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)\(^1\) 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 cell 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.

\(^1\)Abbreviations used in this paper: BCR, B cell receptor; HEL, hen egg lysozyme; sHEL, soluble HEL; CFSE, 5- (and 6-) carboxyfluorescein diacetate succinimidyl ester.

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 congenic mice (gift of Dr. I. Weissman, Stanford University, Stanford, CA) to generate TCR+Ly5+H-2b donor mice. Transgenic mice expressing a HEL-specific BCR (IgHEL) and double transgenic mice expressing both the HEL-specific BCR and soluble HEL (HEL×IgHEL; reference 32) on the B6 background were bred with B10.BR mice to generate IgHEL+H-2b and HEL×IgHEL-H-2b donor mice. Double transgenic (HEL×IgHEL) 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 5 wk before use in experiments. Polyoxymyxin 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 (Milteny Biotec, Auburn, CA) as per the manufacturer’s instructions, and passing over MiniMACS columns (Milteny 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.

Western blot for HEL quantitation. B cells were purified from the spleens of HEL-specific IgHEL-transgenic and double transgenic (HEL×IgHEL) mice and pulsed in vitro with 10 ng/ml HEL as above. Cells were pelleted and lysed in 1% NP-40 under lys conditions previously described (34). Lysates of 3 × 10^5 cells were run on a 15% SDS-polyacrylamide gel under reducing conditions. HEL was detected by probing with H+ HEL9-biotin, followed by Streptavidin–horseradish peroxidase (N ycomed Amersham, Buckinghamshire, UK) and Renaissance detection system (New England Nuclear Life Sciences, Boston, MA). Quantitation was accomplished by adding titered amounts of exogenous HEL (Sigma Chemical Co., St. Louis, MO) to nontransgenic spleen cell lysates.

Results

The experimental system used to visualize the consequences of specific B-T cell interactions in vivo is dia-
grammed in Fig. 1 A. Lymph node and spleen cells were isolated from 3A9 TCR-gene transgenic mice of (B6-Ly5<sup>a</sup> × B10.BR)F1 background (13) providing a source of Ly5<sup>a</sup>-marked T cells specific for HEL peptide 46-61 bound to I-A<sup>z</sup>. Approximately 3 × 10<sup>7</sup> cells, which include ~3 × 10<sup>6</sup> HEL-specific CD4<sup>+</sup> T cells, were transferred into normal congenic Ly5<sup>b</sup> (B6 × B10.BR)F1 recipients that had not been immunized, irradiated, or treated in any way that might change the lymphoid microenvironment or provide adjuvant effects. The transferred cells were distinguished from the excess of host cells by flow cytometry using antibodies specific for allotypic markers particular to the transferred cells (Ly5<sup>a</sup> for the transgenic T cells and IgD<sup>a</sup> for the transgenic B cells; Fig. 2). Flow cytometric analysis of the recipient mice demonstrated that HEL-specific T cells were seeded into the diverse immune repertoire at a low frequency of 1 in 1,000–2,000 splenocytes (see below), allowing us to track the cells and study their functions in a physiologically relevant context.

One day after T cell transfer, naïve B cells were isolated from the spleens of Ig<sup>HEL</sup>-gene transgenic mice, exposed to intact HEL at 37°C in vitro for 2 h, and washed before transferring 3 × 10<sup>6</sup> cells into each of the T cell recipient mice. The brief exposure to HEL in vitro loads the naïve B cells with antigen for presentation and activates them through BCR signaling (data not shown). Alternatively, HEL-tolerant B cells were isolated from the spleens of sHEL/Ig<sup>HEL</sup> double transgenic mice, yielding a population that have been chronically exposed to HEL so that they are loaded with HEL peptides without becoming activated through their BCR (36, 37). Flow cytometric analysis of the recipient mice demonstrated that HEL-specific B cells were also seeded into the repertoire at a low frequency of 1 per 1,000–2,000 cells (see below).

