Antigen-Specific B Cells Are Required as APCs and Autoantibody-Producing Cells for Induction of Severe Autoimmune Arthritis

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*J Immunol* 2005; 174:3781-3788; doi: 10.4049/jimmunol.174.6.3781
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B cells play an important role in rheumatoid arthritis, but whether they are required as autoantibody-producing cells as well as APCs has not been determined. We assessed B cell autoantibody and APC functions in a murine model of autoimmune arthritis, proteoglycan (PG)-induced arthritis, using both B cell-deficient mice and Ig-deficient mice (mIgM) mice that express an H chain transgene encoding for membrane-bound, but not secreted, IgM. The IgH transgene, when paired with endogenous L chain, recognizes the hapten 4-hydroxy-3-nitro-phenyl acetyl and is expressed on 1–4% of B cells. B cell-deficient and mIgM mice do not develop arthritis after immunization with PG. In adoptive transfer of PG-induced arthritis into SCID mice, T cells from mIgM mice immunized with PG were unable to transfer disease even when B cells from PG-immunized wild-type mice were provided, suggesting that the T cells were not adequately primed and that Ag-specific B cells may be required. In fact, when PG was directly targeted to the B cell Ig receptor through a conjugate of 4-hydroxy-3-nitrophynyl acetyl-PG, T cells in mIgM mice were activated and competent to transfer arthritis. Such T cells caused mild arthritis in the absence of autoantibody, demonstrating a direct pathogenic role for T cells activated by Ag-specific B cells. Transfer of arthritic serum alone induced only mild and transient arthritis. However, both autoreactive T cells and autoantibodies are required to cause severe arthritis, indicating that both B cell-mediated effector pathways contribute synergistically to autoimmune disease. The Journal of Immunology, 2005, 174: 3781–3788.

B cells are critically required for the development of several spontaneous and induced murine models of autoimmune disease (1–14). One way that B cells contribute to autoimmune disease is through the production of autoantibodies. These autoantibodies induce disease directly and indirectly in the form of immune complexes (IC) and their internalization through FcγR expressed on dendritic cells (DC) or macrophages (33–39).

The relevance of B cells in disease pathogenesis has been shown for several experimental models of autoimmune disease, including systemic lupus erythematosus (3, 9, 16) and diabetes mellitus (5, 6, 40). Chan et al. (17) recently demonstrated that mice deficient in the secretion of circulating Ig develop lupus nephritis. These data exclude the simple explanation that B cells influence this autoimmune disease solely through the production of autoantibody, and instead suggest a potential function for B cells as APCs for T cell activation. Although these results clearly defined a critical role for B cells independent of circulating IC, they did not determine whether the Ag specificity of the B cell contributes to their APC function. In a separate model of autoimmune disease, several investigators have examined the role of B cells in the NOD mouse, and their results suggest a role for Ag-specific B cells as APCs (7, 18, 41–43). However, because B cells were still competent to secrete Ab, IC-mediated Ag presentation by DCs or macrophages could have contributed to the level and type of T cell priming necessary for disease.

To clarify the role of Ag-specific B cells as APCs independent of circulating ICs in autoimmune disease, we examined the requirement for Ag-specific B cells in proteoglycan (PG)-induced arthritis (PGIA) using Ig-deficient (mIgM) mice. PGIA is induced in BALB/c mice after immunization with the human PG aggrecan, resulting in clinical manifestations and histology of the diarthrodial joints with many similarities to rheumatoid arthritis (RA) (44–47). CD4 T cells are necessary for the development of PGIA (48), and the higher ratio of IFN-γ to IL-4 indicates that these are Th1 cells by two separate mechanisms. One mechanism involves direct binding and internalization of Ag through the BCR (26–32) whereas a second mechanism involves the formation of immune complexes (IC) and their internalization through FcγR expressed on dendritic cells (DC) or macrophages (33–39).

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Received for publication August 31, 2004. Accepted for publication December 24, 2004.

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1 This work was supported by National Institutes of Health Grants AR47657 and AR47652 (to A.F.), AI43603 (to M.J.S.), and AR47652 (to T.T.G.).

