The Role of B Cells in lpr/lpr-induced Autoimmunity

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Summary

The primary roles of T cells and B cells in the initiation of systemic autoimmunity are unclear. To investigate the role of B cells, we crossed the "Jh knockout" mutation onto the autoimmune lpr/lpr background. Animals homozygous for both traits were obtained. As expected, these animals lack B cells. These animals also show no signs of autoimmune kidney destruction nor vasculitis, in spite of carrying the lpr/lpr mutation. In contrast, lpr/lpr littermates that had B cells had severe nephritis and vasculitis, as well as autoantibodies. These results demonstrate a primary role for B cells and/or (auto)antibodies in initiating several types of autoimmune-mediated tissue destruction.

Systemic autoimmune diseases are the result of complex interactions among T cells, B cells, and target tissues. However, it has been difficult to distinguish the contributions of each cell type in both the initiation of the autoimmune response and the induction of specific pathologic lesions. This is in large part because the dependence of B cell activation on T cells and, reciprocally, the ability of B cells to act as potent APC for T cells.

Evidence of direct roles of T cells and B cells (or secreted Ig) in human lupus has been largely circumstantial. Examples include the presence of T cells in lesions or the presence of autoantibody at sites of inflammation (1–3). Animal models, on the other hand, have provided direct evidence for a role of both cell types (4–8). Intrinsic T cell defects have been clearly shown in lpr (9), BXSB, and NZB-based (10) models. Furthermore, in autoimmune MRL mice homozygous for the lpr mutation, thymectomy (11), anti-Thy1 (12), or anti-CD4 (13) antibody treatment ameliorates lymphoproliferation and delays autoimmune-mediated inflammation. T cells have also been observed within lesions of affected organs (14), most notably within the renal interstitium, surrounding vessels, and, to a lesser extent, glomeruli of MRL-lpr/lpr mice, coincident with the development of nephritis (8, 15).

There is also evidence of a primary role for autoantibodies and B cells in autoimmune pathogenesis. For example, some but not all anti-DNA antibodies cause nephritis upon injection into normal mice (6, 16, 17), whereas certain RF cryoglobulins (particularly of the IgG3 subclass) cause vasculitis and nephritis upon injection (18, 19). The role of B cells in initiating autoimmunity, perhaps by priming or expanding autoreactive T cells, is less clear. Using immunization of normal mice with cross-reactive variants of self-proteins, Lin et al. and Mamula et al. (20, 21) have suggested a key role for B cells in initiating T cell autoreactivity followed by a cascade of additional B cell autoreactivity. B cells from both lpr/lpr and NZB mice are intrinsically prone to autoantibody secretion (22–25). However, genetic studies have separated the secretion of certain autoantibodies from autoimmune manifestations, indicating that the role of B cells may be complex (26, 27).

Experiments using strategies to inactivate B cells have suggested their importance in autoimmunity. NZB.xid mice, which have developmentally arrested B cells owing to the xid defect, have a milder form of disease than their NZB counterparts (10). Similarly, C57Bl/6-lpr/lpr mice, treated with anti-IgM from birth, had few B cells and minimal glomerulonephritis; systemic vasculitis was unaffected (28). However, because anti-IgM treatment may have effects aside from reduction of B cells, genetic approaches would be helpful to determine whether Ig, B cells, or both are required for disease expression.

For this purpose, the lpr/lpr model of autoimmunity (29, 30) is ideal because fulminant autoimmunity and accumulation of abnormal T cells (31, 32) are controlled by a single recessive gene. Genetic studies are further facilitated by the recent discovery that lpr is a mutation in the fas gene (33).
The fact that the wild-type fas gene product transduces a signal for apoptosis (33, 34) has important implications for lpr/lpr autoimmunity. However, how a global fas defect causes autoimmunity remains unclear, in part because fas is expressed in many cell types and its regulation is activation dependent (35, 36). Thus, further work on the cellular contributions to autoimmunity in lpr/lpr mice is required.

To examine the role of B cells in lpr-induced autoimmunity, in the current studies we have used a novel genetic approach whereby lpr/lpr mice were deprived of B cells from birth without exogenous manipulation. We found that these lpr/lpr mice that lacked B cells failed to develop nephritis or vasculitis. Thus B cells and/or antibody play an important role in the initiation of a wide variety of autoimmune manifestations in lpr/lpr mice.

Materials and Methods

Mice

Jh knockout mice were produced as described (37) and had background genes of 129/Sv and C57Bl/6 origin. Mice carrying a single mutant Jh allele were intercrossed at an early stage of propagation, and homozygous mice were identified by PCR assay (see below). These mice were maintained usually by brother-sister matings. Henceforth, the mutated Jh allele will be referred to as JhD. F1 mice were produced from two separate matings of these homozygous JhD mice with MRL/lpr/lpr mice. The MRL/lpr/lpr mice were bred at Fox Chase Cancer Center (Philadelphia, PA) from an original breeding pair obtained from The Jackson Laboratory (Bar Harbor, ME). Three separate F2 crosses were set up at the same time. These mice were analyzed at 4.5–6 mo of age, as indicated in the tables and figures.

Genetic Testing

A dual-primer pair PCR assay was used to type for heavy-chain locus genotype. One pair of primers was specific for the Neo gene. These primers were Neo 5' (5'CCATTGGAGGCCTGCTCAGC-GT3') and Neo 3' (5'GCCGATTGACATCACCCATGGATGGA 3'). A second pair of primers amplified a region of the locus deleted by the gene targeting Jh 5' (5'GGACCAAGGGGCCATCGAC-TCAAG 3') and Jh 3' (5'GAGGAGCCGACCTGGTGTGTCCT-CGC 3'). Tail DNA preparation and PCR were carried out essentially as described (38). PCR buffer contained 2.5 mM MgCl₂. The following thermal cycler temperature program was used: 94°C 1-min initial denaturation; and then 30 cycles of 94°C 30 s/65°C 30 s/72°C 30 s, followed by 2 min at 72°C (M J Research, Watertown, MA). Mice positive in both assays were considered heterozygotes, whereas mice positive in only one assay were considered homozygotes. FACS® analysis agreed with genotyping in all cases tested. A Southern blot assay was used to detect the lpr mutation, using the 180-bp Xba-R1 fragment spanning the 5' end of the fas cDNA (35) as a probe. As expected, homozygous mutant mice had a smaller EcoRI fragment than wild-type mice, whereas heterozygotes had both bands at about half the homozygote intensity. Lymphoproliferation occurred in all mice typed as lpr/lpr with this assay with the exception of a single mouse (see Results). This mouse also lacked double negative T cells. Resting by Southern blot clearly showed this mouse to be a heterozygote.

