Novel Immunoregulatory B Cell Pathways Revealed by lpr+ Mixed Chimeras

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lpr, a murine mutation of the Fas apoptosis receptor, causes lymphadenopathy and autoantibody production, with lymphadenopathy primarily due to a population of CD4-CD8- B220+ T cells. Previous in vivo experiments, in which lpr and normal bone marrow cells were coinfused into lpr hosts, have demonstrated that only T cells of lpr origin accumulated abnormally and only B cells of lpr origin produced autoantibodies. Moreover, in these chimeras, B cells of normal origin were unable to respond to conventional, T cell-dependent exogenous Ag. To address the role of lpr B cells in regulation of lpr autoimmunity, we have prepared lpr+ mixed chimeras and selectively eliminated lpr B cells using allele-specific, mAb treatment, thus allowing normal B cells to develop in an environment with lpr T cells. From these data, we arrived at four major conclusions: 1) Compared with control-treated chimeric mice, lpr B cell-depleted mice had greatly reduced total lymph node cell counts; 2) the T cells were derived equally from normal and lpr donors, and the percentage of lpr-derived CD4-CD8- T cells was greatly reduced; 3) despite the presence of the remaining lpr T cells, no autoantibodies were produced by the normal derived B cells; and 4) lpr T cells without lpr B cells were unable to prevent a normal B cell response to conventional Ag. These data demonstrate that B cells can play a critical and expansive regulatory role, not only for T cells, but for other B cells as well. The Journal of Immunology, 1998, 160: 1497–1503.

The lpr and gld models have been very important in understanding the role of apoptosis in maintaining tolerance. Mice homozygous for either the lpr or the gld mutation develop lymphadenopathy and high titers of autoantibodies with a spectrum similar to that of human systemic lupus erythematosus (1). The lymphadenopathy is largely due to the accumulation of a T cell population that lacks expression of either CD4 or CD8 and aberrantly expresses other cell surface Ags such as the B220 isoform of CD45, usually found on B cells. It is now known that lpr is a mutation in the Fas apoptosis receptor, while gld is a mutation of the Fas ligand (FasL) (2). Cross-linking of the Fas receptor on activated lymphocytes in vitro caused induction of programmed cell death, suggesting a major role in maintaining peripheral tolerance (3, 4). Recent in vitro data have also suggested that Fas may augment other mechanisms of central (thymic) tolerance (5, 6), but its in vivo importance is uncertain, as reflected by normal deletion of autoreactive T cells by endogenous superantigen (7, 8).

Previous in vivo experiments have shown that full expression of the lpr phenotype required that the lpr mutation be present in both the T and B cell populations. When a combination of normal and lpr bone marrow was coinfused into an lpr host, only T cells of lpr origin hyperproliferated (9) and only B cells of lpr origin produced autoantibodies (10). In fact, normal derived B cells appeared to be suppressed in their abilities to respond to T cell-dependent Ags (10). In additional experiments, it was shown that lpr T cells were necessary to drive lpr B cells to produce these autoantibodies (11). That is, normal T cells alone in the presence of lpr B cells were insufficient to cause a break in tolerance. In complementary experiments, bone marrow cells of normal origin could largely correct the gld defect, demonstrating that the gld mutation caused a lesion that was extrinsic to the abnormally behaving T and B cells (12, 13). We originally modeled the Fas and FasL interactions as one of interacting molecules, both reciprocally expressed on T and B cells (14). Additional evidence indirectly supporting this model was the observation that lpr mice lacking B cells had decreased lymphadenopathy (15, 16). The paradigm of reciprocal expression was put into question by the later findings that FasL, was expressed on T cells, but not B cells, although Fas was found on both populations (17). Support for the critical role of T cell expression of FasL came from additional in vivo functional experiments, in which gld-+ mixed chimeras were constructed and selectively depleted of either normal T or normal B cell populations (18). Autoimmunity was restored only with depletion of the normal T cell population. Finally, lpr mice homozygous for targeted deletion of class II expression had decreased autoantibody production, but not decreased lymphadenopathy (19), while lpr mice lacking expression of class I showed the converse phenotype (20). Taken together, these data suggested that apoptosis of T cells was being regulated by another T cell population with no direct contribution by B cells. In the past few months, the in vitro expression of FasL on activated B cells has been demonstrated (21, 22). However, the functional significance of this finding in vivo remains uncertain.
To test the specific role of lpr B cells in the development of lymphadenopathy in lpr mice, we constructed lpr+ mixed chimeras and have selectively eliminated lpr B cells. In these mice, there was a dramatic decrease in the number of lpr-derived T cells, with a corresponding major reduction in the CD4+CD8− B220+ population. The presence of the remaining lpr T cells was insufficient to cause autoantibody production and did not suppress the response of normal derived B cells to a T cell-dependent Ag. These data suggest a fundamental regulatory role for B cells in the maintenance of peripheral tolerance by Fas-mediated apoptosis.