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3 Abbreviations used in this paper: IC, immune complex; DC, dendritic cell; mIgM, Ig-deficient mice; NMS, normal mouse serum; NP, 4-hydroxy-3-nitrophynyl acetyl; NP-OSu, NP succinimide ester; NP-PG, PG conjugated to NP; PG, proteoglycan; PGIA, proteoglycan-induced arthritis; RA, rheumatoid arthritis; WT, wild type; NIP, 4-hydroxy-3-iodo-5-nitrophynyl acetyl.

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mice, (m/H11001 radial immunodiffusion assay (The Binding Site). Furthermore, transgenic IgM cytometry and were found to contain equivalent percentages of splenic control group. The mIgM mice and m/H9262 B cells (54). The mIgM transgenic mice were created using the VH186.2 specificity for the hapten, 4-hydroxy-3-nitrophenyl acetyl (NP), in 2–4% of (0.31 mg/ml). The V H186.2 transgene was detected by PCR using the (m/H11003 Nylon wool-purified T cells (2.5 107) from PG- or NP-PG-immunized WT mice, and 100 [mH11003 penicillin, 100 [mH11003 streptomycin, and 2 mM L-glutamine in the presence of PG (25 [mH11003 IL-2) was measured from the supernatants collected on day 1 by ELISA using an OPT enzyme immunoassay mouse IL-2 kit (BD Biosciences). The samples were run in duplicate on 96-well Maxisorp plates (Nalg Nunc International). Data represent the mean ± SEM of three or four individual mice. Detection of serum Abs titers by ELISA Serum from immunized mice were evaluated for Abs that recognize mouse PG or human PG, using a PG-specific ELISA. Enzyme immunoassay tissue culture 96 “half-area” plates (Costar Corning) were coated overnight at 4°C with 0.5 μg of chondroitinase avidin-biotin peroxidase complex-digested human PG or 0.75 μg of native mouse PG in carbonate buffer. Serum was serially diluted in buffer (PBS/0.5% Tween 20). To detect IgG1 or IgG2a Abs in WT mice, serum dilutions of 1/500, 1/2,500, and 1/12,500 were used to determine the concentration of anti-mouse PG Abs, whereas dilutions of 1/2,500, 1/12,500, and 1/62,500 were used to detect anti-human PG Abs. Samples were run in duplicate. When testing for the presence of PG-specific Abs in the serum of mIgM mice, (m/H11003 mIgM transgenic mice, B cell-deficient mice, serum was diluted at 1/10, 1/50, and 1/100 for plates coated with either human PG or mouse PG. Ab isotypes were detected with HRP-labeled rabbit anti-mouse IgG1, IgG2a, or IgG3 (Zymed Laboratories), which was then detected with the substrate o-phenylenediamine. Colorimetric change in each sample was measured with a spectrophotometer at 490 nm and were compared with a standard curve of known concentrations of unlabeled murine IgG1, IgG2a, or IgM Abs (Southern Biotechnologies). Data represent the mean ± SEM of three to five individual mice. Ag preparation Human cartilage was obtained after joint replacement surgery, and PG was subsequently isolated as previously described (46). Briefly, liquid nitrogen-frozen cartilage was cut into 20-μm sections and extracted with 4 M guanidinium chloride in 50 M sodium acetate, pH 5.8, containing protease inhibitors at 4°C. High buoyant density PG monomers (aggrecan) were isolated by dissociative cesium chloride gradient centrifugation, followed by sequential digestion with endo-β-galactosidase and chondroitinase ABC (Seikagaku America) overnight at 37°C. Additional purification was performed using a Sephacryl S-200 column (Phar-macia Biotech). Murine PG was isolated after obtaining cartilage from newborn mice and was prepared in a similar method to human cartilage PG, without deglycosylation. PG was reacted with NP succinimidyl ester (NP-OSu; Biosearch Technologies) by dissolving PG (as measured in protein) in 0.1 M borate buffer to 5 mg/ml, pH 9.2. NP-OSu was dissolved in dimethylformamide to 10 mg/ml. To achieve a substitution ratio of ~1 PG:20 NP molecules, 11.6 μl of 10 mg/ml NP-OSu/dimethylformamide was added to 1 ml of PG (5 mg/ml) dissolved in borate buffer at 4°C and stirred overnight. The remaining free hapten was separated from the hapten-protein conjugate by dialysis against TS buffer (50 mM Tris and 150 mM NaCl). Induction and assessment of arthritis Female mice, ≥3 mo of age, were immunized i.p. with either 150 μg of human PG or PG conjugated to NP (NP-PG) measured as protein, emulsified in CFA. Mice received booster immunizations at wk 3 and 6 with 100 μg of PG or NP-PG in IFA. Mice were monitored biweekly for signs of arthritis subsequent to the final immunization, and scoring was blinded. Paws were scored 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; and 4, severe erythema, swelling, and redness affecting the entire paw. Each animal received a cumulative score ranging from 0 to 16, based on individual paw scores of 0–4. Detection of in vitro cytokine secretion by ELISA T cells were purified from spleens of PG- or NP-PG-immunized mice by passage through nylon wool. T cells (2.5 107 cells/well) from PG- or NP-PG-immunized mice plus irradiated (2500 rad) spleen cells from naive WT mice were cultured in triplicate in 96-well Falcon plates (Fisher Scientific) in serum-free HL-1 medium, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine in the presence of PG (25 μg/ml). The binding site. In this study we demonstrate that Ag-specific B cells are indispensable as APCs for the activation of autoreactive T cells. Only when Ag-specific B cells activate T cells are they competent to induce arthritis. Importantly, these studies also reveal that autoreactive T cells and autoantibodies are required to cause severe arthritis, indicating that both aspects of B cell function contribute synergistically to autoimmune disease.