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Cell Preparation. Bone marrow (BM) from a single femur was harvested from the first cohort of mice by flushing the marrow cavities with staining medium (RPMI 1640 deficient in biotin and lacking phenol red, supplemented with 2.5% FCS and 0.04% sodium azide). Spleens, thyms, and inguinal LN were harvested from all mice, and single-cell suspensions were prepared. After washing, ~5–20 × 10⁶ cells were included in staining reactions, which were performed as described (39). LN were weighed in both experiments. Spleens were weighed in the second experiment only; the large size of the spleens from the older lpr/lpr mice precluded disruption of entire spleens via our standard procedure. Therefore, a few representative portions were severed and disrupted.

Staining. The following mAbs were used in this study: 331.3 (40; anti-lgM), RA3-6B2 (41; anti-B220), 30H12 (42; anti-Thyl.2), and 53.7 (42; anti-Ly1.1). GK1.5 (43; anti-CD4) and 53.6 (42; anti-CD8) were prepared and kindly provided by N. Reutsh in the laboratory of M. Bosma (Fox Chase Cancer Center). 50AA12 (anti-CD3; PE conjugated) was purchased from Pharmingen (San Diego, CA) and provided by N. Reutsh. Multicolor FACS® analysis was performed on a FACStar Plus (Becton Dickinson & Co., Mountain View, CA) equipped with dual lasers. 50,000 ungated events were collected. In all plots, dead cells were excluded from analysis by staining with propidium iodide. Percentages given of cells in various FACS® gates are of live cells.

Kidney Analysis

Histology. Kidney sections from each animal were analyzed by direct immunofluorescence and light microscopy, as previously described (6, 44). For direct immunofluorescence, fluoresceinated subclass-specific antisera were used, and the intensity of fluorescence was graded on a scale from 0 to 3+ for the presence and quantity of immune deposits (45). For light microscopy, one entire kidney from each animal was fixed in 10% formalin and embedded in paraffin. Multiple 4-μm sections through the center of the longitudinal axis of each whole kidney were obtained, and they were stained with hematoxylin and eosin.

Microscopic Evaluation. The sections were evaluated by one of us (M. P. Madaio) without knowledge of the donor mouse genotype. The severity of disease in each compartment (glomerular, interstitial, vascular) was graded on semiquantitative scoring of biopsy features (0–3+) according to previously described methods of Austin et al. (46), used in the analysis of human lupus nephritis. For purposes of grading in this study, 5–6-mo-old MRL/lpr/lpr mice with severe disease and 2–mo-old normal CBA/J mice were used for comparison (defined as 3+ and 0, respectively). Morphologic analysis involved assessment of the following: (a) for glomerulonephritis: glomerular hypercellularity (including glomerular cell proliferation, leukocyte exudation), karyorhexis and fibrinoid necrosis, luminal occlusion, and cellular crescents; (b) for interstitial nephritis: infiltration of mononuclear cells, loss of normal architecture, and tubular necrosis; and (c) for vasculitis: perivascular infiltrates, interenal hyperplasia, and luminal occlusion.

ELISA for Ig and RF

ELISA assays were performed as previously described (38). Standard titrations were done on each plate, and concentrations were

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1 Abbreviations used in this paper: BM, bone marrow; DN, double negative for CD4 and CD8; FL, fluorescein; SP, single positive for CD4 or CD8.
Table 1. Analysis of Lymph Nodes

| Genotype* | 4.5-mo-old mice | 5.5-6-mo-old mice |
|-----------|-----------------|------------------|
|           | CD4⁺/CD8⁻ T cells | Total cell count (x 10⁶) | LN weight | CD4⁺/CD8⁻ T cells | Total cell count (x 10⁶) | LN weight |
| lpr       | DN T cells†     | mg              | mg        |
| Jh        |                 |                 |           |
| +/+       | 1 26            | 47              | 0.1       | 8              | 1 6             | 59              | 0.2       | 5          |
| +/+       | 1 4             | 58              | 5.3       | 16             | 1 9             | 36              | 0.4       | 4          |
| lpr       | 2 72 (12)       | 15 (5)          | 4.8 (4.1) | 85 (48)        | 1 87            | 7               | 40        | 292        |
| Jh        | 2 63 (19)       | 20 (8)          | 2.2 (1.5) | 55 (30)        | 5 84 (4)        | 10 (2)          | 23 (9.6)  | 537 (277)  |
| +/-       | 3 78 (12)       | 13 (7)          | 17 (9.1)  | 113 (41)       | 3 74 (6)        | 13 (2)          | 4 (1.6)   | 59 (34)    |

Numbers are percentages of live lymphocytes, rounded to the nearest whole number. When multiple mice have been analyzed in a group, the SEM is given in parentheses.

* Genotype was determined by Southern blot for lpr and PCR for Jh loci. +, wild-type allele; lpr, mutant allele; -, inactivated Jh locus allele.
† DN T cell values are percentages of total T cells (as determined by CD3 or Thy1 expression in multicolor staining) to facilitate direct comparison of mice with and without B cells.
† Total number of nucleated cells recovered from two inguinal LN.

Results

Analysis of F2 Progeny and Experimental Design. F2 mice provided all possible genotypic combinations. The genetic background of these mice was half MRL and half from the Jh background, which was itself a mixture of 129/Sv and C57Bl/6. Individual background genes segregate independently of Jh and lpr types in such crosses and thus should not induce a bias between the groups, except those genes closely linked to the Jh and lpr loci themselves.

From 3 F1 males crossed to 9 F1 females, 81 F2 progeny were genotyped. The distribution of the nine possible genotypes (not shown) does not differ from the expected frequency (p = 0.86, χ², eight degrees of freedom). Two cohorts of mice, each containing three mice of the lpr/lpr JhD/JhD genotype along with randomly chosen individuals of other genotypes, were analyzed, the first at 4.5 mo and the second at 5.5-6 mo.

