Th1 and Th2 CD4⁺ T Cells Provide Help for B Cell Clonal Expansion and Antibody Synthesis in a Similar Manner In Vivo

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The relative ability of Th1 and Th2 T cells to help B cells remains controversial as do the mechanisms by which both T cell subsets provide help in vivo. Whether this help affects the clonal expansion and/or differentiation of B cells has been difficult to assess due to the low frequency of Ag-specific T and B lymphocytes. We have employed a novel technique to directly monitor the clonal expansion of Ag-specific T and B lymphocytes in vivo. OVA-specific TCR transgenic T lymphocytes were polarized toward a Th1 or Th2 phenotype in vitro. These cells were then transferred into syngeneic recipients, along with B cell receptor transgenic hen egg lysozyme-specific B lymphocytes. Our results indicate that Th1 and Th2 cells support B cell responses to a similar extent in vivo and that they achieve this in the same manner by migrating into B cell follicles to promote CD154-dependent B cell clonal expansion and Ab production. The Journal of Immunology, 2000, 165: 3136–3144.

The B cell response to protein Ags requires cognate interactions between Ag-specific B cells and activated Ag-specific Th cells within the microenvironment of secondary lymphoid tissues (1–3). T cell activation involves the presentation of Ag by professional APCs, such as dendritic cells (DC), to naive Ag-specific T cells. Once activated, T cells clonally expand and move to the outer edge of B cell follicles where they can interact with Ag-specific B cells, which have also encountered Ag in the lymph node and have moved to the same location (3). The B cell is then able to present Ag to the T cell, which, in turn, provides the B cell with stimulation via surface molecules, such as CD154 (4–6), and soluble cytokines. These signals drive the various components required for an effective B cell response, which include clonal expansion, differentiation, Ab production, and isotype switching (7).

Th cells can be divided into at least two subsets, Th1 and Th2, determined by which cytokines they produce upon activation (8), with Th1 cells producing IL-2, IFN-γ, and TNFβ, and Th2 cells producing IL-4, IL-5, IL-6, IL-10, and IL-13. The balance of cytokines produced by these subsets is a key factor influencing the character of an immune response (9–13). Although a number of studies have demonstrated the ability of both Th1 and Th2 cells to support B cell responses (14–17), the latter are widely believed to be particularly adept in this respect, as the cytokines they characteristically secrete have all been implicated in various stages of B cell proliferation and differentiation (11, 18–20). However, many of the studies noted above were performed in vitro, employed T cells clones or lines, relied upon different Ags to induce Th1 or Th2 responses, or could not identify the Ag-specific cells in question. Recent studies employing the adoptive transfer of TCR transgenic (tg) T cells have indicated that, in contrast to Th2 cells, CCR7 expression on Th1 cells reduces their migration to follicles and hence their ability to help B cell responses in vivo (21). However, Th2 cells may not be essential for B cell proliferation and differentiation, as IL-4 or IL-5 knockout (KO) mice, which have deficient Th2 responses, have readily detectable Ab production and germinal center formation (22–24). Furthermore, whereas Th1 cells are associated with cell-mediated immunity, and generally regarded as less important for B cell responses, IgG2a isotype switching is promoted by IFN-γ (20, 25, 26), the prototypical Th1 cytokine. These results suggest that Th2 cells are not unique in their ability to induce T-dependent B cell responses and that the mechanisms by which Th1 and Th2 cells provide B cell help remain to be defined, particularly in vivo. Understanding the relative roles of Th1 and Th2 cells in providing help for B cells and the mechanisms they employ to achieve this will be important for the rational design of vaccines and therapies for immune-mediated disorders.

To study the role of Th1 and Th2 cells in providing B cell help in vivo, we have modified a recently developed system to investigate the interactions and requirements underlying T cell help for B cell responses. By polarizing Ag-specific TCR tg T cells toward a Th1 or Th2 phenotype in vitro and transferring them into naive syngeneic recipients along with Ag-specific B cell receptor (BcR) tg B cells, we have been able to examine the effect Th1 and Th2 cells have on B cell expansion, differentiation, and Ab production in vivo. Our results indicate that, following immunization, both Th1 and Th2 cells migrate into B cell follicles to support B cell clonal expansion and Ab production in a CD154-dependent manner.

