Antigen-Primed T Cells from B Cell-Deficient JHD Mice Fail to Provide B Cell Help

Anne E. Macaulay, Rosemarie H. DeKruyff and Dale T. Umetsu

*J Immunol* 1998; 160:1694-1700; ;
http://www.jimmunol.org/content/160/4/1694

**References**
This article cites 32 articles, 18 of which you can access for free at:
http://www.jimmunol.org/content/160/4/1694.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Antigen-Primed T Cells from B Cell-Deficient JHD Mice Fail to Provide B Cell Help

Anne E. Macaulay, Rosemarie H. DeKruyff, and Dale T. Umetsu

B cells are effective in presenting Ag to primed T cells, but a specific role for B cells in priming naive T cells has not been clearly established. In this report we demonstrate that Ag administration to B cell-deficient JHD mice primes T cells for Ag-specific proliferative responses, but such primed T cells fail to provide help for isotype switching and IgG production in B cells. Although T cells primed in the absence of B cells could proliferate in response to Ag presented by B cells and could induce Ag-specific IgM production, such T cells failed to produce high levels of IL-4 as are normally induced in T cells by Ag-presenting B cells. These findings suggest that while B cells are not absolutely required for T cell priming, they provide signals to T cells that are not replicated by other APCs and that influence the subsequent ability of T cells to interact with B cells.


The importance of B cells as APC has been debated for a number of years. Early experiments using mice depleted of B cells by treatment with anti-IgM suggested that T cells could not be primed in the absence of B cells (1–3). More recent studies have used mice genetically depleted of B cells, created by targeted deletion of the μ region (μMT) (4) or JH region (JHD) (5) of the IgM locus. Constant et al. reported that T cells from μMT mice could be primed with peptide Ags, but not with some protein Ags (6), whereas Vella et al. reported that there was a weak proliferative response to peptide Ag in μMT mice (7). However, in both of these studies, control T cell populations were only 85 to 90% pure and may have contained contaminating primed B cells. In the one report investigating priming in JHD mice, Liu et al. found that unfractionated lymph node (LN) cells from these mice could not be primed (8). In contrast to these studies, Epstein et al. found that thoroughly purified CD4+ T cells from μMT mice responded as well as normal controls in a number of assays, including recall responses to Ags and schistosome eggs, rejection of skin grafts, and CTL responses (9).

The experiments described here were undertaken to determine whether CD4+ T cells from JHD mice could be primed normally in vivo and whether such T cells were capable of providing help for B cells. We show that when rigorously purified, primed CD4+ T cells from JHD and normal BALB/c mice are compared, there is no difference in their proliferative responses to Ag. However, we also found that JHD T cells provided little or no help for Ab production. Additionally, we discovered that JHD T cells produce less IL-4 than normal T cells when B cells, but not splenic adherent cells (SpAC), are used as APCs, suggesting that the defect in providing B cell help may be due in part to a failure to up-regulate IL-4 synthesis in response to B cells.

Materials and Methods

Animals

MD4 mice transgenic for hen egg lysozyme (HEL)-specific IgM and IgD expressed in B cells (10) were backcrossed to BALB/cBy (11). After seven generations, mice that screened positive for I-Eα4 were selected and interbred. BALB/cByJ mice were obtained from either The Jackson Laboratory (Bar Harbor, ME) or the Stanford Medical Center Division of Laboratory Animal Medicine (Stanford, CA). B cell-deficient JHD mice (5) were obtained from Dr. D. Huszar (GenPharm International, Mountain View, CA) and were backcrossed to BALB/cBy for seven generations. JHD mice were maintained on 1% Sulfatrim pediatric suspension (Butler Co., Hayward, CA) for 4 days/wk. Homozygous B cell-deficient mice were generated by interbreeding and identified by PCR specific for the JH region (12). Animals were used between 8 and 16 wk of age and were sex and age matched within each experiment. All animal protocols were approved by the Stanford University committee on animal welfare.

