Expression of CD80/86 on B Cells Is Essential for Autoreactive T Cell Activation and the Development of Arthritis

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Depletion of B cells in rheumatoid arthritis is therapeutically efficacious. Yet, the mechanism by which B cells participate in the inflammatory process is unclear. We previously demonstrated that Ag-specific B cells have two important functions in the development of arthritis in a murine model of rheumatoid arthritis, proteoglycan (PG)-induced arthritis (PGIA). PG-specific B cells function as autoantibody-producing cells and as APCs that activate PG-specific T cells. Moreover, the costimulatory molecule CD86 is up-regulated on PG-specific B cells in response to stimulation with PG. To address the requirement for CD80/CD86 expression on B cells in the development of PGIA, we generated mixed bone marrow chimeras in which CD80/CD86 is specifically deleted on B cells and not on other APC populations. Chimeras with a specific deficiency in CD80/CD86 expression on B cells are resistant to the induction of PGIA. The concentration of PG-specific autoantibody is similar in mice sufficient or deficient for CD80/CD86-expressing B cells, which indicates that resistance to PGIA is not due to the suppression of PG-specific autoantibody production. CD80/CD86-deficient B cells failed to effectively activate PG-specific autoreactive T cells as indicated by the failure of T cells from PG-immunized CD80/CD86-deficient B cell chimeras to transfer arthritis into SCID mice. In vitro secondary recall responses to PG are also dependent on CD80/CD86-expressing B cells. These results demonstrate that a CD80/CD86:CD28 costimulatory interaction between B cells and T cells is required for autoreactive T cell activation and the induction of arthritis but not for B cell autoantibody production. The Journal of Immunology, 2007, 179: 5109–5116.

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease that affects ~1% of the population. Recent studies demonstrate a significant improvement in clinical symptoms of disease in patients treated with rituximab, an anti-CD20 mAb that depletes pre-B cells and mature B cells but not plasma cells (1–3). This finding indicates that B cells significantly contribute to the pathogenesis of RA. Autoantibodies produced by B cells may be directly pathogenic in RA or may indirectly contribute to disease through the formation of immune complexes and the activation of the complement cascade and Fcy receptors (4, 5). However, the pathogenic consequences of autoreactive B cell activation are not limited to Ab production. B cells have the capacity to promote T cell activation through their ability to process and present Ag, to 10-fold more effectively than other APCs, suggesting that B cells may be important for the presentation of autoantigens that are at low concentrations (6). The role of B cells as APCs has been extensively studied (6–12). In a murine model of RA, proteoglycan (PG)-induced arthritis (PGIA), we have demonstrated that the activation of autoreactive T cells is dependent on the specific recognition of PG by the BCR. In the absence of BCR recognition of PG, the development of arthritis is inhibited (13). This interaction between B and T cells is direct and does not involve the presentation of Ag by immune complexes taken up by macrophages or dendritic cells (DCs), because PG-specific T cell activation occurs in mice deficient in circulating Ig (13).

Activation of naive T cells requires signaling through the Ag-specific TCR and costimulatory molecules. The best-characterized costimulatory pathway is CD28 on T cells and CD80/CD86 on APCs (14). Ab blocking studies and studies in CD28- and CD80/CD86-deficient mice support the importance of this pathway in CD4+ T cell activation. In autoimmune disease models where T cells are critical, combined blockade of CD80 and CD86 inhibits the development of disease (15–20).

Mouse naive B cells express low levels of costimulatory molecules that are up-regulated upon LPS, BCR, anti-CD40, or IL-4 stimulation (16, 21–26). Once the B cells are activated and express CD80/86, they have the potential to receive signals from T cells or reciprocally signal T cell activation. A recent study demonstrated that CD80/86 costimulatory-dependent signaling on B cells is not essential for Ab production (27). However, because CD28+ T cells interact with CD80/86 on several different APCs it has been difficult to determine in vivo whether selective expression of CD80/86 on B cells versus other APCs is essential for T cell activation. In PGIA, we have shown that engagement of the BCR with PG induces B cell proliferation and up-regulation of CD86 expression, suggesting that B cell costimulatory molecule expression may be important for T cell activation. The precise requirement for B cell CD80/86 signaling to T cells has not been established in autoimmunity under physiological in vivo conditions.