Materials and Methods

Animals
BALB/c (H-2d/d, IgMb), C57BL/6 (H-2b/b, IgMa/b), and BALB/c × C57BL/6 F1 (H-2b/b, IgMa/b) mice were purchased from Harlan Olac.

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2 Address correspondence and reprint requests to Dr. Paul Garside, Department of Immunology and Bacteriology, University of Glasgow, Western Infirmary, Glasgow, G11 6NT, U.K. E-mail address: paul.garside@clinmed.gla.ac.uk

3 Abbreviations used in this paper: DC, dendritic cell; tg, transgenic; KO, knockout; BcR, B cell receptor; cOVA, chicken OVA; HEL, hen egg lysozyme.
Mice were injected with 250 μg anti-CD154 mAb (clone MR-1, kind gift of Dr. R. Noelle, Department of Microbiology, Dartmouth Medical College, Lebanon, NH) or 250 μg hamster IgG (ICN Biochemicals, Aurora, OH) i.p. on the day of immunization and 2 days later. 

### Results

#### Phenotype of polarized T cells

CD4⁺ T cells were purified from DO11.10 mice by negative selection and polarized in vitro to become Th1 or Th2 cells. After 3 days of culture, these cells were harvested for adoptive transfer. Before transfer, an aliquot of each cell type was cultured in vitro with OVA323-339 peptide (Vector) for 45 min, and then washed. Sections were next incubated with biotinylated anti-CD45R/B220 (RA3-6B2) (1/500) (PharMingen) for 30 min before washing. Avidin-biotin complex-labeled peroxidase (Vector) was then added for 30 min after washing. Sections were then cut on a cryostat microtome, fixed in acetone, and allowed to dry before being blocked in PBS-2% Goat serum (Vector Laboratories, Bicester, U.K.). Sections were left to dry before being blocked in PBS-2% Goat serum (Vector Laboratories, Bicester, U.K.).

### Statistics

Results are presented as the mean ± the range where groups contained two animals. If groups contained at least three animals, results are represented as the mean ± SEM.
BALB/c × C57BL/6 F1 mice, which were then immunized with cOVA-HEL in CFA. The draining lymph nodes were removed from recipients 3, 5, and 14 days after immunization, and flow cytometric analysis performed. Th1, Th2, and unpolarized T cells could all be detected in vivo (Fig. 2). In agreement with our previous studies (3), peak clonal expansion was observed on day 3 after immunization with unpolarized TCR tg T cells and this was similar for the Th1 and Th2 groups. Unpolarized and Th1 cells expanded to similar levels, whereas Th2 cells proliferated to a lesser extent. All immunized groups displayed expansion above unimmunized control levels, indicating that in vitro polarized Th1 and Th2 TCR tg T cells can survive and respond to Ag in vivo.

**Phenotype of polarized T cells in vivo**

To confirm the phenotype of the polarized tg T cells following their adoptive transfer, we examined their effect on isotype switching of the endogenous anti-OVA Ab response of the recipients. The endogenous anti-OVA Ab responses of animals transferred with naive, Th1, or Th2 cells was assessed as the HEL-specific B cells are unable to switch their heavy chains. The serum level of anti-OVA IgG2a was determined 14 days postimmunization (Fig. 3A). OVA-specific IgG2a could be detected in immunized mice transferred with Th1 or unpolarized T cells, but not in those transferred with Th2 cells. No OVA-specific IgG2a could be detected in any unimmunized groups. There was no difference between immunized mice transferred with Th1 and Th2 cells when anti-OVA IgG1 levels were examined (data not shown); this may be explained by the recent report that this isotype is not entirely Th2-dependent (30).

