Prevention of Nephritis in Major Histocompatibility Complex Class II-deficient MRL-*lpr* Mice

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Summary

MRL-*lpr* mice develop aggressive autoimmune kidney disease associated with increased or de novo renal expression of major histocompatibility complex (MHC) class II molecules and a massive systemic expansion of CD4$^-$CD$^-$ double negative (DN) T cells. Whereas non-MHC linked genes can have a profound effect on the development of nephritis, lymphadenopathy, and anti-DNA antibody production in MRL-*lpr* mice, the role of MHC molecules has not been unequivocally established. To study the role of MHC class II in this murine model of systemic lupus erythematosis, class II–deficient MRL-*lpr* mice (MRL-*lpr* $-/-$) were created. MRL-*lpr* $-/-$ mice developed lymphadenopathy but not autoimmune renal disease or autoantibodies. This study demonstrates that class II expression is critical for the development of autoaggressive CD4$^+$ T cells involved in autoimmune nephritis and clearly dissociates DN T cell expansion from autoimmune disease initiation.

M RL-Mp-*lpr/lpr* (MRL-*lpr*)[1] mice spontaneously develop an autoimmune disorder characterized by an immune complex glomerulonephritis, arthritis, vasculitis, and autoantibodies to nucleic acids (1). Although these mice do not precisely represent human autoimmune disease, shared immunopathologic and serologic features make this an accepted model of human SLE or lupus (2). MRL-*lpr* mice homozygous for the *lpr* gene develop massive lymphadenopathy due to the influx of abnormal double negative (DN) T cells which express CD3 but not CD4 or CD8 accessory molecules (3, 4). The *lpr* gene represents a loss of function mutation in the *fas* gene located on murine chromosome 19, resulting in defective apoptosis and DN T cell expansion (2, 5–7). Although data exists to support defects in both positive and negative thymic selection, detailed TCR Vβ repertoire analysis of CD4$^+$, CD8$^+$, and DN T cell populations would support the suggestion that normal thymic selection processes exist despite the absence of *fas* (8–10). DN cells may originate from early CD4$^-$CD8$^-$ T cell precursors or more likely from "neglected" CD4$^+$, CD8$^+$, dull TCR$^+$ T cells, with loss of accessory molecules and proliferation after escape to LN or liver mediated by a peripheral *fas* defect (3, 10, 11). The *lpr* mutation does not directly cause autoimmune disease, as the congenic strain MRL +/+ develops late

onset autoimmune syndrome, and homozygous expression of the *lpr* gene backcrossed to nonautoimmune susceptible strains leads to lymphadenopathy and autoantibodies, but not to aggressive and rapid renal disease (12). It is therefore apparent that whereas the MRL background is required for autoimmune disease to develop, *lpr* accelerates rather than causes disease, and other as yet unidentified factors influence the expression of disease.

DN T cells do not appear to be essential for initiation of disease. Mice homozygous for the *lpr* mutation and transgenic for a class I–restricted α/β TCR specific for male (H-Y) antigen do not generate CD4$^-$CD8$^-$ T cells yet have similar levels of autoantibodies, although the effect on lymphadenopathy may vary with mouse strains (3, 4, 13). Whereas in vitro studies of freshly isolated DN cells from MRL-*lpr* mice demonstrate transcription of IFN-γ and TNF-α mRNA (14), DN T cells may not produce these as biologically active cytokines in vivo (11, 15). However, these cells may not be totally functionally inert as DN T cell clones have been shown to induce MHC class II and intercellular adhesion molecule 1 (ICAM-1) expression on renal tubular cell epithelia, suggesting a role in promoting renal disease (16). Despite the massive expansion of DN T cells, increased absolute numbers of CD4$^+$ T cells in adult MRL-*lpr* mice could accelerate nephritis by inducing renal MHC expression and augmenting antibody production with low-level expression of cytokines such as IFN-γ (15). Consistent with this concept, lupus

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[1] *Abbreviations used in this paper:* DN, double negative; MRL-*lpr*, MRL-Mp-*lpr/lpr*; wt, wild type.

nephritis is associated with increased or de novo renal expression of both adhesion and MHC class II molecules (17, 18). Although clinical improvement occurs with treatments leading to reduced class II expression or limiting CD4+ T cell interaction, the benefit from such treatments is limited, and a critical role for class II molecules in the development of renal immune injury has not been unequivocally established (19–22).

