Successful T Cell Priming in B Cell-deficient Mice

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Summary

B cells are an abundant population of lymphocytes that can efficiently capture, process, and present antigen for recognition by activated or memory T cells. Controversial experiments and arguments exist, however, as to whether B cells are or should be involved in the priming of virgin T cells in vivo. Using B cell-deficient mice, we have studied the role of B cells as antigen-presenting cells in a wide variety of tests, including assays of T cell proliferation and cytokine production in responses to protein antigens, T cell killing to minor and major histocompatibility antigens, skin graft rejection, and the in vitro and in vivo responses to shistosome eggs. We found that B cells are not critical for either CD4 or CD8 T cell priming in any of these systems. This finding lends support to the notion that the priming of T cells is reserved for specialized cells such as dendritic cells and that antigen presentation by B cells serves distinct immunological functions.

Over the years and in spite of a myriad of studies, the issue of whether B cells play a role in the initiation and/or maintenance of T cell immune responses has remained unresolved. There are both theoretical and experimental reasons for the persistence of this controversy. Theoretically, the concern revolves around the definition of a “professional” APC, a term first coined to distinguish between those APC that can initiate the activation of a virgin T cell and those that are only able to restimulate previously primed cells (1). There are two reasons, both having to do with T cell tolerance, why B cells, despite their abundance and ability to handle antigen, should not act as professional APC. The first is that they present their own idiotypes (2). As N. K. Jerne pointed out, T cells can neither be tolerant of each idiotype, because there would be no cells left to see other antigens (3), nor can they be responsive to every idiotype, lest such introspection paralyze the immune system. Second, because B cells tend to hypermutate during immune responses, their superbly efficient ability to concentrate and present antigens is a recipe for disaster. Though T cells are generally tolerant of self antigens, that tolerance has an affinity cutoff set by the normal concentration with which the self proteins are presented by normal APC. However, newly mutated autoreactive B cells can concentrate self proteins to far greater densities than those to which the T cells are tolerant. Both types of theoretical problems are dealt with if T cells cannot be activated by B cells until they have first interacted with a professional APC.

When B cells were tested for their ability to activate T cells, the results were inconsistent. In some studies, B cells appeared to be crucial for T cell priming in vivo (4–10) or were able to stimulate T cells in vitro (11–23), whereas in others they were not able to activate T cells (1, 24–30) or were tolerogenic (26, 29–32). Since each of these experimental systems either involved manipulations of the responding T cells or of the presenting cells or required specially targeted antigens, we reexamined the question using the µMT mice, which are genetically B cell deficient. To determine the impact of the B cell deficiency on in vivo T cell priming, we assessed T cell responses in the µMT mice with the soluble protein KLH, the eggs from *Schistosoma mansoni, major histocompatibility antigens, and the minor H transplantation antigens, H-Y and BALB.B minors. Whether tested in vitro or in vivo, by proliferation, cytokine production, CTL activity, graft rejection, granuloma formation, or eosinophilia, we found that the absence of B cells had little impact on T cell responsiveness.

Materials and Methods

Mice. C57BI/6 mice (B6) (6–8 wk old) were purchased from Charles River Laboratories (Wilmington, MA). µMT mice (33) were bred at the National Institute of Allergy and Infectious Diseases, which is an accredited American Association for Accreditation laboratory animal care facility. When we received the mice, they had been partially backcrossed from the original 129 × B6 strain to B6 and expressed a mixture of genes from the two strains.
To allow for experiments calling for cell mixes, we backcrossed the mice to the B6 strain for seven generations. We then intercrossed them to generate homozygous B cell–deficient animals.