**Cytokine profile of OVA-specific cells ex vivo**

To further confirm the functional phenotype of the transferred polarized TCR tg T cells, lymph nodes from immunized and unimmunized transferred mice were removed 7 and 14 days postimmunization, and single cell suspensions were prepared. These cells were then cultured in vitro with or without OVA323–339 peptide, and their cytokine production profile was analyzed. OVA323–339 peptide-specific T cells from animals receiving Th1 polarized cells and OVA/CFA continue to produce IFN-γ (Fig. 3B) but little IL-4 (Fig. 3C) or IL-5 (Fig. 3D) after peptide stimulation, whereas peptide-specific T cells from animals transferred with Th2 polarized cells and immunized with OVA/CFA produce IL-4 (Fig. 3C) and IL-5 (Fig. 3D) but little IFN-γ (Fig. 3B). Unpolarized cells produced a mixture of both Th1 and Th2 cytokines when restimulated in vitro (Fig. 3). Little or no cytokine production was apparent in the absence of peptide stimulation.

**Effect of tg Th1 and Th2 cells on tg B cells in vivo**

The relative abilities of Th1 and Th2 cells to provide B cell help could be reflected in differences in B cell clonal
expansion, Ab production, or both. However, it has previously been difficult to make such comparisons due to the inability to track Ag-specific T and B cells in vivo. Therefore, we employed an adaptation of a recently described adoptive transfer system in which the response of BcR tg B cells is dependent upon cognate help from TCR tg T cells, as noted above, to directly assess Ag-specific B cell clonal expansion and Ab production in vivo in the context of help from Th1 or Th2 cells. The kinetics and magnitude of the B cell response to immunization with OVA-HEL/CFA was similar in animals receiving unpolarized, Th1 or Th2 TCR tg cells. In agreement with our previous studies (3), B cell clonal expansion peaked 5 days postimmunization before declining toward control levels by day 14 (Fig. 4A). Indeed, even though the Th2 cells themselves did not expand as well as unpolarized or Th1 cells (Fig. 2), they displayed a similar capacity to support B cell expansion (Fig. 4A). Thus, animals adoptively transferred with unpolarized, Th1, or Th2 cells and immunized with OVA-HEL/CFA all appeared capable of supporting similar levels of clonal expansion of HEL-specific BcR tg B cells. As we have shown previously (3) and confirmed here (Table I), the BcR tg B cells do not respond in the absence of cognate help from the TCR tg T cells.

**Ab production.** To give some indication of the functional status of the clonally expanded tg B cells, we assessed their Ab production (Fig. 4B). Serum was taken from animals transferred with unpolarized, Th1, or Th2 T cells together with tg B cells at 3, 5, 7, and 14 days postimmunization. All tg T cells could support Ab production by MD4 BcR tg B cells as measured by anti-HEL IgM. Serum levels of anti-HEL IgM peaked at 5 days postimmunization in Th1 and Th2 recipients but not until day 7 in recipients of unpolarized T cells.
unpolarized TCR tg T cells. By day 14 after immunization, HEL-specific IgM Ab levels had declined to control levels in all groups. Thus, when Th1 or Th2 cells were transferred and the recipients immunized, anti-HEL IgM Ab was detected earlier compared with recipients receiving unpolarized tg T cells. Little or no anti-HEL IgM Ab was detected in serum from any unimmunized control animals.

Localization of unpolarized, Th1, and Th2 cells in vivo

It has been shown previously that T and B cells alter their anatomical location within lymph nodes in response to immunization to facilitate cognate interactions with each other (3). In the case of the T cells, adoptively transferred, unpolarized DO11.10 TCR Tg T cells disperse throughout the T cell-rich paracortex of the lymph nodes. The B cells, however, preferentially migrate into the B cell follicles of lymph nodes (3).