The advent of gene targeting in embryonic stem cells has allowed the inactivation of specific genes in the immune system to examine their function. Mice devoid of cell surface expression of MHC class II molecules have been created by introducing a loss of function mutation of the A_β^b gene in ES-D3 (H-2^b) cells, which have a naturally occurring mutation in their E$_\alpha$ gene (23). Thus, the subsequent phenotype of these mice is a deficiency in the expression of both I-A and I-E molecules. To definitively test the importance of these molecules in autoimmune nephritis, we produced class II–deficient MRL-lpr mice and followed these mice for the development of kidney disease and autoantibody production. Despite the development of lymphadenopathy and DN T cell expansion, class II–deficient MRL-lpr mice did not produce IgM or IgG autoantibodies, and were protected from the aggressive autoimmune renal injury associated with this model.

Materials and Methods

Mice. Class II–deficient MRL-lpr mice were created by repeatedly backcrossing mice heterozygous for the disrupted A_β^b allele (23) to MRL-lpr/lpr mice (H-2^k) (Jackson Laboratory, Bar Harbor, ME). Mice were screened for the disrupted allele by Southern blotting of BamHI-digested tail genomic DNA, using chemiluminescent detection (Boehringer Mannheim Canada, Laval, Quebec, Canada) of a digoxigenin-labeled genomic probe for A_β^b as previously described (23). An F6 heterozygous intercross which was more than 98% homozygous for non-MHC linked loci, produced mice homozygous for the disrupted class II A_β gene (MRL-lpr –/–) and homozygous wild-type (MRL-lpr wt/wt) control mice as well as heterozygous (MRL lpr –/wt) mice. Initial littermate cohorts of 13–15 mice per group were maintained in laminar flow hoods, given sterile food and water, and treated according to Canadian Council on Animal Care guidelines.

Materials and Histology. Reagents were obtained from GIBCO BRL (Gaithersburg, MD), and chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). The mAbs 10-2.16 (I-A^k), B21-2 (I-A^b), GK1.5 (CD4), and 2.43 (CD8) (American Type Culture Collection, Rockville, MD) were prepared by protein G purification and biotinylated using a standard protocol. Supernatant from the 145-2C11 hybridoma (CD3) was kindly provided by Dr. B. Singh (University of Western Ontario, London, Canada). Histology of kidney tissue was performed using hematoxylin and eosin staining of formalin (10%)-fixed kidney sections. For immunoperoxidase, 4-μm cryostat sections of OCT (Miles, Etobicoke, Ontario, Canada)-embedded tissue were acetone fixed, blocked with 4% horse serum, and incubated with saturating concentrations of biotinylated primary antibody, followed by Vectastain ABC reagent (Vector Laboratories, Burlingame, CA). Peroxidase activity was detected by a 4–6-min incubation with 3,3' diaminobenzidine (0.5 mg/ml) containing 0.02% H$_2$O$_2$. Counterstaining was with methyl green and alcian blue. Irrelevant mAb of the same isotype were used for negative controls.

