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An MRL/MpJ-*lpr/lpr* substrain with a limited expansion of *lpr* double-negative T cells and a reduced autoimmune syndrome

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Abstract

The autosomal recessive mutant gene, *lpr*, has been shown to accelerate the progression of lupus-like autoimmune disease, which is associated with a massive expansion of a unique CD4$^-$CD8$^-$ double-negative T cell subset, in MRL/MpJ mice. Here we report a substrain of MRL/MpJ-*lpr/lpr* (MRL-*lpr*) mice which live almost twice as long with delayed development of glomerulonephritis, compared with conventional MRL-*lpr* mice. This substrain, termed MRL-*lpr.ll* (*ll* for long-lived), develops generalized lymphadenopathy characteristically seen in MRL-*lpr* mice. However, the expansion of a double negative *lpr* T cell subset is markedly limited with a mean value of 15% in their lymph nodes compared to about 70% in conventional MRL-*lpr* mice. Overall production of autoantibodies, such as anti-DNA and rheumatoid factors, does not significantly differ between the two MRL-*lpr* mice. However, serum levels of cryoglobulins, whose major component is IgG3, are markedly diminished in MRL-*lpr.ll* mice with a parallel decrease in IgG3. Since MRL-*lpr.ll* mice still carry the *lpr* mutation, as documented by the presence of defects in the Fas antigen, a possible new mutation in this substrain may play a significant role in the pathogenesis of lupus-like autoimmune syndrome.

Introduction

MRL/MpJ-*lpr/lpr* (MRL-*lpr*) mice spontaneously develop a lupus-like autoimmune syndrome associated with massive lymphadenopathy due to the expansion of a unique T cell subset expressing Thy-1, CD3, B220, but not CD4 or CD8 antigen (1,2). More recently, it has been demonstrated that the *lpr* mutation causes defects in the Fas antigen which mediates apoptosis (3). It is speculated that because of the absence of functional Fas antigen, autoreactive T cells could escape thymic selection, resulting in the autoimmune disease. However, it remains unclear how the *lpr* mutation results in a massive expansion of the *lpr* CD4$^-$CD8$^-$ double-negative (DN) T cell subset and whether this DN T cell subset is indeed involved in the pathogenesis of the *lpr* gene-induced autoimmune syndrome.

We have observed that a fraction of MRL-*lpr* mice, originally obtained from the Jackson Laboratory (Bar Harbor, ME), occasionally exhibited an abnormally prolonged life span, although they developed typical generalized lymphadenopathy. Offspring of long-lived male and female MRL-*lpr* founder mice were bred by rigorous brother sister mating to establish a MRL-*lpr* substrain with significantly delayed disease expression. Reported here are the generation and cellular, histological and serological characteristics of this MRL-*lpr* substrain with a prolonged survival, termed MRL-*lpr.ll* (*ll* for long-lived).

Methods

Mice

MRL-*lpr* and MRL-+/+ mice were originally obtained from the Jackson Laboratory in 1978 and were maintained at Centre de Service des Animaux de Laboratoire (Orléans, France). In 1988, offspring of a single pair of long-lived male and female MRL-*lpr* founder mice were bred by rigorous brother – sister mating. At

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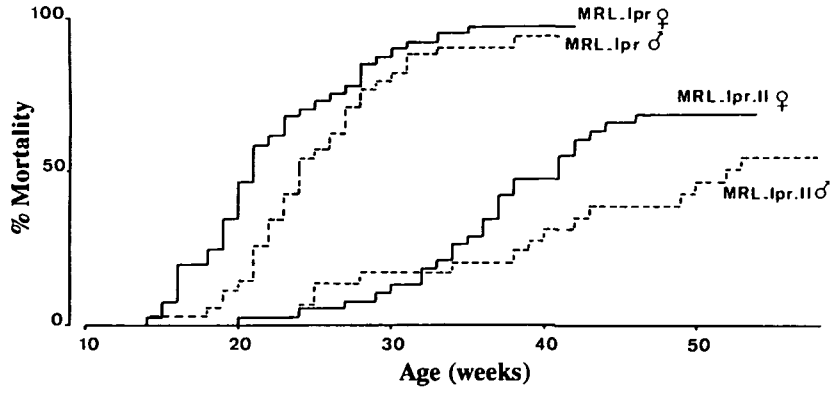

Fig. 1. Cumulative mortality with glomerulonephritis in 28 MRL-*lpr.II* female, 19 MRL-*lpr.II* male, 29 MRL-*lpr* female, and 24 MRL-*lpr* male.

the sixth generation, a subline with a prolonged survival, referred to as MRL-*lpr.II*, was obtained. Both lines of MRL mice have been kept under the same condition and studied in parallel.