Table 2. Analysis of Spleens

| Genotype* | 4.5-mo-old mice | 5.5-6-mo-old mice |
|-----------|-----------------|------------------|
|           | CD4⁺/B220⁺ T cells | Total cell count (x 10⁶) | Spleen weight | CD4⁺/B220⁺ T cells | Total cell count (x 10⁶) | Spleen weight |
| lpr       | DN T cells†     | mg              |             | mg          |
| Jh        |                 |                 |             |
| +/+       | 1 12            | 1               | 59          | 7           | 1 29             | 21              | 44         | 77         |
| +/+       | 1 13            | 1               | 3           | 6           | 1 11             | 7               | 3          | 53         |
| lpr       | 2 56 (8)        | 22 (9)          | 43 (14)     | 33 (19)     | 1 68             | 37              | 25         | 605        |
| Jh        | 2 40 (12)       | 16 (4)          | 43 (2)      | 27 (13)     | 5 68 (5)         | 28 (10)         | 20 (7)     | 1,475 (566)|
| +/-       | 3 64 (6)        | 39 (1)          | 7 (3)       | 6 (2)       | 3 67 (3)         | 42 (2)          | 4 (1)      | 108 (3)    |

Numbers are percentages of live lymphocytes, rounded to the nearest whole number. When multiple mice have been analyzed in a group, the SEM is given in parentheses.

* Genotype was determined by Southern blot for lpr and PCR for Jh loci. +, wild-type allele; lpr, mutant allele; -, inactivated Jh locus allele.
† DN T cell values are percentages of total T cells (as determined by CD3 or Thy1 expression in multicolor staining) to facilitate direct comparison of mice with and without B cells.
† Total number of nucleated cells recovered from the whole spleen. This was determined only for the 4.5-mo-old mice. Spleen weight was determined for the second cohort.
5.5–6 mo of age. In the first cohort, 12 mice were analyzed for autoantibodies and renal disease; nine were also subjects of FACS® analysis. In the second cohort, 14 mice were analyzed, 12 by FACS® as well. The data from the two cohorts are presented separately in the tables.

**Peripheral Lymphoid Organs.** Lymphadenopathy was observed in LN of all lpr/lpr mice (Table 1). Among the younger cohort of mice, lymphadenopathy (both weight and cell number) was similar regardless of whether mice lacked B cells. However, in the older cohort, lymphadenopathy was more severe among mice that had B cells. Spleens of the 4.5-mo-old lpr/lpr mice that had B cells contained ~5-fold more cells on average than mice heterozygous for lpr or +/+ mice (Table 2). Spleen weight averaged 15-fold higher in the older (5.5–6 mo) lpr/lpr mice with B cells. Thus, for splenomegaly, a difference was already apparent in the younger cohort, but it became more pronounced in the older cohort, whereas a difference in LN hyperplasia was apparent only among the older cohort of B cell-containing lpr/lpr mice. That lymphoaccumulation fails to progress with age in lpr/lpr mice lacking B cells suggests a role for B cells in augmenting the accumulation of lpr/lpr T cells.

**Phenotypes of Peripheral Lymphoid Cells.** lpr/lpr mice >4 mo are known to harbor large numbers of T cells with unusual phenotypes. The majority of these are Thy1+/CD3+D−/ B220+/CD4+/CD8−, termed double negative (DN) T cells (32). Lymphoaccumulation also includes an increase in the absolute numbers of CD4+ and CD8+ single positive (SP) cells, although to a lesser degree. Since B cells are required for efficient priming of T cells during immunization (47, 48), we investigated by FACS® whether the absence of B cells would affect T cell accumulation. As shown in Fig.

![Figure 1](image-url)

**Figure 1.** FACS analysis of CD4 and CD8 expression in CD3+ LN cells of 4.5-mo-old mice. Single cell suspensions of inguinal LN were stained with fluorescein (FL)-anti-CD8, PE-anti-CD3, and allophyocyanin anti-CD4, followed by multicolor FACS® analysis. Shown are 5% probability plots from representative animals. Only live cells (unstained with propidium iodide) with forward and side scatter characteristics of lymphocytes that were positive for CD3 are shown. Genotypes of the animals are as follows: (A and B) lpr/lpr JhD/JhD; (C and D) lpr/lpr +/+ JhD/JhD; and (E and F) +/+ JhD/JhD. Percentages of total CD3+ cells in quadrants are given in each panel. Note the increased frequency of CD4+/CD8− T cells in lpr mice. Animal-to-animal variability in DN T cell frequency is typical at this early stage of disease.
1 and Tables 1 and 2, absence of B cells had no effect on the proportions of these cells in LN or spleen. Whether defined as Thy1+ or CD3+ cells, there were no differences in the proportions of DN, CD4+, or CD8+ SP T cell subsets. In addition, there were similar frequencies of cells that were dull for Thy1, CD3, and B220 (Fig. 2 and Tables 1 and 2). Thus the presence of B cells does not affect the proportional nature of lymphoproliferation caused by the homozygous lpr mutation. However, because the number of accumulated cells was greater in lpr/lpr mice that had B cells, the absolute numbers of both DN and SP T cells were greater in such mice than in lpr/lpr mice lacking B cells. This was particularly evident in the oldest cohort of mice tested.

We also performed staining with anti-B220 and anti-IgM to enumerate B cells. As revealed by bright anti-B220 staining and (in the second cohort) anti-IgM staining, mature B cells were present only in those mice genotyped as heterozygous or wild type at the Jh locus (Fig. 2 and Tables 1 and 2). Some lpr/lpr mice with a wild-type Jh allele had a relatively small proportion of splenic B cells, presumably due to extensive T cell accumulation. However, in all such lpr/lpr mice, the absolute number of splenic B cells was increased.

Two curiosities were noted in the FACS® experiments. First, in some lpr/lpr JhD/JhD mice, a small population of B220Alld Thy1- cells was found in LN (data not shown). Three-color staining with anti-Thy1, anti-IgM, and anti-B220 staining in the second experiment showed that these cells were sIgM+. Thus, these cells may have reflected seeding of the LN by pre-B cells, which do develop in JhD mice (37, our unpublished data). However, similar cells have also been reported in previous studies of lpr/lpr mice; it was speculated that such cells are in the T lineage (49). This latter conclusion is consistent with the observation in separate staining reactions that nearly all lymphoid cells from the same LN were CD3+ (data not shown). Further multicolor staining will be required to define the lineage of these cells.