Clinical and Histological Scoring. Weights and urinary protein levels were assessed weekly. Urinary protein was monitored by albumin reagent strips (Albustix; Miles) and recorded as 0–4+ (1+ = 0.3 g/liter, 2+ = 1 g/liter, 3+ = 3 g/liter and 4+ = >4 g/liter). Values >1+ are pathological in mice. Arbitrary clinical scoring of nodes was assigned by an observer blinded to group identities, using a scale of 0–4 (0 = none; 1 = a single node anywhere; 2 = bilateral axillary; femoral, or cervical nodes; 3 = generalized femoral, axillary, and cervical nodes; and 4 = massive generalized adenopathy). Serum was obtained by retro-orbital plexus sampling at 20 wk and serum creatinine levels were determined by a modified Jaffe method using an automated CX5 clinical analyzer (Beckman Instruments, Fullerton, CA). Hematoxylin and eosin–stained kidney sections were scored for histopathological glomerular damage by a blinded observer, using a scale of 0–4 as previously described (20) (0 = no involvement; 1 = mild changes in <25% of glomeruli; 2 = mild to moderate changes in 25–50%; 3 = moderate to severe changes in 50–75%, with crescent formation, and vasculitis; 4 = severe glomerulonephritis with changes in >90%, and with sclerotic glomeruli). Additionally, the degree of mononuclear cell infiltration was scored in arbitrary units 0–4 (0 = none; 1 = few in some fields; 2 = moderate in most fields; 3 = moderate in most fields; 3 = moderate in all fields; 4 = severe infiltrates with loss of normal surrounding histology).
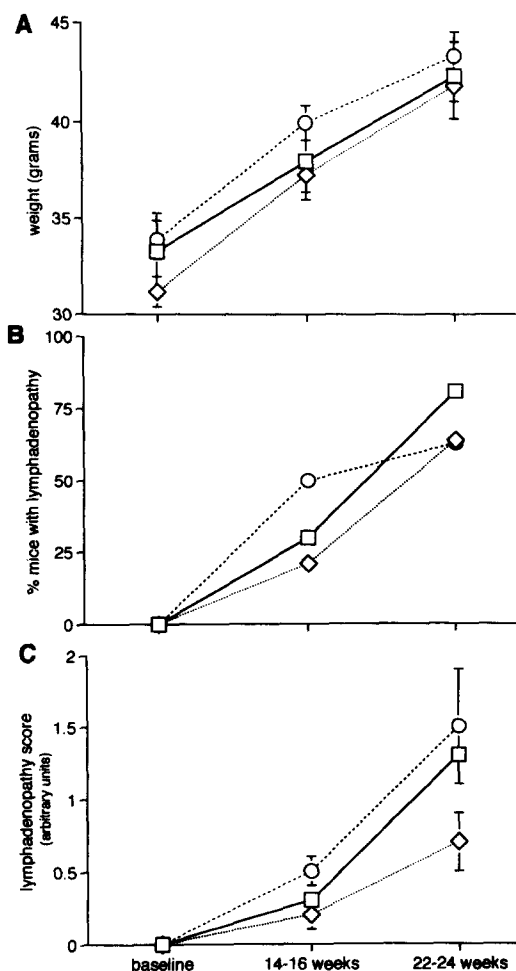
Flow Cytometry. Single cell suspensions were prepared from spleens and LN of MRL-lpr wt/wt and MRL-lpr –/– mice. 1.5 × 10^6 cells were dual labeled with biotinylated mAb 10-2.16 (I-A^k), GK1.5 (CD4) or 2.43 (CD8), and nonbiotinylated 145-2C11 (anti-mouse CD3). Cells were washed with HBSS, 1% FCS, and 0.1% NaN$_3$, and then incubated with streptavidin-PE (Jackson ImmunoResearch, Avondale, PA) and a fluorescein-conjugated F(ab')$_2$ fragment of goat anti-hamster antibody (Jackson ImmunoResearch). Cells were washed, fixed in 2% paraformaldehyde, and analyzed by flow cytometry (FACStar Plus®; Becton Dickinson & Co., Mountain View, CA) using 20,000 events for each analysis.

ELISA. Flat bottom 96-well microtiter plates (Falcon, Mississauga, Canada) were coated with single-stranded (ss)DNA (50 μg/ml) overnight at 4°C in PBS, blocked at room temperature with 1% gelatin, incubated with sera from 20-wk-old mice, labeled with biotinylated anti-Ig (Jackson ImmunoResearch) at a 1:5,000 dilution, followed by phosphatase-conjugated streptavidin (Jackson ImmunoResearch). Absorbance of color products was read at 405 nm after addition of substrate (p-nitrophenyl phosphate disodium), fixation with 3 N NaOH, and washing with 0.01 M PBS, 0.05% Tween, 0.2% gelatin, and 0.02% Na azide between steps. Each sample was run as a serial dilution and compared with standard curves of IgG or IgM (Sigma Chemical Co.) dilutions run on the same plate.

Statistics. Statistical analyses, where applicable, were performed using Statview IV software (Abacus Concepts, Berkeley, CA) on a Macintosh LC computer (Apple Computer, Inc., Cupertino, CA). Differences between groups were compared by one way analysis of variance and unpaired t tests. All results are expressed as mean ± SEM.