The experiments described in this report test whether B cell-specific expression of CD80/86 is required for the development of
PGIA. We generated mixed bone marrow (BM) chimeras in which only the B cells were defective in CD80/86 expression. Our results demonstrate that a deficiency in B cell expression of CD80/86 prevented the development of PGIA despite normal production of PG-specific autoantibodies. However, autoreactive T cells from PG-immunized CD80/CD86−/− B cell chimeric mice were less effective at transferring disease into SCID recipients. Thus, B cell expression of the CD80/CD86 costimulatory molecules is critical for the generation of autoimmunity by regulating autoreactive T cell activation.

Materials and Methods

Mice

Female BALB/c and SCID on BALB/c background (NCr.C.B-17-scid/scid) mice were purchased from the National Caner Institute (Frederick, MD). B cell-deficient (B cell−/−) mice contain homozygous deletion of the joining chain region Ig Jα (Jα2) due to a neo insertion in the Jα locus and are incapable of developing mature B cells (28). B cell−/− mice were generously provided by Dr. M. Shlomchik (Yale University, New Haven, CT). CD80/CD86-deficient (CD80/86−/−) mice were generously provided by Dr. A. Sharpe (Harvard University, Boston, MA) (29).

Induction and assessment of arthritis

Human cartilage was obtained after joint replacement surgery and PG (aggrecan) was subsequently isolated as described (30). Female mice, >3 mo of age, were immunized i.p. with 150 μg of human PG measured as protein, emulsified in dimethyldioctadecylammonium bromide (DDA) (Sigma-Aldrich) as previously described (13), and boosted with 100 μg of PG/DDA at 3 and 6 wk. Mice were monitored for arthritis and scored in a manner previously described (13), and boosted with 100 μg of PG/DDA at 3 and 6 wk. Following the second dose, this was continued for 1 mo. Mice were lethally irradiated with two separate doses of 450 rad 3 h apart using a 137Cs source. Before immunization with PG, peripheral blood leukocytes were surface stained with Abs specific for B220 and CD86 and the percentages of B cells (B220+ CD69− and B220+ CD69+) and non-B cells (B220− CD69− and B220− CD69+) were determined. We measured CD86 expression, because resting B cells express CD86 whereas CD80 is undetectable. Cells were analyzed by flow cytometry (FACSCalibur cytometer and CellQuest software; BD Biosciences). Table I describes how chimeric mice were made.

Adoptive transfer of PGIA into SCID mice

CD80/CD86−/− mice were tested for their ability to transfer arthritis into SCID recipients. Nylon wool-purified T cells (1 × 107) from PG-immunized chimeras were mixed with T cell-depleted (Thy1.2 (CD90) microbeads (Miltenyi-Biotec)) spleen cells (2 × 109) from WT arthritic mice. Cells and 10 μg of PG were injected i.p. into female SCID mice. SCID mice were examined for arthritis onset and severity every 3 days after cell transfer.

Detection of anti-PG Ab by ELISA

Mice immunized with human PG were bled from the orbital plexus and serum was obtained and examined for Abs against mouse PG and human PG by ELISA. Enzyme immunoassay tissue culture 96-well “half area” plates (Costar; Corning) were coated overnight at 4°C with 0.5 μg of chondroitinase avidin-biotin-peroxidase (ABC)-digested human PG or 0.75 μg of native mouse PG in carbonate buffer. Serum was serially diluted in buffer (PBS with 0.5% Tween 20). Serum dilutions of 1/50 and 1/100 were used to determine the titer of IgG1 Abs against mouse PG. Dilutions of 1/2,500, 1/12,500, and 1/62,500 were used to determine the titer of IgG1 Abs against human PG. Samples were run in duplicate. Abs were detected with HRP-labeled rabbit anti-mouse IgG1 (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 the relative concentration was determined from a standard curve of known concentrations of unlabeled murine IgG1 Ab (Southern Bio-technology). Data represent the mean ± SEM of Abs from 4–6 individual mice.