Second, we noted in a single +/- +/- mouse a population of cells staining brightly for CD3 and B220 (Fig. 2 D). These B220+ /CD3+ cells also stained with anti-IgM but not anti-Thy1. Thus, they probably represent B cells. The reason for the bright CD3 staining is unclear; it does not represent reagent interaction, as most B220+ cells were negative for CD3. It is possible that a subpopulation of B cells expressed high levels of FcR, which bound the anti-CD3 antibody. Unfortunately, because of the experimental design, it was not possible to repeat the staining with different reagents and controls. Since cells falling in the B220+CD3+ area were IgM+ Thy1- in Fig. 2 D but IgM+ Thy1+ in Fig. 2, A and B, we gated on the B220+CD3+ population characteristic of DN T cells to quantitate comparable cell populations in each mouse.

Central Lymphoid Organs. Single-cell suspensions derived from thymi and BM were examined by FACS®. Similar quantities of thymic cells were recovered from all mice. lpr/lpr mice with B cells had higher proportions of CD4-/CD8- thymocytes than lpr/lpr mice that lacked B cells (Fig. 3 and

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**Figure 2.** FACS® analysis of B220 and CD3 expression on splenocytes of 5.5-mo-old mice. Cells were stained with FITC anti-B220 and PE-anti-CD3. Gating and plots are as in Fig. 1. The lpr and Jh genotypes of the animals are as follows: (A) lpr/lpr JhD/JhD; (B) lpr/lpr JhD/+; (C) +/- JhD/JhD; and (D) +/- +/- . Note the virtual absence of B220+/CD3- B cells (lower right box in each panel) in the JhD/JhD animals (A and C). The upper left box in each panel shows a population of B220+/CD3+ cells. These cells are rare in +/- mice (C and D) but are common in lpr/lpr mice (A and B), and accumulate in similar proportions regardless of the Jh genotype (compare A and B). The population of cells brighter for both CD3 and B220 seen in A and B likely comprises an additional abnormal population of T cells. See text for details. The 3% of B220+ cells in A and C do not express IgM (not shown) and thus resemble pre-B cells.
Figure 3. FACS analysis of CD4 and CD8 expression among Thy1+ thymocytes of 4.5-mo-old mice. Single-cell suspensions were stained with biotin anti-Thy1, FL-anti-CD8, and allophycocyanin-anti-CD4, followed by Texas Red-avidin. Gating and plots are as in Fig. 1. Percentages of total Thy1+ cells are given for each quadrant. In all thymi, >96% of live lymphocytes were Thy1+.

The Ipr and Jh genotypes of the animals are as follows: (A) Ipr/lpr JhD/JhD; (B) Ipr/Ipr +/+; (C) +/+ JhD/JhD; and (D) +/+ +/+ . Note the large fraction of DN Thy1+ cells in the lpr/lpr +/+ animal (B) not present in any of the other genotypes. The thymi of the mice used for A and B yielded similar numbers of total cells.

Table 3. Analysis of Thymus

| Genotype* | 4.5-mo-old mice | 5.5-6-mo-old mice |
|-----------|-----------------|------------------|
|           | DN Tcells | CD4+/CD8- | CD4+/CD8- | CD4+/CD8+ | CD4+/CD8+ | CD4+/CD8+ | CD4+/CD8+ |
| lpr Jh    | n        | Tcells   | Tcells   | Tcells   | Tcells   | Tcells   | Tcells   |
| +/+       | 1        | 4       | 13       | 11       | 72       | 1        | 2        | 10       | 6        | 82       |
| +/+ -/-   | 1        | 5       | 10       | 50       | 33       | 1        | 2        | 10       | 7        | 82       |
| l/l +/+   | 2        | 15 (8)  | 15 (2)   | 15 (5)   | 55 (1)   | 1        | 26       | 23       | 8        | 41       |
| l/l +/ -  | 2        | 19 (12) | 23 (13)  | 19 (7)   | 39 (32)  | 5        | 34 (9)   | 21 (3)   | 11 (1)   | 33 (9)   |
| l/l -/ -  | 3        | 2 (0)   | 14 (2)   | 12 (0)   | 71 (2)   | 3        | 3 (0)    | 13 (1)   | 10 (2)   | 74 (2)   |

Numbers are percentages of live lymphocytes, rounded to the nearest whole number. When multiple mice have been analyzed in a group, the SEM is given in parentheses. Percentages are of T lymphocytes as determined by gating on Thy1 as a third color.

* Genotype was determined by Southern blot for lpr and PCR for Jh loci. +, wild-type allele; l, lpr mutant allele; - , inactivated Jh locus allele.
were gated for the expression of Thy1 (which included usually >95% of live T cells). The significance of the B cell dependence of DN thymic T cells is discussed below. BM staining were gated for the expression of Thyl (which included usu-

compared with mice with B220+ cells but homozygous for JhD mutation lacked mature (B220high) or immature (B220low) splenic B cells, although most had detectable pre-B cells (data not shown). As expected, mice homozygous for the JhD mutation lacked mature (B220high) or immature (B220low) splenic B cells, although most had detectable pre-B cells (data not shown).

Autoantibody Production. Serum RF activity as well as serum IgG1 and κ levels were measured in all mice (Fig. 4). As expected, mice that lacked B cells lacked serum RF and Ig. lpr/lpr animals with B cells had markedly elevated Ig and RF levels compared with mice with B cells but homozygous for lpr. Elevations of RF (two to five orders of magnitude compared with non-lpr controls) were proportionally much greater than general hypergamma globulinemia (about one order of magnitude; see Fig. 4), consistent with antigen-selective autoantibody production in lpr/lpr mice (52). Thus, age-related lpr/lpr-controlled autoantibody production resembling that of MRL-lpr/lpr mice had ensued among the cohorts of F2 mice.

Nephritis and Vasculitis. Renal pathology was interpreted without knowledge of mouse genotypes. The mice could be readily distinguished into two groups with strikingly different morphologies: a group with normal architecture and no immune deposits and a group with severe glomerulonephritis, interstitial nephritis, vasculitis, and both glomerular and interstitial immune deposits. Representative examples are illustrated in Fig. 5. Details of the pathology are described in the legends. Severe nephritis always segregated with the presence of both the lpr/lpr genotype and B cells, and this was observed for all animals in both cohorts (a total of 10 lpr/lpr mice with B cells). By contrast, none of the six lpr/lpr mice that lacked B cells and circulating Ig developed nephritis; their histology was indistinguishable from the four +/+ littermates that had B cells and with normal control CBA/J mice.