Cytofluorometric analysis

The expression of different cell surface antigens was analyzed using anti-Thy-1.2 (30-H12), anti-CD4 (GK1.5), anti-CD8 (H35-17.2), anti-CD3 (145-2C11), anti-B220 (RA3-3A1/6.1), anti-CD44 (IM7.8.1), and anti-IgM (LO-MM-9) mAbs with a FACScan (Becton-Dickinson, Mountain View, CA), as described previously (4,5).

Preparation of DN T cells

Lymph node cell suspensions were prepared from a pool of axillary, mediastinal, mesenteric, retroperitoneal, and inguinal lymph nodes. To purify DN T cells from lymph nodes, surface Ig-positive B cells were first depleted by adsorption on Petri dishes coated with affinity purified rabbit anti-IgG antibodies, and $CD4^+$ and $CD8^+$ cells were then eliminated by cytotoxicity with anti-CD4 (RL 172.4), anti-CD8 (31M), and rabbit complement, as described previously (4,6).

T cell proliferation assays

Flat-bottomed microtiter wells (Falcon Oxnard, CA) were incubated for 3 h at 37°C with 100 μl of PBS containing 20 μg/ml anti-TCR mAb specific against $V_\beta 8$ segment (F23.1) or control mAb of the same isotype (IgG2a), and then washed three times before use. Lymph node cells or DN T cells (10^5) were incubated for 3 days in 200 μl of Dulbecco's modified Eagle's medium supplemented with additional amino acids, 10 mM HEPES, 5×10^{-5} M 2-mercaptoethanol, and 10% FCS at 37°C in a humidified incubator containing 5% CO_2 in air (4). Cells were harvested after a 6 h pulse label with 1 μCi of [^{3}H]thymidine. For concanavalin A (Con A)-induced proliferative response, lymph node cells or DN T cells were incubated with 5 μg/ml of Con A for 3 days.

Northern blot analysis

Total cellular RNA from lymph nodes, livers, and DN T cells of MRL-*lpr*, MRL-*lpr.II* and MRL-+/+ mice were extracted using the guanidine isothiocyanate – CsCl method. Samples of 10 or 20 μg RNA were glyoxalated and subjected to agarose gel electrophoresis. RNA was transferred to nylon membrane (GeneScreen Plus, DuPont, Boston, MA) and hybridized to ^{32}P-labeled cDNA corresponding to *fas* (a 1.5 kb *Eco*RI fragment of pMF1, kindly provided by Dr Shigekazu Nagata, Osaka Bioscience Institute, Osaka, Japan) (7), *fyn* (a 0.6 kb *Hin*dIII – *Hin*cII fragment of pUC-FYN, kindly provided by Dr Kentaro Semba, Tokyo University, Tokyo, Japan) (8), *Eta-1* (a 0.95 kb *Hin*dIII fragment from pXM-Eta-1, kindly donated by Dr Harvey Cantor, Harvard Medical School, Boston, MA) (9), c-*myb* (10), and *β-actin*, as described (8).

Southern blot analysis

High molecular weight genomic DNA was prepared from the liver (11). Genomic DNA (10 μg) was digested with *Eco*RI, electrophoresed on a 0.8% agarose gel, and transferred to a nylon membrane as described (11). Hybridization was carried out under high stringency with a full-length mouse Fas antigen cDNA (7) as probe.

Morphological studies

Weights of lymph nodes (axillary, mediastinal, mesenteric, retroperitoneal, and inguinal) and spleens from MRL-*lpr* and MRL-*lpr.II* mice were measured. Samples of all major organs were obtained at autopsy and histological sections were stained with either the periodic acid – Schiff (PAS) reagent or with hematoxylin & eosin. Glomerulonephritis was scored on a 0 – 4 scale based on the intensity and extent of histopathological changes as described previously (12).

Serological assays

Serum levels of IgG subclasses and IgG anti-DNA antibodies were determined by ELISA as described (13). The anti-IgG1 and anti-IgG2a RF activities were determined by ELISA according to Wolfowicz *et al.* (14). Briefly, microtiter plates were initially coated with 5 μg/ml of (4-hydroxy-3-iodo-5-nitrophenyl)acetyl (NIP)-conjugated BSA and then with 1 μg/ml of C.18 (IgG1, λ1) or NIP-23 (IgG2a, λ1) anti-NIP mAb (a kind gift of Dr A. Marshak-Rothstein, Boston, MA). The plates were then incubated with appropriately diluted sera, and the assay was developed with alkaline phosphatase-labeled rat anti-mouse κ-chain mAb (H139.52.1). The results are expressed in titration units referring to standard curves obtained by anti-IgG1 RF (411H12) and

anti-IgG2a RF (AM2) mAb, provided by Dr D. Nemazee, Denver, CO, and Dr A. Marshak-Rothstein respectively. Cryoglobulins were separated from sera as described previously (15).