3140 Th1 AND Th2 CELLS HELP B CELLS

Table I. Th1 and Th2 cells help B cells

<table>
<thead>
<tr>
<th>Group</th>
<th>% KJ1.26</th>
<th>% HEL</th>
<th>Anti-HEL IgM Ab (OD 630 nm)</th>
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<tr>
<td>Unpolarized T cells + B cells (U)</td>
<td>0.17, 0.19</td>
<td>0.29, 0.25</td>
<td>0.015, 0.016</td>
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<tr>
<td>Unpolarized T cells + B cells (I)</td>
<td>1.52, 2.06</td>
<td>0.66, 2.19</td>
<td>0.372, 0.497</td>
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<tr>
<td>Th1 T cells + B cells (U)</td>
<td>0.81, 0.60</td>
<td>1.10, 0.46</td>
<td>0.060, 0.033</td>
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<td>Th1 T cells + B cells (I)</td>
<td>0.88, 0.76, 0.49</td>
<td>0.94, 1.30, 0.93</td>
<td>0.290, 0.438, 0.074</td>
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<tr>
<td>Th2 T cells + B cells (U)</td>
<td>0.53, 0.26</td>
<td>0.39, 0.30</td>
<td>0.030, 0.031</td>
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<tr>
<td>Th2 T cells + B cells (I)</td>
<td>0.94, 1.46, 1.12</td>
<td>2.28, 2.56, 2.30</td>
<td>0.245, 0.340, 0.124</td>
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<tr>
<td>B cells only (U)</td>
<td>–</td>
<td>1.28, 0.45</td>
<td>0.006, 0.028</td>
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<tr>
<td>B cells only (I)</td>
<td>–</td>
<td>0.57, 0.48, 0.38</td>
<td>0.021, 0.022, 0.035</td>
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Adoptive transfer (T+B or B alone) recipients were immunized as noted above. Clonal expansion of CD4+ KJ1.26+ T cells was assessed by flow cytometry on day 5 and B220+ HEL-specific B cell expansion was assessed day 5 postimmunization. Serum was collected on day 5 and analyzed for anti-HEL-specific IgM Ab by ELISA. U, Unimmunized; I, immunized.

FIGURE 5. Migration of Ag-specific Th1 and Th2 cells in vivo. Lymph nodes from transferred recipient mice were removed at days 3 and 5 postimmunization and stained as described in Materials and Methods. Unimmunized mice, which received unpolarized (data not shown), Th1 (A), or Th2 (data not shown) cells, showed paracortical localization of KJ1.26+ T cells that did not migrate into B cell follicles. Three days after immunization, KJ1.26+ T cells could be observed in the B cell follicles of unpolarized (data not shown), Th1 (B), and Th2 (data not shown) transferred groups. Treatment with anti-CD154 at day 0 and day 2 did not prevent this migration of unpolarized, Th1, or Th2 T cells at 3 (unimmunized, data not shown; Th1, data not shown; Th2, C) or 5 days (unimmunized, data not shown; Th1, D; Th2, E) after immunization. Where data are not shown, the pattern of migration is the same as that illustrated by the representative micrograph shown.
and CD154 (3, 31). Therefore, we decided to investigate whether T cell help for B cells is dependent on interactions between CD40.

**Effect of anti-CD154 on Th1 and Th2 cell-mediated help in vivo**

Pape, and M. K. Jenkins, unpublished observations).

Hamster IgG

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Anti-CD40L

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**FIGURE 6.** Effect of anti-CD154 on CD4<sup>+</sup> KJ1.26<sup>+</sup> T cell and B220<sup>+</sup> HEL-specific B cell responses in vivo. Adoptive transfer recipients of Th1 (A, C, E, G, I, K, M, and O) or Th2 (B, D, F, H, J, L, N, and P) TCR tg T cells together with BcR tg B cells (A–P) cells were unimmunized (A–D, I–L) or immunized (E–H, M–P) and received 125 μg of anti-CD154 (C, D, G, H, K, L, O, and P) or hamster IgG (A, B, E, F, I, J, M, and N) on day 0 (day of immunization) and 2 days later. CD4<sup>+</sup> KJ1.26<sup>+</sup> T cell expansion (A–H) was assessed by flow cytometry on day 3, and B220<sup>+</sup> HEL-specific B cell expansion (I–P) was assessed on day 5 postimmunization.