Discussion

To further elucidate the role of B cells in MRL-lpr/lpr mice, a series of F2 mice were created from crosses of lpr/lpr homozygotes and mice homozygous for a mutation (JhD) that prevents development of mature B cells. Strikingly, in the absence of B cells, lpr/lpr mice developed neither glomerulonephritis, vasculitis, nor interstitial nephritis. In contrast, all of the lpr/lpr littermate mice with B cells developed severe nephritis and autoantibodies. These results indicate that B cells and/or autoantibodies play a primary role in the initiation of lupus nephritis in this genetic background. Severe nephritis was present in B cell-containing lpr/lpr mice at 4 mo of age, a time when lymphoaccumulation was equivalent in mice lacking B cells and animals with B cells. Thus, suppression of nephritis was not the result of decreased lymphoaccumulation. For each form of nephritis observed in B cell-sufficient mice, it is formally possible that the role of B cells is as (auto)antibody-forming cell precursors, as APC in causing T cell activation, or both. However, likely roles of B cells for each form of nephritis are suggested by previous work on pathogenesis.

The requirement of B cells for induction of glomerulonephritis substantiates previous work suggesting a role of immune deposits in glomerulonephritis (1, 5, 6, 16–18, 45) and indicates that immune deposit formation is likely a pivotal event in the initiation of the glomerular lesion. Furthermore, although other systemic and local cellular and cytokine perturbations are present in these animals (53, 54), they are by themselves insufficient to induce even microscopic disease. In this regard, it will be of particular interest to evaluate cytokine levels within the glomeruli of mice lacking B cells to determine the influence of B cells and Ig deposition on cytokine disorganization reported to occur before the onset of overt disease (54).

Absence of interstitial nephritis in lpr/lpr mice lacking B cells is also interesting and surprising, particularly because
of the hypothesis that T cells and macrophages are the primary initiators (4, 55). It has been reported that intense cellular inflammation often precedes Ig deposits along tubular basement membranes (56). In addition, T cell lines have been isolated that are nephritogenic upon injection (15). Our results thus suggest that B cells may be required for the activation of autospecific T cells that infiltrate the kidney. Local cytokine release due to glomerular inflammation could also be required to promote interstitial inflammation (57).

The role of Ig in the pathogenesis of vasculitis has been controversial (6–8, 58). Vasculitis was not detected in mice lacking B cells, indicating that the vascular lesions were dependent on either the deposition of Ig or the presence of B cells. Definite, but relatively small, quantities of Ig were de-
ected within the vessel walls of diseased mice (when glom-
merular immune deposits were abundant; data not shown).
This observation is consistent with Berden et al. (58), who
found Ig within inflamed vessels but not within normal
vessels of the same MRL-lpr/lpr mice. However, Moyer et
al. (8) reported that perivascular lymphocytes and macrophages
were present before vascular immune deposits; antibody depo-
sition coincided with the development of necrotizing vascu-
laritis in older MRL-lpr/lpr mice. These workers emphasized
the cell-mediated nature of lpr/lpr vasculitis. However, they
did find B cells in the outer zone of the inflammatory infiltrate.
Cerny et al. (28) observed that anti-IgM treatment of C57BL/6-
lpr/lpr mice ameliorated nephritis, but did not affect the
vasculitis, suggesting, in contrast to our results, that B cells/Ig
are not required for the development of vasculitis. It is difficult
to reconcile the differences observed between JhD mice and
anti-IgM treatment. The presence of low levels of B cells,
heterologous rabbit antiserum, and presumably, immune com-
plexes in the anti-IgM–treated animals may have been sufficient
to allow for the development of vasculitis. These potential
artifacts were not a problem in the JhD mice. Interestingly,
previous work suggests that the role of B cells in vasculitis
may differ from that in glomerulonephritis. Genetic (59) and
passive autoantibody injection studies (18) have suggested that
vasculitis and glomerulonephritis can occur and be induced
separately, implying that they arise by distinct mechanisms.
However, further work will be required to distinguish the
underlying mechanisms.

Aside from its effect on nephritis, it is interesting that a
lack of B cells was associated with reduced accumulation of
T cells, which was prominent in the older cohort of mice.
It has been proposed by Huang et al. (60) that activation of
CD4+ and/or CD8+ SP cells is required for accumu-
lation of DN T cells. Based on the present data, we propose
that B cell interaction is an important pathway for such acti-
vation. Accumulation of both DN and SP T cells was re-
duced in mice lacking B cells, suggesting that B cells either
interact equally with these subsets to promote their accumu-
lation or else interact with a precursor that contributes equally
to these T cell subsets. T cell accumulation in not, however,
completely abrogated in the absence of B cells, especially in
young animals. This suggests that other cell types, perhaps
dendritic cells or macrophages, are also competent to acti-
te precursors of accumulating T cells. The role of B cells
in promoting further accumulation would become more ap-
parent as macrophages and dendritic cells become more lim-
itng amid the accumulating T cells in mice lacking B cells.
The requirement for B cells to induce maximal lymphoac-
cumulation further suggests that the fas defect prevents an
apoptotic event, which would normally occur only after T
cell interaction (either stimulatory or tolerogenic) with an
APC. Whether the fas deficiency is also required in the B
cell in order to promote T cell accumulation could be tested
via reconstitution of B cells in this model system.

In contrast to the periphery, DN T cells were dispro-
portionately overrepresented in the thymi of lpr/lpr mice that
had B cells. This curious observation could be explained by
either of two mechanisms. First, DN T cells may be gener-
ated in the periphery and home to the thymus. The differ-
ence between B cell–sufficient lpr/lpr mice and those lacking
B cells would then be attributed to a difference in the overall
number of DN T cells, which in the mice lacking B cells
would be two small to permit an “overflow” into the thymus.
However, young lpr/lpr mice lacking B cells have as much
lymphadenopathy as the B cell–sufficient mice, yet have a lower
proportion of thymic DN T cells. Alternatively, B cells may
directly affect the generation of some DN T cells in the
thymus. This is plausible since small numbers of B cells are
present in thymus (61). As in the periphery, a lack of B cells
required for DN T cell expansion would limit DN T cell
generation in the thymus of mice lacking B cells.

