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A New Role for B Cells in Systemic Autoimmunity: B Cells Promote Spontaneous T Cell Activation in MRL-\(lpr/lpr\) Mice

Owen Chan* and Mark J. Shlomchik2*†

A conventional view of the pathogenesis of systemic lupus erythematosus is emerging. The role of B cells is to secrete pathogenic autoantibodies, while the role of T cells is to provide help for autoantibody-producing B cells. A problem with this view is that spontaneous T cell activation as well as T cell infiltration of organs such as kidney and skin are prominent features in systemic lupus erythematosus patients and murine models of lupus. The identification of T cell infiltrates, in particular, suggests that autoantibody-mediated damage may be only part of the story and that T cells could also play a primary role in immune-mediated pathology. To test the role of B cells directly, we previously generated autoimmune-prone MRL-\(lpr/lpr\) mice that lack B cells. The complete absence of T cell infiltrates in these mice was surprising, and it prompted us to examine whether a key role of B cells in disease evolution is to prime autoreactive T cells. Here we demonstrate, by comparing B cell-deficient and control mice, that the expansion of activated and memory T cells in the MRL-\(lpr/lpr\) mouse is indeed highly dependent on B cells. These results suggest a novel role for B cells in autoimmune disregulation. The Journal of Immunology, 1998, 160: 51–59.

**Materials and Methods**

Construction of B cell-deficient MRL-\(lpr/lpr\) mice

Mice bearing a targeted deletion in the J\(H\) locus (J\(H\)) were bred with MRL-\(lpr/lpr\) mice (35). Animals homozygous for the deletion (J\(H/JH\)) are unable to assemble Ig H-chain genes and are thus devoid of mature B cells (36). The resultant F\(1\), J\(H/JH\) mice were backcrossed (BC) onto the MRL-\(lpr/lpr\) background, fixing \(lpr\) homozygosity after BC\(1\), PCR was used to type for the \(lpr\) allele (see below). At each BC, J\(H/JH\) \(lpr/lpr\) animals were intercrossed to yield J\(H/JH\) \(lpr/lpr\) mice (one-fourth of the progeny), which were used in this study. Mice in this study ranged from F\(2\) mice to BC\(1\) mice (99.9% MRL genes). No differences were observed between animals at different BC generations; thus, data from all are pooled in the figures and tables.

B cell-deficient (J\(H/JH\)) animals were identified by an anti-IgM ELISA and/or a PCR to detect the J\(H\) deletion. The deletion was detected via the combination of two PCRs. The Neo PCR detected the Neo insertion in the J\(H\) locus. The oligonucleotides used for this PCR were Neo\(^{5'}\) (CTTTGCGACGTGTGCAGCGTGGTG (5\(^{5'}\) primer)) and Neo\(^{3'}\) (GGCGATTGATGCACCGATGAGG (3\(^{3'}\) primer)). The J\(H\) PCR detected the wild-type J\(H\) locus. The oligonucleotides used for this
PCR were I$_{Pr}^0$ (GAGCAGGGGGCTAGTCATTCCAG) (5’ primer) and I$_{Pr}^3$ (GAGGAGACGGTGACCGTGGTCCCTGC) (3’ primer). Amplification conditions were: 94°C for 2 min; 35 cycles of 30 s each at 94°C, 62°C, and 72°C; followed by a 7-min incubation at 72°C. Homozygosity for lpr was detected by two PCRs. The oligonucleotides used for these PCRs were FAS-12FOR (AGCATAGATTCCATTTGCT (5’ primer)), FAS-12BAK (CAAATTITTTGTTCCCTGCACA (3’ primer)), and FAS-12BFK (AGAAATGGGCTCAATTGCGA (3’ primer)). Amplification conditions were: 94°C for 2 min; 35 cycles of 30 s each at 94°C, 58°C, and 72°C; followed by a 7-min incubation at 72°C.