Antigens

HEL, OVA, BSA, and conalbumin were obtained from Sigma Chemical Co. (St. Louis, MO). OVA (Sigma lot 54F-8150) was found by ELISA and SDS-PAGE to be free of contaminating HEL. An HEL-OVA conjugate was prepared as previously described (11). KLH was purchased from Calbiochem (La Jolla, CA). TNP-coupled BSA, KLH, and conalbumin were prepared as previously described (13). Briefly, the protein of interest was dissolved inborate-buffered saline and reacted with trinitrobenzenesulfonic acid (ICN, Cleveland, OH) at a ratio of 38 mg trinitrobenzenesulfonic acid/g of protein, followed by extensive dialysis.

Immunizations

To generate OVA-primed CD4+ T cells, mice were immunized with OVA (200 μg/mouse) in CFA (Difco Laboratories, Detroit, MI) in the footpads. Draining popliteal, inguinal, and axillary LNs were removed 8 days after immunization. To generate KLH-primed Th cells, mice were immunized with KLH (150 μg/mouse) in CFA i.p., and spleens were removed 7 to 9 days later. For TNP-primed B cells, mice were immunized with 300 μg of TNP-conalbumin in CFA i.p., and spleens were harvested 3 to 4 wk later. In some experiments, mice were boosted at 4 wk with 300 μg of TNP-conalbumin in PBS and used 2 wk later. B cells from boosted and unboosted mice produced similar levels of Ab.

Medium

Cells were cultured in DMEM (Life Technologies, Grand Island, NY), supplemented as previously described (14) and containing 10% FCS (HyClone Laboratories, Logan, UT).
LN cell purification
In some experiments, LN cells were depleted of most B cells by passage over goat anti-mouse IgG- and IgM-coated (Jackson Immunoresearch, West Grove, PA) plates as previously described (16). This was performed so that the number of T cells in the cultures from normal and JHD LNs would be roughly equivalent. No cells from the JHD mice bound to such plates.

T cell purification
Highly purified CD4+ T cells from LNs or spleens of OVA- or KLH-primed mice were prepared as previously described (16). Briefly, cells were passed over goat anti-mouse IgG- and IgM-coated (Jackson Immunoresearch Laboratories) plates followed by two treatments with Abs against MHC class II CD8, and heat-stable Ag plus baby rabbit C (Pel-Freez, Brown Deer, WI). T cells purified in this way failed to proliferate in response to Ag or Con A (Sigma Chemical Co.) in the absence of added APCs.

Preparation of SpAC
SpAC were prepared by adhering spleen cells from BALB/c by mouse on plastic tissue culture dishes as previously described (11). The resulting cells were irradiated at 2500 rad before addition to cultures with T cells.

Preparation of Tg B cells
Purified B cells from MD4+/+ Ig receptor transgenic mice were prepared as previously described (11). Briefly, spleen cells were allowed to adhere to plastic tissue culture dishes, and nonadherent cells were removed and treated with anti-Thy-1, anti-Mac-1, and mitomycin C (Sigma Chemical Co.). B cells prepared in this way were routinely 80% B220+ as determined by FACS.

Preparation of TNP primed B cells
Spleen cells from TNP-conalbumin primed mice were adhered to goat anti-mouse Ig-coated plates (15). Nonadherent cells were washed off, and the plates were incubated with warm PBS and 2% FCS for 5 min at 37°C. B cells were recovered by washing off with a stream of warm PBS and 2% FCS.

Proliferation assays
CD4+ T cells (2.7 x 10^6 cells/ml) were cultured in 96-well plates in 150 μl of medium in triplicate in the presence of a range of concentrations of either OVA or HEL-OVA. Either SpAC (2.7 x 10^5/ml) or B cells (2.7 x 10^5 or 3.3 x 10^5/ml) were added as APCs. Wells to which no APCs were added were used to control for T cell purity. After 72 h, cells were pulsed with 1 μCi of [3H]thymidine for 18 to 22 h. Cultures were harvested with a PHD harvester (Cambridge Technology, Cambridge, MA), and [3H]thymidine incorporation was measured using standard liquid scintillation counting techniques.

LN cell proliferation
To compare the proliferation of unprimed LN cells from normal and JHD mice, LN cells were passed over goat anti-mouse Ig-coated plates, and the remaining LN cells were plated at 2.7 x 10^5/ml in 150 μl of medium in 96-well plates. Cultures were pulsed with [3H]thymidine after 72 h and harvested 18 to 22 h later.

