG or NP-PG all responded to intact PG (Figure 5). However, T cells from mIgM mice only responded to PG

peptides when immunized with NP-PG and not PG. These data suggest that antigen-specific B cells may present a unique subset of peptides that are essential for the activation of arthritogenic T cells.

## 5.2. B cell co-stimulatory and co-inhibitory molecules in arthritis

The immune system has developed powerful mechanisms to activate T cells and prevent unnecessary T cell activation that could lead to autoimmunity. One mechanism is the expression of positive and negative costimulatory signals delivered to the T cell after interaction with antigen. These second signals are reliant on the interaction between cell surface receptors and their ligands expressed on APCs and on nonlymphoid cells. The most well studied positive costimulatory interactions are CD80/CD86: CD28 and ICOSL: ICOS whereas the negative costimulatory interactions are CD80/CD86: CTLA-4 and PD-L1/PD-L2: PD-1 (reviewed in (102)).

The importance of the CD80/CD86: CD28 interactions in arthritis has been demonstrated in CIA where blockade of CD80/CD86: CD28 interaction prevents disease (103-105). Consistent with the blocking data, CD28-deficient mice on the DBA/1 background are resistant to CIA (106) and a deficiency in both CD80 and

CD86 prevent PGIA and autoantibody production (data not shown). Defects in the CD80/CD86: CD28 interactions not only prevent signaling to T cells but the reciprocal CD80/CD86 signal to dendritic cells, macrophages, and B cells. For B cells, it has recently been shown that CD80/CD86 signaling on B cells is not required for antibody production, Ig class switch, or memory B cell development (107). Whether the reciprocal interaction, CD80/CD86 expression on B cells is required for T cell activation, remains to be determined. However, antigen recognition by B cells rapidly upregulates expression of CD86 suggesting a role in B cell activation of T cells (84, 108).

A new member of the CD28 family; ICOS (inducible stimulator) is expressed on activated T cells and the ICOS ligand, B7RP-1, is expressed on several cell populations including B cells, macrophages, dendritic cells, fibroblasts, endothelial cells, and epithelial cells (102). Initially it was thought that ICOS engagement selectively stimulates IL-10 and IL-4 production but later studies indicated that ICOS activates both Th1 and Th2 cytokines (109, 110). The role of ICOS in arthritis was examined in CIA where ICOS-deficient mice were resistant to disease. Reduction in arthritis was associated with decreased collagen-specific antibodies and decreased IL-17 (111). Administration of anti-ICOS mAb in CIA during the induction and effector phases ameliorated disease, which also resulted in a decrease in collagen-specific antibodies (112). It is unclear whether the decrease in antibody was due to suppression of T cell helper activity or a requirement for T cell/B cell collaboration via ICOS: B7RP-1. These data demonstrate a requirement for ICOS: B7RP-1 interactions in the induction of arthritis, however, whether B7RP-1 signaling on the B cells is required for antibody production or B cell B7RP-1 activates ICOS signaling for T cell responses remains to be determined.

PD-1 (programmed death-1) belongs to the CD28 and CTLA-4 family and is expressed on activated T cells, B cells, NK-T cells and monocytes (113). PD-1 belongs to a family of regulatory molecules containing an immunoreceptor tyrosine-based inhibitory motif in its cytoplasmic domain (114). PD-1 interacts with two ligands PD-L1 and PD-2. PD-L1 is expressed on a wide variety of hematopoietic (activated B cells, T cells, dendritic cells, and macrophages) as well as nonhematopoietic cells (endothelial cells, heart, lung, pancreas, muscle, and placenta) whereas PD-L2 expression is limited to macrophages and dendritic cells (102, 115-117). PD-1: PD-L1 interaction functions to inhibit T cell and B cell activation (118-120). It has been postulated that the expression of PD-L1 on nonlymphoid tissue may regulate T cell and B cell activity in peripheral tissues via engagement with PD-1 on lymphoid cells (102). PD-1: PD-L1/ PD-L2 interactions play a major negative regulatory role in autoimmune diseases. Mice deficient in PD-1 or neutralization of PD-1 and PD-L1 accelerate the onset and severity of autoimmune dilated cardiomyopathy, experimental autoimmune encephalomyelitis, and autoimmune diabetes, (121-123). Also, C57Bl/6 mice deficient in PD-1 develop late onset lupus-like proliferative

