Abortive Proliferation of Rare T Cells Induced by Direct or Indirect Antigen Presentation by Rare B Cells In Vivo

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Summary

Antigen-specific B cells are implicated as antigen-presenting cells in memory and tolerance responses because they capture antigens efficiently and localize to T cell zones after antigen capture. It has not been possible, however, to visualize the effect of specific B cells on specific CD4+ helper T cells under physiological conditions. We demonstrate here that rare T cells are activated in vivo by minute quantities of antigen captured by antigen-specific B cells. Antigen-activated B cells are helped under these conditions, whereas antigen-tolerant B cells are killed. The T cells proliferate and then disappear regardless of whether the B cells are activated or tolerant. We show genetically that T cell activation, proliferation, and disappearance can be mediated either by transfer of antigen from antigen-specific B cells to endogenous antigen-presenting cells or by direct B–T cell interactions. These results identify a novel antigen presentation route, and demonstrate that B cell presentation of antigen has profound effects on T cell fate that could not be predicted from in vitro studies.

Key words: peripheral tolerance • antigen-presenting cell • interactions • B cell • T cell

A variety of both beneficial and pathological immune responses involve B cell presentation of antigen to Ths. Memory B cells carry high affinity B cell receptors (BCR) that allow them to concentrate trace amounts of antigen and these cells often localize to sites of antigen entry or filtering, such as beneath the mucosal epithelia and the splenic marginal zone (1, 2). Once they bind antigen, they rapidly move to the T cell zones of the spleen and lymph node, where there is a large traffic of recirculating T cells (1). Similarly, self-reactive B cells are concentrated in the T zones as a result of binding autoantigens (3–6). Once located in this site, memory B cells which present foreign antigens to Ths proliferate and produce antibody, whereas self-reactive B cells that present self-antigens are killed by Ths expressing Fas ligand (7, 8). Although the outcome of these B–T cell interactions is known for the B cell, it is not known if the Th is primed, proliferates, or is tolerized, and how B cell presentation may impact regulatory phenomena such as immune deviation in allergy or epitope spreading in autoimmunity.

In vitro studies have shown that activated, antigen-specific B cells present antigen to T cells and trigger proliferation and lymphokine secretion, although this presentation may be less efficient than presentation by activated dendritic cells (9–12). By contrast with activated B cells, presentation of antigen by tolerant B cells does not induce T cell proliferation and lymphokine secretion in vitro, due to deficient expression of, and costimulation by, B7.2 (13). Based upon in vitro studies with T cell clones, presentation of antigen by costimulation-deficient cells might be predicted to induce a state of T cell anergy (14–16).

B cells have been shown to be tolerogenic for T cells in vivo, but it remains unclear whether the basis for tolerance lies in T cell deletion, anergy, or other regulatory processes (17–22). It has been suggested that B cells cannot activate T cells in vivo, but rather induce a state of anergy (17, 21, 23–25). Studying the fate of helper T cells after B cell antigen presentation in vivo represents a substantial technical challenge. Approaches that use irradiated or lymphocyte-deficient mice as vessels to track specific cells produce inflammatory conditions or disrupted lymphoid microenvironments that induce nonspecific proliferation in T cells (26–28 and Townsend, S.E., data not shown). Conversely, experiments in TCR-transgenic mice involve unphysiologically high frequencies of specific T cells that can obscure regulatory effects (29, 30). An elegant solution to the...
problem of tracking T cell fate in a physiological setting is the adoptive transfer of TCR-transgenic T cells into intact, unirradiated mice using unique cell surface markers to visualize the subsequent responses of the T cells to antigen (31).

Here we show, using an adoptive transfer system, that naive antigen-specific T cells efficiently find tolerant or naive antigen-specific B cells in intact, unirradiated recipients, in the context of a normal immune repertoire and an unperturbed microenvironment. Naive B cells that have acutely captured antigen are helped to antibody production, whereas tolerant B cells that have chronically captured antigen are killed. In addition to direct presentation of antigen to T cells, we describe a novel and efficient process of antigen transfer by B cells to an endogenous APC population. In the absence of adjuvant, presentation of antigen by tolerant B cells, naive B cells, or after transfer of antigen to endogenous APCs in each case leads to the initial activation and proliferation of the T cells, followed by rapid disappearance.