Induction of lymphokine production by LN cells
After passage over goat anti-mouse Ig-coated plates, LN cells (2.7 x 10^6/ml) were cultured in triplicates of six in 150 μl of medium with a range of concentrations of OVA. After 96 h, supernatants were harvested, pooled, and analyzed for cytokine content.

Induction of lymphokine production in primed CD4+ T cells
CD4+ cells (2.7 x 10^5/ml) were cultured in 24-well plates in 1 ml of medium in the presence of a range of concentrations of either OVA or HEL-OVA. Either SpAC (2.7 x 10^5/ml) or B cells (2.7 x 10^5/ml) were added as APCs. In some experiments, supernatants were harvested after 96 h. In other experiments, the cells were removed from the plates after 96 h, washed once with PBS and 2% FCS, counted, resuspended to 1.35 x 10^5/ml, and restimulated with fresh Ag and APCs at concentrations identical with those used in the primary stimulation. This restimulation was performed to optimize the production of IL-4 (17). Supernatants were harvested 24 h after restimulation and analyzed for cytokine content. Parallel cultures containing T cells with Ag but no APCs produced negligible IL-4 or IFN-γ.

Help assays
TNP-primed B cells (3.3 x 10^5/ml) and KLH-primed T cells (1 x 10^5/ml) were cultured in 1 ml of medium in 24-well plates with a range of concentrations of TNP-KLH. Supernatants were harvested after 8 days and assayed for TNF-specific IgG. Wells containing T cells with no added B cells or B cells without T cells produced no detectable Ab.

Cytokine ELISAs
IL-4 and IFN-γ were assayed by ELISA as previously described (11). The limits of detection were 7.8 pg/ml for IL-4 and 300 pg/ml for IFN-γ. IL-5 was assayed using the same protocol, except that plates were coated with 1 μg/ml anti-IL-5 mAb TRFK-5 (Dr. T. Mosmann, University of Alberta, Edmonton, Canada) and biotinylated anti-IL-5 mAb TRFK-4 (Dr. T. Mosmann, University of Alberta) was used as the second Ab. Supernatant from TH2 clone D10 that had been standardized against recombinant murine IL-5 (Genetics Institute, Cambridge, MA) was used as a standard, and the limit of detection was 780 pg/ml.

TNF-IgG ELISA
Ninety-six-well plates were coated with 5 μg/ml of TNF-BSA in bicarbonate buffer at 4°C overnight. After blocking for 1 h with PBS and 0.5% BSA, samples and standards were added and incubated overnight at 4°C. Plates were then incubated for 2 h at room temperature with horseradish peroxidase-labeled anti-murine IgG (Southern Biotechnology Associates, Birmingham, AL). Finally, plates were developed with o-phenyl-diamine (Sigma Chemical Co.) for 5 to 20 min, stopped with 2 N H₂SO₄, and read at 492 nm. Affinity-purified TNF-specific ascites from a TNF-BSA-hyperimmunized mouse was quantified for IgG by ELISA and used as a standard (18). The limit of detection was 1.95 ng/ml.

Results
LN cells from JHD mice proliferate weakly and produce lower levels of cytokines than normal BALB/c LN cells
Whether T cells from B cell-deficient mice can be primed with Ag is controversial. To address this question, we primed JHD or normal BALB/c mice with OVA in CFA. Eight days later, the cells from the draining LNs were examined. In normal mice, B cells constitute 50 to 60% of mononuclear cells in the spleen. Therefore, it was necessary to normalize the number of T cells obtained from JHD and BALB/c primed LNs. This was accomplished by passing the LN cells over anti-Ig-coated plates, which depleted the majority of B cells. Figure 1A shows that primed JHD LN cells proliferated poorly in response to Ag compared with primed normal BALB/c LN cells. In addition, primed JHD LN cells secreted much less IL-4 and IFN-γ compared with primed normal LN cells (Fig. 1, B and C).

