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We generated MRL/lpr mice deficient in activation-induced deaminase (AID). Because AID is required for Ig hypermutation and class switch recombination, these mice lack hypermutated IgG Abs. Unlike their AID wild-type littermates, AID-deficient MRL/lpr mice not only lacked autoreactive IgG Abs but also experienced a dramatic increase in the levels of autoreactive IgM. This phenotype in AID-deficient mice translated into a significant reduction in glomerulonephritis, minimal mononuclear cell infiltration in the kidney, and a dramatic increase in survival to levels comparable to those previously reported for MRL/lpr mice completely lacking B cells and well below those of mice lacking secreted Abs. Therefore, this study wherein littermates with either high levels of autoreactive IgM or autoreactive IgG were directly examined proves that autoreactive IgM Abs alone are not sufficient to promote kidney disease in MRL/lpr mice. In addition, the substantial decrease in mortality combined with a dramatic increase in autoreactive IgM Abs in AID-deficient MRL/lpr mice suggest that autoreactive IgM Abs might not only fail to promote nephritis but may also provide a protective role in MRL/lpr mice. This novel mouse model containing high levels of autoreactive, unmutated IgM Abs will help delineate the contribution of autoreactive IgM to autoimmunity. The Journal of Immunology, 2007, 178: 7422–7431.

MRL-Fas replication (MRL/lpr) mice develop a systemic autoimmune syndrome that shares many characteristics of human systemic lupus erythematosus (SLE) (1–2). Like the human disease, the MRL/lpr syndrome is characterized by polygenic inheritance, the presence of circulating autoantibodies, particularly to nuclear components, and lupus nephritis development through glomerular disease, mononuclear cell infiltration, and immune complex deposition (1–2). MRL/lpr mice also develop splenomegaly and lymphadenopathy, with mononuclear cell infiltration in lungs, liver, and other tissues (3–4). Unlike human SLE with low monozygotic twin concordance (5), all MRL/lpr mice eventually develop the syndrome (2). Multiple loci contribute to autoimmunity in MRL/lpr mice, suggesting the involvement of various systems. Implicated are defects in B and T cell tolerance, complement activation, cytokine regulation, endothelial cell function, and apoptotic clearance (6–21).

There is compelling evidence for a role of B cells in the MRL/lpr syndrome, particularly affecting glomerulonephritis. B cell-deficient-MRL/lpr mice failed to develop glomerulonephritis (22–24). Also important is a diverse lymphocyte repertoire, because MRL/lpr mice lacking terminal deoxynucleotidyl transferase, an enzyme that adds nucleotides to the V(D)J segments during recombination, have decreased glomerular disease (25–29). However, how B cells contribute to lupus nephritis might be more complicated than previously appreciated. In addition to secreting autoantibodies, B cells might contribute to lupus nephritis as APCs to autoreactive T cells and by promoting an inflammatory environment (23, 30–31). MRL/lpr mice lacking secreted Abs but with B cells bearing IgM receptors still develop a milder form of kidney disease and experience higher mortality rates than mice completely lacking B cells (32). A hallmark feature of MRL/lpr mice lacking B cells is a dramatic increase in the proportion of naive CD4+ T cells with a concomitant decrease in memory or activated T cells that was reconstituted to levels similar to those of conventional MRL/lpr mice in mice with B cells but without secreted Abs (32). These results suggest an additional, autoantibody-independent B cell role in the development of lupus nephritis in MRL/lpr mice, likely through the activation of autoreactive T cells.