Results

Generation of the MRL-lpr.ll strain

Using MRL-*lpr* mice which developed lymphadenopathy but survived much longer than conventional MRL-*lpr* mice as founders, we obtained a new strain of MRL-*lpr* mice with a longer life span, designated MRL-*lpr.ll*. Conventional MRL-*lpr* female and male mice have a 50% mortality rate at 5 and 6 months of age respectively, and more than 90% of mice of both sexes died by 8 months of age. In contrast, the MRL-*lpr.ll* strain exhibits a 50% female survival at 9 months and a 50% male survival at 12 months of age (Fig. 1). The difference in their mortality rates of both sexes at 6 months of age was highly significant by χ^2 analysis ($P < 0.005$). An intermediate mortality was observed in F_1 hybrid mice between conventional MRL-*lpr* and MRL-*lpr.ll* mice (50% mortality rate: females, 7 months; males, 8 months), suggesting that the long-lived phenotype was inherited as an autosomal semidominant trait.

Analysis of 14 polymorphic loci in three of each *lpr* mice has revealed the identity between MRL-*lpr.ll* and MRL-*lpr* mice (*Akp-1^b, Apoa-1^b, Es-1^b, Es-3^c, Gpd-1^b, Gpi-1^a, Hbb^d, Idh-1^a, Len-1^a, Mod-1^a, Pep-3^a, Pgm-1^a, Svp-1^a*, and *Svp-2^c*). This supports the idea of the absence of a genetic contamination in MRL-*lpr.ll* mice.

Table 1. Surface staining of lymph node cells from 5 month old MRL mice

Surface phenotype	*lpr*	*lpr.ll*	+/+
$CD4^-CD8^-Thy\text{-}1^+$	71.1 ± 9.0[a]	15.1 ± 10.0	<2.0
$CD4^+$	13.0 ± 5.1	40.0 ± 7.1	44.4 ± 7.2
$CD4^+B220^+$	5.3 ± 1.5	5.0 ± 1.6	ND[b]
$CD8^+$[c]	6.2 ± 3.2	13.2 ± 6.4	26.2 ± 9.1
IgM^+	3.7 ± 1.9	18.5 ± 5.4	17.1 ± 9.3

[a]Mean (±1 SD) of four to seven mice.
[b]Not detectable.
[c]$B220^+$ cells were not detectable in the $CD8^+$ population.

Lymphoproliferation in the MRL-lpr.ll strain

Conventional MRL-*lpr* mice exhibited a marked lymphadenopathy and splenomegaly, with the mean weight of lymph nodes and spleen being 2.4 ± 1.6 and 0.44 ± 0.19 g respectively at 5 months of age. Although the lymphoproliferation was delayed, MRL-*lpr.ll* mice similarly developed lymphadenopathy and splenomegaly; their lymph node and spleen weights at 7 months of age (2.1 ± 0.8 and 0.74 ± 0.13 g respectively) were comparable to those of 5 month old conventional MRL-*lpr* mice.

As described previously (1,2,4,16), the predominant phenotype (approximately 70%) of lymph node cells in MRL-*lpr* mice was $Thy\text{-}1^+CD3^{lo}CD4^-CD8^-$ double negative (DN). However, this *lpr* DN population represented a mean value of only 15% of lymph node cells from MRL-*lpr.ll* mice at 5 months of age (Table 1 and Fig. 2). Notably, this DN population highly expressed CD44 and B220 at levels comparable to those found in MRL-*lpr* mice (data not shown). Although the relative number of $CD4^+$ cells in MRL-*lpr.ll* mice was higher, there was no increase in $CD3^{lo}CD4^{lo}B220^+$ cells, which have been postulated to be a transitional stage between $CD4^+$ and $CD4^-CD8^-$ populations (17,18), and the majority (more than 90%) of $CD4^+$ cells expressed CD3 and CD4 at a density identical to those of