Materials and Methods

Mice. Transgenic mice expressing the hen egg lysozyme (HEL)-specific 3A9 transgenic TCR (13) on the B10. BR background were bred with B6 Ly5. 1 congenic mice (gift of Dr. I. Weissman, Stanford University, Stanford, CA) to generate TCR+Ly5.2 H-2b donor mice. Transgenic mice expressing a HEL-specific BCR (IgαIgδ; reference 32) on the B6 background were bred with B10. BR mice to generate IgαIgδ-H-2b and shEL-IgαIgδ-H-2b donor mice. Double transgenic (shEL-IgαIgδ) mice bearing the lpr/lpr mutation in Fas and expressing H-2b were bred and screened as described (7, 33). Recipient mice were unmanipulated (B10. BR ×B6)F1 mice bred at the Research Animal Facility at Stanford (Stanford, CA). The transgenic phenotypes of all donor cells were verified by flow cytometry before transfer into recipient mice. Donor cells and recipient mice were sex-matched for all transfers >3 d. Mice were used between 6 and 20 wk of age. Radiation bone marrow chimeras were made as described previously (3, 4) using T cell–depleted bone marrow from B6 donors to reconstitute lethally irradiated (B10. BR ×B6)F1 recipients. The recipients were left to reconstitute for 9 wk before use in experiments. Polyomavirus B (110 mg/liter) and neomycin (1.1 g/liter) were added to the drinking water weekly during reconstitution.

B Cell Purification and Antigen Loading. Whole spleen cell suspensions from B cell donors were transferred, except where indicated in the figure legends. B cells were purified for some experiments by incubating spleen cell suspensions with B220+ microbeads (Miltenyi Biotec, Auburn, CA) as per the manufacturer’s instructions, and passing over MiniMACS columns (Miltenyi Biotec). Preparations of whole spleen or purified B cells were loaded with HEL in vitro by incubating cells at <5 × 10^6 cells/ml in RPMI medium, 10% FCS, and 10 mM Hepes, with either 1 μg/ml (Figs. 2 and 3) or 10 ng/ml (Figs. 1, 5, 6, and 7) HEL for 2 h at 37°C in polypropylene tubes. Cells were washed with complete medium, counted, and resuspended in PBS for injection.

Flow Cytometry. Flow cytometry was performed using FACSCalibur and Vantage cytometers (Beckton Dickinson, Mountain View, CA) and FACSdating and FLOJO analysis software (Becton Dickinson, Stanford, CA). The staining reagents used were: Ly5.1, AS-20–biotin or –PE; CD4, GK1.5-allophycocyanin (gift of Dr. J. Altman, Emory University, Atlanta, GA); CD69-FITC or -biotin (PharMingen, San Diego, CA); B220, RA3-6B2–PE (Caltag, Burlingame, CA); Streptavidin-Triculum (TR; Vector Laboratories, Burlingame, CA), or –allophysocyanin (Biomed, Foster City, CA); IgG1, SM9.1-FITC; HEL, HyHEL9-biotin or -Tricolor (custom conjugation; Caltag). Some samples were live-gated on target populations to collect sufficient data; all negative gating was performed after analysis, as described in the figure legends.

Histology. Cryostat sections (10 μm) of spleen were stained for immunofluorescence using CD4–allophysocyanin and HEL followed by HyHEL9-biotin and Streptavidin-Cy3 (Jackson Immunoresearch, Bar Harbor, ME) in the presence of normal mouse serum, normal rat serum, and 0.1% bovine serum albumin. Images were produced using a confocal microscope (Mulliteployte 2010; Molecular Dynamics, Eugene, OR), ImageSpace software (Molecular Dynamics), and an Indigo 2 computer (Silicon Graphics, Palo Alto, CA) at the Cell Sciences Imaging Facility, Stanford University (Stanford, CA).