An aspect of B cell biology that impacts autoimmunity is the memory B cell response. B cells jointly activated by Ag and CD4+ T cells seed germinal centers (GCs) (3) in secondary lymphoid tissues wherein their affinity to foreign Ag is enhanced by Ig somatic hypermutation (SHM) and cellular selection (33–38). Isotype class switch recombination (CSR) also occurs in the GC environment, although not exclusively. In SHM, base pair substitutions are introduced into the DNA encoding the V regions of Ig receptors. Follicular dendritic cells provide foreign Ag to B cells in the GCs, selecting B cells with affinity-enhancing mutations to Ag in their receptors. Multiple rounds of division, mutation, and selection generate highly specific memory B cells. Interestingly, a majority of autoantibodies in patients with SLE and in MRL/lpr mice are hypermutated and isotype switched (39–46). In MRL/lpr mice, Abs with mutations in the H chain Ig V region correlate with anti-dsDNA specificity, particularly those introduc-
self-Ags drive the affinity maturation reaction. Evidence of the latter scenario is found in diseases such as rheumatoid arthritis, myasthenia gravis, and Sjögren’s syndrome with ectopic GC formation resulting in high affinity autoantibodies against local self-Ags (48–50). The recent discovery of activation-induced deaminase (AID), a molecule critical to SHM and CSR (51), can now provide a direct approach at examining the contribution of mutated, class-switched Abs to the MRL/lpr mice syndrome. Because AID is required for SHM and CSR (51–55), AID deficiency blocks the formation of high affinity, isotype-switched Abs in activated B cells without impacting B or T cell development (51). In this study, we generated MRL/lpr mice lacking AID and examined the impact of the lack of hypermutated, switched autoantibodies in the lupus syndrome of these mice.

Materials and Methods

Generation of AID-deficient MRL/lpr mice

AID-deficient C57BL/6 mice were provided by T. Honjo (Kyoto University, Kyoto, Japan) and D. Schatz (Yale University School of Medicine, New Haven, CT). MRL-lpr (C57Bl/6-lpr/lpr MRL/lpr), C57BL/6/HBlcr, and BALB/c strains were purchased from The Jackson Laboratory. AID−/− mice were backcrossed to MRL/lpr mice and, at the fifth and sixth generations AID−/− MRL/lpr mice (with >96% MRL/lpr background) were intercrossed to generate AID−/+; AID−/-, and AID−/− MRL/lpr mice (n = 34, 33, 34, respectively). The mice were housed in specific pathogen-free facilities, maintained in microisolation cages on hardwood bedding, and provided with autoclaved food and reverse osmosis, deionized water.

AID alleles were examined by PCR with primers 5′-CTGGAGATGGAACCCCTAACCTCACG-3′ plus G4 (5′-CAGGATTTCTTCTAAAATGTATCCAGCTC-3′) for the wild-type allele and G3 (5′-GGGCCAGCTATTTCCCTACCTC-3′) plus G4 for mutant allele detection as described (51). Fas alleles were amplified by PCR following The Jackson Laboratory web site protocol with the primers FASf1 (5′-GTTAAATTATTTGTGCTTGT-3′/H11032) and the wild-type allele and G3 (5′-GGGCCAGCTATTTCCCTACCTC-3′) plus G4 for mutant allele detection. Both reagents were purchased from The Jackson Laboratory.

Histology

Formalin-fixed tissues were embedded in paraffin, cut into-5 μm sections, and stained with H&E. The severity of any abnormalities observed was graded as follows: 1, minimal; 2, mild; 3, moderate; and 4, marked. Additional sections of kidney were stained with periodic acid-Schiff stain. Glomerular change severity was graded based upon an increase in the size of affected glomeruli due to increased cellularity and the mesangial matrix. The severity of mononuclear cell infiltrate was graded based upon the total amount of infiltrate present. The number of cells in each of 20 glomeruli per mouse was scored for the kidneys of each mouse. C57BL/6 and BALB/c mice of similar age were used as controls; the amount of mesangial matrix present in the glomeruli of controls, (~10% of glomeruli), was considered the amount normally present. Lungs, lymph nodes, spleen, liver, and bone marrow from each animal were examined for mononuclear cell infiltration.

Electron microscopy

Kidneys from 16- to 18 wk-old mice were collected in 3% paraformaldehyde and embedded in Spurr’s resin. Approximately 80-mm sections from epoxy blocks were cut, mounted on 200-mesh copper grids, stained with methanolic uranyl acetate and Reynolds lead citrate, and examined on a Zeiss 900 transmission electron microscope. A total of 40 photomicrographs from two representative mice of each genotype were evaluated.