Reagents and Abs

The following reagents were used: CD4 (H129.19-Quantum Red, Sigma Chemical Co., St. Louis, MO), CD8a (53-6.7-Quantum Red, Sigma Chemical Co.), CD44 (Pgp-1-FITC), CD45RB (C363.16A-PE, Pharmingen, San Diego, CA), CD62L (M24-14-biotin), Streptavidin-conjugated PE was added as a secondary step for the biotinylated reagents. Pgp-1, Mel-14, 145-2C11 (anti-CD3), and RA3-6B2 (anti-B220) were purified from hybridoma supernatants on protein G columns (Pharmacia, Piscataway, NJ) after ammonium sulfate precipitation and were conjugated as described (37). The Abs were verified by comparison to commercially available Abs with the same specificities.

Cell preparation and flow cytometry

This was performed essentially as described (37). Spleens and the inguinal LN were removed and disrupted in complete RPMI. Red blood cells were lysed in Tris-buffered ammonium chloride. Cells (1 × 10$^6$) were stained for 20 min with the primary Ab and for 15 min with a secondary Ab (where necessary) on ice. Cells were analyzed on a FACSscan (Becton Dickinson Immunocytometry Systems, San Jose, CA).

Anti-CD3 proliferation assay

Splenocytes were isolated and pooled two to three mice sharing the same phenotype. Red blood cells were removed by treatment with Tris-buffered ammonium chloride. The resultant cells then underwent two rounds of purification via magnetic beads in a VarioMacs Column (Miltenyi Biotec, Auburn, CA). First, the cells were depleted of B220$^+$ cells via negative selection with anti-B220 conjugated with biotin followed by MACS beads conjugated with streptavidin (Miltenyi Biotec, Auburn, CA). Then, the negative fraction underwent positive selection of CD4$^+$ cells using MACS beads conjugated directly with anti-CD4 (Miltenyi Biotec). This protocol usually yielded a purification of >90% CD4$^+$ cells. The cells then were sorted via flow cytometry (FACStarPlus, Becton Dickinson Immunocytometry Systems) into naive (CD44high, CD62Llow) and memory subsets (CD44low, CD62Lhigh). Typical purities as determined by sorting were >97%. Cells from the individual subsets were then cultured in a 96-well plate (Falcon, Franklin Lakes, NJ) that had been coated overnight with various concentrations of anti-CD3 (145-2C11). PMA (Sigma Chemical Co., St. Louis, MO) was added as a secondary step for the biotinylated reagents. Pgp-1, Mel-14, 145-2C11 (anti-CD3), and RA3-6B2 (anti-B220) were verified by comparison to commercially available Abs with the same specificities.

Statistics

Values from all groups were compared by the non-parametric Mann-Whitney U statistic, using StatView 4.5 (Abacus Software, Berkeley, CA) for the Macintosh. A two-tailed p value of <0.05 was considered significant. Comparisons with p values of >0.05 but <0.1 are discussed as approaching significance.

Results

Cell number and lymphoid organ weight

Splenomegaly and lymphadenopathy are characteristics of older MRL-lpr/lpr mice (7). This cell expansion is primarily composed of a TCR-$\alpha$-$\beta^+$, CD3$^+$, CD4$^+$, CD8$^-$, B220$^+$, CD24$^+$ subset of thymus-derived cells but also includes conventional CD4$^+$ and CD8$^+$ T cells (38–40). We previously reported that 6-mo-old B cell-deficient F$_2$ MRL-lpr/lpr mice have fewer splenocytes and reduced lymphoid organ weight than their B-intact counterparts (35). In the current study using 4- to 8-mo-old F$_2$ and up to BC$_{10}$ mice, we confirm and extend our previous findings (Table 1).

<table>
<thead>
<tr>
<th>Age/Genotype</th>
<th>Spleen Weight (mg)</th>
<th>Spleen Cell number (millions)</th>
<th>Lymph Node Weight (mg)</th>
<th>Lymph Node Cell number (millions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3–6 mo</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>B$^+$ lpr/lpr</td>
<td>452 ± 270*</td>
<td>1138 ± 82*</td>
<td>120 ± 140</td>
<td>43 ± 40</td>
</tr>
<tr>
<td>B$^-$ lpr/lpr</td>
<td>150 ± 30</td>
<td>55 ± 48</td>
<td>30 ± 30</td>
<td>30 ± 49</td>
</tr>
<tr>
<td>6–8 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B$^+$ lpr/lpr</td>
<td>720 ± 370*</td>
<td>226 ± 169*</td>
<td>190 ± 150*</td>
<td>63 ± 69*</td>
</tr>
<tr>
<td>B$^-$ lpr/lpr</td>
<td>180 ± 90</td>
<td>41 ± 44</td>
<td>40 ± 50</td>
<td>5 ± 5</td>
</tr>
</tbody>
</table>

