ABNORMALITIES INDUCED BY THE MUTANT GENE, *lpr*

Patterns of Disease and Expression of Murine Leukemia Viruses in SJL/J Mice Homozygous and Heterozygous for *lpr*

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The *lpr* locus was defined by an autosomal recessive mutation initially observed in strain MRL (1). Whereas nonmutant mice of this strain develop late-onset autoimmune disease characterized by chronic glomerulonephritis, MRL-*lpr*/*lpr* mice experience severe early-onset subacute glomerulonephritis, arteritis, and arthritis associated with massive lymphadenopathy and splenomegaly (1–3). The lymph nodes and spleens of *lpr* homozygotes are expanded by an abnormal population of cells that coexpress cell surface determinants usually restricted to T cells (Thy-1, Ly-1) or B cells [Ly-5(B220)] (4–9), and are deficient in their ability to produce interleukin 2 (IL-2)[1] (9–12). These aberrant cells were clearly shown not to be B cells, in that they had no rearrangements of Ig heavy chain genes (6). MRL-*lpr*/+ resemble MRL-+/+ mice in terms of disease patterns, longevity, and phenotypic and functional characteristics of their lymphocytes, indicating that the mutation is fully recessive in this strain.

To determine if this mutation would produce similar abnormalities when present on other genetic backgrounds, *lpr* was backcrossed onto strains NZB/BlNJ, SJL/J, C3H/HeJ, AKR/J, C57BL/6J, and BALB/cJ. Ongoing phenotypic, functional, and histopathologic studies of mice (from these strains) homozygous for *lpr* have shown that, although aberrant T cells were detected in the lymphoid tissues of mice from all strains (6, 9, and H. C. Morse III, W. F. Davidson, and J. B. Roths, unpublished observations), *lpr*/*lpr* mice differed considerably among strains in the degree of lymphadenopathy they exhibited, as well as in their longevity and rates of development, and severity of the autoimmune disease they incurred (5, 9–13, and J. B. Roths and E. D. Murphy, unpublished observations).

During the course of these studies, it was noted that the survival of SJL-*lpr*/+ mice was shortened in comparison to SJL-+/+ mice, suggesting that, in this strain, *lpr* had an effect in the heterozygous state. To evaluate this possibility,

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[1] *Abbreviations used in this paper:* FMF, flow microfluorimetry; IL-2, interleukin 2; MCF, mink cell–focus inducing; MuLV, murine leukemia virus; sIg, surface Ig.

SJL-*lpr/lpr*, -*lpr*/+, and -+/+ mice were compared for longevity, histopathology, expression of murine leukemia viruses (MuLV), and phenotypic characteristics of lymphocytes.

Materials and Methods

Mice. All mice were bred by J. B. Roths and E. D. Murphy or were purchased from the Animal Resource colonies of The Jackson Laboratory, Bar Harbor, ME, except for NFS/N, which was obtained from the colonies of the National Institutes of Health. Mice of different strains bearing the *lpr* mutation had been through at least seven cycles of cross-intercross matings at the time of study.

Histopathology. Moribund mice were sacrificed and tissues were fixed in Fekete's formal-acetic-ethanol solution. Sections (8 μm) were stained with hematoxylin and eosin for microscopic examination. Sections of kidney were also stained with PAS/Schiff reagent.

Virus Studies. Infectious center assays for ecotropic, xenotropic, and MCF MuLV were performed with mitomycin-C–treated lymphoid cells, as previously described in detail (14, 15). Infectious ecotropic MuLV in 2% (wt/vol) extracts of tail biopsies (16) was quantitated by UV-XC tests of SC-1 cells inoculated with 0.2 ml of extract.