Detection of urine protein level

Urine protein levels, collected monthly by expressing urine from the urethra directly, were tested with Multistix 10 SG (Bayer), and scored as follows: 0, negative; 1, trace; 2, 30 mg/dl; 3, 100 mg/dl; 4, 300 mg/dl; and 5, 2000 mg/dl or more.

Blood urea nitrogen and creatinine levels in the serum

Blood urea nitrogen and creatinine levels were determined by urease with the glutamate dehydrogenase reaction and alkaline picate (Jaffe Reaction), respectively. Both reagents were purchased from Olympus America and the determinations were run using an Olympus AU400e clinical analyzer (Olympus America).

Immunofluorescence and immunohistochemistry

To examine complement component 3 (C3) staining in glomeruli, kidneys from 16- to 18-wk-old mice were frozen in Tissue-Tek OCT (Sakura) and frozen sections (6 μm) were stained with a FITC-conjugated goat serum (CalTag Laboratories) and with a FITC-conjugated anti-mouse Ab (Sigma-Aldrich) at a 1/100 dilution.

To examine GC morphology, biotin-labeled peanut agglutinin (PNA) (Vector Laboratories) was used following standard avidin-biotin-peroxidase protocols. Briefly, frozen spleens from 16- to 18-wk-old F5 mice were sectioned on a cryostat (6 μm), affixed to slides, and placed into Rapid Fix solution (Shandon-Lipshaw) and 1× automation buffer solution. The sections were placed in 3% H2O2 and rinsed in 1× automation buffer. Protein blocking was done with an avidin-biotin blocking kit (Vector Laboratories). Incubation with PNA was done at a 1/1000 dilution (1 mM CaCl2, MgCl2, and MnCl2) and labeling with Biogenex streptavidin label. The stain was developed with diaminobenzidine chromogen (DakoCytonation) and the slides were counterstained with hematoxylin, dehydrated with graded ethanol, and visualized with a fluorescence microscope.

Flow cytometry

The following conjugated Abs from BD Pharmingen were used following the manufacturer’s instructions (1 μg/million cells): rat anti-mouse CD19 PE-Cy7; rat IgG2a, kappa isotype control PE-Cy7; rat anti-mouse CD45R/B220-allophycocyanin-Cy7; allophycocyanin-Cy7-conjugated rat IgG2a, kappa isotype control; FITC-conjugated rat anti-mouse CD21/CD35 mAb; FITC-conjugated rat IgG2b, kappa isotype control; biotin-conjugated rat anti-mouse CD23 (FcγII/III) mAb; biotin-conjugated IgG2a, kappa isotype control; streptavidin-allophycocyanin conjugate; rat anti-mouse CD4 PE; rat IgG2a, kappa isotype control R-PE; rat anti-mouse CD8 allophycocyanin, rat IgG2a, kappa isotype control allophycocyanin; rat anti-mouse CD44 PE-Cy5; rat IgG2b, kappa isotype control PE-Cy5; anti-mouse CD62L FITC; rat IgG2a, kappa isotype control FITC; anti-mouse CD40 R-PE'; rat IgG2a, kappa isotype control R-PE; rat anti-mouse I-A^b; streptavidin-allophycocyanin conjugate; mouse IgG2b, kappa isotype control; pan anti-mouse CD3 PE-Cy5; and rat IgG2b, kappa isotype control PC-5.

Splenic suspensions were made by squashing spleens between two frosted slides. RBC were lysed by ACK lysis buffer (0.15 M NH4Cl, 1.0 mM KHCO3, and 0.1 mM Na2-EDTA). After washing with Dulbecco’s PBS, cells were resuspended in staining buffer (Dulbecco’s PBS plus 3% FBS and 0.09% sodium azide) at 1 × 109 cells/100 μl, incubated with anti-FcγIII/IIIA Ab (BD Pharmingen), and stained with the corresponding conjugated Abs in the dark. Cells were resuspended in 1 ml of staining buffer and were either analyzed on a BD LSR II flow cytometer (BD Biosciences) or fixed with 1 ml of 1% paraformaldehyde in PBS and stored at 4°C for next day analysis.