* Spleen and lymph node organ weight and total cell numbers were obtained from B-intact ($B^+$) and B-deficient ($B^-$) mice. Averages and 1 SD were calculated to the fraction of naive T cells and a decrease in the fraction of memory T cells (data not shown).

Spleen and lymph nodes (LN) of B cell-deficient mice have fewer cells; similarly, in B cell-deficient MRL-lpr/lpr animals, the peripheral lymphoid organs are heavier than B-intact, nonhomozygous lpr mice (35). Thus, overall lymphoid organomegaly is highly B cell dependent. However, the magnitude of the differences (3- to 6-fold (Table I)) could not be accounted for simply by the absence of B cells per se, since they normally comprise less than one-half of the cells in LN and spleen and usually less than 20% in an MRL-lpr/lpr mouse (and see below).

B Cell role in spontaneous CD4$^+$ T cell activation

By 15 wk of age, more than one-half of the CD4$^+$ T cells in MRL-lpr/lpr mice are CD44high, a phenotype which suggests prior activation (41). To assess whether B cells play a role in the activation of T cells in spontaneous autoimmunity, we used this and other phenotypic markers to identify T cell differentiation state via flow cytometry. With the CD44 and CD62L cell surface markers, CD4 and CD8 T cells were identified phenotypically as being naive (CD44low, CD62Lhigh), activated (CD44high, CD62Lhigh), or memory (CD44high, CD62Llow) (42–44). Similarly, CD44 and CD45RB were used to identify naive (CD44low, CD45RBhigh) and memory (CD44high, CD45RBlow) activation states (42, 43, 45).

CD4$^+$ cell percentages. At 4 to 5 mo of age, only a small percentage of CD4$^+$ T cells in B-intact MRL-lpr/lpr mice had a naive phenotype in the spleen and LN, as assessed by CD44 and CD62L (Figs. 1 and 2). Activated cells comprised the second largest group of CD4$^+$ T cells, while the memory cells occupied the greatest fraction. These data are consistent with other reports on MRL-lpr/lpr mice. However, in the absence of B cells, naive cells comprised a markedly greater percentage of CD4$^+$ T cells as compared with the B-intact animals (spleen: 5.5-fold increase, p < 0.003; LN: 3.9-fold increase, p < 0.0005). Reciprocally, in B-deficient animals, memory cells comprised a smaller fraction in both spleen (39% decrease, p < 0.0005) and LN (69% decrease, p < 0.0002). There was no statistical difference in the percentage of activated cells. The CD44 and CD45RB activation markers similarly demonstrated that the absence of B cells causes a dramatic increase in the fraction of naive T cells and a decrease in the fraction of memory T cells (data not shown).

CD4$^+$ cell numbers. Cell counts revealed that the B-deficient lpr/lpr mice had fewer numbers of CD4$^+$ memory T cells than their B-intact counterparts, as determined by CD44 and CD62L (spleen: 42, 43, 45).
81% decrease, \( p < 0.005; \) LN: 82% decrease, \( p < 0.01 \) (Fig. 2, C and D). Thus, at 4 to 5 mo of age in MRL-\( lpr/lpr \) mice, the majority (>75%) of memory CD4\(^+\) T cells depend on the presence of B cells. Also, the B-deficient mice had fewer cells in the activated subset (spleen: 66% decrease, \( p < 0.03; \) LN: 40% decrease, \( p < 0.08 \)), where statistical significance is approached for the LN. However, the number of naive cells in the B-deficient mice was statistically indistinguishable from that of B-intact mice.