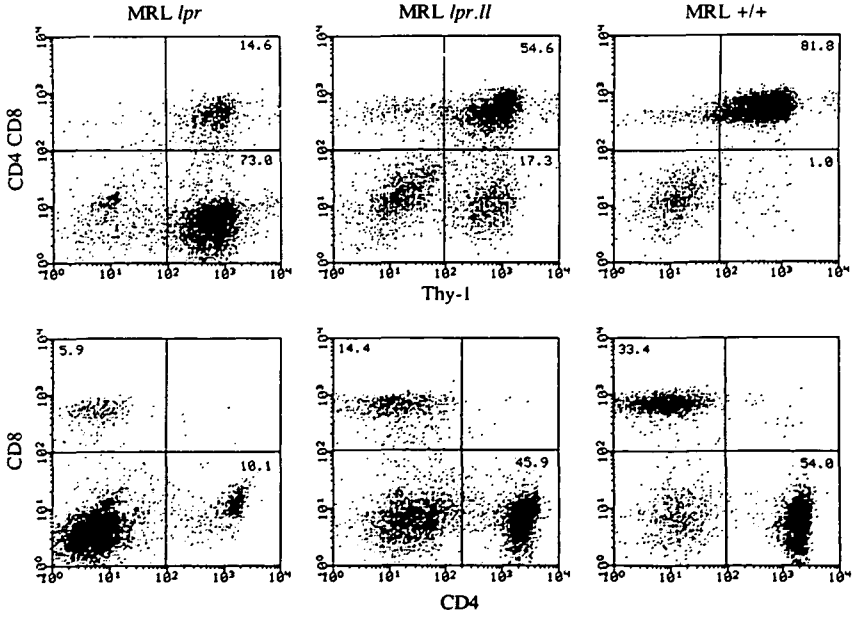

Fig. 2. Cytofluorometric analysis of lymph node cells from 5 month old MRL-*lpr*, MRL-*lpr.ll*, and MRL-+/+ mice. Lymph node T cells were first stained with anti-CD4 (GK1.5) and anti-CD8 (H35-17.2) mAb, followed by FITC-labeled goat anti-rat IgG conjugates, and then incubated with biotinated anti-Thy-1 (30-H12) mAb, followed by phycoerythrin-conjugated avidin. $CD4^+$ and $CD8^+$ cells were first stained with anti-CD8 mAb, followed by goat anti-rat IgG FITC conjugates, and then incubated with biotinated anti-CD4 mAb, followed by phycoerythrin-conjugated avidin.

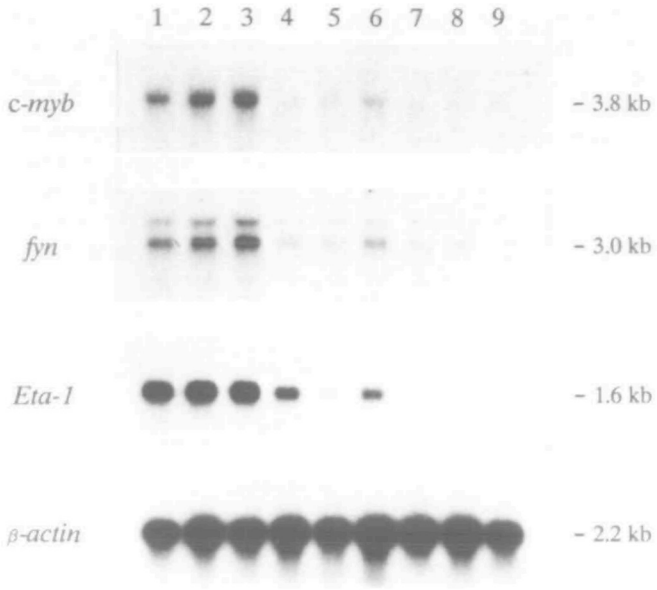

Fig. 3. Detection of c-*myb*, *fyn*, and *Eta-1* mRNA in lymph nodes from MRL-*lpr*, MRL-*lpr.ll*, and MRL-+/+ mice by Northern blot analysis. RNA (20 μg) from lymph nodes of three different individual MRL-*lpr* (lanes 1 – 3), MRL-*lpr.ll* (lanes 4 – 6), and MRL-+/+ (lanes 7 – 9) female mice at 5 – 7 months of age. The percentages of DN T cells in these *lpr* lymph nodes are 73.3% (no. 1), 77.9% (no. 2), 69.7% (no. 3), 23.4% (no. 4), 8.0% (no. 5), and 32.3% (no. 6).

Table 2. Lack of anti-TCR or Con A-induced proliferative responses in DN T cells from MRL-*lpr* and MRL-*lpr.ll* mice

Mice	Population	[^{3}H]Thymidine uptake			
		anti-$V_\beta 8^a$	control mAb[a]	Con A[b]	none
lpr	CD4$^-$CD8$^-$DN	604	496	288	311
	unseparated	12934	635	13679	287
lpr.ll	CD4$^-$CD8$^-$DN	1662	1839	838	824
	unseparated	30695	4284	10333	1476
+/+	unseparated	50229	500	29122	333

[a]10^5 DN T cells or unseparated lymph node cells from 4 month old MRL mice were cultured in microtiter wells coated with anti-$V_\beta 8$ or control IgG2a mAb. Cultures were pulsed with 1 μCi [^{3}H]thymidine for the final 6 h of 72 h cultures. Results are expressed as mean c.p.m. of triplicate cultures.