Materials and Methods

Mice

C57BL/6 (B6), C57BL/6.Thy-1IgG1-Gpt (B6.TC), and C57BL/6.MRL/lpr (B6/lpr) mice were originally obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained in our breeding colonies at University of North Carolina at Chapel Hill and/or at University of Florida (Gainesville, FL). C57BL/6.I-Eα+ mice were originally obtained from Dr. M. Kimoto (Saga Medical School, Saga, Japan), and B6.C20 (B6.Ighb) from Gayle Bosma (Philadelphia, PA). B6/lpr-I-Eα+ and B6/lpr-IgG1 mice were produced as previously described (10, 23). The former strain has decreased lymphadenopathy and decreased autoantibody titers relative to normal B6, B6.Ighb, and B6.TC mice (23).

Preparation of mAb for in vivo treatment

HB63 (murine IgG2a anti-human IgA) and the cytolytic Ab 14-4-4s (murine IgG2a anti-I-Eα) were obtained from American Type Culture Collection (Rockville, MD). MmT1 and HB63 were prepared as ascites from hosts, as previously described (11), and were used as ascites, as hosts, previously described (11), while the 14-4-4s mAb was prepared in irradiated (450 rad) BALB/c mice. Ascites were concentrated and partially purified as the precipitate of a 40% saturated ammonium sulfate cut, dialyzed against PBS, and stored frozen at 9 mg/ml until used.

Production of mixed chimeras

One week before cell transfer, 2- to 3-mo-old B6/lpr-IgG1 (Exp. 1) or B6/lpr (Exp. 2) host mice were housed in autoclaved cages in an isolation cubicle and placed on either neomycin (0.2% w/v)- or trimethoprim-sulfamethoxazole (1% w/v; Lemmon Company, Sellererville, PA)-treated water. On the day before cell transfer, the mice received 900 rad of γ-irradiation in a Cs137 source (Atomic Energy of Canada, Ontario, Canada). In later experiments, the mice received two doses of 525 rad of γ-irradiation separated by 3 h (24). Pilot experiments showed that the latter protocol enhanced survival, yet permitted complete engraftment by donor bone marrow cells. Bone marrow cells were prepared from the femora and tibiae of age- and sex-matched congenic mice. The procedure was as previously described, including the use of mAb and complement to eliminate contaminating T cells (11). Mice received an equal number of bone marrow cells from the two donor strains, as indicated in Results. For each transfer, a total of 107 cells in 0.5 ml of HBSS was given by tail vein injection.

Beginning 1 wk after cell transfer, mice received twice weekly injections of either 14-4-4s or the irrelevant control mAb HB63. Dose was 1 mg/injection and was continued for the duration of the experiments. Mice were bled at monthly intervals for flow cytometric and/or ELISA analysis.

Immunization with human IgG

Four months after preparation of the chimeras, mice were given 50 μg of human γ-globulin (HGG) purified from Cohn Fraction II of human serum (Sigma Chemical Co., St. Louis, MO). Injections were given s.c. in CFA and boosted with an additional 50 μg s.c. in CFA 10 days later. On day 17, the mice were bled by tail vein.