Both the percentage and number of memory cells CD4\(^+\) T cells are greater in B-intact mice than in B-deficient mice. The number of activated cells is also greater in B-intact mice, although B-intact and B-deficient animals do not differ statistically in the percentage of activated cells. Conversely, the percentage of naive cells is less in B-intact mice, although the total naive cell population number is similar. The different patterns observed with cell number vs cell percentage (especially in the naive subset) can be attributed to the differing total lymphoid organ cell counts between the two mouse phenotypes (Table I). The significance of these patterns will be discussed below.

**B cell role in spontaneous CD8\(^+\) T cell activation**

To assess whether other cell populations were affected by the absence of B cells, CD8\(^+\) T cell activation state was determined using the CD44 and CD62L markers. (A preliminary analysis of CD8 cells from a smaller cohort of mice has recently been reported as part of a review (46).) Analysis of this T cell population demonstrated trends similar to those of the CD4\(^+\) T cells. At 4 to 5 mo, in the absence of B cells, there was a decrease in the percentage of activated (spleen: 67% decrease, \( p < 0.05; \) LN: 70%, \( p < 0.05 \)) and memory (spleen: 45% decrease, \( p < 0.01; \) LN: 40%, \( p < 0.04 \)) cells (Fig. 3, A and B). There was no statistically significant difference in the percentage of naive cells.

The number of naive CD8\(^+\) T cells remained similar in the absence of B cells (Fig. 3, C and D). However, there was a decrease in the number of activated (spleen: 76% decrease, \( p < 0.05; \) LN: 96% decrease, \( p < 0.04 \)) and memory (spleen: 77% decrease, \( p < 0.07; \) LN: 89% decrease, \( p < 0.08 \)) cells, where statistical significance is approached for the memory subset of the spleen and LN. Thus, the results for CD8\(^+\) T cells mirrored that of CD4\(^+\) T cells.

**Age comparison of spontaneous T cell activation**

Lymphoaccumulation of CD44\(^{\text{high}}\), CD4\(^+\) T cells in MRL-\( lpr/lpr \) mice increases with age (Ref. 41; unpublished observations). To determine whether this progression was B cell dependent, we analyzed older cohorts (6–8 mo), in addition to the 4- to 5-mo-old cohorts. Here, we compared lymphoid organ weights and, in particular, cell numbers between the young and old cohorts with the same genotype. In B cell-intact MRL-\( lpr/lpr \) mice, there was an age-dependent increase in peripheral lymphoid organ weight (spleen: 1.4-fold increase; LN: 1.6-fold increase) (Table I). Organ weight in B-deficient mice also increased but to a lesser degree (spleen: 1.2-fold increase; LN: 1.3-fold increase). Although, there were trends to increased weight in spleen and LN in both groups, this difference did not reach statistical significance in any one group, probably due to the inherent mouse-to-mouse variation in \( lpr/lpr \) mice.

However, there was a statistically significant increase in memory CD4\(^+\) T cell numbers with age in the spleens of B-intact mice (1.9-fold increase, \( p < 0.03 \)) (Fig. 2C). This was not the case for
B-deficient mice, in which numbers of splenic memory cells remained stable, showing that the continued accumulation of memory T cells in the spleen is B cell dependent. The change in memory CD4 T cell number was attributable to the overall increased total splenic cell number and the increase in the percentage of memory cells (1.1-fold increase, \( p < 0.006 \)).

Memory CD4 T cells did not continue to expand in the LN after 4 to 5 mo of age. The reasons for this are not clear, but it is possible that the LN at 4 to 5 mo are not structurally capable of encompassing more cells. Consistent with this idea, there was no significant change in LN average weight between the 4- to 5-mo cohort and the 6- to 8-mo cohort of B-intact mice (Table I). Differences in homing patterns of CD62L-negative cells may also contribute to the difference between spleen and LN in this regard (47, 48).