Materials and Methods

Mice Female BALB/c and SCID mice were purchased from the National Cancer Institute. The mIgM, (m/H11001 Ig transgenic mice contain a transgene with a rearranged VDJ region ligated to an IgM a (BALB/c-derived) H chain. B cell-deficient mice contain a homozygous deletion of the joining H chain region of Ig DNA (JH) due to a neo insertion in the JH locus and are incapable of developing mature B cells (53). B cell deficiency was validated by confirming the absence of surface IgM and low levels of B220+ spleen cells by flow cytometry; additionally, neither IgM nor IgG was detectable in the serum of B cell-deficient mice by immunofluorescence assays (The Binding Site). Variable H chain (Vγ186.2) Ig transgenic mice contain a transgene with a rearranged VDJ region ligated to an mIgM (BALB/c-derived) H chain. From cells from Vγ186.2 transgenic mice express an H chain that can pair with any endogenous Ig L chain; however, pairing with a L chain confers Ig specificity for the hapten, 4-hydroxy-3-nitrophenyl acetyl (NP), in 2–4% of B cells (54). The mIgM transgenic mice were created using the Vγ186.2 transgene with an in-frame deletion of the DNN2 domain and polyadenylation sequence (pA) of the Ig M chain (17). Additionally, a transgenic control strain, referred to as (m/H11001 mIgM, contains an intact transgene encoding for both membrane-bound and secreted IgM. The transgenic mice (mIgM transgene expression on a CB-17 background) were backcrossed to the B cell-deficient (Jγ127.1) strain to fix homozygosity of the Jγ1 mutation, which eliminates the expression of endogenously rearranged IgH chains as previously described (54). CB-17 mice normally express IgM on the BALB/c genetic background; thus, IgM transgenic mice more closely resemble BALB/c animals, which were used as the control group. The mIgM mice and (m/H11001 mIgM mice were assessed by flow cytometry and were found to contain equivalent percentages of splenic IgM and B220+ cells compared with wild-type (WT) BALB/c animals (54) (our unpublished observation). Total serum IgM levels in the (0.01 mg/ml) compared with nonimmune WT mice (8.4 107/mouse) from NP-PG-immunized mIgM mice or WT mice on days 0–3 after antigen injection (The Binding Site). Furthermore, transgenic mice, (m/H11001 mIgM or mIgL, had significantly diminished serum IgG (0.01 ± 0.01 mg/ml) compared with nonimmune WT mice (8.4 ± 2.2 mg/ml). The Vγ186.2 transgene was detected by PCR using the Vγ186.2–5′ primer (5′-TCTCTTCTTGGACGACAC-3′) and the Vγ186.2–3′ primer (5′-TGAAGGACTTGGTGAAGTG-3′), as previously described (17).