**Antibodies.** mAbs were either purified from ascites or were purchased. The following mAbs were used in this study: anti-CD4 (GK1.5 [34] and YTA3.1.2 [35]), anti-CD8 (53-6.72; Pharmingen, San Diego, CA) (36), anti-Thy.1.2 (J11.10; American Type Culture Collection [ATCC], Rockville, MD) (37), anti-IE (14.4.4; ATCC) (38), antimacrophage (anti-Mac-1, M1/70; Boehringer Mannheim Corp., Indianapolis, IN) (39), anti-dendritic cell (3D1, ATCC) (40), anti-IA (M5/114.15.2 [41] and AF6–120.1 [42]; Pharmingen), anti-B cell (anti-B220, anti-CD45R; Pharmingen) (43), and anti-IL-4 (11B11; ATCC) (44). For FACS® analysis, the following mAbs were used: anti-CD4–PE (RM4-4; Pharmingen), anti-CD8α–Red613 (53-6.7; Life Technologies, Inc., Grand Island, NY), anti-CD45R/B220–PE (RA3-6B2; Pharmingen), and anti-FcyR (2.4G2; ATCC) (45).

**Cytokine Assays.** At 72 h, supematants were removed from Peyer’s patches and [3H]thymidine was added 18 h before harvesting. Proliferative responses to schistosome egg antigens were tested 14 d after intraperitoneal injection of 5,000 S. mansoni eggs intraperitoneally, 20 d later, recipient spleen cells from each mouse were stimulated in vitro with B6 male or CBA/J female spleen cells and tested 5 d later for killing activity by the JAM Test (49). Briefly, 4 × 10⁶ responder spleen cells were cultured for 5 d against 2 × 10⁵ irradiated (1,500 rad) stimulator spleen cells in 2 ml of IMDM, containing additives as above for KLH proliferation. They were then harvested and tested for cytotoxic activity against [3H]thymidine-labeled B6 male, B6 female, and CBA/J female Con A–activated blasts. The response to CBA/J cells, bearing the H-2d allantigen and the Mls superantigen, was used as a general control of responsiveness. To study priming to H–Y in the absence of CD4 cells, B6 and μMT females were either untreated or were injected with 0.5 mg of GK1.5 and 0.1 mg of YTA3.1.2 intraperitoneally, two synergistic anti-CD4 mAbs (50), daily for 5 d, and were then injected intraperitoneally with 2 × 10⁶ μMT male spleen cells. 2 wk later, responder spleen cells in separate cultures were stimulated in vitro with B6 male and CBA/J female spleen cells, and they were tested 5 d later for killing activity. Optimal concentrations of conditioned supernatant (Rat T–Stim without Con A; Collaborative Biomedical Products) were added to all of the anti–H–Y cultures so that the conditions in wells with and without CD4 T cells were both supportive of CTL development. Staining and FACS® analysis of spleen cells on the day of culture revealed that there were <0.6% CD4 T cells remaining in the spleens of the anti–CD4 mAb–treated mice. The staining was performed using the anti-CD4 mAb RM4-4, which is not blocked by either GK1.5 or YTA3.1.2 at the concentrations used.

**Tail-to-tail Skin Grafts.** μMT and B6 mice were grafted with autologous and BALB.B tail skin by Bailey’s method (51) with the modification that each graft was dabbed with a drop of super glue before casting. The grafts were examined daily and considered rejected when they fell off.