Results and Discussion

Lymphadenopathy in Class II–deficient Mice. To prevent exposure to pathogens, all mice were isolated in laminar flow facilities and given sterile food and water. Under these conditions, mice were generally healthy and all had equivalent weight gains during the first 5–6 mo (Fig. 1). As expected,

Figure 1. Analysis of weight gain and lymphadenopathy in MRL-*lpr* −/− mice. Littermate cohorts of equal numbers of male and female MRL-*lpr* wt/wt (*circles*), MRL-*lpr* −/wt (*diamonds*), and MRL-*lpr* −/− (*squares*) (*n* = 13 – 15 mice per group) were followed. Mice were examined daily for clinical evidence of illness, and weekly for weight gain and the presence of lymphadenopathy. (*A*) Weight gains were equal between groups. (*B* and *C*) Most mice in all groups developed lymphadenopathy between 4 and 6 mo, and all had nodes by 7 mo (data not shown). Arbitrary clinical scoring of nodes was assigned by an observer blinded to group identities, using a scale of 0–4 in Materials and Methods. Results are represented as mean ± SEM.

>50% of the MRL-*lpr* −/wt and MRL-*lpr* wt/wt had advanced disease by 6 mo and most were dead or required euthanasia by 7–8 mo, because of severe renal failure. Several class II–expressing homozygous MRL-*lpr* wt/wt and heterozygous MRL-*lpr* −/wt mice were euthanized before 6 mo as they demonstrated advanced signs of disease. One homozygous (1/13) MRL-*lpr* −/− mouse was killed at 4.5 mo because of a large facial abscess, and several mice (3/12) lost weight with fur ruffling at the age of 6–7-mo, and were therefore killed. Previous reports have noted an accelerated mortality rate in nonautoimmune strains of mice homozygous for the *lpr* gene, possibly due to hemorrhagic necrosis of enlarged LN with secondary infection (12). None of the MRL/*lpr* −/− mice that were euthanized had evidence of renal failure, systemic pathogen infection, or other organ failure at the time of death.

All mice developed lymphadenopathy by 6–7 mo which was similar in magnitude in homozygous wild type, heterozygous, and class II–deficient mice (Figs. 1 and 2). The pheno-

Figure 2. Development of lymphadenopathy in MRL-*lpr* −/− mice. Class II–deficient MRL-*lpr* −/− (*left*, aged 4.5 mo) and class II expressing MRL-*lpr* wt/wt (*right*, aged 5 mo) mice developed massive cervical and axillary lymphadenopathy typical of mice homozygous for the *lpr* mutation.
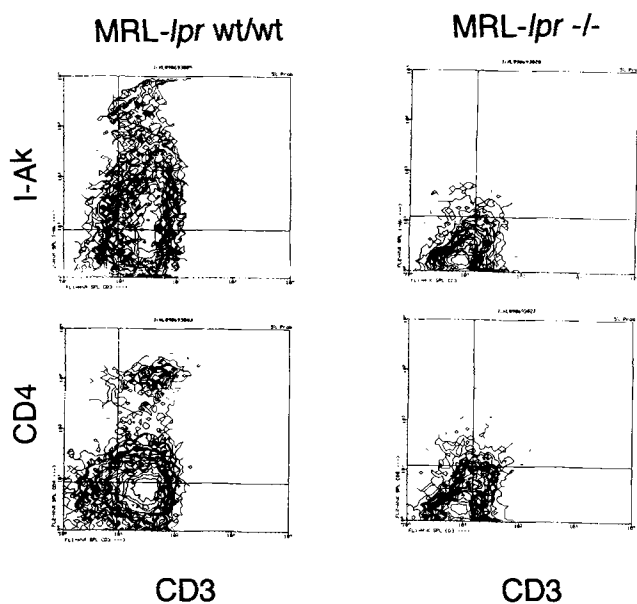
MRL-*lpr* wt/wt   MRL-*lpr* -/-   **A**

CD3   CD3

Figure 3. Flow cytometric analysis of spleen lymphocyte subsets in class II–deficient MRL-*lpr* -/- mice. Percentage of I-A^k positive cells (*top, upper right quadrants*) decreased from >70% in MRL-*lpr* wt/wt mice to <1% in MRL-*lpr* -/-. CD3$^+$CD4$^+$ T cells (*bottom, upper right quadrants*) in MRL-*lpr* wt/wt mice was <10% and not detectable in MRL-*lpr* -/- mice. CD3$^+$CD8$^+$ T cells were not detected in 5-mo-old MRL-*lpr* wt/wt or MRL-*lpr* -/- mice (data not shown). Results are representative of three experiments, twice using one *lpr* wt/wt and *lpr* -/- mouse, and once with a single MRL-*lpr* -/- mouse.