Adoptive transfer of PGI A into SCID mice Nylon wool-purified T cells (1 × 107) from PG- or NP-PG-immunized WT or mIgM mice or naive WT mice plus Thy1.2 (CD90)-microbead (Milenyi Biotec)-depleted spleen cells (2 × 107) from arthritic WT mice, and 100 μg of Ag (PG or NP-PG) were mixed in saline and adoptively transferred i.p. into female SCID mice. SCID mice were examined for arthritis onset and severity every 3 days after cell transfer. Enriched T cells were >90% CD3+ and CD90-depleted cells were <2% CD3+ by flow cytometry. In separate experiments, SCID mice were injected i.p. with splenocytes (3 × 107/mouse) from NP-PG-immunized mIgM mice or WT mice on days 0 and 16. SCID mice that received spleen cells from NP-PG-immunized mIgM mice were injected i.p. with 500 μl of pooled sera from arthritic WT mice or normal mouse serum (NMS) every 3 days for 4 wk after the initial cell transfer. SCID mice were administered arthritic serum alone (500 μl) according to the same transfer schedule.

Flow cytometry Spleen cells from WT and mIgM mice were depleted of RBCs, washed, and stained for 20 min on ice with an optimal dilution of the relevant Ab. Cells were analyzed by flow cytometry (FACSCalibur and CellQuest software; BD Biosciences). Vγ186.2 IgH chain transgenic B cells from mIgM

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mice recognize 4-hydroxy-3-iodo-5-nitrophenyl acetyl (NIP) with high affinity when paired with a L chain (54). PE (Molecular Probes) was haptenated with NIP-hydroxy succinimide ester (Biosearch Technologies), and the NIP-PE conjugate was used to identify V₄₁₈₆.2 IgM⁺ B cells. Naive WT and mlgM mice spleen cells were additionally stained with rat anti-mouse β2-FITC (Southern Biotechnology Associates). Day 12 spleen cells from PG- or NP-PG-immunized mlgM mice were stained with a combination of NIP-PE and biotin rat anti-mouse CD86 (B7-2)-biotin, then washed and stained with streptavidin-allophycocyanin (BD Biosciences).

Results

B cells are required for the development of arthritis

To evaluate whether B cells are necessary for the development of PGIA, we used B cell-deficient (J_HD) mice. WT and B cell-deficient mice were immunized with PG and assessed for clinical signs of arthritis. Arthritis developed in a few WT mice before the third injection with PG and progressed until 100% (n = 14) of the animals were arthritis with a maximum mean arthritic score of 6.70 ± 1.13 (Fig. 1, A and B). B cell-deficient mice (0 of 26) were completely resistant to disease. B cell-deficient animals remained resistant to arthritis even 100 days after the final immunization (data not shown), confirming an absolute requirement for B cells in the initiation of PGIA.

The mlgM mice are resistant to arthritis

B cells may function as autoantibody-producing cells and as APCs in the development of arthritis. To address whether B cells contribute to the development of PGIA independent of autoantibody, we used mlgM mice. B cells in the mlgM mice express a membrane-bound Ig H chain transgene in association with an endogenously generated L chain, but do not secrete Ig (17). The V₄₁₈₆.2 transgene contains a deletion of the DNA encoding secretion (μs) and polyadenylation sequence (pa₅) of the IgM H chain (17). Control animals, termed (m+s)IgM mice, similarly express a transgenic IgM H chain that is both expressed on the B cell surface and secreted. WT, mlgM, and (m+s)IgM mice were immunized with PG and examined for disease progression. None of the mlgM mice (n = 15) or (m+s)IgM mice (n = 21) mice developed arthritis compared with 100% of WT mice that developed severe disease (Fig. 1).

To determine whether B cells specific for PG are present in mlgM mice, we used the (m+s)IgM mice that secrete Ig and assayed serum from the PG-immunized mice. PG-specific IgM levels in (m+s)IgM mice to human PG (0.55 ± 0.16 μg/ml) or mouse PG (0.98 ± 0.19 μg/ml) were not statistically different from those in naive WT animals (human PG, 1.33 ± 0.56 μg/ml; mouse PG, 1.13 ± 0.46 μg/ml), in contrast to significantly increased Ab levels in PG-immunized WT mice (human PG, 11.89 ± 3.14 μg/ml; mouse PG, 7.51 ± 0.44 μg/ml). These data demonstrate that PG-specific Abs are undetectable and suggest that (m+s)IgM and mlgM mice are deficient in PG-specific B cells.