Rigorously purified CD4+ T cells from JHD and normal BALB/c mice respond equivalently to Ag
The reduced proliferative response of LN cells from JHD mice was not due to a lack of APC activity in these cultures, since removal of the B cells leaves sufficient numbers of non-B cell APCs. Rather, we hypothesized that the presence of primed B cells in the BALB/c LN cultures greatly enhanced the responses of the BALB/c T cells and falsely exaggerated the difference between BALB/c and JHD responses. Therefore, highly purified CD4+ T cells were prepared by passage over goat anti-mouse Ig-coated plates followed by two treatments with Abs against MHC class II, CD8, and heat-stable Ag plus complement. This treatment resulted in T cells that were completely unresponsive to Ag or to Con A in the absence of added APCs. Figure 2A shows that the proliferative response of primed CD4+ T cells from JHD mice was equal to that of BALB/c T cells when stimulated with Ag presented by SpAC, which consist of macrophages and dendritic cells. Cytokine responses were also examined in cultures with CD4+ T cells plus...
We found that purified JHD and normal BALB/c T cells secreted similar levels of IL-4 and IFN-γ (Fig. 2, C and D). Figure 2B shows the response of LN cells from the same mice before purification, demonstrating that, as in Figure 1, proliferation was greater in the LN cultures from normal mice, which contained primed B cells. Primed B cells could enhance the total proliferative response by proliferating themselves, by stimulating increased T cell proliferation, or by carrying over Ag from in vivo, as suggested by the higher proliferation in normal compared with JHD LN cultures when no Ag was added (Fig. 2B).

**FIGURE 1.** JHD LN cells proliferate less and produce less IL-4 and IFN-γ than LN cells from normal BALB/c mice. LNs from two individual OVA-primed BALB/c or JHD mice were depleted of most B cells by passage over anti-Ig-coated plates. The remaining cells were cultured at $2.7 \times 10^5$/ml in 150 µl of medium in 96-well plates with the indicated concentrations of OVA. A. Cells were pulsed with [³H]thymidine after 72 h and harvested 18 h later. B and C. Supernatants were harvested at 96 h and assayed for IL-4 (B) and IFN-γ (C) by ELISA. Each point represents the mean of triplicate wells ± SD. This experiment is representative of four similar experiments.

**FIGURE 2.** Purified JHD LN T cells proliferate and produce cytokines equivalently to T cells from normal BALB/c mice. A, CD4⁺ T cells ($2.7 \times 10^5$/ml) were cultured in 150 µl of medium in 96-well plates with the indicated concentrations of OVA. Irradiated SpAC ($2.7 \times 10^5$/ml) were added as APCs. Cells were pulsed with [³H]thymidine and harvested 18 h later. Background proliferation from wells containing no Ag, which is always found to be high when irradiated SpAC are used, was subtracted. B, BALB/c or JHD LN cells from the same mice as those in A were passed over anti-Ig-coated plates, cultured as described in Figure 1 for 72 h, pulsed for 18 h with [³H]thymidine, and then harvested. C and D, T cells ($2.7 \times 10^5$) and $2.7 \times 10^5$ irradiated SpAC were cultured in 24-well plates in 1 ml of medium with the indicated concentrations of HEL-OVA. After 96 h, T cells ($1.35 \times 10^6$) were washed and restimulated with the same APCs and Ag as those in the initial culture. Supernatants were harvested 24 h later and analyzed for IL-4 (C) and IFN-γ (D) by ELISA. Each point represents the mean of triplicate wells ± SD. These experiments are representative of two similar proliferation experiments and four similar cytokine experiments.
JHD T cells provide little or no help for Ab production

To determine whether Ag-primed T cells from JHD mice were effective in inducing Ig synthesis, we set up an in vitro help assay. JHD or normal BALB/c mice were injected i.p. with KLH in CFA, and 7 to 9 days later the primed, CD4\(^+\) T cells were purified as described above. TNP-conalbumin primed mice were used as a source of B cells. The T and B cell populations were cultured together for 8 days in vitro with varying concentrations of TNP-KLH. As shown in Figure 3 and Table I, normal KLH-primed BALB/c mice were used as a source of B cells. The T and B cell populations were cultured together for 8 days in vitro with varying concentrations of TNP-KLH. As shown in Figure 3 and Table I, normal KLH-primed BALB/c T cells consistently provided help for TNP-specific Ab production, while primed JHD T cells induced little or no detectable TNP-specific IgG secretion. Because we used purified CD4\(^+\) T cells, and because T cells and B cells were primed with different Ags, it is unlikely that the TNP-specific IgG was produced by contaminating T cells in the B cell population induced Ig synthesis. Despite the inability of primed JHD T cells to provide help, the cultures with JHD T cells proliferated normally and induced amounts of TNP-specific IgM equivalent to control cultures with primed BALB/c T cells (data not shown). The failure of Ag-primed JHD T cells to help was not due to suppression, since mixtures of BALB/c and JHD T cells were very effective at inducing TNP-specific Ab production (Fig. 4). Optimal Ig production was observed when low numbers of BALB/c T cells were used (1.5 \( \times 10^6 \)), and Ig production declined with higher numbers of T cells (3 \( \times 10^6 \)). As shown in Figure 4, when equal numbers of BALB/c and JHD T cells totaling 3 \( \times 10^6 \) were used as helper cells, the amount of TNP-specific IgG produced was similar to that observed with 3 \( \times 10^6 \) BALB/c T cells.