Detection of serum Abs by ELISA

Beginning at 2 mo of age, mice were bled monthly by retro-orbital puncture. Serum IgM, IgG, and IgA levels were determined with commercial ELISA kits (Bethyl Laboratories) following the manufacturer’s instructions. Sera were diluted at 1/10,000, 1/50,000, and 1/100,000 for the detection of total IgM, IgG, and IgA, respectively. Mouse anti-dsDNA IgM and IgG Abs were measured as previously reported with modification (47). Briefly, Costar 96-well enzyme immunoassay/radioimmunoassay (ELIA/RIA) plates (Corning) were coated with streptavidin (Sigma-Aldrich) in PBS at 100 μl/well at 4°C, washed with PBS (pH 7.4) and 0.05% Tween 20), Salmon sperm DNA (Sigma-Aldrich) was phenol/chloroform extracted, treated with S1 nuclease, and digested with HaeIII. The DNA was biotinylated with Photoprobe biotin reagent (Vector
Laboratories) following the manufacturer’s instructions. One hundred microliters of biotin-dsDNA in PBS at 400 ng/ml were added to wells and incubated at 4°C overnight. Two hundred microliters of blocking buffer (PBS (pH 7.4) with 1% BSA) at room temperature for 2 h. Mouse sera were diluted with a sample diluent (blocking buffer plus 0.05% Tween 20) at 1/2,000 (IgM) and 1/1,000 (IgG) dilutions and added to wells at 100 μl/well. Pooled sera from four moribund MRL/lpr mice were serially diluted with a sample diluent from 1/200 to 1/51,200 (IgM) and 1/400–1/102,400 (IgG) dilutions and added to wells at 100 μl/well. Following incubation and washing, 100 μl of a 1% BSA blocking buffer was added (PBS (pH 7.4) with 1% BSA) at room temperature for 2 h. Mouse sera were incubated at 4°C overnight. Two hundred microliters of biotin-dsDNA in PBS at 400 ng/ml were added to wells and incubated at 100 μl/well. Pooled sera from four moribund MRL/lpr mice were serially diluted with a sample diluent from 1/200 to 1/51,200 (IgM) and 1/400–1/102,400 (IgG) to be used as standards. Background binding to streptavidin was determined. Goat anti-mouse IgG-HRP and goat antimouse IgM-HRP conjugators (Bethyl Laboratories) were diluted at 1/100,000 and added at 100 μl/well. Following incubation and washing, tetramethylbenzidine enzyme substrate (Kirkegaard and Perry Laboratories) was added at 100 μl/well and incubated for 30 min at room temperature. The reaction was stopped by adding 100 μl of 1 M H2SO4. The absorbance at 450 nm was measured in a Multiskan Ascent microplate reader (Thermo Electron). The amount of anti-dsDNA IgM and IgG in wells was calculated according to the standard curve in which the pooled standard sera were defined as a value of 1.

Antinuclear Abs (ANA) test

ANAs were examined using a commercial indirect fluorescent Ab assay (ANA HEP-2 Ag substrate slide; Bion Enterprises) following the manufacturer’s instructions. A drop of diluted mouse serum (1/200 in PBS) was applied to slide wells. After incubation for 30 min at room temperature, the slide was washed twice with PBS in a Coplin jar. FITC-conjugated goat anti-mouse IgG (γ-chain specific) or IgM (μ-chain specific) Abs (Sigma-Aldrich) was added to wells at 1/200 in PBS. After two washes, slides were mounted with a coverslip and examined under a fluorescence microscope.