Assessment of T cell activation

Nylon wool-purified T cells (2.5 × 104 cells/well) from peritoneal exudates (pooled from 4–6 mice) of PG-immunized or CD4+ T cells from spleen of SCID (adoptive transfer) or PG-immunized mice were purified by negative selection using microbeads (Miltenyi-Biotec) and stimulated with PG (10 μg/ml) and irradiated (2500 rad) spleen cells from naive WT mice. In other experiments as indicated in figure legends, CD4+ T cells from PG-immunized mice were stimulated with irradiated purified B cells (Miltenyi-Biotec), either naive or activated overnight with 10 μg/ml LPS. Cells were cultured in quadruplicate in 96-well Falcon plates ( Fisher Scientific) in RPMI 1640 medium containing 10% FCS, 100 μg/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. Cultures were pulsed on day 4 with [3H]thymidine overnight and examined for proliferation. CD4+ T cells cytokines (IFN-γ, IL-4, and IL-17) were measured from the spleens of SCID mice stimulated with PG (10 μg/ml) and irradiated (2500 rad) spleen cells from naive WT mice by ELISA (BD Biosciences).
FIGURE 1. Inhibition of PGIA and PG-specific immune responses in CD80/86−/− mice in comparison with WT mice. A and B, BALB/c WT and CD80/86−/− mice were immunized i.p. with human PG in adjuvant three times at 3-wk intervals and monitored for the incidence and severity of PGIA at given time points. Severity (A) (cumulative arthritis score) is the sum of the paw inflammation scores in individual mice divided by the number of arthritic mice. Incidence (B) denotes the percentage of mice that developed PGIA. Values are the mean ± SEM. *, p < 0.01 for WT (n = 9) vs CD80/86−/− (n = 10). C, T cell proliferation was measured by the incorporation of [3H]-thymidine for 18 h into proliferating T cells activated with PG in the presence of irradiated naive spleen cells for 5 days. T cells were isolated from the peritoneal cavity of PG-immunized mice (pooled from 4–6 mice) and purified by nylon wool. Values are the mean ± SD of quadruplicate wells. p < 0.01 for WT vs CD80/86−/−. D, The concentration of PG-specific anti-mouse IgG1 (mIgG1) and anti-human IgG1 (hIgG1) in serum was measured by ELISA. Values are the mean ± SD. p < 0.01 for WT (n = 9) vs CD80/86−/− (n = 10).

Statistical analysis
The nonparametric Mann-Whitney U test was used to compare data for statistical significance. Significance is based on p < 0.05.

Results
CD80/CD86-deficient mice (CD80/86−/−) are resistant to PGIA
The costimulatory molecules CD80 and CD86 are essential for T cell-dependent autoimmune responses, and because PGIA is a T cell- and B cell-mediated autoimmune disease we anticipated that CD80/86-deficient (CD80/86−/−) mice would be resistant to PGIA. WT and CD80/86−/− mice were immunized with PG and the development of disease was monitored over time. The WT mice developed severe inflammatory arthritis with 100% incidence of disease, whereas the CD80/86−/− mice failed to develop arthritis (Fig. 1, A and B). Thus, the expression of CD80/86 costimulatory molecules by APCs is required for the induction of PGIA.

It has been previously shown that both T cell and B cell responses are defective in CD80/86−/− mice. To determine whether resistance to PGIA was due to reduced B and T cell responses, we examined PG-specific Ab production and PG-specific T cell proliferation. Both PG-specific T cell and B cell responses were decreased in CD80/86−/− mice (Fig. 1, C and D). These data confirm previous reports on the requirement for a CD80/86-CD28 pathway in the induction of arthritis and the generation of T and B cell responses (31–33).