JHD T cells proliferate normally in response to B cell APCs, but secrete low levels of IL-4

As expected, IgG1 was the predominant isotype present in the supernatants from help assays with normal Ag-primed T cells (data not shown). Because isotype switching to IgG1 is mediated by IL-4 (19), we hypothesized that the JHD T cells produced less IL-4 in response to B cells than did T cells from normal mice. We examined JHD T cell responses to B cell Ag presentation using mitomycin C-treated MD4 Ig receptor transgenic B cells, which express surface Ig specific for the Ag HEL (10). These B cells were used to present an HEL-OVA protein conjugate to T cells primed to OVA in a unique system that allows highly efficient Ag presentation by Ag-specific B cells (11). Using this system, we found that the JHD T cells only secrete 30 ± 8.3% \( (p < 0.05, \) by Student’s paired t test) as much IL-4 as normal BALB/c T cells (Fig. 5). Previously, using SpAC as APCs, we found that IL-4 production by OVA-primed JHD T cells was similar to that by OVA-primed T cells from normal mice (Fig. 2C), although the absolute amount of IL-4 induced with SpAC was much lower than that induced with Ag-specific B cells as APCs (11). Therefore, we directly compared the responses of JHD and BALB/c T cells to Ag presented by SpAC or B cells. The OVA-primed JHD T cells proliferated to a similar extent as OVA-primed normal T cells in response to SpAC or B cells (Fig. 6A). However, while IL-4 production by JHD T cells was equivalent to that of normal T cells when SpAC were used as APC, the JHD T cells produced significantly less IL-4 than did normal T cells when B cells presented Ag (Fig. 6B). We also assayed IFN-\( \gamma \) and IL-5 in these cultures. We found that, like IL-4, IFN-\( \gamma \) was reduced in JHD cultures with B cells as APC, but not in those with SpAC (Fig. 7A). Surprisingly,

**Table I. JHD T cells provide little or no help for Ab production**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Normal T Cells</th>
<th>JHD T Cells*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80.37</td>
<td>&lt;1.95</td>
</tr>
<tr>
<td>2</td>
<td>63.85</td>
<td>&lt;1.95</td>
</tr>
<tr>
<td>3</td>
<td>50.14</td>
<td>11.01</td>
</tr>
<tr>
<td>4</td>
<td>6.73</td>
<td>&lt;1.95</td>
</tr>
<tr>
<td>5</td>
<td>14.31</td>
<td>5.86</td>
</tr>
<tr>
<td>6</td>
<td>12.07</td>
<td>3.30</td>
</tr>
<tr>
<td>7</td>
<td>3.16</td>
<td>&lt;1.95</td>
</tr>
<tr>
<td>8</td>
<td>4.16</td>
<td>&lt;1.95</td>
</tr>
<tr>
<td>9</td>
<td>34.69</td>
<td>&lt;1.95</td>
</tr>
<tr>
<td>10</td>
<td>85.30</td>
<td>&lt;1.95</td>
</tr>
</tbody>
</table>

*JHD or normal BALB/c T cells were used to provide help to B cells as described in Figure 3 using 0.1 \( \mu \)g/ml of TNP-KLH.

\*p < 0.02 by Student’s paired t test.
IL-5 was consistently increased in all cultures with JHD T cells (Fig. 7B).