FIGURE 1. AID deficiency-associated reduction in glomerulonephritis and mononuclear cell infiltrates in MRL/lpr mice. A. Average severity scores for amount of mesangial matrix, mononuclear cell infiltrates, and overall glomerulonephritis in kidneys of AID+/+ MRL/lpr (n = 19), AID+/− MRL/lpr (n = 18), and AID−/− MRL/lpr (n = 19) F5 mice. The increase in the mesangial matrix and glomerular change severity (due to increased cellularity and matrix) was graded as follows: 0, no increase (matrix occupied up to 10% of the glomerulus; 10% increased cellularity and mesangial matrix); 1, minimal (up to 25%); 2, mild (up to 50%); 3, moderate (up to 75%); and 4, marked (>75%). B. The average number of glomerular cells in similar-size glomeruli is depicted. Nonautoimmune AID-deficient mice (n = 6), C57BL/6 mice (n = 4), and BALB/c mice (n = 3) were used as controls. Mesangial matrix, glomerulonephritis, and mononuclear cell scores in control mice were set at 0 for comparison. C, Periodic acid-Schiff stain from representative AID wild-type and AID-deficient MRL/lpr mice are shown at ×20 magnification. Arrows point to glomeruli. D, H&E stains of kidney cross-sections from representative AID wild-type and AID-deficient MRL/lpr mice. The arrows indicate areas of dense infiltrates.

Statistical analysis

Pairwise associations between the outcomes (lymphoid hyperplasia, glomerulonephritis, and mononuclear cell infiltrate) were examined with the Kruskal-Wallis ANOVA and Spearman’s correlation coefficient. When significant differences were detected, Mann-Whitney tests were used to compare them to the mild severity group. To assess which combination of measures best predicted outcome severity, stepwise linear regression analysis was used. Kruskal-Wallis ANOVA was used to test for differences among genotypes followed by Mann-Whitney tests to identify the differing pair of genotypes. For urine data, paired measurements on wk 12–14 and wk 17–19 were compared using the Wilcoxon signed-ranks test.

Differences were considered statistically significant at the 0.05 level using the Bonferroni correction for multiple testing where appropriate.

Results

To examine the role of high affinity isotype-switched Abs in the lupus-like syndrome of MRL/lpr mice, we backcrossed AID−/− mice to MRL/lpr mice and generated fifth (F5) and sixth (F6) generation backcrossed MRL/lpr mice that were wild-type, heterozygous, or deficient in AID. In analyses where both F5 and F6 mice were examined (kidney weights, urine protein, lymphocyte populations, and anti-dsDNA IgG and IgM), the trends were nearly identical between them.

Within each genotype, lesion severity was not significantly associated with Ab levels. However, among all genotypes combined
the following trends were consistently detected for glomerulonephritis scores: with increasing glomerulonephritis severity, anti-dsDNA IgG, urine protein, and mononuclear cell infiltrate levels increased. Among genotype comparisons, highly significant differences were detected for most measures and are discussed below.

**AID deficiency in the MRL/lpr background alleviated glomerulonephritis and mononuclear cell infiltration in the kidneys**

Multifocal mononuclear cell infiltration and glomerulonephritis were prominent findings in the kidney of MRL/lpr mice. The average severity of glomerulonephritis and mononuclear cell infiltrates among the F5 mice was significantly higher in the AID-wild type and AID-heterozygous MRL/lpr mice than in the AID-deficient MRL/lpr mouse littermates (Fig. 1; Kruskal-Wallis ANOVA, \( p < 0.0001 \)).

Glomerulonephritis was characterized by varying increases in the mesangial matrix due to a homogeneous eosinophilic material filling the mesangial spaces between glomerular capillary loops (Fig. 1C). In glomeruli with severe glomerulonephritis, mesangial matrix increase was associated with increasing numbers of glomerular cells. Both the mesangial matrix average score and the number of glomerular cells were dramatically reduced with AID deficiency (Fig. 1, A and D) wherein glomerular cell numbers in AID-deficient MRL/lpr mice were similar to those observed in C57BL/6 and BALB/c mice (Fig. 1B). The glomerular cell increase was due primarily to inflammatory cells, particularly mesangial macrophages.