Creation of mice with a CD80/86 deficiency restricted to the B cell compartment
Although complete deficiency of CD80 and CD86 prevents arthritis and accompanying T cell-dependent responses, it is unclear whether the expression of these costimulatory molecules on B cells is essential for the induction of PGIA. We have previously shown that Ag-specific B cells are important as APCs for the activation of autoreactive T cells and that the expression of CD86 is up-regulated on Ag-specific B cells after priming with PG in vivo (13). To determine whether B cell expression of CD80/86 is required for PGIA, we created mice in which CD80/86-deficient B cells coexist with other APC populations that express CD80/86. Lethally irradiated WT recipient mice were reconstituted with a mixture of BM from B cell-deficient (B cell−/−) and CD80/86−/− mice (B cell:CD80/86−/−). In these chimeric mice the APC compartment will be derived partially from the B cell−/− BM and thus give rise to CD80/86-positive non-B cell APCs, whereas the B cells will arise from CD80/86-negative BM. Positive control chimeras were a mixture of BM from B cell−/− and WT mice (B cell:CD80/86+/+), and negative control chimeras were BM from either B cell−/− or CD80/86−/− mice injected into lethally irradiated WT recipient mice. Chimeras were analyzed for CD86 expression by flow cytometry on B220− and B220+ cells in peripheral blood 3 mo after reconstitution (Fig. 2). The percentage of B220− B cells in B cell:CD80/86−/− chimeras was similar to that of positive control B cell:CD80/86+/+ and negative control CD80/86−/− chimeras but, as expected, substantially higher than that of B cell−/− chimeras (Fig. 2A). The percentage of B220− non-B cells was similar in B cell:CD80/86−/−, B cell:CD80/86+/+, and CD80/86−/− chimeras and elevated in B cell−/− chimeras (Fig. 2B). CD86 expression was substantially reduced in B220− cells in B cell:CD80/86−/− and CD80/86−/− mice in comparison to positive control B cell:CD80/86+/+ chimeras (Fig. 2C). The percentage of B220− CD86+ cells, the non-B cell compartment comprised of monocytes and DCs, was surprisingly similar in B cell:CD80/86−/− and B cell:CD80/86+/+ mice (Fig. 2D). These data suggest that CD86 expression might confer a survival advantage on the non-B cell APC.

Development of PGIA requires B cell CD80/86 expression
To determine whether the expression of CD80/86 on B cells is required for the development of PGIA, nonchimeric WT and chimeric groups were immunized with PG and evaluated for arthritis.
incidence and severity (Fig. 3). B cell:CD80/86 chimeras developed arthritis with 100% incidence and similar severity as that of nonchimeric WT mice (Fig. 3, A and B). B cell:CD80/86 chimeras with a specific deletion of CD80/86 expression on B cells and not other APCs were resistant to arthritis as were chimeras with either no B cells (B cell−/−) or a complete deficiency in CD80/86 expression (CD80/86−/−). Similar results were obtained in a second experiment (Fig. 3, C and D). These data demonstrate that expression of CD80/86 on B cells is required for the development of PGIA.

To determine the extent of inflammation, we examined ankle joint histology in B cell:CD80/86+/+, B cell:CD80/86−/−, CD80/86−/−, and B cell−/− chimeras as depicted in Figs. 3, C and D. The histologic picture in B cell:CD80/86+/+ chimeras was characteristic of acute arthritis. Mononuclear and polymorphonuclear cell infiltration was abundant in the tissues and joint spaces. There was edema of the synovial and periarticular tissues accompanied by synovial hyperplasia. Cartilage erosion and disintegrating chondrocytes were also seen in the remaining layer of the articular surface (Fig. 4A). Conversely, cellular infiltrates and histopathological signs of arthritis were completely absent in B cell:CD80/86−/−, B cell−/−, and CD80/86−/− chimeras (Figs. 4, B–D). In these mice, the lack of paw erythema and swelling correlated with the absence of cellular infiltrate and joint destruction (Figs. 3D and 4, B–D).

**FIGURE 2.** CD86 expression in the B cell and non-B cell compartments in chimeric mice. The percentages of B220+ cells (A), B220− cells (B), B220+ CD86+ cells (C), and B220− CD86+ cells (D) were identified by surface staining with anti-B220 and anti-CD86 on cells isolated from the peripheral blood of B cell CD80/86+/+, B cell CD80/86−/−, B cell−/−, and CD80/86−/− chimeras before immunization. Values are the mean ± SD. *, p < 0.01 for B cell CD80/86+/+ (n = 9) vs B cell CD80/86−/− (n = 11), B cell−/− (n = 7), and CD80/86−/− (n = 10). E, Representative flow cytometric analysis of CD86 expression on B cells and non-B cells in peripheral blood of chimeric mice. The percentages of B cells are indicated in the lower right quadrants, the percentages of B cells expressing CD86 are in the upper right quadrants, and the percentages of non-B cells expressing CD86 are in the upper left quadrants.
B cell expression of CD80/86 is not required for Ab production