In the recipient mice, small numbers of HEL-specific T and B cells seed the white pulp of the spleen and migrate effectively to the T cells zones and B cell follicles, respectively (Fig. 1 C). The previously described TCR<sup>+</sup> cells that express neither CD4 nor CD8 (7, 13) did not traffic to the lymphoid organs (data not shown). B cells that had been exposed to HEL in vitro before transfer were preferentially retained in the outer T cell zone, consistent with the previously described migration pattern for cells whose BCRs have recently captured antigen and signaled (4, 5). The amount of antigen that had been captured by the B cells and transferred into the recipient mice was quantitated by Western blot analysis of whole cell lysates of the antigen-pulsed naïve B cells and tolerant B cells (Fig. 1 B). Approximately 2 ng of HEL is associated with the 3 × 10<sup>6</sup> B cells transferred to each mouse. This amount of antigen is 100–100,000-fold lower than the dose of antigen previously used to induce low-zone humoral tolerance (19, 20, 23, 38) or peripheral tolerance of CD4<sup>+</sup> T cells in vivo (31, 39–41).

Despite the low frequency of transferred T and B cells, the lack of adjuvant and irradiation, and the small amount of antigen, HEL-pulsed naïve B cells were helped to differ-
Abortive Proliferation of Rare T Cells In Vivo

Naive B (Ag-pulsed)
T + Naive B (Ag-pulsed)
T Only

Figure 2. Fate of B cells after T-B cell interactions in vivo. (A) Antigen-pulsed, acutely activated B cells downregulate IgD in the presence of transferred T cells. Transgenic Ly5a-marked T cells (3 × 10⁷ total cells containing 3 × 10⁶ CD4⁺ T cells) were transferred into intact recipient mice, followed 24 h later by transfer of purified (94% B220⁺) acutely activated B cells that had been pulsed with 1 μg/ml HEL or sham pulsed for 2 h in vitro. 60 h after transfer, the surface phenotype of the transferred B cells in the spleen was analyzed by flow cytometry using stains for HEL binding, IgD⁻, and negative gating for CD4. Each plot is representative of two or three individual recipients from each of three experiments. Similar results were obtained using B cells pulsed with 10 ng/ml HEL. (B) Antigen-pulsed, acutely activated B cells are helped by transferred T cells to become antibody-forming cells. Transfer as in A, except that mice were killed 5 d after B cell transfer. Antibody forming cells (AFC) in the spleen were quantitated by spot ELISA (66). Data are representative of two separate experiments. (C) Tolerant B cells are killed by transferred T cells via Fas-mediated lysis. Transgenic T cells were transferred as in A and B, followed by either wild-type or lpr/lpr tolerant B cells (1.8 × 10⁷ total cells containing 3 × 10⁶ HEL-binding cells). 60 h after B cell transfer, the number of HEL-binding B cells in the spleen was analyzed by flow cytometry, using stains for HEL binding, IgD⁻, and negative gating for CD4. The background value in a mouse that received no transferred B cells was zero. Dots, the number of HEL-binding B cells from the spleen of an individual recipient mouse. These results are representative of four separate experiments for the wild-type tolerant B cells, and two separate experiments for the lpr/lpr tolerant B cells.
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 Ig^HEL^ B cells of the H-2^bb^ MHC haplotype, which lack the I-A^k^-presenting element recognized by the HEL-specific TCR. These B cells could not directly interact with HEL/I-A^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^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.

Figure 3. Transfer of antigen-pulsed HEL-specific B cells or HEL-tolerant B cells induces the activation of most transferred HEL-specific CD4^+^ 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^a^, and CD69, with negative gating for B220. The histograms represent CD69 staining on the small boxed CD4^+^Ly5^a^ population. The numbers indicate the percentage of CD4^+^Ly5^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^bb^ or H-2^kb^). 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^bb^ in two additional experiments.
from the spleen, the lymph node, and the blood by 5 d after transfer. Transfer of H-2\textsuperscript{bb}-tolerant B cells resulted in a similar degree of T cell proliferation and disappearance, demonstrating that cross-presentation of antigen by endogenous APCs was sufficient to mediate the aborted proliferative response. Increasing the number of transferred tolerant B cells did not decrease the number of remaining CFSE high T cells, suggesting that the remaining cells may not be capable of proliferation.