Flow cytometry

Two to three months after initiation of the experiment, mice were checked routinely for effectiveness of Ab treatment by IgM allelically specific immunofluorescence on PBMCs. Blood was collected into heparinized tubes immediately before the next scheduled injection, and mononuclear cells were isolated on a Lymphocyte M (Cedarlane, Ontario, CA) density gradient. At the completion of each experiment, the mice were killed and lymph nodes and spleens were collected, weighed, and placed into single cell suspension in buffered HBSS supplemented with 5% FCS and 0.1% NaN3. RBCs were lysed with NH4Cl, and the total cell number was determined by hemocytometer or Coulter counter (Coulter Corp., Hialeah, FL). Immunofluorescence staining was routinely performed in 96-well round-bottom microtiter plates (Corning Glass Works, Corning, NY). For cell surface staining of B cells, saturating amounts of fluoresceinated affinity-purified DS-1 (murine IgG1 anti-IgM M with biotinylated AF6-78.25.2 (murine IgG1 anti-IgM M) were added as first step. The second step consisted of avidin-coupled phycocerythrin (Jackson ImmunoResearch Laboratories, West Grove, PA). In the studies depicted in Figure 1, biotinylated MmT1 (murine IgG2a anti-Thy-1.1) were added as first step. The second step consisted of avidin-coupled phycocerythrin (Jackson ImmunoResearch Laboratories, West Grove, PA). In the studies depicted in Figure 1, biotinylated MmT1 (murine IgG2a anti-Thy-1.1), the generous gift of Dr. Elisabeth Kremmer, Munich, Germany) was used for two-color immunofluorescence of T cells. For allele-specific Thy-1 staining of Figure 2, biotinylated anti-CD90.1 (Thy-1) and anti-CD90.2 (Thy-1) (PharMingen, San Diego, CA) was used along with the combination of directly fluoresceinated anti-CD4 and anti-CD8 (PharMingen). For determination of B220+ T cells, directly fluoresceinated anti-B220 (PharMingen) was substituted for the combination of anti-CD4 and anti-CD8. On the last step, the cells were suspended in PBS and fixed with an equal volume of 2% paraformaldehyde in PBS. Analysis was performed on a Becton Dickinson FACScan (San Jose, CA) equipped with Cicerco software (Cytomation, Fort Collins, CO) or by collection using B-D software, followed by analysis with WinMDI version 2.1A software (available as freeware from Dr. Joseph Trotter at http://facs.scripps.edu). The lymphocyte population was identified by size gating.

Allotype-specific ELISA

The protocols used for measurement of allotype-specific serum total IgM, IgM anti-IgG2b rheumatoid factor, IgG2a anticharcinoma, and IgG2a anti-HGG have been described in detail (10). The only modification is that the concentration of serum total IgM is now reported in equivalent dilution factors of standardized reference B6/lpr and B6/lpr-IgG1 sera. This is defined by the formula: equivalent dilution factor = (dilution of standard reference sera, which gives the equivalent OD of the test serum) × 105.

Results

Elimination of lpr B cells from lpr+ mixed chimeras resulted in greatly decreased lymphadenopathy and loss of CD4+CD8− T cells

Previous experiments had documented the crucial role of lpr T cells in the development of lymphadenopathy. To determine the role of lpr B cells, lpr+ mixed chimeras were prepared using B6/lpr-IgG1 host mice and lpr-derived B cells selectively eliminated. In the first set of experiments (data not shown), elimination of lpr B cells was attempted with b allotype-specific anti-IgM (AF6-78.25). However, probably due to the presence of competing circulating IgM in the host (also b allotype) and the less effective complement-fixing properties of an IgG1 MAb (25, 26), depletion was incomplete. To circumvent this problem, B6 and B6/lpr mice expressing the I-Eα transgene were utilized as donors, and B cells were eliminated with the IgG2a anti-I-E MAb 14-4-4s. As shown in Figure 1, this treatment was highly effective in eliminating b allotype B cells of either normal (C) or lpr (G) origin. Moreover, with the elimination of lpr B cells, there was a marked decrease in percentage of CD4+CD8− Thy-1+ T cells (F vs H) to levels comparable with mice given only normal bone marrow (B and D). Not only were the percentages of CD4+CD8− T cells decreased, but lymphadenopathy was reduced such that total lymphocyte count from lymph nodes and spleen was again comparable with mice receiving only normal bone marrow (Table I).

With elimination of lpr B cells, normal T cells accumulated in appropriate numbers in the periphery

Although the experiment described in the paragraph above demonstrated that lpr B cells were crucial to the full development of lymphadenopathy, it could not be determined whether the remaining T cells were of lpr or +/- origin. Accordingly, additional bone marrow reconstitution experiments were performed in which both lpr and +/- T and B cells were differentially marked by allotype-specific cell surface Ags. To accomplish this, the B6.TC (Thy-1+, Igδ+) strain was used as normal bone marrow donor. In control-treated lpr+ mixed chimeras, very few CD90.1 (Thy-1+) T
cells of normal origin were found 5 mo later (Fig. 2C). This contrasted somewhat with our findings published earlier using donors with single-congenic strain differences, which showed marked but incomplete skewing toward the lpr donor (9–11). The poor representation of normal derived T and B cells using the B6.TC strain is consistent with our previous experience in gld-1 mixed chimeras, suggesting that the additional congenic loci placed the B6.TC strain at a competitive disadvantage relative to single congenic strains (18). Despite this disadvantage, elimination of lpr B cells from these mixed chimeras resulted in development of appropriate numbers of normal B cells (Fig. 2B). More surprisingly, the T cells were now of both lpr and normal origin, and in comparable numbers (Fig. 2, D vs F, upper quadrants). Again, few