In PGIA, Abs specific for PG are an essential component in the pathogenesis of disease. Hence, it is possible that the expression of CD80/86 on B cells is required for the development of PG-specific Abs. Nonchimeric WT and chimeras immunized with PG were assessed for PG-specific Abs (Fig. 5). Chimeric B cell:CD80/86+/+ and B cell:CD80/86−/− mice produced PG-specific IgG1 Abs equivalent to those of PG-immunized nonchimeric WT mice, whereas PG-specific Abs in B cell:CD80/86−/− (n = 7), B cell−/− (n = 7), and CD80/86−/− (n = 6). In D, values are the mean ± SEM. * p < 0.01 WT mice (n = 5) vs B cell:CD80/86−/− (n = 9), B cell−/− (n = 11), B cell−/− (n = 7), and CD80/86−/− (n = 10).

B cell:CD80/86+/+
B cell:CD80/86−−

FIGURE 3. Suppression of PGIA in B cell:CD80/86−/− chimeras with a specific deletion of CD80/86 expression on B cells and not on other APCs. WT, B cell:CD80/86+/+, B cell:CD80/86−/− B cell−/−, and CD80/86+/+ mice were immunized with PG as described in Fig. 1. A and C, Incidence represents the percentage of mice that developed PGIA. B and D, Severity (cumulative arthritis score) is the sum of paw inflammation scores in individual mice divided by the total number of arthritic mice. In B, values are the mean ± SEM. * p < 0.01 WT mice (n = 5) and B cell:CD80/86+/+ (n = 7) vs B cell:CD80/86−/− (n = 5), B cell−/− (n = 7), and CD80/86−/− (n = 6). In D, values are the mean ± SEM. * p < 0.01. B cell:CD80/86+/+ (n = 5), vs B cell:CD80/86−/− (n = 11), B cell−/− (n = 7), and CD80/86−/− (n = 10).

FIGURE 4. Suppression of histopathology in the joint of B cell:CD80/86−/− chimeras. Hind limbs of mice were dissected, fixed in formalin, decalcified, and embedded in paraffin. The tissue sections were stained with H&E. Sections are from representative ankle joints from B cell:CD80/86+/+ (A), B cell:CD80/86−/− (B), B cell−/− (C), and CD80/86−/− (D) at ×10 magnification. Arrow indicates an area of cartilage destruction.

FIGURE 5. Similarity of PG-specific Ab response in WT, B cell:CD80/86+/+, and B cell:CD80/86−/− chimeric mice. The concentration of serum PG-specific human IgG1 (hIgG1) and mouse IgG1 (mIgG1) were measured by ELISA. Values are the mean ± SD. * p < 0.01 WT mice (n = 5) and B cell:CD80/86+/+ (n = 7) vs B cell:CD80/86−/− (n = 5), B cell−/− (n = 7), and CD80/86−/− (n = 6). Data are representative of two separate experiments.
costimulatory molecules must be expressed in vivo by non-B cell
APC populations to generate a humoral immune response.

B cell expression of CD80/86 critically regulates autoreactive T
cell activation

To determine whether expression of CD80/86 by B cells is nec-

cessary for autoreactive T cell activation, we tested whether primed
T cells in the chimeras were able to transfer disease. To do this, we
transferred purified T cells from PG-immunized B cell:CD80/86+/+, B cell:CD80/86−/−, CD80/86−/−, or B cell−/− chimeras and B cells and APCs (T cell-depleted spleen) from WT arthritic
mice into SCID recipients. SCID mice that received T cells from
B cell:CD80/86+/+ chimeras were significantly more effective
at transferring arthritis than T cells from B cell:CD80/86−/−,
CD80/86−/−, or B cell−/− chimeras. Arthritis was delayed by sev-
eral weeks in SCID mice that received T cells from B cell:CD80/86−/−, CD80/86−/−, or B cell−/− chimeras. Although 100% of
SCID mice that received T cells from B cell:CD80/86−/− event-
ually developed arthritis, the degree of inflammation was signif-
icantly reduced in comparison to SCID mice receiving T cells from
B cell:CD80/86+/+ chimeras. These data demonstrate that B cell
expression of CD80/86 is necessary to adequately activate T cells
to transfer arthritis.