Flow Microfluorometry (FMF). Single-cell suspensions from thymus, spleen, and lymph nodes were prepared and stained for FMF analyses using established procedures (14). The antibodies used in this study and their specificities for normal hematopoietic cells have previously been described in detail (6, 9, 12, 17–21). Antibodies to Thy-1, surface Ig (sIg), Ly-5(B220), ThB, Ly-5, XenCSA (14), and "8C5" were fluorescein labeled. Antibodies to "H-11", Ly-24 and Mac-1 were sandwiched with affinity-purified, fluorescein-labeled rabbit anti–rat Ig. Antibodies to Ly-1, Ly-17, Ia and Lyb-2 were sandwiched with affinity-purified goat anti–mouse IgG2. Fluorescence profiles were generated on a FACS II (B-D FACS Systems, Sunnyvale, CA) from 3×10^4 viable nucleated cells (as determined by combined gating with propidium iodide to exclude dead cells, and narrow forward-angle light scatter to exclude red cells), using logarithmic amplification of signals from the photomultiplier tubes.

DNA Analyses. High molecular weight DNA was prepared, cleaved with restriction endonucleases, and separated by electrophoresis on 0.6% agarose gels as previously described (22). After transfer to nitrocellulose membranes, the blots were probed with ^{32}P-labeled pBR322 containing an ecotropic envelope-specific DNA fragment, pEc-B4 (23) using established techniques (22).

Results

Lymphoproliferation in Mice Homozygous for lpr. In earlier studies, we found that *lpr* induced lymphadenopathy in different inbred strains homozygous for the mutation (13), and that the nodes of these mice were expanded with an abnormal population of Thy-1$^+$,Ly-1$^+$,Ly-2$^-$ or Thy-1$^-$,Ly-1$^+$,Ly-2$^-$ T cells bearing the B cell surface marker, Ly-5(B220) (6–9). The effect of *lpr* on lymphadenopathy in SJL mice homozygous for the mutation was compared at 10 and 26 wk with that in C3H-, MRL-, AKR-, and C56BL/6-*lpr/lpr* mice (Fig. 1). At 10 wk, nodes of SJL-*lpr/lpr* mice were significantly larger than those of other strains homozygous for *lpr*. At 26 wk, however, SJL-*lpr/lpr* nodes were only slightly larger than those of MRL-*lpr/lpr* mice, and were smaller than those of C3H-*lpr/lpr* mice.

Lymph node and spleen cells from 4.5–6-mo-old SJL-+/+ and SJL-*lpr/lpr* mice were tested by FMF for expression of a series of cell surface antigens (Table I). As for other strains homozygous for *lpr*, the predominant phenotype of LN cells from SJL-*lpr/lpr* mice was Thy-1$^+$, H-11$^+$, Ly-5(B220)$^+$, Ly-5$^+$, Ly-2$^-$, sIg$^-$,

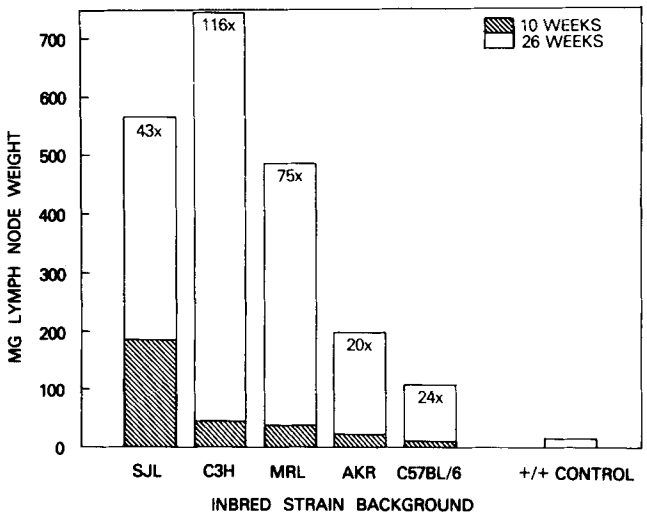

FIGURE 1. Lymphadenopathy in SJL/J, C3H/HeJ, MRL/MpJ, AKR/J, and C57BL/6J mice homozygous for *lpr* at 10 wk (hatched) and 26 wk of age (open). Bars indicate the mean weight of pooled axillary, renal, lumbar, and mesenteric lymph nodes of five to six mice at each time point. Numbers at the top of the open bars indicate the relative increase in the weight of *lpr/lpr* lymph nodes as compared with +/+ nodes for each strain at age 26 wk. The mean weight of +/+ lymph nodes from all strains is indicated by the open bar at the right side of the figure.