Surprisingly, HEL-pulsed naive B cells induced a comparable cycle of T cell proliferation and disappearance from the spleen, lymph node, and blood (Figs. 5 and 7), despite the fact that the acute antigen pulse triggers BCR signaling and activates the naive B cells. In contrast, when HEL antigen was given as a depot in adjuvant, 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 BCR's 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 aborted 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 lacked I-A\textsuperscript{k}–expressing bone marrow–derived APCs, thus eliminating cross-presentation of antigen. For this purpose, recipient mice were constructed by lethally irradiating (B10.BR × B6)F1 mice (H-2\textsuperscript{kb}) and reconstituting them with parental B6 (H-2\textsuperscript{kb}) 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 liable or depleted. The chimeric mice were held for 9 wk before use in these experiments to allow time for the loss of liable or depleted. The chimeric mice were held for 9 wk before use in these experiments to allow time for the loss of liable or depleted.

The HEL-tolerant B cells were killed by the T cells as efficiently as they were killed in conventional H-2\textsuperscript{bb}-recipient mice where I-A\textsuperscript{k}–expressing bone marrow–derived APCs, but remain tolerant to H-2\textsuperscript{bb} due to expression of H-2\textsuperscript{k} on radioresistant cells such as thymic epithelium.

Transfer of antigen-specific T cells with tolerant B cells expressing H-2\textsuperscript{bb} or H-2\textsuperscript{kb} into chimeric recipients [bb→kb] showed that, under these circumstances, antigen presentation to the T cells depended upon B cells bearing H-2\textsuperscript{k}. Thus, HEL-specific T cells were not induced to express CD69 nor to proliferate after transfer of H-2\textsuperscript{bb}-tolerant B cells in bb→kb chimeric recipients, whereas they were triggered if the B cells expressed I-A\textsuperscript{k}–presenting molecules (Fig. 8). The absence of T cell activation or proliferation demonstrates that H-2\textsuperscript{bb}-tolerant B cells do not transfer antigen to residual I-A\textsuperscript{k}–expressing B cells, thus 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. T cells proliferate and disappear after transfer of tolerant B cells. Transfer as in Figs. 2 and 5 with CFSE-labeled transgenic TCR\textsuperscript{a} cells and purified transgenic B cells (B220\textsuperscript{hi} for all donor types 92–98%) that were I-A\textsuperscript{k}– (H-2\textsuperscript{bb}) or I-A\textsuperscript{k}– (H-2\textsuperscript{kb}) and time points taken at days 3 and 5. Dots, the value from an individual recipient; bars, the mean value for the group. IgG\textsubscript{2a}–antigen-pulsed IgHEL–transgenic B cells, Dbl Tg, double transgenic shHEL × IgHEL–tolerant B cells. One group of mice was injected subcutaneously with 100 \textmu g HEL emulsified in complete Freund's adjuvant (HEL/CFA) as a positive control for T cell proliferation. The number of HEL-specific T cells was analyzed by flow cytometry and the number of CFSE high (above fluorescence channel 160) and CFSE low (below fluorescence channel 160) were counted. The number of HEL-specific T cells per 10\textsuperscript{5} cells for each individual was normalized to the number of nonspecific CFSE high B cells for that individual divided by the average number of nonspecific CFSE high B cells in order to correct for any small differences in injection efficiency between different recipients.
Figure 7. HEL-specific T cells disappear to the same degree after transfer of antigen-pulsed B cells, tolerant B cells, or excess tolerant B cells. Transfer as in Fig. 2 using CFSE-labeled transgenic TCR Tg cells and unpurified splenocytes from B cell donors. Dots, the number of HEL-specific T cells of each phenotype from an individual recipient; bars, the mean value for the group. IgTg, antigen-pulsed IgHEL-transgenic B cells; Dbl Tg, double transgenic tolerant B cells. One group of mice was injected subcutaneously with 100 μg HEL emulsified in RIBI adjuvant (HEL/RIBI) as a positive control for T cell proliferation. Values are flow cytometric data analyzed as in Fig. 6.