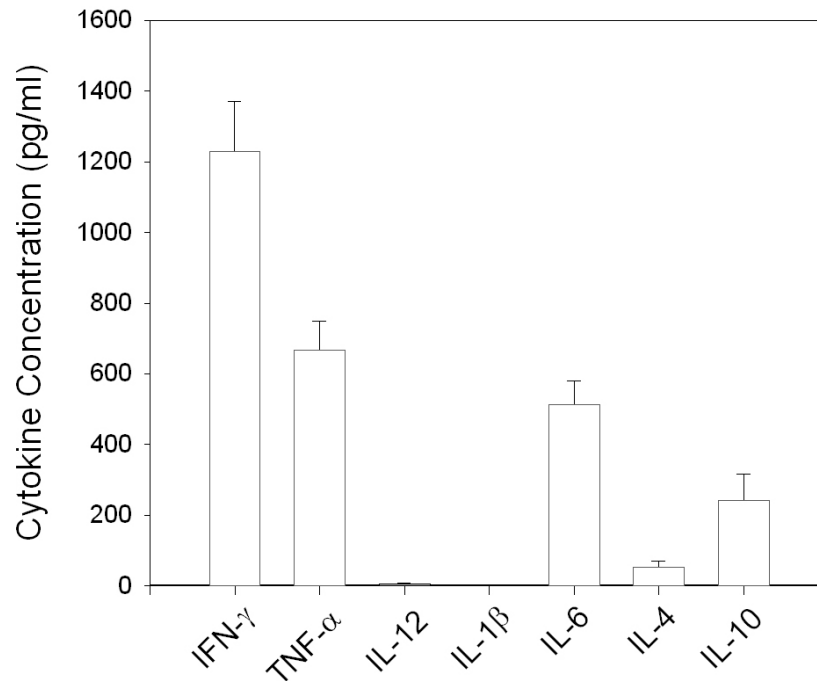
arthritis and glomerulonephritis (114). These studies demonstrate multiple systemic consequences to the absence of PD-1: PD-L1/PD-L2. The role of PD-1 and PD-L1 ligands in models of RA remain to be determined; however, data from other autoimmune models suggest that arthritis severity will be enhanced in the absence of PD-1: PD-L1/ PD-L2 interactions. In addition, recent reports suggest that PD-1 is a susceptibility gene for RA (124, 125). Whether T cell/B cell reciprocally regulate each other function through PD-1: PD-L1/PD-L2 has not been addressed.

## 6. B CELL CYTOKINES IN ARTHRITIS

At sites of chronic inflammation, such as synovial tissue in RA joints, secretion of cytokines is upregulated for an extended period of time contributing to cell activation and release of MMPs. The contribution of proinflammatory cytokines, in particular TNF- $\alpha$ , to the pathogenesis of RA, was established based on the evidence that depletion of TNF- $\alpha$  with neutralizing antibodies or soluble receptor reduced the symptoms of disease (126). More recently, the IL-1 receptor antagonist, antibodies specific for IL-6, and IL-15 have been introduced. Treatment with these anti-cytokine agents is effective in suppressing symptoms of RA and in many cases reducing the progression of bone erosion [reviewed in (127)]. T cells, macrophages, fibroblasts, and neutrophils are thought to be the main source of these pro-inflammatory cytokines in the joint. However, B cells are also present in synovial tissue and may be a potential source of cytokines.