[b]10^5 DN T cells or unseparated lymph node cells were cultured in the presence of 5 μg/ml Con A.

MRL-+/+ mice. Notably, these phenotypic characteristics of lymph node cells in MRL-*lpr.ll* mice remained constant even later in their life.

The diminished number of *lpr* DN T cells in MRL-*lpr.ll* mice was reflected by a markedly decreased expression in their lymph nodes of mRNA specific for the c-*myb*, *fyn*, and *Eta-1* genes (Fig. 3), all of which were shown to be highly expressed by *lpr* DN T cells (9,19,20). Analysis on purified *lpr* DN T cells revealed that levels of the c-*myb* and *Eta-1* transcripts were comparable in DN T cells from both *lpr* mice, although the *fyn* expression was somehow less in DN T cells from MRL-*lpr.ll* mice (data not

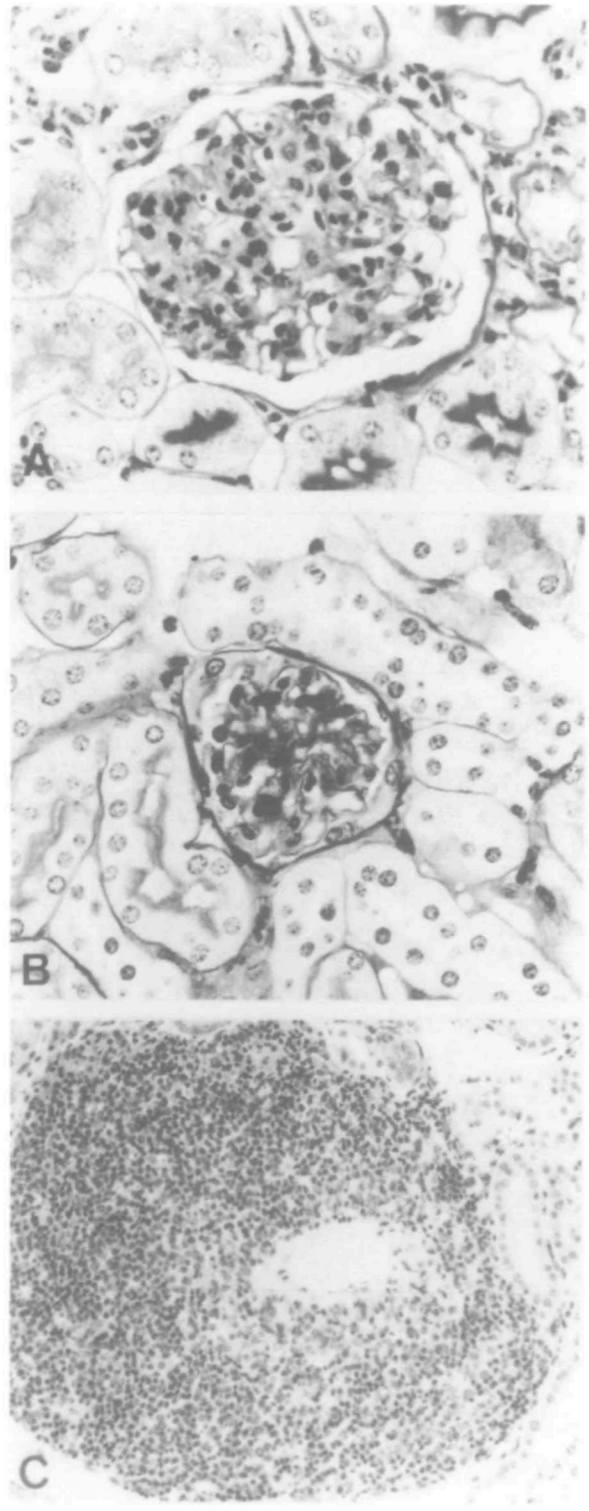

Fig. 4. Representative histological appearance of glomeruli from 5 month old MRL-*lpr* (A) and MRL-*lpr.ll* (B) female mice. Note increased glomerular cellularity, increased mesangial matrix, and markedly increased size of glomerulus from MRL-*lpr* mice, as compared with that of MRL-*lpr.ll* mice (periodic acid – Schiff, ×170). (C) Representative histological appearance of arteritis of renal artery in 5 month old MRL-*lpr.ll* mice, characterized by granulomatous lesions extending into perivascular tissue associated with massive mononuclear cell infiltration (hematoxylin & eosin, ×128).