node and do not enter B cell follicles (3) in naive animals. However, following immunization, the Ag-specific T cells undergo clonal expansion and migrate into B cell follicles, presumably to provide help to B cells. To determine whether Th1 and Th2 cells alter their localization within the lymph node in response to immunization in vivo, and if they do so in a similar manner, we transferred each cell type into naive mice and immunized with cOVA-HEL/CFA. In contrast to recent studies (21), we found that unpolarized, Th1, and Th2 TCR tg T cells all displayed paracortical localization in naive animals (Fig. 5A) and all displayed a similar follicular migration following immunization (Fig. 5, B–E).

There was no difference in the kinetics of the migration of unpolarized, Th1, and Th2 TCR tg T cells into B cell follicles as this occurred within 3 days of immunization and was still apparent after 5 days in all groups. These results confirmed earlier findings describing the follicular migration of unpolarized T cells in response to immunization (3) and significantly extend them by showing that both Th1 and Th2 T cells undergo similar migration with similar kinetics. Furthermore, the presence of the BcR tg B cells has no apparent effect on the response or localization of the TCR tg T cells; no differences were detected when similar studies to those described above were performed using immunization with cOVA-HEL (targets OVA to BcR tg B cells) vs cOVA + turkey OVA-HEL (no targeting of OVA), or in μMT KO mice, or intact MD4 BcR tg mice immunized with cOVA or cOVA-HEL (targeting OVA to a large population of BcR tg B cells, (P. Garside, K. A. Pape, and M. K. Jenkins, unpublished observations).

**Effect of anti-CD154 on Th1 and Th2 cell-mediated help in vivo**

T cell help for B cells is dependent on interactions between CD40 and CD154 (3, 31). Therefore, we decided to investigate whether both Th1 and Th2 cells displayed similar requirements to deliver T cell help to B cells in vivo. As we have reported previously (3), treatment with anti-CD154 affected the clonal expansion of T and B cells. The effect of anti-CD154 on T cell expansion was measured at the peak of T cell expansion 3 days after immunization. The Th1 and Th2 responses were similarly affected in the anti-CD154-treated animals as the cells only expanded to 49% and 47% of their respective untreated groups (Th1 clonal expansion = 1.03% vs 0.51% in untreated vs anti-CD154-treated groups; Th2 clonal expansion = 0.68% vs 0.32% (representative FACS profile, Fig. 6, A–H; mean values ± range for two to three animals per group, Fig. 7A). We assessed the effect of anti-CD154 treatment at the peak of B cell clonal expansion on day 5 after immunization. B cells from Th1-transferred anti-CD154-treated and immunized mice only achieved 26% (representative FACS profile, Fig. 6, I–P; mean values ± SEM for two to three animals per group, Fig. 7B) of the clonal expansion of untreated Th1-transferred and immunized mice (1.69% vs 0.44%), whereas B cells from Th2-transferred mice only achieved 32% (Fig. 6, I–P) of the clonal expansion exhibited by untreated Th2-transferred and immunized mice (2.44% vs 0.79%). Ab responses were also decreased in the presence of anti-CD154. Serum anti-HEL IgM<sup>+</sup> was analyzed by ELISA 5 days after immunization. It was found that anti-CD154-treated mice transferred with Th1 cells only produced 58% of the HEL-specific serum IgM<sup>+</sup> of untreated mice and anti-CD154-treated mice transferred with Th2 cells had only produced 56% of the anti-HEL serum IgM<sup>+</sup> of untreated Th2-transferred and immunized mice (Fig. 7C). These results clearly demonstrate that in response to antigenic challenge in vivo previously primed Th1 and Th2 cells require interactions between CD40 and CD154 to expand fully and to mediate B cell help. Interestingly, treatment