Results

The experimental system used to visualize the consequences of specific B–T cell interactions in vivo is dia-
Figure 1. Tracking antigen-specific B cells and Ths in normal lymphoid tissue. (A) Schematic of experiments. (B) Quantitation of transferred antigen. NP-40 lysates of $3 \times 10^8$ antigen-pulsed, HEL-specific B cells purified from naive (N) IgHEL-transgenic or tolerant (T) HEL/IgHEL-transgenic mice were subjected to SDS-PAGE, blotted to nitrocellulose, and probed with antibodies specific for HEL and actin as indicated. Serial dilutions of HEL were added to nontransgenic spleen lysates to give HEL titrations of 50 ng, 10 ng, 2 ng, and 400 pg. The amount of HEL associated with each B cell population was $\sim 2$ ng. (C) Transferred T and B cells traffic to the T and B cell regions, respectively, of the spleen. Three-color immunofluorescent analysis of spleen cryostat sections 12 h after transfer of sham-pulsed IgHEL transgenic B cells. Transgenic TCR$^+$ lymph node and spleen cells were labeled with CFSE and 3 $\times 10^7$ total cells containing $3 \times 10^6$ CD4$^+$ T cells were transferred into intact (B6 × B10.BR $\times$ B6)$F_1$ recipient mice, followed by IgHEL-transgenic spleen cells ($9 \times 10^6$ total cells containing $3 \times 10^6$ HEL-binding cells). CFSE-labeled cells (red) localize to the white pulp, CD4-expressing cells (blue) define the T cell zone. Colocalization analysis (ImageSpace software; Molecular Dynamics of CD4 and CFSE identifies the HEL-specific CD4$^+$CFSE$^+$ cells (yellow; see also Fig. 3) within the T cell zone. HEL-binding cells (green; HEL/HyHEL9-biotin/Streptavidin-Cy3) localize to the white pulp outside the T cell zone.

Despite the low frequency of transferred T and B cells, the lack of adjuvant and irradiation, and the small amount of antigen, HEL-pulsed naive B cells were helped to differ-
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entiate into antibody-forming cells by the HEL-specific T cells (Fig. 2, A and B). By 3 d after transfer, the HEL-pulsed naive B cells lose surface IgD expression in the presence, but not the absence, of HEL-specific T cells, consistent with activation (Fig. 2 A). By 5 d after transfer, HEL-specific antibody-forming cells were readily detectable in the spleen in the presence, but not the absence, of HEL-specific T cells (Fig. 2 B).

In contrast, tolerant B cells transferred under the same conditions were killed by the rare HEL-specific T cells within 5 d, but not by the excess of host T cells. Elimination of the tolerant B cells required B cell expression of the Fas molecule, as Fas-deficient B cells homozygous the lpr mutation were not killed (Fig. 2 C). This T cell-dependent killing of tolerant B cells demonstrates that despite their low frequency, the HEL-specific B and T cells interact efficiently in the context of a normal immune system, and that the outcome for tolerant B cells is identical to that found previously in irradiated hosts where the cells are more frequent and the microenvironment perturbed (7).

Because B–T cell interactions occurred efficiently under these circumstances, we explored the consequences of these interactions for the T cells in the recipients. 12 h after the transfer of either HEL-pulsed naive B cells or HEL-tolerant B cells, most of the HEL-specific T cells in the spleen had been induced to express the early activation marker, CD69 (Fig. 3). T cell activation was antigen specific because CD69 expression was not induced on the endogenous T cells, which are not HEL reactive (data not shown), nor on HEL-reactive T cells transferred with sham-loaded HEL-specific B cells. CD69 induction required the presence of HEL-specific B cells, as depletion of B220+ splenocytes from double transgenic sHEL/IgHEL donor inoculum eliminated CD69 induction on the T cells, and HEL-pulsed nontransformed B cells were not able to induce CD69 (data not shown). Tolerant B cells selectively induced CD69 expression on lymph node T cells (Fig. 3), suggesting differences in trafficking between recently activated and tolerant cells. Very few CD69-expressing T cells were detectable in the blood after transfer of either type of B cells, suggesting that T cell activation may occur within the lymphoid organs.

Although HEL-specific B cells were required for activation of the T cells, this T cell activation was not solely due to direct presentation of antigen by HEL-specific B cells, but...
but also to transfer of antigen to another APC. We distinguished T cell–endogenous APC interactions from T–B cell interactions by transferring antigen-pulsed IgHEL B cells of the H-2\textsuperscript{bb} MHC haplotype, which lack the I-A\textsuperscript{k}–presenting element recognized by the HEL-specific TCR. These B cells could not directly interact with HEL/I-A\textsuperscript{k}–specific T cells, but were nevertheless able to induce comparable CD69 expression on the T cells in the spleen (Fig. 4). In contrast to the spleen, tolerant H-2\textsuperscript{bb} B cells did not induce CD69 expression by T cells in lymph node (Fig. 4), raising the possibility that the endogenous APC responsible for this very efficient presentation of antigen transferred from B cells is resident in the spleen.