type of LN and spleen cells in the three groups was assessed by FACS® analysis and confirmed that by 20 wk in both spleen and LN, the predominant phenotype in all three groups of mice was CD3$^+$CD4$^-$ (Fig. 3), and CD3$^+$CD8$^-$ (data not shown). These results are consistent with previous reports defining the phenotype of DN T cells in wild-type MRL-*lpr* mice, and the present data demonstrate that thymic MHC class II expression is not essential for the development of these abnormal DN T cells. Expression of CD4 during T cell maturation in the thymus does not require either I-A or I-E, but progression from CD4$^+$CD8$^+$ to single positive CD4$^+$ T cells is dependent on class II expression (23). In MRL-*lpr* wt/wt mice, a small number of CD3$^+$CD4$^+$ cells (<10%) persisted in spleen and LN cell populations at 5 mo (Fig. 3), but CD3$^+$CD8$^+$ cells were not detectable at this age (data not shown). Previous reports have similarly demonstrated that by 20 wk of age, the massive expansion of DN cells can dilute the number of normal CD4$^+$ and CD8$^+$ T cells, which are actually increased in absolute numbers, to nearly nondetectable levels in spleen or LN (15, 24). In contrast to MRL-*lpr* wt/wt mice, MRL-*lpr* -/- mice did not have detectable levels of CD4$^+$ T cells in either spleen (Fig. 3) or LN (data not shown). We have previously demonstrated the failure of CD4$^+$ T cell development in "normal" class II–deficient mice, and this holds true in the presence of the abnormal thymic microenvironment produced by the absence of the *fas* gene (5, 23).
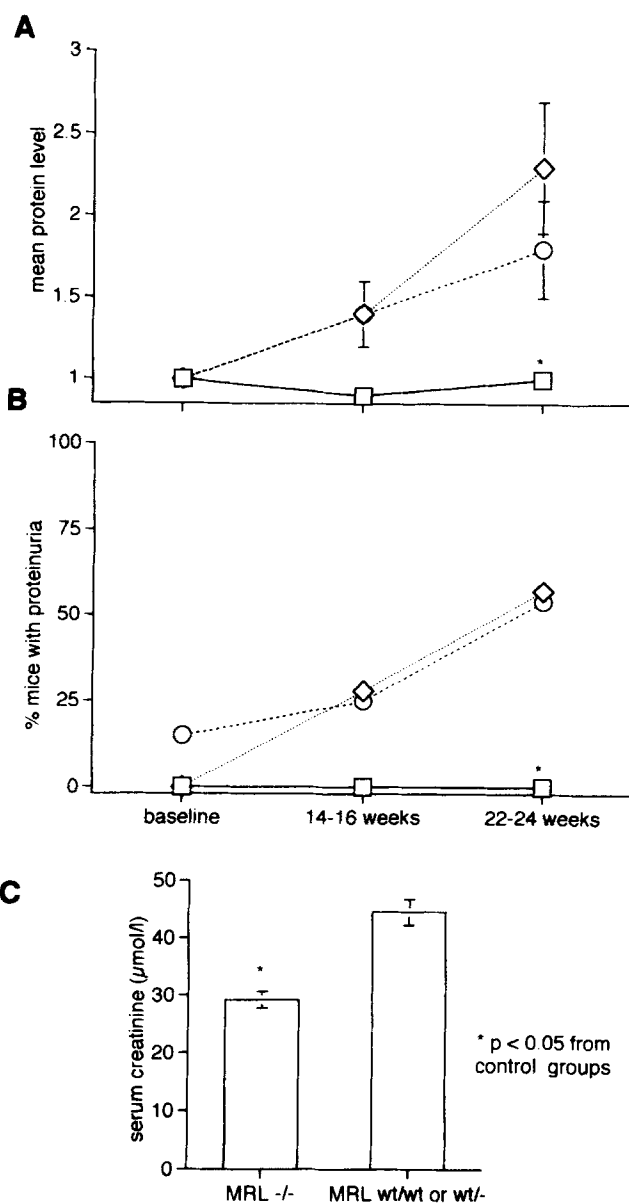
Figure 4. Analysis of kidney function in MRL-*lpr* -/- mice. (*A* and *B*) MRL-*lpr* wt/wt (*circles*), MRL-*lpr* -/wt (*diamonds*), and MRL-*lpr* -/- (*squares*) mice (*n* = 13–15/group) were followed weekly for urinary protein. None of the MRL-*lpr* -/- mice developed significant proteinuria, whereas >50% of control MRL-*lpr* wt/wt and MRL-*lpr* -/wt mice developed proteinuria by 24 wk. (*C*) MRL-*lpr* -/- mice had lower serum creatinine levels compared with control mice (*p* <0.05). Serum creatinine levels were determined in serum samples obtained from random MRL-*lpr* -/- mice (*n* = 7) and MRL-*lpr* wt/wt or MRL-*lpr* -/wt mice (*n* = 7). Results are given as mean ± SEM; and (*) difference from control groups (*p* <0.05).