Table I. Selective elimination of lpr B cells in lpr +/− mixed chimeras greatly reduced lymphadenopathy

<table>
<thead>
<tr>
<th>Donor Composition</th>
<th>n</th>
<th>Treatment</th>
<th>Cell Number $\times 10^6$ (SD)</th>
<th>Splenic Phenotype (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lymph node</td>
<td>Spleen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>% IgM</td>
<td>% DNT</td>
</tr>
<tr>
<td>Expt. 1$^d$</td>
<td>4</td>
<td>Control</td>
<td>43 (15)</td>
<td>113 (30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-I-E</td>
<td>29 (12)</td>
<td>100 (19)</td>
</tr>
<tr>
<td>B6.Igh$^b$ and B6.I-Eo$^d$</td>
<td>5</td>
<td>Control</td>
<td>119 (40)</td>
<td>180 (30)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-I-E</td>
<td>35 (18)</td>
<td>76 (20)</td>
</tr>
<tr>
<td>Expt. 2$^e$</td>
<td>4</td>
<td>Control</td>
<td>N.D.</td>
<td>250 (45)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Anti-I-E</td>
<td>N.D.</td>
<td>20 (20)</td>
</tr>
</tbody>
</table>

* Number of mice.
$^d$ Double-negative T cell, defined as CD90.2 CD4^- CD8^-.
$^b$ Ig heavy chain allotype.
$^e$ B6/lpr-1gh$^b$ host.
$^f$ B6/lpr host.
CD4⁺ CD8⁻ B220⁺ T cells were present in mice depleted of lpr B cells to produce autoantibodies. Accordingly, in mixed chimeric mice depleted of B cells, allotype-specific IgM anti-IgG2b rheumatoid factor (Fig. 4A) and IgG2a antichromatin (Fig. 4B) ELISAs were performed. In mice given only normal bone marrow, autoantibody titers were low, as expected and in keeping with previous experience (10). Control-treated mice given a mixture of normal and lpr bone marrow produced high titers of autoantibodies, all of lpr donor-derived b allotype. The lack of a allotype autobody production in either of the control-treated groups suggested that there was little recovery of a allotype host-derived lpr B cells in these chimeras. Interestingly, even in the absence of competing lpr B cells and despite the presence of lpr-derived T cells, B cells of normal origin failed to produce autoantibodies. However, these B cells were present and producing IgG2a (Fig. 3).

In the absence of lpr B cells, lpr T cells did not down-regulate a conventional T cell-dependent Ab response

It had been observed previously that in lpr⁺ mixed chimeras, normal derived B cells failed to respond to immunization with T cell-dependent foreign Ags (10). To determine whether lpr T cells alone would be sufficient to suppress a normal B cell response, we immunized control- and anti-I-E-treated chimeras with HGG, a potent T cell-dependent Ag. In the absence of lpr B cells, normal B cells responded vigorously to immunization (Fig. 5). In fact, titers were equal to or greater than found in mice given only normal bone marrow. Thus, the functional suppression by lpr-derived donor cells required the presence of lpr B cells.

Discussion

Our understanding of the role of apoptosis in the regulation of peripheral tolerance has exploded in the past 3 yr following the discovery of the Fas receptor and its ligand. Inasmuch as lpr is a mutation of Fas, the importance of Fas in regulating autoimmunity was quickly apparent. What has more recently become clearer, however, is that Fas also has an expanded role as a down-regulator of a normal immune response, a process that has been termed...
activation-induced cell death (27–29). In the absence of functional Fas, mice accumulate large numbers of lymphocytes in the periphery, the majority of which are CD4+CD8− T cells. These T cells show evidence of previous activation in vivo (30), although they proliferate poorly to typical mitogens in vitro (1). Although less emphasized, in absolute but not relative terms, the B cell compartment in lpr and gld mice is also greatly expanded. Thus, Fas has an active role in maintaining T and B cell homeostasis. The exact cellular mechanisms by which Fas maintains homeostasis are still unclear. There is in vitro (31) and in vivo (13) evidence for autocrine function of FasL and Fas for T cells. However, the dominant pathway is probably mediated through cognate, cell-to-cell interactions (32, 33). In vitro data have demonstrated functional expression of FasL on CD4+ (particularly Th1) (34) and CD8+ (35) T cells, macrophages (36), dendritic cells (37), and B cells (21), but the relative role of each population is as yet unknown. In vivo experiments with gld mice showed that normal derived T, but not normal derived B cells could prevent autoimmunity in mixed chimeras, suggesting that functional FasL can be expressed only on T cells (18). However, it is known that the lpr mutation causes massive up-regulation of functional FasL (17, 38), and an important role for participation of B cells in Fas-mediated homeostasis has been suggested by a reduction in lymphadenopathy in lpr mice lacking B cells, either through anti-IgM treatment (15, 39) or by induced mutation (16).