- **FIGURE 6.** Effect of anti-CD154 on CD4<sup>+</sup> KJ1.26<sup>+</sup> T cell and B220<sup>+</sup> HEL-specific B cell responses in vivo. Adoptive transfer recipients of Th1 (A, C, E, G, I, K, M, and O) or Th2 (B, D, F, H, J, L, N, and P) TCR tg T cells together with BcR tg B cells (A–P) cells were unimmunized (A–D, I–L) or immunized (E–H, M–P) and received 125 μg of anti-CD154 (C, D, G, H, K, L, O, and P) or hamster IgG (A, B, E, F, I, J, M, and N) on day 0 (day of immunization) and 2 days later. CD4<sup>+</sup> KJ1.26<sup>+</sup> T cell expansion (A–H) was assessed by flow cytometry on day 3, and B220<sup>+</sup> HEL-specific B cell expansion (I–P) was assessed on day 5 postimmunization.
with anti-CD154 did not prevent movement of Ag-specific Th1 or Th2 (Fig. 5) cells into B cell follicles after immunization.

**Discussion**

We have generated Th1 and Th2 cells of identical Ag specificity and observed their interactions with Ag-specific B cells in vivo. Our study has demonstrated in vivo that both Th1 and Th2 cells are capable of supporting Ag-specific B cell clonal expansion and Ab production in a CD154-dependent manner by migrating into B cell follicles.

To analyze cognate interactions between Ag-specific Th1, Th2, and B cells in vivo, it was first necessary to circumvent the obstacles presented by the extremely low frequency of Ag-specific lymphocytes present in a normal naive animal. To do this, we modified an adoptive transfer system in which we have recently shown that BcR tg B cells require cognate help from TCR tg T cells for clonal expansion and germinal center formation (3). In our current study, naive TCRtg T cells were stably polarized toward a Th1 or Th2 phenotype in vitro, as has been described previously (13, 32–37), before adoptive transfer. Our transferred T cells secreted the expected pattern of cytokines (IFN-γ for Th1 and IL-4 and IL-5 for Th2) before adoptive transfer and maintained their phenotypes following restimulation after recovery from immunized animals. Furthermore, in contrast to TCRtg Th2 cells, the TCRtg Th1 cells supported the generation of OVA-specific IgG2a-secreting endogenous B cells, indicating that the Th1 and Th2 cells maintained their adopted phenotypes in vivo. Though there was no difference in OVA-specific serum IgG1 levels between Th1 and Th2 recipients, others (38) have observed IgG1 production in the absence of IL-4 suggesting that the absence of IgG2a, rather than the presence of IgG1, may be a better indicator of a Th2 response. Furthermore, a recent report has indicated that IgG1 may not be entirely Th2-dependent (30).

Although early in vitro studies indicated that both Th1 and Th2 cells could support B cell responses (15–17), the capacity of Th1 cells to provide cognate help for B cells is controversial, with some studies reporting that they are relatively deficient in this function (21, 39, 40). However, care must be taken when interpreting such in vitro studies, as examination of several Th1 and Th2 clones revealed considerable variation in their ability to help B cells with some Th2 clones failing to support Ab synthesis at all (15). Recent studies have also attempted to address this issue in vivo (41) and support a role for Th1 cells in helping B cell responses. However, the Th1 and Th2 cells were of differing Ag specificities and induced by different immunizing regimes as this study was more concerned with help for isotype switching than B cell clonal expansion. We have developed a physiologically more relevant system where the TCRtg Th1 and Th2 cells recognize the same Ag and can be tracked in vivo using mAbs, as can the BcRtg B cells, thus allowing analyses of Ag-specific responses.

We found that both Th1 and Th2 cells clonally expanded in response to immunization. Interestingly, whereas the kinetics of T cell clonal expansion were similar, Th2 cells expanded significantly less than Th1 cells. However, both cell types were able to support a similar degree of B cell clonal expansion and Ab production. This might imply that Th2 cells are more adept at providing B cell responses on a per cell basis, as was suggested by earlier studies (17, 42). Although this issue will require further detailed analysis, it is clear from our studies that Th1 cells are perfectly able to support B cell clonal expansion and Ab production. Alternatively, it may be that Th1 and Th2 cells achieve optimal B cell help in different ways with Th1 cells helping less well on a per cell basis but expanding to provide more cells, whereas Th2 cells provide more help per cell but expand less, thus the net effect is the same.