Prevention of Autoimmune Nephritis in MRL-lpr -/- Mice. Although autoimmune disease can progress independently of lymphadenopathy (4, 25), several reports have shown that neonatal thymectomy, cyclosporine, or dexamethasone, reduces lymphadenopathy and ameliorates nephritis, suggesting that a link exists between peripheral DN T cell expansion and the magnitude of autoimmune responses (4, 20, 24). Addi-
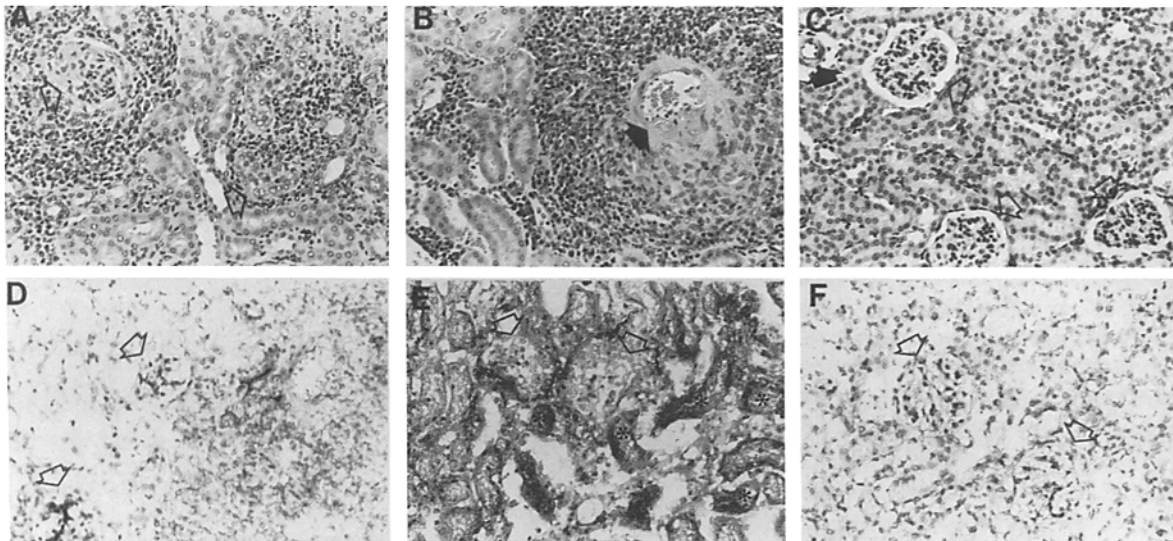
Figure 5. Comparison of kidney histology in MRL-*lpr* wt/wt and MRL-*lpr* −/− mice. (*A*) Sections from 24–28-wk-old MRL-*lpr* wt/wt kidneys showed marked periglomerular infiltrates, necrotizing glomerulonephritis (*open arrows*) and (*B*) massive perivascular infiltrates with vasculitis (*solid arrow*), which are typical in this strain by this age. Sections from MRL-*lpr* −/wt mice had similar changes (data not shown). (*C*) In contrast, kidney structures were normal in MRL-*lpr* −/− mice with normal glomeruli (*open arrows*) and blood vessels (*solid arrow*), with no marked infiltrates. Kidney MHC class II expression was assessed by immunoperoxidase using 10-2.16 (I-A^k) or B21-2 (I-A^b) mAb. Irrelevant mAb of the same isotype were used for negative controls, and control staining shown for MRL-*lpr* −/− was not greater than shown for MRL-*lpr* wt/wt. (*D*) Expression of class II was increased in tubular cells, endothelium, mesangial cells, and infiltrating cells in MRL-*lpr* wt/wt mice (*E*), but absent in MRL-*lpr* −/− (*F*). Staining with antibodies to I-A^b in MRL-*lpr* −/− mice was not greater than background shown in (*D*). Figures are representative of five to seven mice in each group, with mean histopathological scores of mice presented in Table 1. ∼ ×400.