An assumption inherent in our conclusions is that the
phenotypes of homozygous JhD-mutant mice are the result of
the lack of B cells. An alternative interpretation is that a
genetic locus linked to the JhD mutation on chromosome 12,
other than the mutation itself, is responsible for our
findings. A candidate for such a locus, mapped by Watson
et al. (62) to chromosome 12, modifies lpr/lpr renal disease
in the MRL background. Although this locus is linked to
the Jh locus (most likely ∼40 cM away), it is unlikely that
it—that rather than the Jh knockout itself—is responsible for
the present observations. The locus described by these workers
is a “modifying locus,” which, along with another unlinked
locus, has an effect on the “renal index” that accounts for
only ∼50% of the variance in renal disease. By contrast, the
difference in nephritis in our studies was dramatic: disease
was either severe or absent. Thus it is unlikely that this locus
accounts solely for our observations. The observation that
lpr/lpr mice with either one (i.e., JhD/+ or two (i.e., +/+ )
copies of the MRL-derived chromosome 12 had equally se-
vere disease also supports this conclusion.

Very recently, Jevnikar et al. (63) have shown that MRL-
lpr/lpr mice that cannot express class II MHC molecules do
not get nephritis. The authors considered that nephritis was
abrogated either because of blocking of autoaggressive
CD4+ T cell generation within the thymus or lack of T cell
activation within the kidney in the absence of class II expres-
sion on renal cells. Class II knockout lpr/lpr mice lack CD4+
T cells, autoantibodies and MHC expression on B cells and
tissues, complicating interpretation of why these mice do not
get nephritis. Mice lacking B cells, on the other hand, have
CD4+ T cells and can express MHC class II in tissues, yet
still do not get nephritis. In light of our data, we suggest
that in class II–deficient mice, suboptimal B cell activation
because of lack of class II expression may also play a role in
preventing nephritis in these mice. Indeed, the MRL-lpr/lpr
class II–deficient mice lacked serum autoantibodies (63). In
addition, we would predict that, after restoration of CD4+
T cells in these mice through cell transfer, disease would not
ensue because of the inability to activate B cells. In any case,
it is of great interest that in both class II–deficient lpr/lpr mice
and JhD/JhD lpr/lpr mice, B cells are directly affected by the
genetic alteration, and nephritis is prevented.

Our work underscores the importance of B cells in gener-
ating autoimmune-mediated tissue damage. The results indi-
cate that B cells are critical for multiple components of
autoimmune-mediated inflammation, including those previously thought to be primarily mediated by humoral autoimmunity (glomerulonephritis), cellular autoimmunity (interstitial nephritis) or both (vasculitis). These data demonstrate that B cells could be an important target for therapy of systemic autoimmunity. Elimination of B cells or B cell subsets would have distinct advantages over removal of Ig alone (as in plasmapheresis). Pathogenic autoantibodies would be removed more efficiently than in plasmapheresis; moreover, B cell suppression would also ameliorate immune-mediated events requiring cell–cell interaction, such as interstitial nephritis and vasculitis, which would be unaffected by removal of Ig alone. A better understanding of the role of B cells and antibody in inducing autoimmune pathology will be necessary to design appropriate B cell–directed therapy.

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References

1. Winfield, J.B., I. Faiferman, and D. Koffler. 1977. Avidity of anti-DNA antibodies in serum and IgG glomerular eluates from patients with systemic lupus erythematosus: association of high avidity anti-native DNA antibody with glomerulonephritis. J. Clin. Invest. 59:90.

2. Couser, W.G., D.J. Salant, M.P. Madaio, S. Adler, and G.C. Groggel. 1982. Factors influencing glomerular and tubulointerstitial injuries in SLE. Am. J. Kidney Dis. 2(Suppl. 1):126.

3. Foster, M.H., B. Cizman, and M.P. Madaio. 1993. Nephritogenic autoantibodies in systemic lupus erythematosus: immunochemical properties, mechanisms of immune deposition, and genetic origins. Lab Invest. 69:494.

4. Kelley, V.R., G.C. Diaz, A.M. Jevnikar, and G.G. Singer. 1993. Renal tubular epithelial and T cell interactions in autoimmune renal disease. Kidney Int. 39(Suppl.):S108.

5. Dixon, F.J., M.B.A. Oldstone, and G. Tonietti. 1971. Pathogenesis of immune complex glomerulonephritis of New Zealand mice. J. Exp. Med. 134:65.

6. Vlahakos, D.V., M.H. Foster, S. Adams, M. Katz, A.A. Ucci, K.J. Barrett, S.K. Datta, and M.P. Madaio. 1992. Anti-DNA antibodies form immune deposits at distinct glomerular and vascular sites. Kidney Int. 41:1690.

7. Jabs, D.A., and R.A. Prendergast. 1987. Reactive lymphocytes in lacrimal gland and vasculitic renal lesions of autoimmune MRL/lpr mice express L3T4. J. Exp Med. 166:1198.

8. Moyer, C.F., J.D. Strandberg, and C.L. Reinisch. 1987. Systemic mononuclear-cell vasculitis in MRL/Mp-lpr/lpr mice. A histologic and immunocytochemical analysis. Am. J. Pathol. 127:229.

9. Katagiri, T., P.L. Cohen, and R.A. Eisenberg. 1988. The lpr gene causes an intrinsic T cell abnormality that is required for hyperproliferation. J. Exp. Med. 167:741.

10. Tawrog, J.D., E.S. Raveche, P.A. Smathers, L.H. Glimcher, D.P. Huston, C.T. Hansen, and A.D. Steinberg. 1981. T cell abnormalities in NZB mice occur independently of autoantibody production. J. Exp. Med. 153:221.

11. Steinberg, A.D., J.B. Roths, E.D. Murphy, R.T. Steinberg, and E.S. Raveche. 1980. Effects of thymectomy or androgen administration upon the autoimmune disease of MRL/Mp-lpr/lpr mice. J. Immunol. 125:871.

12. Wofsky, D., J.A. Ledbetter, P.L. Hendler, and W.E. Seaman. 1985. Treatment of murine lupus with monoclonal anti-T cell antibody. J. Immunol. 134:852.

13. Santoro, T.J., J.P. Portanova, and B.L. Kotzin. 1988. The contribution of L3T4+ T cells to lymphoproliferation and autoantibody production in MRL-lpr/lpr mice. J. Exp. Med. 167:1713.

14. Rajagopalan, S., T. Zordan, G.C. Tokos, and S.K. Datta. 1990. Pathogenic anti-DNA autoantibody-inucing T helper cell lines from patients with active lupus nephritis: isolation of CD4+ 8+ T helper cell lines that express the γδ T-cell antigen receptor. Proc. Natl. Acad. Sci. USA. 87:7020.