Discussion

The results presented here demonstrate that Ag administration to B cell-deficient JHD mice primes T cells for subsequent proliferative responses. T cells taken from Ag-primed JHD mice proliferated and produced cytokines when stimulated with Ag presented by SpAC to the same extent as did T cells from Ag-primed normal mice. However, when Ag-primed T cells from JHD mice were stimulated with Ag presented by B cells, they produced reduced quantities of IL-4 and IFN-γ compared with primed T cells from normal mice and were unable to induce Ag-specific IgG production from the Ag-presenting B cells.

We undertook these experiments with JHD mice to resolve conflicting reports concerning the importance of B cells in T cell priming and the ability of T cells primed in the absence of B cells to provide B cell help. Older studies using anti-IgM-treated mice suggested that B cells are required for T cell priming (1–3), but more recent studies with B cell-deficient mice have given disparate results (6–9). Likewise, there have been conflicting reports as to whether T cells primed in B cell-deficient mice can provide help for B cells (8, 9, 20).

Our studies addressed the issue of T cell priming in the absence of B cells using clearly defined experimental systems with highly purified populations of T cells and B cells. First, we used JHD mice, which have a targeted deletion of the JH region and have been found to be completely free of both B cells and serum Ab (5). In contrast, previous in vivo experimental systems using mice treated from birth with anti-IgM Ab were much less likely to be completely free of B cells or Ig. More recently, researchers have used μMT mice, which have a targeted deletion of the μ region, but are reported to have low levels of κ-chain rearrangements (21). Second, our in vitro studies are highly reliable because we used extremely pure populations of Ag-primed T cells, devoid of functional APC. Contaminating B cells in control populations of primed normal T cells can potentially be very effective in presenting Ag and can falsely increase the proliferative response of such T cells from normal mice. We, therefore, used a three-step protocol to purify T cells, which produced T cell populations that failed to proliferate in response to Ag in the absence of added APCs. When fully purified Ag-primed CD4+ T cells primed to OVA were cultured as described in Figure 5, except that in some wells SpAC (2.7 × 10^5/ml) were used instead of B cells (2.7 × 10^5). A, Cells were cultured in 150 μl of medium in 96-well plates for 72 h, pulsed with [3H]thymidine for 18 to 22 h, and then harvested. B, Cells were cultured in 1 ml of medium in 24-well plates for 96 h, washed, and restimulated as described in Figure 5 and assayed for IL-4. For both A and B, SpAC and Tg B cells were used in parallel cultures but with different Ag concentrations, which have been found to be optimal for each APC type. Data represent the mean ± SD for triplicate wells and are representative of two experiments.
immune responses. The data were reproduced in multiple independent experiments (Table I), and in all instances, significantly reduced IgG production was noted when Ag-primed T cells from JHD mice were used. The TNP-specific IgG synthesis we observed in help assays using normal control T cells was dependent upon cognate T cell-B cell interactions, and only occurred when T cells, B cells, and specific Ag were all present. Additionally, the capacity of Ag-primed T cells to induce Ig synthesis was determined using a hapten carrier system that avoided the possibility that the B cell population was contaminated by Th cells. Finally, it is unlikely that the Ag-primed JHD T cells were cytotoxic for B cells, since JHD T cells did not inhibit the ability of normal T cells to induce Ig synthesis in mixing experiments (Fig. 4).

The failure of Ag-primed T cells from JHD mice to induce IgG synthesis appears to be due to a failure of such T cells to induce isotype switch, since the proliferation and induction of TNP-specific IgM production occurred normally in these cultures. The two major signals required for B cell isotype switch from IgM to IgG1 are IL-4R engagement and CD40 cross-linking (19). These signals are provided by Th cells, but may not be provided by primed T cells from JHD mice. As shown in Figure 6, JHD T cells proliferated normally when stimulated with Ag plus SpAC or Ag plus B cells, but these T cells secreted much less IL-4 in response to Ag presented by B cells (Fig. 6). These results suggest that a decrease in IL-4 secretion caused the failure of JHD T cells to provide adequate help for B cells. Although the reduction in IL-4 synthesis by JHD T cells is less dramatic than the decrease in help for Ab production, a linear relationship between IL-4 and isotype switch to IgG1 may not exist. Furthermore, for the induction of IgG1, high concentrations of IL-4 may be required only in the immediate B cell microenvironment, a hypothesis that is supported by our inability to detect IL-4 in the supernatants of our help assay cultures (data not shown).