Mononuclear cell infiltrates consisted of mixed mononuclear inflammatory cells, primarily lymphocytes and macrophages in the kidney interstitium. AID-deficient MRL/lpr mice mononuclear cell infiltrate scores were reduced compared with those of AID wild-type and heterozygous MRL/lpr littermates (Fig. 1, A and D; Kruskal-Wallis ANOVA, \( p < 0.0001 \)). These cells accumulated adjacent to the renal pelvis, and in AID-deficient MRL/lpr mice they were seen only in that location (Fig. 1D). As the amount of infiltrate increased, the cells formed large cuffs around arcuate arteries in the cortex, and in AID wild-type MRL/lpr mice the cells were also scattered in the interstitium between clusters of tubules. Mononuclear cell infiltration in AID-deficient MRL/lpr mice, although reduced over that in wild type littersates, was above background compared with AID/C57BL/6, C57BL/6, and BALB/c mice. The kidney weights of F5 and F6 AID \( ^{-/} \) MRL/lpr mice were significantly reduced compared with those of AID wild-type littermates (data not shown).

Consistent with the reduced kidney pathology observed in the histology, urine protein levels in F5 AID-deficient MRL/lpr mice older than 10 wk of age were significantly lower than those in AID wild-type MRL/lpr mice (Fig. 2A; Kruskal-Wallis ANOVA, \( p = 0.0002 \)) and undistinguishable from those in either nonautoimmune AID-deficient mice in the B16 background or conventional C57BL/6 mice. Urine protein scores from AID wild-type MRL/lpr mice of the F6 backcross generation were also significantly higher than those from AID-deficient MRL/lpr siblings at 17–19 wk (\( n = 24 \), mean of 2.8 ± 1.32 in wild type compared with 1.39 ± 0.41 for AID-deficient mice; Wilcoxon signed-ranks test, \( p < 0.01 \)). Similarly, F7 and F9 combined data from mice with >99.22% MRL/lpr background that were between 20 and 30 wk old displayed the same trend (\( n = 17 \), mean of 3.11 ± 1.19 for AID-competent mice vs 1.5 ± 0.40 for AID-deficient MRL/lpr mice; Wilcoxon signed-ranks test, \( p < 0.01 \)). Furthermore, this difference in correlates of kidney pathology persisted over time, as significantly higher levels of blood urea nitrogen and creatinine in the serum of AID wild-type and heterozygous MRL/lpr mice were detected when compared with AID-deficient MRL/lpr siblings of the F5 generation at 52 wk of age (Fig. 2B; \( p < 0.05 \) for creatinine analysis and \( p < 0.01 \) for blood urea nitrogen analysis; Wilcoxon ranks test).

AID-deficient MRL/lpr mice kidneys revealed lower C3 levels in their glomeruli than AID wild-type MRL/lpr littermates (Fig. 3A; Kruskal-Wallis ANOVA, \( p < 0.02 \)), suggesting that the abrogation of glomerulonephritis is associated with a reduction in immune complex deposition. Also, IgG deposition in the glomeruli of AID wild-type MRL/lpr mice was detected at 16- to 18-wk of age but, as expected, was absent in glomeruli from AID-deficient MRL/lpr mice (Fig. 3B). IgM deposition was detected in AID-wild type, heterozygous, and deficient MRL/lpr mice, but no differences among the groups were seen (data not shown). To determine whether any evidence of the early stages of glomeruli damage could be observed in AID-deficient MRL/lpr mice, electron micrographs of glomeruli from representative mice were taken. Although the glomeruli from AID-wild type MRL/lpr mice had severe lesions consisting of fusion of the glomerular podocytes’ foot processes and infiltration by intravascular macrophages, the glomeruli from AID-deficient MRL/lpr mice were intact and undistinguishable from those of nonautoimmune C57BL/6 mice (Fig. 3C).

Glomerulonephritis scores were similar between males and females (Kruskal-Wallis ANOVA, \( p > 0.15 \)), but females tended to have more severe mononuclear cell infiltrate scores (Kruskal-Wallis ANOVA, \( p = 0.007 \)). Gender differences in mononuclear cell
infiltrates cannot account for the differences among genotypes because similar gender ratios were used and, when analyzed separately for gender, the differences between the various MRL/lpr littermates remained intact.