**In Vivo Granuloma Formation to Schistosome Eggs.** B6 and μMT mice were primed with 5,000 S. mansoni eggs intraperitoneally, 20 d later, recipient spleen cells from each mouse were stimulated in vitro with B6 male or CBA/J female spleen cells and tested 5 d later for killing activity by the JAM Test (49). Briefly, 4 × 10⁶ responder spleen cells were cultured for 5 d against 2 × 10⁵ irradiated (1,500 rad) stimulator spleen cells in 2 ml of IMDM, containing additives as above for KLH proliferation. They were then harvested and tested for cytotoxic activity against [3H]thymidine-labeled B6 male, B6 female, and CBA/J female Con A–activated blasts. The response to CBA/J cells, bearing the H-2d allantigen and the Mls superantigen, was used as a general control of responsiveness. To study priming to H–Y in the absence of CD4 cells, B6 and μMT females were either untreated or were injected with 0.5 mg of GK1.5 and 0.1 mg of YTA3.1.2 intraperitoneally, two synergistic anti-CD4 mAbs (50), daily for 5 d, and were then injected intraperitoneally with 2 × 10⁶ μMT male spleen cells. 2 wk later, responder spleen cells in separate cultures were stimulated in vitro with B6 male and CBA/J female spleen cells, and they were tested 5 d later for killing activity. Optimal concentrations of conditioned supernatant (Rat T–Stim without Con A; Collaborative Biomedical Products) were added to all of the anti–H–Y cultures so that the conditions in wells with and without CD4 T cells were both supportive of CTL development. Staining and FACS® analysis of spleen cells on the day of culture revealed that there were <0.6% CD4 T cells remaining in the spleens of the anti–CD4 mAb–treated mice. The staining was performed using the anti-CD4 mAb RM4-4, which is not blocked by either GK1.5 or YTA3.1.2 at the concentrations used.

**Results and Discussion.** The μMT mice were created by a gene-targeted knock-out of the transmembrane portion of the IgM molecule, leading to a failure of B cell precursors to progress beyond the early pre–B stage (33). Analysis of the surface phenotype of splenocytes (Fig. 1) showed that homozygous μMT mice do not have mature B220+ cells, although they have a few B220lo immature pre–B cells. Our μMT mice have small spleens (~30 × 10⁶ cells) compared with B6 controls.
Figure 1. Two-color cytofluorimetric analysis of splenic CD4, CD8, and B220 expression in B6 and μMT mice. The upper panels represent CD4 versus CD8 cytograms, and the lower panels are histograms of B220 staining. More than 30 mice were analyzed per group, and two representative profiles are shown from each group.

(∼100 × 10⁶), but the total number of CD4 and CD8 T cells per spleen is normal (therefore, the proportions of T cells are increased). The original description of the μMT mice showed that the frequency of T cells was normal, but, because the total numbers of spleen cells were not reported, it is unclear whether the numbers of T cells were also normal. The differences in staining patterns in these two reports might easily be due to such differences as mouse strain

Figure 2. Responses of CD4 T cells in μMT and B6 mice to KLH, S. mansoni egg antigens, and allogeneic cells. (A) CD4 T cells from B6 and μMT mice were tested for proliferation to graded doses of KLH. PPD responses were the same for both groups. B was performed as in A, with the addition of anti-B220 mAb during the purification. These are representative data of seven experiments with a total of 71 mice. (C) Graded numbers of μMT and B6 CD4 splenic T cells were incubated with irradiated BALB/c splenocytes for 72 h and tested for proliferation. Neither responded to syngeneic stimulators. (D) Proliferation was tested to graded doses of schistosome egg antigens. (E) At 72 h, supernatants were removed from wells with 50 µg/ml KLH or with medium alone as in B and tested for IL-2, IL-4, and IFN-γ. No cytokines were detected in the absence of KLH. (F) Supernatants from mediastinal lymph node cultures as in D with 20 µg/ml soluble egg antigens were harvested at 24 h to measure IL-2, and at 72 h to analyze IL-4 and IFN-γ. In A–D, each demarcation of the x-axis represents a point in the titration of antigen or cells.
or mouse colony. In any case, there does not seem to be an intrinsic deficiency in T cell numbers in our μMT mice.