Our results show that simply restoring B cells is not sufficient to induce arthritis. There are two potential explanations for why B cells in the mlgM and (m+s)IgM mice were not capable of inducing arthritis. First, a deficiency in PG-specific autoantibodies in mlgM and (m+s)IgM mice may have prevented the development of arthritis, because we have previously shown that autoantibodies enhance arthritis onset (47, 51). The second possibility is that a lack of Ag-specific B cells that function as APCs in the mlgM and (m+s)IgM mice prevented adequate priming of autoreactive T cells that are competent to induce arthritis.

PGIA is reduced in the absence of Ag-specific B cells in mlgM mice

Because T cells are required for the development of PGIA, it is possible that autoreactive T cells are not effectively primed in mlgM mice immunized with PG. To assess whether the T cells from PG-immunized mlgM mice were capable of inducing arthritis, we performed a SCID adoptive transfer experiment to test the function of the T cells in an environment where B cells were capable of producing PG-specific autoantibody. Purified T cells from PG-immunized WT or mlgM mice and Thy1.2 (CD90)-depleted spleen cells (B cells plus APCs) from arthritic WT mice were mixed with 100 μg of PG and transferred into SCID recipients. SCID mice that received T cells from PG-immunized WT mice developed persistent severe arthritis in 100% of cases; in contrast, T cells from PG-immunized mlgM mice developed very mild and transient disease in only 40% of mice (Fig. 2A). These results indicate that T cells in the PG-immunized mlgM mice were not adequately primed to induce severe arthritis, suggesting that Ag-specific B cells may be required for sufficient T cell priming.

Ag-specific B cells restore autoreactive T cell activation

Specific recognition of Ag by B cells through the BCR leads to cell activation and efficient endocytosis of Ag, which substantially enhances Ag presentation to CD4⁺ T cells. To address whether Ag-specific B cells were essential for the activation of autoreactive T cells, we targeted PG to NP-specific B cells in mlgM mice by conjugating PG to the hapten NP. Because ~2–4% of B cells from mlgM mice are NP specific (54), immunization with NP-PG allows B cells from mlgM mice to specifically recognize PG through
the NP-specific BCR. Although NP-PG-immunized WT mice developed severe disease as anticipated (maximum arthritis score, 12.3 ± 1.7), we observed unexpectedly that mlgM mice immunized with NP-PG developed mild, transient arthritis (maximum arthritis score, 3.0 ± 0.7) in 60% of the animals (Fig. 2B). These data suggest that even in the absence of Ab, autoreactive T cells activated by Ag-specific B cells could induce some degree of arthritis. We next investigated whether T cells from NP-PG-immunized mlgM mice were competent to induce severe arthritis in the presence of WT B cells that are capable of secreting autoantibody. Purified T cells from WT or mlgM mice were mixed with CD90-depleted spleens (B cells plus APCs) from NP-PG-immune WT mice, along with 100 μg of NP-PG, and transferred into SCID mice. T cells efficiently induced disease when derived from either WT or mlgM mice, but not from naive WT mice (Fig. 2C). These data suggest that targeting PG to the BCR (in mlgM mice) using NP-PG led to priming of arthritis-inducing T cells. Activation of the T cells in mlgM mice occurred in the absence of circulating Ig, indicating that presentation of Ag by ICs was not necessary to prime T cells for arthritis.

**B cells contribute to arthritis by two distinct, but synergistic, mechanisms: autoantibody production and APC function**

We have shown that PG-specific autoreactive T cells were activated in mlgM mice using NP-PG as the immunizing Ag, although these mice only developed mild and transient arthritis. However, when the T cells from the NP-PG-immune mlgM mice were transferred to SCID mice with B cells from PG-immune WT mice severe arthritis developed, suggesting that autoantibodies were needed for severe disease (Fig. 2C). Therefore, we investigated whether PG-specific autoantibodies were essential for the development of severe arthritis. We first assessed whether autoantibodies contributed to arthritis by blocking the T cell response. T cells from NP-PG-immune mlgM mice, which provided a source of primed T cells, were adoptively transferred into SCID mice in conjunction with either serum from arthritis WT mice or normal mouse serum. SCID mice that received arthritis serum and spleen cells from NP-PG-immune mlgM mice developed severe arthritis, resembling mice that received NP-PG WT arthritis splenocytes alone (Fig. 3). In contrast, no disease was apparent in those mice from NP-PG-immunized mlgM mice, which provided a source of primed T cells, but no autoantibody. SCID mice that received NP-PG WT arthritic serum alone (Fig. 3). In contrast, no disease was apparent in those mice.
that received spleen cells from the NP-PG-immunized mIgM mice treated with normal mouse serum (Fig. 3). Thus, generation of severe arthritis required a source of PG-specific autoreactive T cells in addition to PG-specific autoantibody. From these data, it is evident that Ag-specific B cells contribute to arthritis by two discrete mechanisms, autoantibody production and APC function, each of which is necessary for severe and persistent arthritis.