The cytokine environment controls the differentiation of T cells into Th1 and Th2 cells (128, 129). IL-12 and IFN- $\gamma$  induce the differentiation of Th0 into Th1 cells whereas IL-4 is necessary for Th2 differentiation (130, 131). These polarized Th1/Th2 cells produce distinct cytokine profiles that differ greatly in their biological activity. Recently, it has been shown that B cells can also be divided into B cell effector-1 cells (Be1) secreting IFN- $\gamma$  and IL-12 and B cell effector-2 (Be2) cells secreting IL-4, IL-6, and IL-10 (132). Be1 and Be2 cells induce the differentiation of naïve T cells into polarized Th1 and Th2 cells, respectively. Harris et al. demonstrated that there is a reciprocal relationship between Th1 cells and B cell production of IFN- $\gamma$ . T cell and B cell IFN- $\gamma$  are essential for the maintenance of IFN- $\gamma$  producing B cells (133). B cells producing IFN- $\gamma$  have been identified in mice infected with pathogens such as *Toxoplasma gondii*, *Borrelia burgdorferi*, *Staphylococcus aureus* (132, 134, 135). It has also been observed that B cells stimulated with IL-12 and IL-18 produce robust IFN- $\gamma$  (136, 137). Thus infectious agents may activate macrophages and dendritic cells through TLR to produce IL-12 and IL-18, which in turn activates B cells to produce IFN- $\gamma$ . Similarly, we have shown that direct activation of naïve B cells by LPS or peptidoglycan in the presence of IL-12 results in IFN- $\gamma$  production (138).

In examining the profile of cytokines produced by B cells in PGIA, we observed high levels of IFN- $\gamma$ , TNF- $\alpha$ , and IL-10 whereas the production of IL-12, IL-1 $\beta$ ,



**Figure 6.** Splenic B cells from arthritic mice were purified by negative selection. Purified B cells were stimulated with PMA (5ng/ml) and ionomycin (12.5  $\mu$ m). Supernatants were harvested on day 3 and assayed for cytokines by ELISA. Similar responses were observed from 3 different groups of PG-immunized mice.

and IL-4 were minimal (Figure 6). In PGIA, neutralization of IFN- $\gamma$  or deletion of the IFN- $\gamma$  gene results in mild and delayed disease onset (139). B cell production of IFN- $\gamma$  may provide a source of IFN- $\gamma$  for macrophage activation and the differentiation of Th1 cells.

In addition to activating cytokines, B cells producing IL-10 have been shown to play a regulatory role in several autoimmune models (140-142). Specifically in CIA, Mauri et al. demonstrated that B cells isolated from arthritic mice and activated with agonist anti-CD40 give rise to a population of B cells that produce elevated levels of IL-10 and reduced levels of IFN- $\gamma$ . These B cells could actively prevent the development of arthritis and suppresses established disease (142). It is clear in CIA and PGIA that deficiency in IL-10 exacerbates arthritis (9, 143). Thus, B cells as a source of stimulatory and inhibitory cytokines may contribute to the regulation of arthritis.