To further evaluate T cell activation, T cells were recovered
from spleens of SCID recipient mice and reactivated in vitro with
PG (Fig. 6C). T cells purified from SCID recipients were stimu-
lated with PG in the presence of irradiated naive WT spleen cells.
Proliferation was substantially higher from B cell:CD80/86+/+
chimera T cells than from either B cell:CD80/86−/−, CD80/86−/−,
or B cell−/− chimera T cells. To determine whether CD80/86 de-

ciciency on B cells skewed T cell cytokine subsets, we evaluated T

cell cytokine production. IFN-γ, IL-4, and IL-17 were not signif-
icantly different between the experimental groups. Both IL-4 and
IL-17 production were low whereas IFN-γ production was ele-

tivated but not decreased in comparison (Fig. 6D). These data pro-

devidence that B cell expression of CD80/86 is important for
the in vivo activation of PG-specific T cells but did not alter T cell
cytokine production.

CD80/86 expression on B cells is necessary for optimal
secondary PG-specific T cell responses

It is generally accepted that naive T cells require recognition of
costimulatory molecules on APCs for activation, whereas memory
T cells do not require a costimulatory signal for secondary recall
responses (34). To assess whether PG-specific CD4+ T cells from
arthritic WT mice require recognition of costimulatory molecules
on B cells, we activated CD4+ T cells in the presence of B cells
from WT or CD80/86−/− mice (Fig. 7). B cells were either naive
or stimulated with LPS overnight to up-regulate costimulatory
molecule expression. The proliferation of PG-specific CD4+ T
cells was 3–6 times higher in the presence of WT B cells activated
with LPS in comparison to unstimulated WT B cells (Fig. 7). How-
ever, B cells from CD80/86−/− mice, regardless of whether or not
they were stimulated with LPS, were significantly less effective at
stimulating PG-specific T cell proliferation. These data demon-
strate that PG-specific T cell recall responses require B cell ex-
pression of CD80/86 and suggest that in PGIA, chronic restimu-
lation of the T cells may also require B cells to express CD80/86.
Discussion

The present study was designed to define the role of CD80/86 expression on B cells in autoimmune arthritis and to determine whether the expression of CD80/86 on B cells is necessary for the development of arthritis and autoreactive T cell activation. Previous studies indicate that CD80/86-CD28 costimulatory signals are required for the induction of arthritis and for the development of specific T cell activation and B cell Ab responses (31–33, 35). We confirm in the PGIA model that in the complete absence of CD80/86 expression, induction of arthritis is inhibited and PG-specific T cells and Ab responses are suppressed. Because CD28-expressing T cells interact with CD80/86 that is expressed on several different APCs, it has been difficult to determine in vivo whether selective expression of CD80/86 on B cells is essential for autoreactive T cell activation. To address this question, we created mice with CD80/86 specifically deleted on the B cells but expressed on other APC populations, permitting the T and B cells to interact under physiological in vivo conditions. In mixed BM chimeras, CD80/86-deficient B cells coexist with other APCs that express CD80/86 (B cell:CD80/86−−/− chimeras). Our results clearly demonstrate that B cell:CD80/86−−/− chimeras are unable to develop arthritis similar to that of B cell−−/− and CD80/86−−/− chimeras. In contrast, B cell:CD80/86+/− chimeras develop robust disease similar to that of nonchimeric WT mice. Our findings indicate that the expression of CD80/86 on B cells is essential for the development of arthritis. Because PGIA does not develop in the absence of PG-specific Abs, we assessed B cell autoantibody production. There was a similar concentration of serum IgG1 Abs specific for human and mouse PG in B cell:CD80/86−−/− chimeras, B cell:CD80/86+/− chimeras, and nonchimeric WT mice. These results indicate that B cell expression of CD80/86 is not necessary for B cell Ab production, confirming a similar report under physiological conditions (27).