We have attempted to reconstitute help by adding IL-4 (5–2500 pg/ml) to help assays with either normal or JHD T cells. IgG production increased greatly in response to IL-4 in the cultures with normal T cells, but was only slightly increased in cultures containing JHD T cells. Addition of IL-4 to JHD cultures did not increase IgG production to the level seen in cultures with normal BALB/c cultures that did not have IL-4 added (data not shown). This suggests that reduced IL-4 production is only partially responsible for the inefficient induction of IgG synthesis by JHD T cells.

The second signal required by B cells for isotype switch is CD40 cross-linking, which coincidentally also provides a costimulatory signal to T cells via CD40L cross-linking, which enhances IL-4 synthesis (22). We compared up-regulation of CD40L expression in both normal and JHD T cells in response to anti-CD3 stimulation and found that CD40L is up-regulated to the same extent (data not shown). However, we were unable to determine whether primed JHD T cells up-regulate CD40L in response to Ag presented by B cells because Ag-specific T cells make up a very small percentage of the cells in a primed LN and cannot be readily identified by FACS. In a different approach, we added anti-CD40 mAb 1C10 to cultures of B cells and JHD T cells as a substitute for CD40L stimulation, since this mAb has been shown to stimulate B cell proliferation (23). We found that a range of concentrations (0.1–10 μg/ml) of the mAb blocked TNP-specific IgG production in help assays with normal T cells and did not increase IgG production in help assays with JHD T cells (data not shown). It is possible that this Ab did not provide an effective signal to the B cell or that it interfered with help by blocking T-B interactions. Alternatively, it is possible that CD40 stimulation of B cells with IL-4 must be provided more directly to Ag-specific B cells, presumably by T cells, for TNP-specific IgG production to be observed. Therefore, we cannot as yet conclude whether CD40L expression is normal in Ag-primed JHD T cells responding to B cell APCs or whether primed JHD T cells are defective in signaling B cells through CD40.

Our demonstration that T cells from JHD mice are unable to provide effective help for B cells is in agreement with two other reports. Liu et al. reported that JHD T cells could not induce Ig synthesis; however, they did not use highly purified CD4+ T cells and reported that primed T cells from JHD mice did not proliferate after secondary stimulation with Ag (8). Epstein et al. cited unpublished work by Roes and Rajewsky suggesting that T cells from μMT mice cannot be primed to help (9). In contrast, Öxenius et al. reported that neither CD40 expression nor the presence of B cells is necessary for the development of Th cells (20). Using a T cell transfer system, Öxenius et al. showed that T cells from either μMT or CD40 knockout mice infected with LCMV could help to mediate isotype switching when transferred into normal naive mice. These conflicting results may reflect a difference in the predominant cytokines and isotypes present during LCMV infection (Th1) vs priming with Ag (Th0). It is also possible that viral infection activates APCs in such a way as to give different signals to responding T cells than adjuvant activation does.
The defects we have observed in the interaction of primed IHD T cells with B cells in vitro may be due to the absence of a signal normally provided by B cells during in vivo priming. Given van Essen et al.'s finding that T cells primed in CD40-deficient mice are unable to provide B cell help (24) and our finding that CD40L signaling can stimulate T cell IL-4 synthesis (22), it is likely that the interaction between CD40 on the B cell and CD40L on the T cell provides a critical signal in the development of Th cells. Signaling via CD40, CD40L, or both could be important in this process. Abs to human CD40L can induce T cell proliferation (25) and IL-4 secretion (22), and blocking the CD40L signal can inhibit T cell IL-4 production (11, 26). Although other APCs express CD40, B cells express higher levels (11, 23, 27), which may be necessary for these responses.

The signal that B cells receive via CD40 during T-B interaction could also be important to Th cell development. Signaling via CD40 on B cells has a number of effects, including stimulation of isotype switch and proliferation (28), and up-regulation of co-stimulatory molecules such as B7-1 and B7-2 (29, 30). Liu's group also reported that signaling via CD40 on B cells up-regulates CD44H, which acts as an early costimulatory signal for T cell proliferation (31, 32). Expression of this isoform of CD44 may be a contributor to Th cell memory and affinity maturation. Induction of Th1 and Th2 T cell clones. J. Immunol. 148:4717.