Proliferation of low frequency T cells in vivo was followed using the fluorescent dye CFSE, which has been shown to be diluted linearly with cell division in vivo (35). Although the transferred T cells remained CFSE high in the absence of antigen-pulsed B cells, many T cells became CFSE low within 3 d after transfer with either HEL-loaded naive or tolerant B cells (Fig. 5). The number of T cells that had proliferated (CFSE low) and the number that had not proliferated (CFSE high) were counted in each lymphoid tissue on days 3 and 5 after B cell transfer (Fig. 6). T cell proliferation after transfer of tolerant B cells was followed by disappearance of many of the CFSE low cells but not of the CFSE high cells, indicating that the tolerant B cells were not able to present the antigen efficiently to the transferred T cells.

**Figure 3.** Transfer of antigen-pulsed HEL-specific B cells or HEL-tolerant B cells induces the activation of most transferred HEL-specific CD4\textsuperscript{+} T cells in the spleen within 8 h. Transfer as in Fig. 2, except that mice were killed 8 h after B cell transfer and analyzed by flow cytometry for CD4, Ly5\textsuperscript{a}, and CD69, with negative gating for B220. The histograms represent CD69 staining on the small boxed CD4\textsuperscript{+}Ly5\textsuperscript{a} population. The numbers indicate the percentage of CD4\textsuperscript{+}Ly5\textsuperscript{a} cells of total cells for each sample. The background values in a mouse that received no transferred T cells were 0.002% in the spleen, 0.004% lymph node, and 0% in blood. Each plot is representative of two or three individual recipients from each of three experiments.

**Figure 4.** Activation of HEL-specific T cells in the spleen can be mediated indirectly by transfer of antigen to host APCs. Transfer as in Fig. 2, with the transfer of purified tolerant B cells (B220; 86–93%) expressing MHC H-2\textsuperscript{kb} or H-2\textsuperscript{bb}. Mice were killed 12 h after B cell transfer and analyzed for CD69 expression on T cells in spleen and lymph node as in Fig. 3. Light lines, CD69 expression by T cells in the absence of transferred B cells; heavy lines, CD69 expression by T cells in the presence of transferred B cells as indicated. Each plot is representative of one of two recipients in each of two experiments. Similar CD69 expression by T cells was obtained after transfer of acutely activated HEL-pulsed naive B cells expressing H-2\textsuperscript{bb} in two additional experiments.
Many HEL-specific T cells have proliferated by 3 d after transfer of either antigen-pulsed HEL-specific or -tolerant B cells. T and B cells were transferred as in Fig. 2, except that TCR transgenic cells were labeled with CFSE before transfer. Mice were killed 60 h after B cell transfer, and CFSE fluorescence of CD4^+Ly5^+ splenocytes analyzed by flow cytometry. Each plot is representative of two or three recipients in each of two (HEL-pulsed naive B cells) or four (tolerant B cells) separate experiments.

The data above showed that ultralow amounts of antigen stimulated T cell proliferation and disappearance regardless of whether acutely activated or tolerant B cells were the source of antigen, and that cross-presentation of antigen transferred from the B cells to endogenous APCs could account for this abortive T cell response. To examine if direct B–T cell interactions on their own could also trigger T cell proliferation, the proliferating CFSE low T cells did not disappear, but accumulated in large numbers by day 5. Transfer of HEL-pulsed B cells that lacked HEL-specific BCRs did not trigger T cell proliferation or disappearance.

The data above showed that ultralow amounts of antigen stimulated T cell proliferation and disappearance regardless of whether acutely activated or tolerant B cells were the source of antigen, and that cross-presentation of antigen transferred from the B cells to endogenous APCs could account for this abortive T cell response. To examine if direct B–T cell interactions on their own could also trigger T cell proliferation, we repeated the experiments in bone marrow chimeras that lack I-A^k on most host APCs, thus eliminating cross-presentation of antigen. For this purpose, recipient mice were constructed by lethally irradiating (B10.BR × B6)F1 mice (H-2^b) and reconstituting them with parental B6 (H-2^b) bone marrow that had been T cell depleted. The chimeric mice were held for 9 wk before use in these experiments to allow time for the loss of labile or depleted. The chimeric mice were held for 9 wk before use in these experiments to allow time for the loss of labile or depleted.