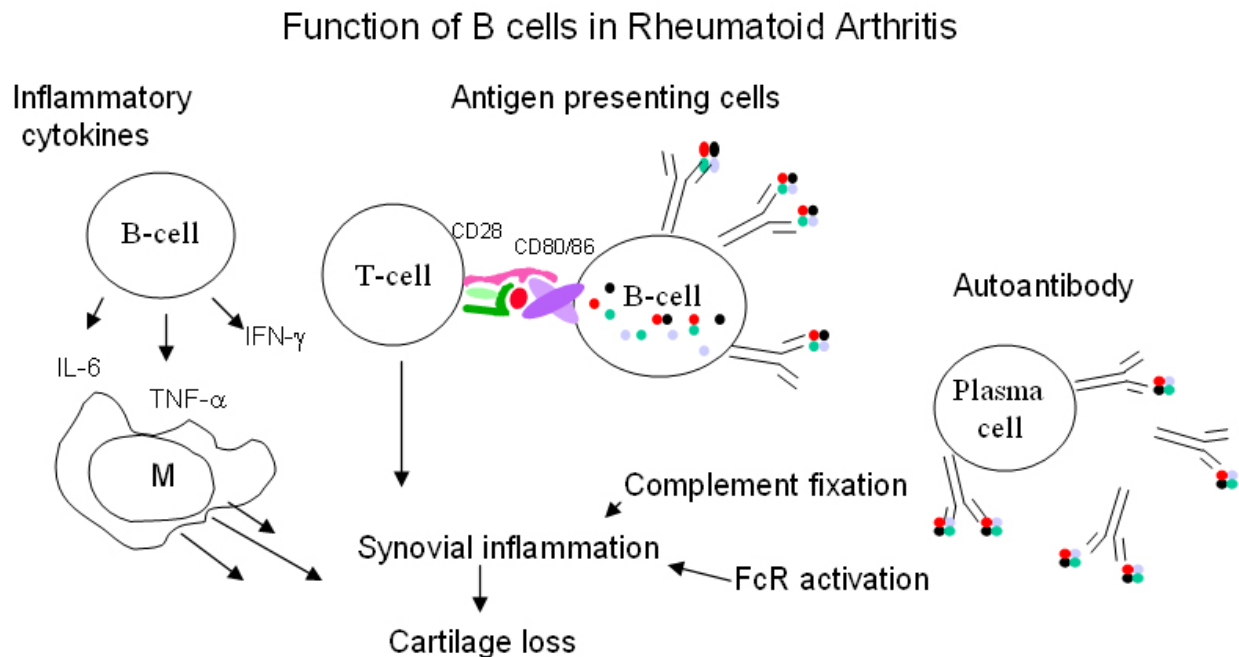
## 7. PERSPECTIVE

In this review we have discussed the involvement of B cells in models of RA where both antibody-dependent and antibody-independent mechanisms may contribute to arthritis. Figure 7 depicts several ways in which B cells may be involved in RA. Based on studies in animal models it is apparent that antibody in the form of IC activate effector pathways that are critical for the induction and recruitment of inflammatory cells. These effector pathways lead to the production of pro-inflammatory cytokines,

chemokines, and cartilage-degrading enzymes that transform the synovial tissue into a tumor-like tissue (pannus) that destroys the cartilage and invades the underlying bone. Many of the autoantibodies that participate in murine arthritis are now recognized in RA, suggesting that antibody-effector mechanisms contribute to inflammation. Therapies to inhibit these effector pathways are under design, for example, therapies are being tested to block complement activation components using monoclonal antibodies to C5 or peptides that bind C5aR inhibiting C5a interaction with C5aR (144-146). Similar approaches might also be used to block Fc $\gamma$ R activation.

The participation of B cells in antigen-presentation, co-stimulatory and co-inhibitory interactions, and cytokine production is not well understood in autoimmune diseases. In systems where it has been examined B cells are necessary for autoreactive T cell activation. We favor the idea that autoreactive B cells are acting as antigen presenting cells and that co-stimulatory interactions are essential to either initiate or amplify the autoreactive T cell response. The presence of germinal centers in synovial tissue is an excellent site for T cell/B cell collaboration. Presentation of new self-antigen from damaged cartilage could lead to expansion of the autoreactive T cell repertoire. An increase in number or specificity of T cells could in turn provide T helper signal for autoantibody secretion. These signals would perpetuate a positive feedback loop and escalate the expansion of autoreactive cells. Co-inhibitory interactions may contribute to resistance to autoimmunity. The association





**Figure 7.** There are several ways B cells may contribute to the pathogenesis of RA. B cells secrete cytokines, which in turn could enhance the pro-inflammatory activity of macrophages. B cells function as antigen presenting cells for T cell activation and may present autoantigens in the synovial tissue to autoreactive T cells maintaining and perpetuating the autoreactive T cell response. The production of autoantibodies and immune complex formation by B cells initiates complement and FcR activation both effector pathway leading to synovial inflammation.

between PD-1 gene polymorphism and the development of RA suggests this possibility. Further studies are needed to analyze whether co-inhibitory interactions between B cells and T cells regulate autoimmunity. Cytokines are well-established effector molecules in RA. Recent findings have shown that B cells produce cytokines, suggesting that they may also contribute to the cytokine milieu that regulates inflammation. If cytokines are produced in synovial germinal centers in close proximity to T cells these effector molecules may have a dramatic effect. Further studies need to assess whether B cell cytokine production contributes to inflammation.

In summary, B cells may act through several different mechanisms in the induction or perpetuation of autoimmune arthritis. A better understanding of antibody-independent mechanisms will permit the development of more direct B cell therapies.

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