tionally whereas mice homozygous for *lpr* have both circulating autoantibodies and immune complexes, B cell activity can be dissociated from immune injury (12, 24). We therefore assessed the effect of class II deficiency on autoimmune disease in an MRL-*lpr* by assessing the development of nephritis. Initially, mice were screened weekly for urinary protein excretion, a sensitive marker of renal injury. By 6 mo, when >50% of MRL-*lpr* wt/wt and MRL-*lpr* −/wt mice had evidence of advanced renal disease, none of the MRL-*lpr* −/− mice (0/12) had elevated urine protein levels (Fig. 4, *A* and *B*). Consistent with these observations, at 5 mo of age, serum samples from MRL-*lpr* wt/wt and MRL-*lpr* −/wt mice had

elevated levels of creatinine in comparison with normal levels in homozygous MRL-*lpr* −/− mice (Fig. 4 *C*). This is noteworthy as serum creatinine reflects glomerular filtration rate, and is increased only after a 50% reduction of normal kidney function has occurred. Histological scoring of kidney sections showed MRL-*lpr* wt/wt and MRL-*lpr* −/wt mice had advanced renal injury consisting of a necrotizing glomerulonephritis with crescents, glomerulosclerosis, and massive periglomerular, interstitial, and perivascular infiltrates (Fig. 5, *A* and *B*, Table 1). In contrast, renal histology was essentially normal in MRL-*lpr* −/− mice, although some glomeruli had mild mesangial expansion and a few periglomerular

Table 1. *Histophathological Scoring in Renal Injury in MRL-lpr wt/wt, MRL-lpr −/wt and MRL-lpr −/− Mice.*

	Glomerular histology*	Infiltrate[‡]			
Type		n	Perivascular	Glomerular	Medulla
MRL-*lpr* −/−	1.2 ± 0.2[§]	5	0.4 ± 0.2[§]	1.0 ± 0.3[§]	0.6 ± 0.2[§]
MRL-*lpr* −/wt	3.3 ± 0.3	7	3.3 ± 0.3	3.0 ± 0.3	2.6 ± 0.5
MRL-*lpr* wt/wt	2.8 ± 0.4	5	3.8 ± 0.2	2.8 ± 0.2	1.8 ± 0.2

24–28-wk-old MRL mice were euthanized, and formalin-fixed kidney tissue was sectioned and stained with hematoxylin-eosin.
* Slides were scored for glomerular injury in a blinded manner, using a scale of 0–4 with 4 representing severe changes.
[‡] Similarly, the degree of infiltration was scored in arbitrary units 0–4. A minimum of 10 fields were examined per section, counting between 20 and 30 glomeruli. Results are reported as mean ± SEM.
[§] Represents difference from MRL-*lpr* −/wt and MRL-*lpr* wt/wt control groups (*p* <0.05).

mononuclear cell infiltrates (Fig. 5, C, Table 1). It is interesting to note that the presence of *lpr*-related lymphadenopathy in nonautoimmune prone strains of mice can result in subtle renal pathology abnormalities without overt nephritis (12). Renal expression of class II precedes overt nephritis in MRL-*lpr* mice and may be related to the local release of cytokines (17). Kidney sections were therefore assessed to confirm loss of class II expression in MRL-*lpr* −/−. In both homozygous and heterozygous control mice, there was abundant expression of class II on tubular epithelial cells, infiltrating mononuclear cells, and glomerular mesangial cells (Fig. 5 *E*). As expected, class II was absent in MRL-*lpr* −/− kidney sections (Fig. 5 *F*). Similarly, splenocytes and LN cells from 5-mo-old MRL-*lpr* −/− mice did not express I-A^b (data not shown).