We next addressed whether autoreactive T cell activation is defective in B cell:CD80/86−−/− chimeras. To address this question, we transferred T cells from the chimeras along with T cell-depleted spleens from arthritis WT mice as a source of B cells and APCs into SCID recipients. Our findings clearly show that only if the B cells express CD80/86 are the T cells able to effectively transfer arthritis. Furthermore, the CD4+ T cell secondary recall response to PG is reduced in T cells recovered from the SCID recipients that were primed in the absence of CD80/86-expressing B cells. These results suggest that there is a reduction in the PG-specific effector or memory T cell pool activated in the absence of CD80/86-expressing B cells.

Our findings indicate that other APC populations, macrophages, and DCs expressing CD80/CD86 in the B cell:CD80/86−−/− chimeras activate T cells, because T-dependent PG-specific autoimmune responses are intact. Paradoxically, these T cells are not sufficient to induce arthritis. The reduction in PG-specific T cell proliferation suggests that fewer PG-specific T cells have been activated. It is possible that a substantially more robust PG-specific T cell response is required to induce arthritis than to activate B cell Ig production.

B cell activation of T cells is generally considered to be restricted to memory T cells. This is based on the dependence of B cells on help from activated CD4 T cells to become competent APCs. B cell maturation into competent APCs requires cross-linking the BCR and signals provided by CD154 on activated T cells and the CD40 receptor on B cells. Signals derived from CD154/CD40 interaction up-regulate the expression of ICAM-1, MHC class II and CD80/86 (36–39). It has been suggested that memory T cells do not require recognition of CD80/86 for recall responses, indicating that B cells might function as APCs independently of CD80/86 expression (40, 41). We demonstrate that the PG-specific T cell recall response in vitro required resting or activated B cells to express CD80/86. Thus, the block in development of arthritis in the B cell CD80/86−−/− chimeric mice is likely due to a reduction in the expansion of PG-specific T cells. This decreased activation and/or expansion of PG-specific T cells was confirmed in T cells recovered from SCID recipients where CD4+ T cell proliferation is reduced in T cells initially activated in the presence of CD80/86-deficient B cells. Our findings are in agreement with others demonstrating that the blockade of CD28/CD80 interaction with CTLA4Ig inhibits secondary responses mediated by memory CD4+ T cells (42). These studies are also in accord with the clinical efficacy in RA patients treated with CTLA4Ig where disease is driven by memory T cells (43).

In RA patients, cells infiltrating the synovium are either diffusely scattered among tissue-resident cells or organized into microstructures (44). In a subset of RA patients, aggregates of T and B cells organize into structures resembling germinal centers found in the lymphoid follicles of secondary lymphoid organs. These synovial lesions may be important sites of T cell and B cell interactions at the site of inflammation (45). In experiments involving the adoptive transfer of RA synovium into SCID mice, activation of T cells was dependent on the presence of B cells (46). Our findings suggest the possibility that interference with costimulatory interaction between T cells and B cells in these synovial lesions would block T cell activation. Thus, clinical efficacy of CTLA4Ig in RA patients may be due to the distribution of these organized structures limiting T cell activation.

We previously reported that PG-specific B cells are required for the development of autoreactive T cells in PGIA. The present studies expand these findings to demonstrate that a CD80/86:CD28 costimulatory interaction between B cells and T cells is required for autoreactive T cell activation and the induction of arthritis. It has now been undoubtedly shown that B cell depletion in RA reduces disease activity (1–3). Although autoantibodies are decreased in many individuals, autoantibody titers do not always correlate with efficacy of treatment. Our findings clearly demonstrate a critical role for B cells as APCs. Similarly, B cells are

![FIGURE 7. The secondary recall response in PG-specific T cells required CD80/86 expression. CD4+ T cells from WT arthritic mice were stimulated with PG in the presence of B cells from WT or CD80/86−−/− mice either naive or activated with LPS. T cell activation was measured by the incorporation of [3H]thymidine. Values are the mean ± SD of quadruplicate cultures * p < 0.05 Data are representative of three separate experiments.](http://www.jimmunol.org/Downloadedfrom)
likely active participants in autoreactive T cell activation in RA. Thus, B cell presentation of Ag and expression of costimulatory molecule is essential for autoreactive T cell activation and the development of arthritis.

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Disclosures
The authors have no financial conflict of interest.

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