**Role of B cells in spontaneous T cell activation is specific to autoimmune mice**

To ensure that the above-described phenomenon was related to autoimmunity in MRL-lpr/lpr mice, 33-wk-old B-deficient and B-intact mice with a BALB/c background (\( >BC10 \) to BALB/c) were investigated by flow cytometry using the same methods. As shown in Figure 4, there were no significant differences in the percentages of T cells in each of the compartments between B-deficient and B-intact mice. This is in marked contrast to percentages in even younger MRL-lpr/lpr mice (Figs. 1 and 2). As expected, there were much higher percentages of naive T cells in the BALB/c mice compared with MRL-lpr/lpr (compare with Figs. 1 and 2). Similar data were obtained for CD4 + cells in spleen and CD8 + cells in both spleen and LN. A similar analysis was conducted for 4-mo-old MRL-+/+ mice, an age at which there is little apparent autoimmune disease (our unpublished observation), again with the finding that B cells had no discernible impact on the percentage of naive, activated, or memory T cells (data not shown). Whether the effect of B cells requires the \( lpr \) mutation will be determined when we have the opportunity to analyze much older MRL-lpr/lpr mice that do have evident systemic autoimmune disease.

**T cell functional analysis**

To corroborate the activation state of the cells as identified by their cell surface markers, a functional assay was conducted on the naive (CD44low, CD62high), activated (CD44high, CD62Lhigh), and memory (CD44high, CD62Llow) subsets as shown in Figure 1. Cell percentages and numbers were obtained by multiplying total cell percentages of each activation subset by the total organ cell number for each mouse. Columns show the average of percentages (A and B) or cell numbers (C and D) from a cohort of mice. Error bars represent 1 SD. Data on splenocytes are indicated in A and C, while LN cells are indicated in B and D. Note the different scales of the y-axis in C and D. Sample sizes: B-intact, 4-to 5-mo-old mice (\( n = 15 \)); B-deficient, 6- to 8-mo-old mice (\( n = 23 \)); B-deficient, 4- to 5-mo-old mice (\( n = 7 \)); B-deficient, 6- to 8-mo-old mice (\( n = 4 \)).

**FIGURE 2.** CD4 + T cell percentages and cell numbers. Cell percentages (A and B) were obtained via FACS analysis for the naive (CD44low, CD62high), activated (CD44high, CD62Lhigh), and memory (CD44high, CD62Llow) subsets as shown in Figure 1. Cell numbers (C and D) were obtained by multiplying total cell percentages of each activation subset by the total organ cell number for each mouse. Columns show the average of percentages (A and B) or cell numbers (C and D) from a cohort of mice. Error bars represent 1 SD. Data on splenocytes are indicated in A and C, while LN cells are indicated in B and D. Note the different scales of the y-axis in C and D. Sample sizes: B-intact, 4-to 5-mo-old mice (\( n = 15 \)); B-intact, 6- to 8-mo-old mice (\( n = 23 \)); B-deficient, 4- to 5-mo-old mice (\( n = 7 \)); B-deficient, 6- to 8-mo-old mice (\( n = 4 \)).
from normal, nonautoimmune mice. This corroborates the surface markers in the FACS analysis as valid indicators of naive and memory T cells.

Discussion

We find that B cells have a major role in the spontaneous activation of T cells in MRL-lpr/lpr mice; in the absence of B cells, the numbers of both activated and memory phenotype T cells were markedly reduced. The work provides in vivo evidence for the hypothesis that B cells are critical for systemic autoimmune disregulation via a direct effect on T cells. Moreover, it begins to define the mechanism by which B cell deficiency completely blocks autoimmune pathology (35), including the infiltration of T cells. This effect on T cell activation is in turn most likely mediated through Ag presentation, although other mechanisms may contribute (see below).