Although MRL-*lpr* −/− mice had lymphadenopathy scores that were indistinguishable from both homozygous and heterozygous control mice (Fig. 1 *C*), the paucity of infiltrates within class II–deficient kidneys might have been expected with a previous demonstration that preferential migration of CD4⁻CD8⁻ B220⁺ T cells to the kidney does not occur and most of the infiltrating cells within the kidneys of diseased MRL-*lpr* mice are CD4⁺ T cells (26). Since CD8⁺ T cells do not require CD4⁺ T cells for normal development and function (27), our data suggest that autoimmune nephritis was abrogated in these class II–deficient MRL mice by blocking autoaggressive CD4⁺ T cell generation within the thymus. Alternatively, since the development of autoreactive T cells from MRL-*lpr* mice in vitro has been shown to be dependent on the presence of class II–bearing APCs (28), it is possible that generation or proliferation of autoreactive CD4⁺ T cells within the kidney may not have occurred in the absence of renal MHC class II expression. The present data also suggest that since lymphadenopathy occurred despite blockage of normal thymic development of CD4⁺ T cells, single positive CD4⁺ T cells are unlikely to be the precursor T cell phenotype giving rise to DN T cells in the periphery. This is consistent with recent data which suggest that DN T cells are positively selected on class I but not class II antigens (10). This hypothesis may be tested using available CD4⁺ and CD8⁺ deficient mice backcrossed to the MRL-*lpr* strain.

Autoantibody Production in Class II–deficient MRL-lpr −/− Mice. The MRL-*lpr* strain of mouse also produces a large number of autoantibodies, including those directed against nucleic acids. The role of antibody in lupus nephritis in this model is not entirely clear as renal disease activity may not correlate with either antibody or immune complex levels (12,
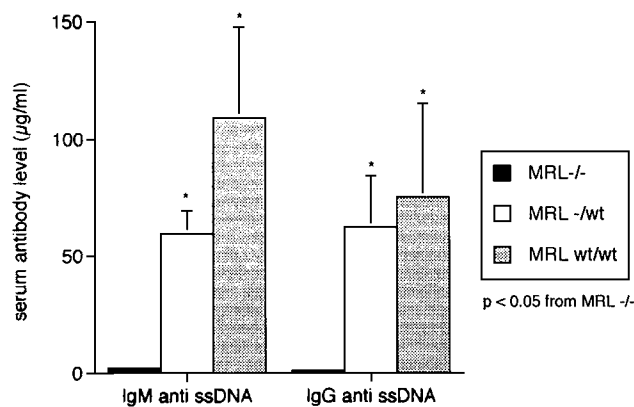

Figure 6. Analysis of anti-ssDNA antibodies was performed by ELISA. Both MRL-*lpr* wt/wt and MRL-*lpr* −/wt mice (n = 7) had high levels of IgM and IgG anti-ssDNA antibodies. In contrast, MRL-*lpr* −/− levels (n = 7) of IgM antibody levels were <1 μg/ml, and IgG levels were not detectable by this assay. Results are reported as mean ± SEM, and represent two experiments.

24). Although CD4⁺ T cell help is required for efficient B cell function and isotype switching, B cell hyperactivity can proceed even if T cell proliferation is suppressed (24), and therefore we assessed IgM and IgG anti-ssDNA antibody levels. Both homozygous and heterozygous MRL control mice had high levels of both antibodies while MRL-*lpr* −/− mice had essentially nondetectable levels (Fig. 6). Whereas it might be expected that with a functional loss of CD4⁺ T helper T cells in class II–deficient mice, IgG levels of all specificities would be severely impaired in MRL-*lpr* −/− mice, the loss of IgM autoantibodies was interesting in that total IgM levels are preserved or even elevated in nonautoimmune prone strains of class II–deficient mice (23, 29).

In summary, MRL-*lpr* mice deficient of MHC class II expression do not develop autoimmune nephritis or autoantibodies. It is likely that disease was prevented in this novel strain by a lack of thymic class II expression which prevented autoaggressive T cell development. Whereas previous models have shown dissociation of disease and autoantibody production and various relationships between nephritis and lymphadenopathy, this report clearly demonstrates that with loss of class II expression, the prevention of nephritis in an autoimmune susceptible strain can occur in the presence of lymphadenopathy. Furthermore, the development of DN T cell–derived lymphadenopathy in a class II– and CD4 helper cell–deficient mouse suggests these mice may become an important model in which to study the thymic and peripheral development of these abnormal T cells.

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