To test the specific role of lpr B cells explicitly, we have constructed lpr−/− mixed chimeras such that lpr−/− derived, but not normal derived cells expressed I-E. We then selectively depleted lpr B cells using a mAb to I-E. By allotype-specific flow cytometric analysis and by total IgM and IgG2a ELISA, α allotype B cells were eliminated effectively from the chimeras. With this elimination, there were a number of dramatic changes. First, there was a marked decrease in lymphadenopathy with both an absolute and relative decrease in CD4+CD8− B220+ T cells. Second, normal derived T cells, which comprised less than 1% of the lymphocyte population in control-treated chimeras, accumulated in numbers similar to those for lpr-derived T cells. What CD4+CD8− T cells that did persist were still of lpr origin. Third, normal derived B cells, which also accumulated in low numbers in control-treated mixed chimeras, were present in percentages comparable with mice receiving only normal bone marrow. Fourth, even in the presence of lpr-derived T cells, normal derived B cells did not produce significant quantities of autoantibodies. Fifth, even in the presence of lpr-derived T cells, normal derived B cells could be stimulated to produce a strong IgG response to a conventional T-dependent Ag.

Elimination of lpr B cells might have been predicted to have a minimal effect on lymphadenopathy and a more pronounced salutary effect on glomerulonephritis. One study of B6/lpr mice treated with rabbit anti-mouse IgM showed a twofold reduction in splenomegaly and a fourfold reduction in lymphadenopathy compared with the control-treated group (15). The anti-IgM group clearly had increased lymphocyte numbers compared with control-treated normal C57BL/6 mice, and the residual lymphadenopathy was still due predominantly to double-negative T cells. A brief report in a second paper confirmed these findings (39). More recently, gene knockout therapy has been used to examine Fas pathway of a cross between mice carrying a homozygous deletion of Jh (and therefore lacking B cells) and the MRL/lpr strain (16). At about 5 to 6 mo of age, there was an eightfold reduction of total lymph node cell number in lpr mice without B cells, although again the numbers were greater than seen in non-lpr littermates. A similar reduction was seen in spleen cell number. At most, there was only a modest decrease in the percentage of CD4+CD8− T cells, although there was a marked reduction in total number. Interestingly, there was no consistent decrease in CD4+ T cells.

Taking all of the above studies together, the magnitude of effect of anti-I-E Ab treatment on lymphadenopathy in our chimeric mice was greater than might have been predicted based on depletion of B cells alone. It is unlikely that this is due to nonspecific toxicity. The anti-I-E-treated mice appeared as healthy as the control group, which also received an isotype-matched mouse mAb. Moreover, the lpr-B cell-depleted mice were able to mount vigorous Ab response to exogenous T cell-dependent Ag. It is possible that some of the differences with the literature are due to strain differences. Much of the cited work was on the MRL background, a strain that, even in the absence of the lpr or gld mutation, develops autoimmunity (1).