To evaluate T cell responses to a common protein antigen, we primed μMT and control B6 mice with KLH and measured the proliferative response of CD4 T cells isolated from the draining lymph nodes 10 d after immunization. Fig. 2A shows that, in our first experiment, T cells from the μMT mice appeared to proliferate less well than the control B6 T cells. However, a closer look showed that the background responses without antigen also differed between the two T cell populations. This difference in background response led us to consider the possibility that the elevated proliferation in the B6 T cell preparation might be due to contaminating B cells that were absent from the μMT cell population. The elevated backgrounds and overall heightened response could have been due to the following: (a) antigen carried over by B cells; (b) more efficient presentation of the titrated antigen by the carried-over antigen-specific B cells (17, 53, 54); and/or (c) proliferation in the cultures of both T and B cells (53, 55). To compare the responses of normal and μMT T cells without the obscuring effects of contaminating B cells, we retested T cells from the two types of mice after rigorously removing any potentially remaining B cells. These cleaner T cell populations generated equivalent responses, both to the KLH protein to which they had been primed (Fig. 2B) and to allo-geneic spleen cells in primary mixed lymphocyte reactions (Fig. 2C). The levels of cytokines produced after immunization with KLH (Fig. 2D) were also equivalent for both T cell populations. Thus, when we tested purified T cells, we found that the in vivo priming events necessary for specific in vitro responses did not seem to be influenced by the presence or absence of B cells.

Although B cells did not appear to be necessary for CD4 T cell activation, we considered the possibility that they might influence the nature of the response. Therefore, we also analyzed a system in which Th2 responses dominate, namely, the reaction to eggs of *S. mansoni*. This system is characterized by a transient Th0 response that matures to Th2 (56–58). Fig. 2D shows the in vitro proliferation of μMT and B6 lymph node cells to egg antigens 2 wk after primary immunization with eggs of *S. mansoni*. Once again, CD4 T cells from μMT mice were able to be primed in vivo. In Fig. 2F, we see that the lack of B cells also had little influence on the pattern of cytokine production by the two types of mice. The overall synthesis of cytokines was higher in the μMT mice, an increase that correlated with the increase in proliferation seen in Fig. 2D. Thus, in the response both to KLH and to *S. mansoni* eggs, the pattern of cytokines depended on the antigen and not on the presence or absence of B cells during priming.

To test the role of B cells in the induction of cytotoxic T cell responses, we evaluated the CD4 helper-dependent CTL response to the male antigen H-Y (59–61). We chose this system because we reasoned that it might be more easily influenced by the presence of antigen-presenting B cells as a helper-independent CTL response. To assess the role of host (female) B cells as well as the donor (male) B cells, we immunized B6 and μMT females with either B6 or μMT male spleen cells. Fig. 3A shows that the CTL responses of the two types of females were indistinguishable, and that both B cell-deficient and normal male spleen cells

![Figure 3. Cytotoxic T cell responses to the male antigen H-Y in μMT and B6 mice in the presence or absence of CD4 cells.](https://jem.rupress.org/content/190/12/8180/F1.large.jpg)
were able to act as antigen-presenting inocula. All responses were also equivalent for the two groups of mice. To check that the anti-H-Y CTL of μMT mice were as dependent on CD4 help as were normal CTL, we depleted the CD4 cells from a set of μMT and B6 females and then immunized them with male μMT splenocytes. Fig. 3 B shows that CD4 depletion abolished the anti-H-Y CTL responses from both the B6 and the μMT mice, showing that CD4 helpers were required in both cases. There were no differences between groups when we titrated the male cell inocula (data not shown). Together, these data suggest that antigen-presenting B cells are not required to prime either CTL or their helpers.

Having determined that B cells do not have much influence on priming for in vitro reactivity, we asked if their absence had more of a consequence when the effector stage was assayed in vivo. We looked at two types of responses, graft rejection and granuloma formation elicited by schistosome eggs.