Transfer of antigen-specific T cells with tolerant B cells expressing H-2^b or H-2^k into chimeric recipients (bb→kb) showed that, under these circumstances, antigen presentation to the T cells depended upon B cells bearing H-2^b. Thus, HEL-specific T cells were not induced to express CD69 nor to proliferate after transfer of H-2^bb–tolerant B cells in bb(kb) chimeric recipients, whereas they were triggered if the B cells expressed I-A^k–presenting molecules (Fig. 8). The absence of T cell activation or proliferation demonstrates that H-2^bb–tolerant B cells do not transfer antigen to residual I-A^k–presenting cells for presentation to T cells in these chimeras. Under these conditions, where antigen presentation was limited to the HEL-tolerant B cells, the T cells proliferated initially, but many of the proliferated cells disappeared by 6 d after transfer. Thus, antigen presented only by tolerant B cells was nevertheless sufficient to drive T cell activation and proliferation in vivo. The HEL-tolerant B cells were killed by the T cells as effectively as they were killed in conventional H-2^b recipients (data not shown), demonstrating that priming on endogenous APCs is not required for this T cell effector function. T–B cell interactions and T cell–endogenous APC interactions induced apparently equivalent functional outcomes in T cells under these noninflammatory conditions in vivo.
The results presented here demonstrate that B cell pre-
sentation of antigen to T cells can have profound effects on
T cell fate in vivo, and that, together with a novel mecha-
nism of antigen transfer to endogenous APCs, such antigen
presentation may be important in limiting T cell responses
to antigen in the absence of inflammation. Infrequent anti-
gen-specific T and B cells efficiently find each other in in-
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context of a normal immune repertoire, unperturbed mi-
croenvironment, and trace amounts of antigen. The conse-
quences of these interactions differ for acutely activated na-
ive B cells and tolerant B cells, such that the T cells help
the naïve B cells to become antibody-forming cells, whereas
the T cells kill the tolerant B cells. In marked contrast with
in vitro experiments, these direct interactions with acutely
activated or tolerant B cells do not have distinct effects on
T cell fate in vivo. Both direct presentation by B cells and
indirect presentation by endogenous APCs induce abortive
proliferation of T cells. This effect on T cells results from
very efficient presentation of minute quantities of antigen.
The process of concentration and presentation of such
small quantities of antigen may be important in establishing
or maintaining low zone tolerance, in epitope spreading
during disease, and in maintaining tolerance to potential al-
lergens.

Minute quantities of antigen (~2 ng) are transferred by
B cells into recipient mice in these experiments. This small
amount of antigen is nevertheless efficiently transferred to
and presented by endogenous APCs. Transfer of H-2b B
cells activated T cells in the spleen, which suggests that B
cell–bound antigen is transferred to endogenous I-A^d–
expressing APCs that then present antigen to T cells. In con-
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CD69 expression by T cells in lymph node (Fig. 4). The ef-
cient cross-presentation of transferred antigen by endog-
enous APCs in the spleen and not the lymph node, as well
as the lack of CD69 expression by T cells in the blood,
rates the possibility that the responsible APC is resident in
the spleen, rather than one that acquires antigen in the pe-
riphery and migrates through the blood to the spleen or
through the lymph to the lymph nodes. The essential prop-
erty of the endogenous APC may be the ability to receive and
present transferred antigen with high efficiency, a proper-
ty that may be linked to its location in a particular
splenic microenvironment. We are actively investigating
the mechanism of antigen transfer from antigen-specific B

Discussion

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cells to endogenous APCs, which could be antibody mediated, such as Fc receptor or complement receptor uptake of antigen–antibody complexes, or cell mediated, such as phagocytosis of whole cells. We have identified the endogenous APC that receives antigen transferred from B cells as a labile or radiation-sensitive cell that is probably bone marrow derived, as cross presentation did not occur in radiation chimeras where bone marrow–derived cells lacked the relevant restriction element, I-A$^\alpha$. Interestingly, cultured myeloid dendritic cells are not capable of T cell stimulation by re-presentation of transferred antigen from HEL-specific B cells in vitro, which suggests that neither immature nor mature activated dendritic cells mediate this activity (data not shown). Candidate APCs are marginal zone macrophages (42), migratory dendritic cells located in the bridging channels between the B cell follicles of the spleen (43), and lymphoid dendritic cells located in the T cell zone (44).

Studies in B cell–deficient mice demonstrate that B cells are not essential for the induction of peripheral T cell tolerance to some superantigens (45), the injection of microgram quantities of peptide (45), or microgram or milligram quantities of intact antigen (46, 47). These models rely on high doses of antigen that could load many types of APCs, obscuring a role for specific B cells in peripheral T cell tolerance. The one model that addressed T cell tolerance in the presence of low serum levels of antigen ($>0.5$ ng/ml) did not exclude a role for thymic deletion in the observed tolerance (47). B cells may be necessary for the maintenance of T cell tolerance in nonobese diabetic (NOD) mice (48) and can induce tolerance in vivo (17–22). The results presented here demonstrate that the mechanism of B cell tolerogenicity could be the induction of T cell abortive proliferation either by direct presentation of antigen or by transfer of antigen to an endogenous APC.