15. Gallo, C.D., A.M. Jevnikar, D.C. Brennan, S. Florquin, S.A. Pacheco, and V.R. Kelley. 1992. Autoreactive kidney-infiltrating T-cell clones in murine lupus nephritis. Kidney Int. 42:851.
16. Dang, H., and R.J. Harbeck. 1984. The in vivo and in vitro glomerular deposition of isolated anti-double-stranded-DNA antibodies in NZB/W mice. Clin. Immunol. Immunopathol. 30:265.

17. Panekiewicz, O.G., P. Migliorini, and M. Madaio. 1987. Polyreactive autoantibodies are nephritogenic in murine lupus nephritis. J. Immunol. 139:3287.

18. Reininger, L., T. Berney, T. Shibata, F. Spertini, R. Merino, and S. Izui. 1990. Cryoglobulinemia induced by a murine IgG3 rheumatoid factor: skin vasculitis and glomerulonephritis arise from distinct pathogenic mechanisms. Proc. Natl. Acad. Sci. USA. 87:10038.

19. Berney, T., T. Fulpius, T. Shibata, L. Reininger, J. Van Snick, H. Shan, M. Weigert, A. Marshak-Rothstein, and S. Izui. 1992. Selective pathogenicity of murine rheumatoid factors of the cryoprecipitable IgG3 subclass. Int. Immunol. 4:93.

20. Lin, R.-H., M.J. Mamula, J.A. Hardin, and C.A. Janeway, Jr. 1991. Induction of autoreactive B cells allows priming of autoreactive T cells. J. Exp. Med. 173:1433.

21. Mamula, M.J., R.-H. Lin, C.A. Janeway, Jr., and J.A. Hardin. 1992. Breaking T cell tolerance with foreign and self-co-immunogens: a study of autoimmune B and T cell epitopes of cytchrome c. J. Immunol. 149:789.

22. Herron, L.R., R.L. Coffman, and B.L. Kotzin. 1988. Enhanced response of autobody-secreting B cells from young NZB/NZW mice to T-cell–derived differentiation signals. Clin. Immunol. Immunopathol. 46:314.

23. Kliman, D.M., and A.D. Steinberg. 1986. Proliferation of DNA-producing NZB B cells in a non-autoimmune environment. J. Immunol. 137:69.

24. Sobel, E.S., T. Katagiri, K. Katagiri, S.C. Morris, E.L. Cohen, and A.D. Steinberg. 1986. Proliferation of autoantibody-secreting B cells from young NZB/NZW mice to T-cell–derived differentiation signals. Clin. Immunol. Immunopathol. 46:314.

25. Nemazee, D., C. Guier, K. Buerki, and A. Marshak-Rothstein. 1991. Lymphocytes from the autoimmune-prone mouse strain MLF/lpr manifest an intrinsic defect in tetraparental MRL/lpr F1 mice. J. Immunol. 147:2536.

26. Raveche, E.S., B.A. Novotny, C.T. Hansen, J.H. Tjio, and A.D. Steinberg. 1981. Genetic studies in NZB mice, V. Recombinant inbred lines demonstrate that separate genes control autoimmune disease and production of autoantibodies in the lpr model of murine lupus. J. Immunol. 127:2322.

27. Eastcott, J.W., K.S. Schwartz, and S.K. Datta. 1983. Genetic analysis of the inheritance of B cell hyperactivity in relation to the development of autoantibodies and glomerulonephritis in NZB x SWR crosses. J. Immunol. 131:2323.

28. Cerny, A., M. Kimoto, A.W. Hugin, R. Merino, and S. Izui. 1989. Anti-IgM treatment of C57BL/6-lpr/lpr mice: depletion of B cells reduces lpr gene–induced lymphoproliferation and mononuclear cell vasculitis. Clin. Exp. Immunol. 77:124.

29. Andrews, B.S., R.A. Eisenberg, A.N. Theofilopoulos, S. Izui, C.B. Wilson, P.J. McConahey, E.D. Murphy, J.B. Roths, and F.J. Dixon. 1978. Spontaneous murine lupus-like syndrome. Clinical and immunopathological manifestations in several strains. J. Exp. Med. 148:1198.

30. Izui, S., V.E. Kelley, K. Masuda, H. Yoshida, J.B. Roths, and E.D. Murphy. 1984. Induction of various autoantibodies by mutant gene lpr in several strains of mice. J. Immunol. 133:227.

31. Wöflsky, D., R.R. Hardy, and W. Seaman. 1984. The proliferating cells in autoimmune MRL/lpr mice lack L3T4, an antigen on “helper” T cells that is involved in the response to class II major histocompatibility antigens. J. Immunol. 132:2586.

32. Davidson, W.F., P.J. Dumont, H.G. Bedigian, B.J. Fowlkes, and H.C.I. Morse. 1986. Phenotypic, functional, and molecular genetic comparisons of the abnormal lymphoid cells of C3H-lpr/lpr and C3H-gld/gld mice. J. Immunol. 136:4075.

33. Watanabe-Fukunaga, R., C.I. Brannan, N.G. Copeland, N.A. Jenkins, and S. Nagata. 1992. Lymphoproliferation disorder in mice explained by defects of Fas antigen that mediates apoptosis. Nature (Lond.) 356:314.

34. Itoh, N., S. Yonehara, A. Ishii, M. Yonehara, S.-I. Mizushima, A. Hase, Y. Seto, and S. Nagata. 1991. The polypeptide encoded by the cDNA for human cell surface antigen fas can mediate apoptosis. Cell. 66:233.

35. Watanabe-Fukunaga, R., C.I. Brannan, N. Itoh, S. Yonehara, N.G. Copeland, N.A. Jenkins, and S. Nagata. 1992. The cDNA structure, expression, and chromosomal assignment of the mouse Fas antigen. J. Immunol. 148:1274.

36. Drappa, J., N. Brot, and K.B. Elkon. 1993. The Fas protein is expressed at high levels on CD4+ CD8+ thymocytes and activated mouse mature lymphocytes in normal mice but not in the lupus-prone strain, MRL/lpr/lpr. J. Immunol. 148:1274.

37. Chen, J., M. Trounstine, F.W. Alt, F. Young, C. Kurahara, J.F. Loring, and D. Huszar. 1993. Immunoglobulin gene rearrangement in B cell deficient mice generated by targeted deletion of the JH locus. Int. Immunol. 5:647.