We have demonstrated previously with this adoptive transfer system that Ag-specific T cells migrate into B cell follicles to provide B cell help. Recent studies have emphasized the importance of cellular localization in the induction of immune responses (43) and this issue has only begun to be directly addressed for Th1 and Th2 cells in vivo. Therefore, we determined the location of TCRtg Th1 and Th2 cells following immunization in vivo. We
found that, whereas few Th1 and Th2 cells were present in B cell follicles in naive adoptive transfer recipients, considerable numbers of both types of cell could be found in this location following immunization. Our findings contrast with recent studies (21, 39), which suggested that, unlike Th2 cells, Th1 cells localize in the paracortex by virtue of their expression of CCR7 and are unable to support the differentiation of naive B cells into germinal center cells. These results do not explain the production of high-affinity IgG2a-secreting B cells that develop with the help of Th1-derived cytokines such as IFN-γ. The discrepancies between the studies may be the result of the in vitro vs in vivo nature of some of the systems employed, our use of primed T cells, the use of different adjuvants, or the fact that, in contrast to the other studies, we have also tracked Ag-specific B cells directly during detailed kinetic studies in vivo. However, as noted above, it seems unlikely that the presence of the BcR tg B cells affects the behavior of the TCR tg B cells, and we have clearly shown that Th1 cells move into the B cell follicle to support B cell clonal expansion, Ab production, and class-switching to IgG2a.

A considerable number of studies have previously shown that CD40-CD154 interactions are an important component of T cell help for B cells, and we confirmed this using the adoptive transfer system (3, 31). Therefore, it was important to determine whether both Th1 and Th2 cell help for B cells were CD154-dependent. Both Th1 and Th2 cells can express CD154 (44), and membranes from each cell type can provide cognate help for B cell responses in vitro, which was shown to be CD154-dependent (31). However, more recently, it has become clear that CD154 may be involved in B cell help directly, or indirectly via effects on the differentiation of T cells or both. Thus the interaction of CD154 on T cells with CD40 on APC may result in the production of cytokines, e.g., IL-12, which polarize or activate the T cell before it interacts with the B cell. Here, we have shown that the clonal expansion of both Th1 and Th2 cells was reduced by ~50% in anti-CD154-treated animals, whereas B cell clonal expansion and Ab production were almost abolished in both situations. This is consistent with our previous findings (3) and suggests that, as reported by others, CD154 is important for the differentiation of Th1 and Th2 cells and their ability to help B cells. We cannot distinguish whether the decreased B cell responses in our anti-CD154-treated animals are a result of blocked interactions between T cells and APC leading to reduced T cell clonal expansion (3), altered T cell differentiation (45), or of a direct blockade of subsequent T cell–B cell interactions. Nevertheless, our results show clearly that Th1 and Th2 cells require CD40-CD154 interactions for maximal T and B cell responses. Interestingly, unpoplarized, Th1 and Th2 cells can still enter B cell follicles after treatment with anti-CD154, suggesting that this interaction is not required for movement into the follicle and that a defect in T cell migration does not underlie the failure of T cell help in this situation, as has been demonstrated in tolerance (46).

Although our studies have provided the first direct in vivo evidence that both Th1 and Th2 cells support Ag-specific B cell expansion and Ab production in a similar manner and location, all of the molecular interactions involved remain to be elucidated. The most likely candidates would be cytokines, which have been widely implicated in T cell help for B cells. However, as we have demonstrated that Th1 and Th2 cells, which secrete very different patterns of cytokines, are both able to support B cell responses and others have been unable to find a role for a battery of the expected cytokines (39), it is clear that further studies are required to define the molecules involved in T–B cell interactions in vivo.

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