Pathological manifestations in other tissues were similar among all MRL/lpr mice and different from those of normal mice

In the liver, a small degree of mononuclear cell infiltrates was observed in some mice of the MRL/lpr background regardless of AID status. Also, nearly all MRL/lpr mice had lymphoid hyperplasia in the spleen, and myeloid hyperplasia of the bone marrow, although the latter was reduced with AID deficiency. There was lymphoid hyperplasia in the lung that was characterized primarily by an increase in the number of lymphocytes normally present around vessels and airways and generally affected primarily one or two lung lobes rather than diffusely affecting all lobes. However, there was no difference in lung hyperplasia in AID-deficient MRL/lpr mice when compared with that of AID-wild type or heterozygous siblings. Also, no significant difference was detected among all MRL/lpr mice in spleen or lymph node weights (data not shown).

Improved survival with AID deficiency in MRL/lpr mice

To examine the impact of AID deficiency on lifespan, a group of F5 mice were allowed to live until multiple signs of impending death were evident as determined by at least two veterinarians (i.e., decreased activity, lowered body temperature, respiratory distress, weight loss, etc.). After 50 wk, ~75% of the AID-wild type MRL/lpr mice, 65% of AID-heterozygous MRL/lpr mice, and 75% of the nonbackcrossed MRL/lpr controls had perished whereas only 22% of the AID-deficient MRL/lpr had died, indicating a dramatic increase in lifespan with AID deficiency in MRL/lpr mice (Fig. 4; Wilcoxon test; p < 0.0001).

B and T cell subsets in AID-deficient MRL/lpr mice

The total numbers of CD19+ B220+ B cells, and, among CD19+ B cells, the percentages of naive and activated B cells (based on the expression of CD40, I-Ak, PNA, or CD44) from spleen and lymph...
nodes were similar among F5 and F6 MRL/lpr mice regardless of AID status (data not shown). As reported previously (7) marginal zone B cells (based on CD21/CD23 expression) in all MRL/lpr mice were increased over those of BALB/c and C57BL/6 mice (~26% in MRL/lpr vs ~10% in C57BL/6 mice) with a concomitant reduction in follicular zone B cells. The germinal centers of AID-deficient MRL/lpr mice were similar in morphology and number to AID wild-type MRL/lpr littermates as revealed by PNA staining of GC B cells (data not shown).

Within T cells, the fractions of CD4+ or CD8+ T cells were similar among MRL/lpr mice regardless of AID status. The intriguing CD4+CD8−B220+ T cell population characteristic of MRL/lpr mice (56) was similar among all MRL/lpr mice, (50% of CD3+ cells in the spleen and lymph nodes). B cell-deficient MRL/lpr mice had been previously reported to have a large increase in the percentage of naive CD4+ T cells with a concomitant decrease in activated or memory T cells (23), suggesting B cell-mediated activation of autoreactive T cells. Because in mice with B cells but lacking secreted Abs the alteration in the proportions of naive, activated, and memory T cells were restored to those seen in MRL/lpr mice, (naive T cell population was reduced by ~90%), this effect on splenic T cells was directly attributed to an Ab-independent role by B cells (32). F5 and F6 AID-deficient MRL/lpr mice consistently displayed a slight increase in the splenic naive CD4+ T cell population that was significant in the F6 mice, but this increase was only 2-fold (3% in AID+/+ vs 6% in AID−/−).
AID deficiency in MRL/lpr mice resulted in a dramatic decrease in glomerulonephritis and monoclonal cell infiltrates in the kidneys, with a concomitant increase in survival. Because these mice cannot undergo SHM or CSR, these results directly implicate high-affinity switched Abs in kidney damage and mortality in MRL/lpr mice. Affinity maturation and SHM in the GC reaction have been implicated in the lupus-like syndrome of MRL/lpr mice because many MRL/lpr mice-derived autoantibodies originate from oligoclonal populations of B cells with mutations selected for self-Ag binding (39, 41, 42, 44, 46, 57–59). These results confirm the observation that Abs derived from B cells activated to undergo CSR and SHM play a critical role in the development of kidney disease in MRL/lpr mice.