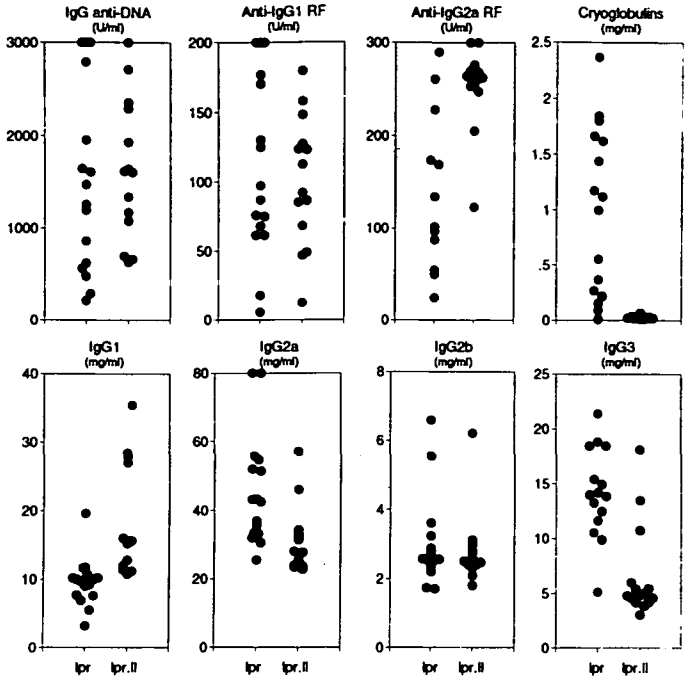

Fig. 5. Serum levels of anti-DNA, anti-IgG1 RF, anti-IgG2a RF, cryoglobulins, and IgG subclasses in 4 month old MRL-*lpr* and MRL-*lpr.ll* female mice. IgG anti-DNA and total (IgM and IgG) anti-IgG1 and anti-IgG2a RF are expressed in U/ml. Serum cryoglobulin and IgG concentrations are expressed in mg/ml.

shown). Moreover, like DN T cells from MRL-*lpr* mice, DN T cells purified from lymph nodes of MRL-*lpr.ll* mice failed to exhibit significant proliferative responses *in vitro* in the presence of anti-$V_\beta 8$ TCR mAb or Con A (Table 2).

Histopathological characteristics in the MRL-lpr.ll strain

When evaluated for renal histopathology at 5 months of age, MRL-*lpr.ll* female mice had less histological evidence of glomerulonephritis than conventional MRL-*lpr* mice (Fig. 4A and B); renal lesions of MRL-*lpr.ll* mice reached a mean grade of only 1.2, whereas lesions of conventional MRL-*lpr* mice were at grade 3.0 or higher. Despite marked differences in the phenotype of T cells expanding in the lymph nodes of both *lpr* mice, MRL-*lpr.ll* mice developed systemic granulomatous arteritis: this is characterized by the destruction of the arterial media and adventitia associated with a massive infiltration of perivascular mononuclear cells (Fig. 4C), similar to that observed in conventional MRL-*lpr* mice.

Serological characteristics in MRL-lpr.ll mice

The spontaneous production of autoantibodies was examined in 4 month old MRL-*lpr.ll* and conventional MRL-*lpr* female mice. Mean levels of IgG anti-DNA antibodies in MRL-*lpr.ll* mice (1766 U/ml) did not significantly differ from those of conventional MRL-*lpr* mice (1870 U/ml) (Fig. 5). MRL-*lpr.ll* mice produced RF, whose mean values (anti-IgG1 RF, 101 U/ml; anti-IgG2a RF, 254 U/ml) were comparable or even higher than those of conventional MRL-*lpr* mice (mean values: anti-IgG1 RF, 110 U/ml; anti-IgG2a RF, 139 U/ml). In contrast, levels of cryoglobulins, one of the most characteristic serological abnormalities of MRL-*lpr* mice (21,22), were markedly limited in MRL-*lpr.ll* mice: their mean level was only 6.3 μg/ml, which was approximately 150 times less than that observed in conventional MRL-*lpr* mice (963 μg/ml). Since murine cryoglobulin activities were almost exclusively associated with the IgG3 subclass (22), serum levels of IgG subclasses were compared between both groups of MRL-*lpr* mice. IgG3 concentrations in MRL-*lpr.ll* mice were approximately 2.5 times lower than those in conventional MRL-*lpr* mice, while their IgG1 concentrations were twice as high. However, a slight decrease and no change was observed in IgG2a and IgG2b levels, respectively. Furthermore, these serological parameters remained unchanged even later in the life of MRL-*lpr.ll* mice (data not shown).