Another major difference between our study and previously reported lpr-B cell-depletion studies is that we constructed our chimeras in such a way that normal B cells were allowed to develop, thus permitting lpr T normal B cell interactions. It may be that the normal B cells were able to provide down-regulatory signals not available in the total absence of B cells. It has been shown recently that infusion of normal B cells could down-modulate some of the B cell abnormalities in autoimmune (NZB × NZW)F1, mice (40). However, a more likely possibility is that the anti-I-E treatment affected APC other than B cells. In the original description of the B6.I-Ea−/− transgenic mouse, adherent cells obtained from the peritoneal cavity demonstrated constitutive expression of I-E (41). Staining for I-E in peripheral blood and spleen was negative in our anti-I-E-treated chimeras (data not shown), suggesting that lpr donor-derived cells of monocyte and dendritic cell origin were also being depleted. Experiments with fully allogeneic chimeras have demonstrated that within a few weeks of reconstitution, most macrophages are of donor origin (42, 43). Whether lpr-derived macrophages are more radioresistant is unknown. However, in vivo data also indicated that MRL/lpr macrophages have an intrinsic defect (44). Moreover, in vitro data have shown that Th1 cells can delete activated macrophages by the Fas pathway (45), suggesting that the intrinsic defect in MRL/lpr macrophages is due to the lpr mutation rather than to the MRL background. Conversely, it also has been shown that dendritic cells cannot only kill CD4+ T cells by Fas/FasL interactions (37), but can also express Fas (46). Thus, these additional interactions involving Fas-deficient macrophages and dendritic cells may be lacking in our anti-I-E-treated lpr−/− mixed chimeras. Attempts to evaluate the relative presence of donor-derived Fas-deficient monocytes/macrophages in the spleen proved inconclusive. In two chimeras receiving a combination of
B6.TC and B6/lpr-E bone marrow without Ab treatment, approximately 30 to 40% of the spleen cells identified as being of the monocyte/macrophage lineage (by F4/80 expression) coexpressed I-E and I-A 5 mo after reconstitution, and were therefore of lpr donor origin (data not shown). However, no marker was present that could distinguish whether the remaining cells were of normal donor or lpr host origin. This same limitation would be expected to restrict any evaluation of dendritic cells. Nevertheless, the fact that lymphadenopathy can be greatly reduced by so many interventions, including CD4 T cell depletion (47), CD8 T cell depletion (47), or B cell depletion (16), strongly suggests that full manifestation of lpr disease requires participation of all subsets in defective activation-induced cell death.

One of the most striking features of our mixed chimeras was the unexpected finding that elimination of lpr B cells allowed normal B cells to develop and function. We had expected that the lpr T cells, which have been shown to express high levels of FasL constitutively (38), would have continued to eliminate or suppress normal B cells following activation by Ag (48). The lack of down-regulation raises several interesting possibilities regarding cellular interactions in our mixed chimeras. One possibility is that, in the absence of lpr B cells, lpr T cells were not abnormally stimulated, and thus failed to excessively up-regulate FasL or other surface Ags and/or cytokines. As mentioned above, other lpr-derived APCs might contribute to abnormal activation of lpr T cells, and these would probably have been affected by our anti-I-E treatment. Our experiments demonstrate the importance of Fas expression on APCs. However, they do not show directly which cell population is regulating peripheral tolerance. It might be that there is T-B-T cross-talk such that the T cells mediating apoptosis of other T cells interact through a common B cell intermediary.

A second, perhaps more intriguing possibility is that suppression of a normal derived B cell response was mediated through B cell/B cell interactions. There is a literature suggesting a role for such interactions in the genesis of both an autoimmune (49) and a normal immune response (50–52), although the basis has been unclear. In the past few years, however, a greatly enhanced understanding of germinal center formation and regulation has emerged (53). CD40/CD40 ligand (CD40L) interactions are crucial for the development of germinal centers (54, 55) and, until recently, it had been thought that CD40L expression was limited to T cells. However, it is known that T cells are absent from the germinal center dark zone, where B cell proliferation and protection from apoptosis occurs when the B cell receptor is engaged by Ag (56). Recent data demonstrate that CD40L can be expressed on activated B cells (56), as can FasL (21, 61). In normal germinal centers, Fas is expressed on B cells, but is not required for apoptotic regulation of the immune response (57). However, when stimulated through CD40, B cells can become sensitive to cross-linking of Fas (58).

Thus, it might be that, in the germinal centers of the mixed chimeras, activated lpr B cells with up-regulated FasL expression engaged activated normal B cells sensitized by CD40 engagement to undergo Fas-mediated apoptosis. Preliminary experiments by flow cytometric analysis have suggested FasL expression on a small population of splenic B cells in both lpr and normal mice (data not shown), but additional experiments by independent techniques will be required to confirm this. Alternatively, normal derived B cells may be at a competitive disadvantage for a limited supply of follicular niches (59), although it is less clear why this should be so for an Ag that is not self reactive. This latter hypothesis might predict that normal B cells, in a mixed chimera, would not be down-regulated in response to a T cell-independent Ag.

In summary, our data illustrate the unexpected finding that abnormal stimulation by Fas-deficient B cells (and possibly other professional APCs) is crucial for the full development of lpr disease, both for lymphoproliferation and autoantibody production, and that T cell abnormalities alone do not suffice. Moreover, presence of the abnormal B cells is required for suppression of nonautoimmune B cells. The pathways causing these profound effects are under active investigation.

Acknowledgments

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