The dependence of activated and memory T cells on B cells is profound; at 6 mo of age, >75% of extant memory CD4 and CD8 T cells in MRL-lpr/lpr mice are B cell dependent by comparison with B-deficient mice. Nevertheless, the absence of B cells does not completely abrogate activated/memory T cells. Other APC, such as dendritic cells or macrophages, may be responsible for initially activating these T cells, whereas B cells are responsible for their subsequent amplification. There is controversy over whether, in normal immunization situations, B cells can prime naive T cells (23, 24), although it seems most likely that this can occur with modest efficiency under some circumstances (27). Whether in spontaneous autoimmunity, activated autoreactive B cells can activate naive T cells is unknown. Thus, at present we favor the idea that B cells principally amplify previously activated T cells, maintaining them in an activated, proliferating state. This would fit with the observation that there are similar total numbers of naive T cells in B-intact and B-deficient mice, yet higher numbers of activated T cells in B-intact mice. The effect of B cells on memory T cells probably occurs through the increased supply of activated precursors. However, since B cells may be required for the maintenance of CD4 memory T cells (R. Ahmed, personal communication), we cannot rule out an effect of B cells on maintenance of memory CD4 T cells. Since B cells are not required to maintain CD8 memory T cells (50, 51), the accumulation of memory CD8 cells is most likely due to B cells promoting entry into the memory compartment. The amplification role of B cells is further suggested by the progressive, age-dependent lymphoaccumulation and increase in memory T cells seen only in the presence of B cells (Figs. 2 and 3). The fractions of residual memory and activated T cells present in B-deficient MRL-lpr/lpr mice are comparable with age-matched non-lpr controls such as BALB/c (Fig. 4), MRL-+/+ and C3H/J (our unpublished observations), suggesting that such residual T cells could

FIGURE 3. CD8+ T cell percentages and cell numbers. Cell percentages (A and B) were obtained via FACS analysis for the naive (CD44low, CD62Lhigh), activated (CD44high, CD62Lhigh), and memory (CD44high, CD62Llow) subsets. Cell numbers (C and D) were obtained by multiplying total cell percentages of each activation subset by the total organ cell number for each mouse. Columns show the average of percentages (A and B) or cell numbers (C and D) from a cohort of mice. Error bars represent 1 SD. Data on splenocytes are indicated in A and C, while LN cells are indicated in B and D. Note the different scales of the y-axis in C and D. Sample sizes: B-intact, 4- to 5-mo-old mice, spleen (n = 10); B-intact, 4- to 5-mo-old mice, spleen (n = 7); B-intact, 6- to 8-mo-old mice, spleen (n = 21); B-intact, 6- to 8-mo-old mice, LN (n = 20); B-deficient, 4- to 5-mo-old mice, spleen (n = 4); B-deficient, 4- to 5-mo-old mice, LN (n = 2); B-deficient, 6- to 8-mo-old mice, spleen (n = 4); B-deficient, 6- to 8-mo-old mice, LN (n = 3).
be accounted for by normal immune system function and maturation, which may be less B cell dependent.

The pivotal role of B cells in T cell expansion likely stems from the ability of B cells to present cognate Ags with remarkable efficiency (52–55). In mice and humans with systemic autoimmune disease, spontaneously activated B cells are highly enriched for autoreactive specificities (56–59). Thus, in established autoimmunity, this pool of B cells likely represents a potent reservoir of APCs for activating autoreactive T cells. This view is certainly consistent with our observed dependence of the vast majority of activated T cells on B cells. If this is indeed the mechanism, our finding that CD8 memory and activated T cells are also dependent on B cells suggests that B cells are a major APC for spontaneous class I-restricted autoimmune responses. Reconstitution of B-deficient MRL-\textit{lpr}/\textit{lpr} mice with various types of B cells should test these ideas further. Although we favor the role of B cells as APC, it remains possible that the effect of B cells on T cells in this model could be mediated through Abs (60) or even B cell-derived cytokines (61–63). We doubt that Abs alone could account for T cell priming in our model, since we have not observed any effect on T cell priming after high dose and prolonged reconstitution of polyclonal serum autoantibodies in B-less MRL-\textit{lpr}/\textit{lpr} mice (our unpublished observation).