**T cells primed in the presence of Ag-specific B cells provide efficient B cell help for Ab production**

Because the above experiments suggested that there is a difference in T cell priming, we directly examined T cell responses in vitro. Purified T cells were isolated from B cell-deficient mice and mIgM mice immunized with either PG or NP-PG, and IL-2 production was assessed. T cell priming in mice lacking B cells was first evaluated. T cells from B cell-deficient animals produced significantly less IL-2 in response to PG stimulation compared with WT T cells (Fig. 4A). These results demonstrate that PG-specific T cell activation was substantially reduced in B cell-deficient animals and could in part account for the lack of arthritis in these mice.

To determine whether the presence of B cells leads to T cell activation, T cell responses in mIgM mice were evaluated. T cells from mIgM mice produced significantly less IL-2 than WT T cells; however, IL-2 levels were significantly higher than those in T cells from B cell-deficient mice (Fig. 4A). These data demonstrate that B cells partially activate T cells, independently of their Ag specificity or secreted Ig. Thus, non-Ag-specific B cells contribute to T cell priming to some degree, although this level or type of activation is not sufficient to transfer arthritis.

Next, T cell responses were examined in NP-PG-immunized WT and mIgM mice to determine whether the presence of Ag-specific B cells reconstitutes T cell responses. T cells from NP-PG-immunized WT and mIgM mice produced similar levels of IL-2 in response to PG, indicating that T cells were efficiently primed in the presence of Ag-specific B cells (Fig. 4B). These in vitro results correlate with the SCID adoptive T cell transfer experiments in which T cells from NP-PG-immunized mIgM mice were competent to transfer arthritis (Fig. 2B).

To determine whether Th activity was affected by the priming of T cells, we assessed autoantibody production. T cells from PG- or NP-PG-immunized WT or mIgM mice and B cells plus APCs from WT arthritic mice were transferred into SCID mice. Serum from the SCID recipients was examined for IgG1 and IgG2a Abs that recognize human PG (hFPG) or native mouse PG (mPG) by ELISA. Ab production is the mean ± SEM of five SCID mice and represents one of two experiments.

**Ag-specific B cells proliferate and are markedly activated in mIgM mice immunized with NP-PG, but not PG**

Activation of B cells and the expression of costimulatory molecules enhance the Ag-presenting function of B cells. To determine whether NP-PG activates proliferation and costimulatory molecule expression, we assessed the NP-specific population of B cells in mIgM mice. As previously shown, ~1.5% of $\gamma\delta$ T cells bind NP and were identified by FACS analysis in naive mIgM mice (Fig. 5B), whereas this population of cells was undetectable in naive WT mice (Fig. 5A). To assess whether NP-specific B cells expand in mIgM mice immunized with NP-PG, we compared the
A and B. Naive WT and mIgM mouse spleen cells were analyzed by FACS for the expression of NIP Ig-λ⁺ double-positive cells. A distinct population of NIP⁺ Ig-λ⁺ double-positive cells is evident in transgenic mIgM mice and is undetectable in WT mice (n = 3). C–F, mIgM mice (n = 3 mice/group) were immunized with either PG (C and E) or NP-PG (D and F) and examined on day 12 for the percentage of NIP⁺ cells (C and D) and the percentage of NIP⁺CD86high cells (E and F; R2 gate) by FACS.