The presence of the lpr mutation in MRL-lpr.ll mice

To prove the preservation of the *lpr* mutation in MRL-*lpr.ll* mice, we have determined using a mouse Fas antigen cDNA probe whether MRL-*lpr.ll* mice carry defects in the Fas antigen. In agreement with recent observation (3), Southern blot analysis of DNA from MRL-*lpr.ll* mice as well as MRL-*lpr* mice showed an extra band of 9 kb in *Eco*RI-digested DNA, as compared with MRL-+/+ mice (Fig. 6A). Furthermore, Northern blot analyses were carried out to determine the expression of Fas antigen in MRL-*lpr.ll* mice. Although a 2.1 kb Fas antigen mRNA was detected in the liver of MRL-+/+ mice, no Fas-specific mRNA was observed at a detectable level in both MRL-*lpr* mice (Fig. 6B). These results clearly indicate the rearrangement of the Fas antigen gene and the absence of Fas antigen expression in MRL-*lpr.ll* mice as described for the molecular defect of the *lpr* mutation (3).

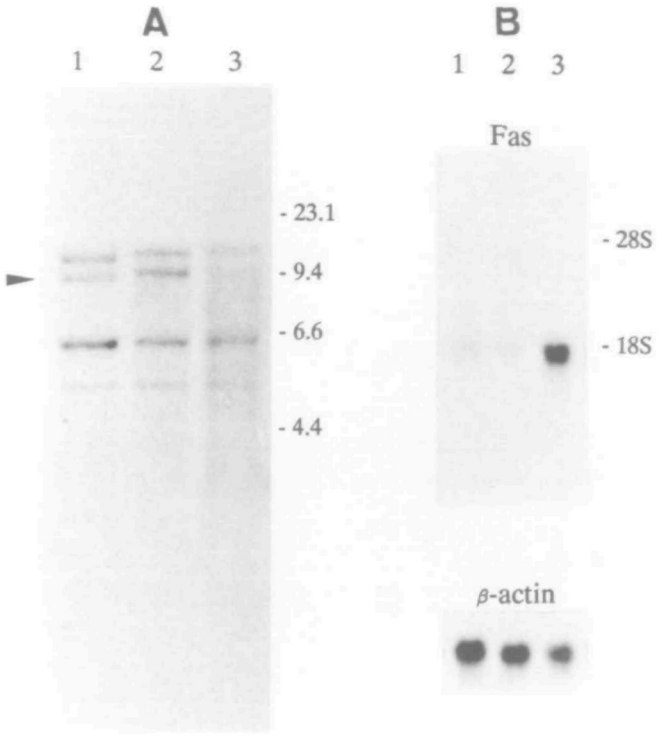

Fig. 6. (A) Southern blot analysis of the Fas antigen gene in MRL-*lpr* (lane 1), MRL-*lpr.ll* (lane 2), and MRL-+/+ (lane 3) mice. Genomic DNA (10 μg) was digested with *Eco*RI and hybridized with a full-length mouse Fas antigen cDNA. Arrowhead indicates DNA fragments rearranged in MRL-*lpr* and MRL-*lpr.ll* mice. (B) Little expression of Fas antigen mRNA in MRL-*lpr* and MRL-*lpr.ll* mice. RNA (10 μg) from the liver of MRL-*lpr* (lane 1), MRL-*lpr.ll* (lane 2), and MRL-+/+ (lane 3) were probed for Fas antigen and β-actin transcripts.

Discussion

In the present study, we describe cellular, histological, and serological characteristics of a substrain of MRL-*lpr* mice which exhibit a prolonged survival. This substrain, termed MRL-*lpr.ll* has been generated by inbreeding of offspring of long-lived MRL-*lpr* founder mice. Although this substrain develops lymphadenopathy characteristically described in mice bearing the *lpr* mutation, phenotypical analysis of their lymph node cells has revealed remarkable differences from that of conventional MRL-*lpr* mice. In addition, MRL-*lpr.ll* mice produce only limited amounts of cryoglobulins accompanied with a substantial decrease in serum levels of IgG3, whereas overall activities of autoantibodies such as anti-DNA and RF do not differ from those of MRL-*lpr* mice. Since MRL-*lpr.ll* mice carry defects in the Fas antigen, thus still bearing the *lpr* mutation, it is likely that MRL-*lpr.ll* mice differ from conventional MRL-*lpr* mice at the level of background gene(s).