The role of B cells in graft rejection is not clear, despite several studies showing the effect of antibody and of reprocessing of donor antigens by host APC (62, 63). In general, B cells are more likely to function as APC in graft rejection than as effectors. To compare B6 and μMT mice for their ability to reject grafts, we transplanted the two types of mice with tail skin from BALB.B mice. We chose to use a skin graft that differed by minor antigens rather than an MHC different allograft for three reasons. First, rejection of minor histocompatibility grafts has been shown to depend on both CTL and Th1 type T helpers (35, 64). Second, since there is no known antibody production to the minor antigens, we could evaluate the in vivo effect of the B cells themselves rather than that of their secreted products. Third, it should be possible to assess subtle differences in responsiveness that might have been missed with antigenically more disparate grafts (65–67). We found that there was no delay in the kinetics with which μMT mice rejected the BALB.B grafts (Fig. 4 A). In fact, the average rejection time was slightly (2 d) faster. Thus, it appears that skin graft rejection occurs equally well, if not better, in an immune system lacking B cells.

To study an in vivo response that depends on Th2 types of cytokines, we looked at granuloma formation in mice primed and later challenged with *S. mansoni* eggs. Fig. 4 B shows that there was no difference between B6 and μMT mice in granuloma size 6 d after secondary challenge with eggs. In Fig. 4 C, both types of mice also produced the typical, IL-5–dependent, tissue eosinophilia characteristic of the Th2 response elicited by schistosome eggs (68).

Overall, when taken together with several earlier studies (1, 24–31, 64), our data confirm the view that B cells are not necessary for a wide variety of in vivo and in vitro T cell responses (69, 70). What then of the studies showing impaired T cell function in mice deprived of B cells by neonatal treatment with anti-IgM antibodies (4–10), and those showing that B cells may have deleterious effects on other APC, either because of cross-reactive antibodies, natural antibodies, or both. Regarding the more recent studies with μMT mice showing a defect in transferable T cell help for antibody production (Roes, J., and K. Rajewsky, personal communication), there exists the possibility that T cells that have matured in the absence of B cells may have defects in tolerance that preclude effective collaboration.

Because B cells have been shown to activate T cell clones in vitro (11–23) and expand memory T cells in vivo (29), we were surprised that their absence had so little effect in the μMT mice. Although we assumed that the lack of B cells would have no impact on the initial priming of virgin T cells (1, 70), we expected that the T cell populations in the μMT mice might not expand as much, or might perhaps make different classes of responses from

![Figure 4](https://jembiosci.org/dxl/fr.png)

**Figure 4.** In vivo effector responses to BALB.B skin graft and schistosome egg challenge. (A) Seven μMT and five B6 mice were grafted with allogeneic and BALB.B tail skin. Kinetics of BALB.B rejection is shown. A single autologous graft fell off early, whereas all other autologous grafts were accepted. (B) B6 and μMT mice were primed with 5,000 *S. mansoni* eggs intraperitoneally, challenged with 5,000 eggs intravenously 3 wk later, and killed after 6 d. Granuloma size was assessed on histological sections from the challenged lungs. The calculated means and the SEM for B6 and μMT were 8.89 ± 0.61 and 7.5 ± 0.73 (mm³ × 10⁻³), respectively. (C) Eosinophils in the granulomas were counted. Mean percentage of eosinophils in the lesions were 67.5 ± 2.16 (SEM) for B6 and 63.75 ± 4.1 (SEM) for μMT mice.
those made by normal T cells, but neither of these responses were affected. We can envisage several possible explanations. The first is that the in vivo microenvironment may ensure that T–B interactions are essentially unidirectional (activated/effector T helping B) and that, therefore, antigen presentation by B cells normally exists only to attract previously activated T cells. The second explanation arises from the view that B cell presentation of antigen to T cells can lead to two different outcomes, depending on the state of the T cell. If B cells tolerize virgin T cells and induce the expansion of previously primed T cells (1, 27–31), the net result may not be measurable. Finally, if B cells do present antigen to activate memory T cells, this may normally occur in germinal centers over greater lengths of time than those we have measured. Late in the response, when the only remaining antigen is attached to and released by follicular dendritic cells (71), the antigen-specific B cell may be the APC best able to capture enough antigen to maintain the response (16, 17, 72). In this case, a deficiency of B cells would have a serious impact on T cell memory (73).

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