AID-deficient MRL/lpr mice displayed a 5-fold increase in anti-dsDNA IgM levels in the serum, and their Abs stained nuclear and cytoplasmic components when incubated with HEp-2 cells. The increased levels of autoreactive IgM Abs required both AID deficiency and the MRL/lpr background, because conventional AID knockout mice had only a 2-fold increase over conventional C57BL/6 mice in autoreactive IgM and both had >60-fold lower levels than AID-deficient MRL/lpr mice. It may be that autoreactive surface IgM-bearing B cells that escape central tolerance are normally mutated away from polyreactivity as they switch to IgG-secreting cells in GCs. If true, then these mice are not capable of undergoing SHM these autoreactive B cells would be predicted to accumulate in AID-deficient MRL/lpr mice. Preliminary data from hybridomas generated from these mice showed a 7-fold increase in the number of autoreactive clones when compared with hybridomas derived from AID wild-type littermates, suggesting that the increase in autoreactive IgM Abs in the serum originates from an increase in autoreactive IgM-secreting B cells (C. Jiang and M. Diaz, unpublished data).

The importance of IgG autoantibodies in kidney pathology is suggested by the reduced glomerular injury in mice deficient for the activating receptors FcγRI and FcγRIII but the increased severity in mice deficient in the inhibitory receptor FcγRIIb (60–63). The role of IgM in lupus nephritis is more controversial. Several studies have identified IgM autoantibodies that significantly contribute to immune complex deposition and are nephritogenic (64–66), whereas others have either found a negative correlation between secreted autoreactive IgM and kidney pathology or proposed that monoclonal autoreactive IgM autoantibodies might even mediate the alleviation of nephritis (67–70). This study provides a direct
have high levels of natural IgM Abs and that these Abs play a role in inflammation, it may be that AID-deficient MRL/lpr mice with high levels of secreted IgG have a limited repertoire. These results suggest, at best, a modest contribution by B cells to the difference in mortality rates for mIgM mice and similar to those of MRL/lpr mice. However, others and we have observed IgM deposition in the glomeruli of kidneys of MRL/lpr mice. More likely explanations for the nonpathogenic effect of autoreactive IgM reported here include the recruitment of different clearance pathways activated by IgM vs IgG and the impact of these pathways on inflammation (see below).

If IgG vs IgM Abs was the entire story, one would expect that the mortality rates of AID-deficient MRL/lpr mice would be similar to those of MRL/lpr mice with B cells but lacking secreted Abs (mIgM) (32). However, the mortality rates of AID-deficient MRL/lpr mice were much lower than the previously reported mortality rates for mIgM mice and similar to those of MRL/lpr mice completely lacking B cells (IgM), even though the mortality for AID-deficient MRL/lpr mice was similar to that of MRL/lpr mice. (IgM) (32). However, the mortality rates of AID-deficient MRL/lpr mice were much lower than the previously reported mortality rates for mIgM mice and similar to those of MRL/lpr mice completely lacking B cells (IgM), even though the mortality for AID-deficient MRL/lpr mice was similar to that of MRL/lpr mice completely lacking B cells (IgM). Despite having high levels of autoreactive IgM, experienced a near complete abrogation of glomerulonephritis, proving that autoreactive IgM Abs derived from a full B cell repertoire are not sufficient to induce kidney disease in MRL/lpr mice. One possibility is that the deposition of IgM-containing immune complexes might be altered and their trapping in glomeruli might be inhibited due to the larger size of the IgM pentamer compared with that of IgG. However, others and we have observed IgM deposition in the glomeruli of kidneys of MRL/lpr mice (65). More likely explanations for the nonpathogenic effect of autoreactive IgM reported here include the recruitment of different clearance pathways activated by IgM vs IgG and the impact of these pathways on inflammation (see below).

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