It is striking that in MRL-*lpr.ll* mice the expansion of T cells with the *lpr* phenotype, i.e. $CD3^{lo}CD4^{-}CD8^{-}B220^{+}$, is markedly limited. This was further confirmed by the fact that lymph nodes from MRL-*lpr.ll* mice expressed lower levels of mRNA specific for the c-*myb*, *fyn*, and *Eta-1* genes, all of which were shown to be constitutively expressed by *lpr* DN cells (9,19,20). Notably, the proportion of T cells carrying the *lpr* phenotype remains constant at low levels after 5 months of age, excluding an age-related phenomenon. Although the cellular origin of *lpr* DN cells is still unclear, we favor a hypothesis that they are derived from single-positive T cells (9,23,24). In fact, we have recently observed that the treatment of MRL-*lpr* mice with anti-CD4 and anti-CD8 antibodies from birth completely blocked the appearance of *lpr* DN cells, and that the activation of T cells may be prerequisite for the possible switching from single positive cells to DN cells (R.Merino *et al.*, manuscript in preparation). If so, one can postulate that the lesser extent of T cell activation occurring in MRL-*lpr.ll* mice, probably related to a new mutation, may result in a limited expansion of DN T cells.

Serological analysis has revealed three significant points: (i) despite the prolonged survival of MRL-*lpr.ll* mice, serum levels of anti-DNA autoantibodies and RF remained virtually unchanged, (ii) cryoglobulin levels were markedly diminished, and (iii) there was a decrease in IgG3, but an increase in IgG1. The lack of correlation between autoantibody levels and survival rate is consistent with the notion that the qualitative aspect of autoantibody responses in murine systemic lupus erythematosus is likely to be important in provoking renal disease. In this respect, diminished production of IgG3 and cryoglobulins in MRL-*lpr.ll* mice is particularly significant. In fact, we have recently demonstrated using RF and anti-DNA mAbs that autoantibodies of the IgG3 subclass are more nephritogenic than others including IgG1 (25 – 29), because of their cryoglobulin activity associated with IgG3 constant region (22), and that they are able to induce 'wire-loop'-like glomerular lesions (28,30), characteristically described in lupus nephritis. The pathogenic relevance of IgG3 autoantibodies has been also suggested by recent genetic studies on MRL-*lpr* × (MRL-*lpr* × C3H-*lpr*) backcross mice (31).

In contrast to limited development of glomerulonephritis, MRL-*lpr.ll* mice develop severe systemic granulomatous arteritis associated with a massive infiltration of mononuclear cells. This further supports the idea that different mechanisms govern the pathogenesis of glomerular and granulomatous vascular lesions in MRL-*lpr* mice. In fact, the dissociation between arteritis and glomerulonephritis has been recently demonstrated in genetic studies on MRL-*lpr* × (MRL-*lpr* × C57BL/6-*lpr*) backcross mice (32). Furthermore, B cell-depleted C57BL/6-*lpr* mice lacking the autoantibody production still developed systemic granulomatous arteritis, but completely failed to produce glomerular lesions (33). Notably, systemic granulomatous vascular lesions seen in mice bearing the *lpr* mutation are different from cutaneous leukocytoclastic vasculitis in mice induced by some of the IgG3 monoclonal cryoglobulins, i.e. those having RF activity; the latter lesions were characterized by perivascular infiltration of polymorphonuclear leukocytes and were only observed in skins which are not well protected by hair from thermic variations (25 – 27).

The mechanism responsible for the diminished production of IgG3 and IgG2a and a parallel increase in IgG1 levels in MRL-*lpr.ll* mice remains to be determined. However, in view of the active role of several lymphokines for the IgG class switchings, one may speculate that limited expansion of *lpr* DN cells and/or modification of $CD4^{+}$ T cell populations may alter a balance of various T cell lymphokines, leading to the observed difference in IgG subclass expression. It has been claimed that *lpr* DN cells preferentially produce interferon-γ (34) and a newly defined T cell cytokine, *Eta-1* (9), both of which appear to enhance the

production of IgG2a (35,36); and interferon-γ has been recently reported to promote the production of IgG3 (37). Further analysis of lymphokine expression may help elucidate the molecular mechanism responsible for the differential expression of IgG subclasses in MRL-*lpr.ll* and conventional MRL-*lpr* mice.

It should be emphasized that MRL-*lpr.ll* mice still carry the *lpr* mutation, as documented by the presence of defects in the Fas antigen (3). The moderate development of autoimmune glomerulonephritis in MRL-*lpr.ll* mice is consistent with the notion that the *lpr* mutation by itself is not sufficient to cause full-blown lupus-like autoimmune syndrome, as in the case of C57BL/6 and C3H/HeJ mice carrying the *lpr* gene (38). Thus, a possible new mutation in MRL-*lpr.ll* mice may play a significant role in the pathogenesis of lupus-like syndrome. Clearly, this new substrain is a useful tool for further understanding of the immunopathogenesis of systemic lupus erythematosus.

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Abbreviations

DN	double negative
ll	long-lived
MRL-*lpr*	MRL/MpJ-*lpr/lpr*
Con A	concanavalin A
NIP	(4-hydroxy-3-iodo-5-nitrophenyl)acetyl

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