Regardless of the mechanism(s) by which B cells promote the spontaneous activation and expansion of T cells in systemic autoimmunity, an implication of this phenomenon is that B cells would be an ideal target for lupus therapy. It would not be sufficient to target autoantibodies alone; in fact, this strategy as executed by plasmapheresis does not work (64). Elimination of previously activated B cells would have the dual effect of ameliorating autoantibodies and of eliminating the reservoir of potent APC for autoreactive T cells. This, in turn, is predicted to delay the progression of disease. It may further be necessary to eliminate activated T cells as well, since they may rely on MHC class II-expressing parenchymal cells for continued Ag presentation when causing damage in tissues. In fact, there is very little in the way of disease-modifying therapy available for systemic autoimmune diseases. A recently described treatment approach that does target autoantibodies and B cells (and probably T cells) is the combination of plasmapheresis and cyclophosphamide (65). Preliminary results suggest that this is indeed a disease-modifying therapy. We speculate that at the heart of the efficacy of this therapy is elimination of autoreactive B cells and their APC function.

Although the current work has demonstrated the inhibition of spontaneous T cell activation in B-deficient mice, the results do not directly prove that this population of T cells is indeed autoreactive. In fact, direct demonstration of autoreactive T cells in systemic autoimmunity has been difficult and is limited to a few notable reports (66–72). The Ag specificity of T cells probably does play a role, since a TCR transgene that restricts specificity leads to a decreased accumulation of memory T cells in MRL-\textit{lpr}/\textit{lpr} mice (73). If self-reactive T cells do exist and play a role in pathogenesis, it is reasonable to assume that they would have the activated/memory phenotype, since effector function is generally associated with activated T cells (49, 74). We would further predict that cell for cell, the activated/memory subpopulations in B-sufficient mice will be enriched for autoreactive specificities. Once generated, such autoreactive T cells could promote autoantibody production in B cells as well as attack target organs (i.e., kidney and skin infiltration). In concert with this view, there is no lymphocytic

\textbf{FIGURE 4.} FACS analysis of CD4$^+$ T cells from LN of B-intact and B-deficient BALB/c mice. LN cells from B-intact and B-deficient mice were analyzed by three-color FACS. Analysis and gating were performed as in Figure 1 and \textit{Materials and Methods}. Two mice of each type are shown, as indicated. Numbers in quadrants are percentages of CD4$^+$ T cells. Note that mice are similar regardless of B cell phenotype.
infiltration in the kidneys (35) and skin (O. Chan, J. McNiff, and M. J. Shlomchik, manuscript in preparation) of MRL-lpr/lpr mice lacking B cells.

A possible role of the Fas lpr defect in this context is to cause the retention of primed autoreactive lpr/lpr T cells which would otherwise undergo Fas-mediated death in the periphery (75, 76). However, such accumulating T cells mainly have the aberrant, DN phenotype (CD4−/CD8−/B220+) whereas the cells we enumerated are SP CD4+ and CD8+, most of which are also B220+ (our unpublished observation). Thus, the Fas deficiency in the T cell may not alone account for the expansion of SP, phenotypically normal T cells. Because the Fas defect must also be present in B cells themselves, at least to promote autoantibody production (77, 78), a similar failure to eliminate postactivated, autoreactive B cells would lead to an increased pool of B cells capable of activating T cells. These in turn may, as discussed, be potent activators of T cells. Since MRL-+/+ mice are autoimmunize prone (79, 80), we suspect that the lpr defect is merely amplifying the autoreactive cell accumulation that takes place at lower levels in Fas-sufficient autoimmune-prone mice. Additional experiments will clarify this point.

Overall, our results prompt a reevaluation of the idea that systemic autoimmune disease is strictly the result of IC-mediated pathogenesis, as recently discussed by Kotzin (1). It further expands the potential functional role of B cells in pathogenesis from Ab-forming cell to APC. This in turn should prompt a reevaluation of the B cell as a therapeutic target in the treatment of systemic autoimmune diseases. This may not apply only to systemic autoimmune diseases, since it was recently shown that B cells are required for the expression of diabetes in the NOD mouse model (81).

Because our genetically based B cell-deficient model is amenable to cell/antibody reconstitution, we hope to use it to evaluate a number of questions raised by the current studies. These include the role of direct B cell APC function vs autoantibody secretion in T cell stimulation, the putative autoreactive specificity and effector functions of B cell-dependent memory T cells, the importance of autoantigen specificity and Fas deficiency of B cells in this role, and the effectiveness of targeting B cells in halting the progress of systemic autoimmune disease.

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References