Discussion

The results presented here demonstrate that B cell presentation of antigen to T cells can have profound effects on T cell fate in vivo, and that, together with a novel mechanism of antigen transfer to endogenous APCs, such antigen presentation may be important in limiting T cell responses to antigen in the absence of inflammation. Influenza antigen-specific T and B cells efficiently find each other in intact recipients, in the absence of inflammation, and in the context of a normal immune repertoire, unperturbed microenvironment, and trace amounts of antigen. The consequences of these interactions differ for acutely activated naive B cells and tolerant B cells, such that the T cells help the naive 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 cell 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 allergens.

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-2kb B cells activated T cells in the spleen, which suggests that B cell-bound antigen is transferred to endogenous I-Ak-expressing APCs that then present antigen to T cells. In contrast to the spleen, tolerant H-2kb B cells do not induce CD69 expression by T cells in lymph node (Fig. 4). The efficient cross-presentation of transferred antigen by endogenous APCs in the spleen and not the lymph node, as well as the lack of CD69 expression by T cells in the blood, raises the possibility that the responsible APC is resident in the spleen, rather than one that acquires antigen in the periphery and migrates through the blood to the spleen or through the lymph to the lymph nodes. The essential property of the endogenous APC may be the ability to receive and present transferred antigen with high efficiency, a property 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...
cells to endogenous APCs, which could be antibody medi-
ated, such as Fc receptor or complement receptor uptake of
antigen–antibody complexes, or cell mediated, such as phago-
cytes of whole cells. We have identified the endogenous
APC that receives antigen transferred from B cells as a la-
bile 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 rel-
vent restriction element, I-$A^a$. Interestingly, cultured my-
eloid 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 chan-
nels between the B cell follicles of the spleen (43), and lym-
phoid 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 tol-
erance to some superantigens (45), the injection of micro-
gram 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 absence of low serum levels of antigen (>0.5 mg/ml)
did not exclude a role for thymic deletion in the observed
tolerance (47). B cells may be necessary for the main-
tenance 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 APCs.

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 unspecified, 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, in-
duce 3A9 TCR transgenic cells to proliferate and secrete
cytokines in vitro (13). Surprisingly, both tolerant and ac-
tivated HEL-specific B cells induce abortive proliferation in
T cells in vivo (Figs. 5 and 7). This difference in B7.2 ex-
pression 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 $bb(-/->)$ 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$-/-$ mice (51, 52) supporting the notion that this def-
ault pathway of abortive proliferation is CD28 indepen-
dent. It is intriguing that antigen-exposed naive B cells and
tolerant B cells induce an equivalent T cell abortive prolif-
erative response in vivo despite eliciting distinct T effector
functions (help versus killing). We are currently testing
whether interaction with activated and tolerant B cells in-
duces different cytokine expression by the T cells.

In these experiments, minute quantities of antigen asso-
ciated with rare antigen–specific B cells elicited a profound
effect on rare antigen–specific T cells. This approach con-
trasts 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 an-
tigen-specific T cells. The proliferation followed by disap-
ppearance 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
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 pre-
cursor frequency. In continuing experiments, we are test-
ing 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 con-
tribute 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, mi-
grate to T zones, and there initiate or amplify immune re-
sponses, which may include allergic responses to nonpatho-
genic antigens (1, 2). The physiological relevance of these
phenomena is highlighted by the presence of autoreactive
B cells in the 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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