38. Shlomchik, M.J., D. Zharhary, S. Camper, T. Saunders, and M. Weigert. 1993. A rheumatoid factor transgenic mouse model of autoantibody regulation. Int. Immunol. 5:1329.

39. Hardy, R.R. 1986. Purification and coupling of fluorescent proteins for use in flow cytometry. In Handbook of Experimental Immunology. D.M. Weir, editor. Blackwell Scientific Publishers, Edinburgh. 31.

40. Kincade, P.W., G. Lee, and T. Watnabe. 1981. Monoclonal rat antibodies to murine IgM determinants. J. Immunol. Methods. 42:17.

41. Coffman, R.L., and I.L. Weissman. 1981. A monoclonal antibody that recognizes B cells and B cell precursors in mice. J. Exp. Med. 153:269.

42. Ledbetter, J.A., and L.A. Herzenberg. 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. Immunol. Rev. 47:63.

43. Draynas, D.P., Z.S. Quan, K.A. Wall, A. Pierres, J. Quintans, M.R. Loken, M.R. Pierres, and F.W. Finch. 1983. Characterization of the murine T cell surface molecule, designated L3T4, identified by monoclonal antibody GK1.5. J. Immunol. 131:2445.

44. Vlahakos, D., M.H. Foster, A.A. Ucci, K.J. Barrett, S.K. Datta, and M.P. Madaio. 1992. Murine monoclonal anti-DNA antibodies penetrate cells, bind to nuclei, and induce glomerular proliferation and proteinuria in vivo. J. Am. Soc. Nephrol. 2:1345.

45. Madaio, M.P., J. Carlson, J. Cataldo, A. Ucci, P. Migliorini, and O. Pankewzycz. 1987. Murine monoclonal anti-DNA antibodies bind directly to glomerular antigens and form immune deposits. J. Immunol. 138:2883.

46. Austin, H.A.I., L.R. Muenz, K.M. Joyce, P.T. Antonowycz, and J.E. Balow. 1984. Diffuse proliferative lupus nephritis: identification of specific pathologic features affecting renal outcome. Kidney Int. 25:689.

47. Ron, Y., and J. Sprent. 1987. T cell priming in vivo: a major role for B cells in presenting antigen to T cells in lymph nodes. J. Immunol. 138:2848.

48. Janeaway, C.A., Jr., J. Ron, and M.E. Katz. 1987. The B cell is the initiating antigen-presenting cell in peripheral lymph nodes. J. Immunol. 138:1051.
49. Budd, L.C., M. Schreyer, G.C. Miescher, and H.R. MacDonald. 1987. T cell lineages in the thymus of lpr/lpr mice: evidence for parallel pathways of normal and abnormal T cell development. *J. Immunol.* 139:2200.

50. Matsuzaki, Y., C. Pannetien, O. Kanagawa, G. Gachelin, and H. Nakachi. 1992. Evidence for the existence of two parallel differentiation pathways in the thymus of MRL lpr/lpr mice. *J. Immunol.* 149:1069.

51. Zhou, T., H. Bluethmann, J. Eldridge, K. Berry, and J.D. Mountz. 1993. Origin of CD4- CD8- B220+ T cells in MRL-lpr/lpr mice. Clues from a T cell receptor beta transgenic mouse. *J. Immunol.* 150:3651.

52. Shlomchik, M.J., A. Marshak-Rothstein, C.B. Wolfowicz, T.L. Rothstein, and M.G. Weigert. 1987. The role of clonal selection and somatic mutation in autoimmunity. *Nature (Lond.)* 328:805.

53. Levine, J., D. Hartwell, and D.I. Beller. 1991. Imbalanced cytokine production by macrophages from autoimmune-prone mice. *Immunol. Lett.* 30:183.

54. Bloom, R.D., S. Florquin, G.G. Singer, D.C. Brennan, and V.R. Kelley. 1993. Colony stimulating factor-1 in the induction of lupus nephritis. *Kidney Int.* 43:1000.

55. Kelley, V.R., R.D. Bloom, M.A. Yui, C. Martin, and D. Price. 1994. Pivotal role of colony stimulating factor-1 in lupus nephritis. *Kidney Int.* 45(Suppl.):S83.

56. Halloran, P.F., J. Urman, V. Ramassar, C. Laska, and P. Auttenried. 1988. Increased class I and class II MHC products and mRNA in kidneys of MRL-lpr/lpr mice during autoimmune nephritis and inhibition by cyclosporine. *J. Immunol.* 141:2303.

57. Yee, J., G.S. Kuncio, and E.G. Neilson. 1991. Tubulointerstitial nephritis following glomerulonephritis. *Semin. Nephrol.* 11:361.

58. Berden, J.H., L. Hang, P.J. McCahey, and P.J. Dixon. 1983. Analysis of vascular lesions in murine SLE. I. Association with serologic abnormalities. *J. Immunol.* 130:1699.

59. Nose, M., M. Nishimura, and M. Kyogoku. 1989. Analysis of granulomatous arteritis in MRL/Mp autoimmune disease mice bearing lymphoproliferative genes. *Am. J. Pathol.* 135:271.

60. Huang, L., K. Sye, and I.N. Crispe. 1994. Proliferation and apoptosis of B220+ CD4- CD8- TCRα/βtransgenic T cells in the liver of normal adult mice: implication for lpr pathogenesis. *Int. Immunol.* 6:533.

61. Miyama-Inaba, M., S.-I. Kuma, K. Inaba, H. Ogata, H. Iwai, R. Uasumizu, S. Muramatsu, R.M. Steinman, and S. Ikehara. 1988. Unusual phenotype of B cells in the thymus of normal mice. *J. Exp. Med.* 168:811.

62. Watson, M.I., J.K. Rao, G.S. Gilkeson, P. Ruiz, E.M. Eicher, D.S. Pisetsky, A. Matsuzawa, J.M. Rochelle, and M.F. Seldin. 1992. Genetic analysis of MLR-lpr mice: relationship of the Fas apoptosis gene to disease manifestations and renal disease-modifying loci. *J. Exp. Med.* 176:1645.

63. Jernikar, A.M., M.J. Grusby, and L.H. Glimcher. 1994. Prevention of nephritis in major histocompatibility complex class II-deficient MRL-lpr mice. *J. Exp. Med.* 179:1137.

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