**Discussion**

Autoreactive B cells are activated in PGIA, but our understanding of how they contribute to disease susceptibility is incomplete. The present work provides direct evidence that B cells are essential for the development of arthritis and provides several different functions by which they contribute to disease. One of the major findings of our studies is that Ag presentation by B cells is critical for the activation of autoreactive T cells. Moreover, the APC function of B cells requires the direct recognition of Ag through the BCR to activate autoreactive T cells. Once activated, the autoreactive T cells can induce a very mild form of arthritis, indicating that T cells subsequent to activation by Ag-specific B cells have a direct pathogenic role in disease. Although DCs and macrophages are present in mIgM mice, they are not sufficient to function as APCs for autoreactive T cell activation. Our second major finding is that B cell production of autoantibody is critical and that autoantibodies alone induce mild and transient disease. However, both autoreactive T cells and autoantibody are required to cause severe arthritis, indicating that both B cell effector pathways contribute synergistically to autoimmune disease. We also show that autoantibody in the form of ICs are not required to activate autoreactive T cells, because T cells competent to induce arthritis are activated independent of circulating Ab.

There is some controversy over whether B cells can prime naive T cells; however, activated and memory T cells are critically dependent on B cells (25). Our results similarly demonstrate that T cell activation is reduced, and arthritis is prevented in B cell-deficient mice. However, in mIgM mice, PG-specific T cell recall responses are restored to some degree. We found that T cells primed to PG in mIgM mice secrete reduced IL-2 compared with WT T cells. However, these T cells are not sufficient to transfer arthritis. The inability to transfer arthritis correlates with a reduction in B cell helper activity for PG-specific autoantibody production. These findings indicate that the presence of B cells allows some degree of T cell activation that is independent of the B cell specificity; however, this level of activation is not sufficient to transfer arthritis.

The inability of B cells in mIgM mice to fully prime autoreactive T cells may be due to the low frequency of B cells specific for PG. In support of this idea, targeting PG to the high frequency, NP-specific B cells by haptenating PG with NP led to the activation of T cells that induced mild arthritis, and upon transfer with autoantibody caused severe arthritis. Development of T cells in mIgM mice that are competent to induce arthritis correlates with an increase in IL-2 production and the ability to provide Th activity for autoantibody-producing B cells.

Several potential mechanisms could explain why Ag-specific B cells are required for the efficient activation of autoreactive T cells. Ag processing and presentation by Ag-specific B cells can stimulate T cells nearly 1,000–10,000-fold more efficiently than nonspecific B cells. Furthermore, engagement of the BCR by Ag leads to B cell proliferation and up-regulation of costimulatory molecules (54). We demonstrate that Ag-specific B cells are expanded to nearly 7% of the spleen population in NP-PG-immunized mIgM mice, and 60% of these B cells are B7-2high. Activation of Ag-specific B cells could also promote the expansion of PG-specific T cells that induce arthritis. It is possible that internalization of Ag through the BCR could generate a different set of epitopes that are presented to T cells (21, 29, 55, 56), thereby activating a unique subset of Ag-specific T cells. Studies of autoantigens have shown that B cells elicited with foreign cross-reactive Ag can present self-peptides, resulting in priming of autoreactive T cells (57, 58).

With regard to PGIA, immunization with human PG activates B cells that cross-react with mouse PG (45, 47). Based on this scenario, PG-specific T cells may be initially activated by DCs and macrophages, but as clones of autoreactive B cells expand, they may increasingly become important APCs for autoreactive T cell activation.
only after receiving both serum from arthritic mice and autoreactive T cells from NP-PG-immunized mIgM mice (Fig. 3). We have shown that autoantibodies are important for initiating inflammation in the joint by binding to the cartilage surface and initiating chemokine and cytokine responses in the joint (59). Development of joint inflammation is at least in part an FcγR-dependent mechanism (59). However, the activation of autoreactive T cells is independent of FcγR expression. In this study we also show that autoreactive T cells are primed in the absence of circulating Ig. Taken together, these data demonstrate that ICs are not necessary for the priming of autoreactive T cells in PGIA.

This study demonstrates that Ag-specific B cells regulate the initiation of autoimmune murine arthritis by functioning as APCs and producing autoantibody. Neither mechanism alone orchestrates robust disease; however, autoreactive T cells and autoantibody contribute synergistically to severe arthritis. The implication of our study is that autoreactive Ag-specific B cells are crucial at multiple stages of PGIA and, by extension, may be regulating human RA by several different mechanisms. This, in turn, could explain the potent therapeutic effects of B cell depletion in RA (60–66).

Acknowledgments

We thank Dr. Jian Zhang and Dr. Marcus Clark for thoughtful review of the manuscript and helpful comments throughout the course of this research, and Dr. Tamas Koreny for preparation of the human PG.

Disclosures

The authors have no financial interest of interest.

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