Previous studies have shown that the activation state of B cells is critical for the activation of T cells in vitro, such that antigen–specific, activated B cells are effective APCs (12), whereas as unresponsive, resting B cells are not (12, 49). Naive HEL-specific B cells are induced to express high levels of the costimulatory molecule B7.2 upon HEL exposure, whereas HEL-tolerant B cells are deficient in their ability to upregulate B7.2 (37, 50). As a result of differences in B7.2, antigen-activated B cells, but not tolerant B cells, induce 3A9 TCR-transgenic cells to proliferate and secrete cytokines in vitro (13). Surprisingly, both tolerant and activated HEL-specific B cells induce abortive proliferation in T cells in vivo (Figs. 5 and 7). This difference in B7.2 expression by the B cells does not seem to affect the initial proliferation of T cells in vivo, even when antigen is only presented by the tolerant B cells in the Bb$^{\rightarrow}$kb chimeric recipients. The initial proliferation of T cells in vivo may either be B7/CD28 independent or bystander APCs may provide this costimulation in trans, even when they cannot present the antigen. A similar, initial proliferative burst has been noted in response to peptides or superantigens in CD28$^{\rightarrow}$ mice (51, 52) supporting the notion that this default pathway of abortive proliferation is CD28 independent. It is intriguing that antigen-exposed naive B cells and tolerant B cells induce an equivalent T cell abortive proliferative response in vivo despite eliciting distinct T effector functions (help versus killing). We are currently testing whether interaction with activated and tolerant B cells induces different cytokine expression by the T cells.

In these experiments, minute quantities of antigen associated with rare antigen-specific B cells elicited a profound effect on rare antigen-specific T cells. This approach contrasts with those used previously in which large quantities of antigen or superantigens were injected (22, 31, 39, 53–57) often into mice containing very high frequencies of antigen-specific T cells. The proliferation followed by disappearance over a 5-d time course that is induced by these small quanta of antigen is nevertheless remarkably similar to what has been observed in the high antigen dose models (31, 51–53, 57). The similarity between our findings using these small quanta of antigen and the previous observations using much higher doses of antigen suggest that abortive proliferation of T cells is a default mechanism for initial T cell responses to antigen. Factors that dictate whether a proliferative response will be sustained, such as TNF or other adjuvant effects, may be additive to this initial default response of abortive proliferation (55).

It seems counterintuitive that a response as potentially dangerous as T cell proliferation would be "hard wired" as a default response. The key controlling factor must be an equally hard-wired default disappearance mechanism, which prevents T cells from sustaining exponential proliferation. A likely candidate mechanism is activation-induced cell death (AICD), a Fas-mediated T cell suicide mechanism that has been shown to limit T cell proliferation in vitro and in vivo (58–62). Activated T cells express both Fas and Fas ligand, and can kill themselves by Fas-mediated lysis. The control of abortive versus sustained proliferation of T cells may be critical for the maintenance of peripheral self-tolerance. The disappearance of T cells after B cell transfer is likely to be tolerogenic, by the reduction in T cell precursor frequency. In continuing experiments, we are testing whether the disappearance is Fas mediated and whether the remaining T cells have been functionally inactivated.

B cells represent a potential threat to the maintenance of self-tolerance because of their well-characterized ability to concentrate a unique subset of antigens determined by their BCR specificity and to present these antigens to T cells (9, 10). Self-reactive B cells can present self-antigen to self-reactive T cells and so initiate autoimmune responses or contribute to epitope spreading (63, 64). Similarly, high affinity memory B cells located in the sites of antigen entry to the mucosa and the lymphoid organs concentrate antigens, migrate to T zones, and there initiate or amplify immune responses, which may include allergic responses to nonpathogenic antigens (1, 2). The physiological relevance of these phenomena is highlighted by the presence of autoantibodies in T cell zones in autoimmune MRL/lpr mice (65) and the association of the presence of B cells with the initiation of diabetes in nonobese diabetic mice (48). T cell
abortive proliferation after encounter with antigen concentrated by B cells may provide a regulatory checkpoint that limits the effects of antigen presentation in the absence of inflammation, and bypassing this checkpoint may result in pathogenic T cell responses in allergy and autoimmunity.

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