ThB$^-$, Ia$^-$ (6–9). The reduced frequency of Ly-1$^+$ cells in LN of these mice contrasts with the increased frequency of these cells in the other *lpr* strains tested (6). The frequency of Thy-1$^+$ and Ly-5(B220)$^+$ cells was also increased in spleen but to a lesser extent than LN, again paralleling studies of other strains homozygous for *lpr* (6–9).

The frequency of cells bearing markers not previously examined in *lpr* mice was also determined. These include Lyb-2 (24), the B cell–specific antigen; Ly-17 (18), the granulocyte-specific marker; "8C5" (R. L. Coffman, H. C. Morse III, unpublished observations), the myelomonocytic determinant; Mac-1 (19), and an antigen expressed by the great majority of hematopoietic cells of all lineages, Ly-24 (20, 21). The frequencies of Lyb-2$^+$ and Ly-17$^+$ cells in both *lpr/lpr* and normal tissues corresponded closely to the frequencies of B cells defined by expression of sIg, ThB or Ia (Table I), emphasizing that the only B cell determinant greatly altered in expression on *lpr* cells is Ly-5(B220).

The frequencies of spleen but not LN cells expressing antigens confined to the myelomonocytic lineage (8C5, Mac-1) were increased slightly in *lpr* mice, corresponding, in part, with reports of increases in macrophages in other strains homozygous for *lpr* (25, 26). By comparison, the frequency of cells bearing Ly-24, the major surface glycoprotein of phagocytic cells (20, 21), and the density of Ly-24 antigens per cell was greatly increased in *lpr* as compared with normal SJL mice (Table I and Fig. 2). In regard to the altered representation of Ly-5(B220) and Ly-24 antigens on SJL-*lpr/lpr* cells, it should be noted that *lpr* clearly is not linked to the loci on chromosome 1 or chromosome 2 that,

TABLE I

Cell Surface Antigens of SJL-lpr/lpr Lymphocytes

Cell source	Genotype of mice	Cell surface determinant (percent positive cells)*												
		Thy-1	Ly-1	Ly-2	H-11	sIg	Ly-5(B220)	ThB	Ia	Lyb-2	Ly-17	8C5	Mac-1	Ly-5	Ly-24
Lymph node	+/+	56 ± 2	78 ± 5	25 ± 3	69 ± 3	34 ± 3	35 ± 8	35 ± 6	38 ± 7	34 ± 5	35 ± 6	2 ± 1	2 ± 1	97 ± 2	64 ± 2
	lpr/lpr	82 ± 4	40 ± 2	2 ± 2	98 ± 1	7 ± 1	76 ± 8	5 ± 1	7 ± 1	9 ± 1	9 ± 2	1 ± 1	1 ± 1	99 ± 1	98 ± 1
Spleen	+/+	46 ± 2	48 ± 2	16 ± 1	46 ± 4	50 ± 1	50 ± 2	50 ± 2	52 ± 3	54 ± 2	52 ± 3	2 ± 1	5 ± 2	99 ± 2	59 ± 6
	lpr/lpr	70 ± 5	36 ± 2	5 ± 1	89 ± 5	12 ± 5	65 ± 10	14 ± 3	25 ± 8	21 ± 6	30 ± 8	7 ± 3	10 ± 3	98 ± 2	94 ± 4

* Values represent means ± SEM for 4–8 individual mice tested in 2–4 separate experiments. Mice were 4½ mo to 6 mo old.

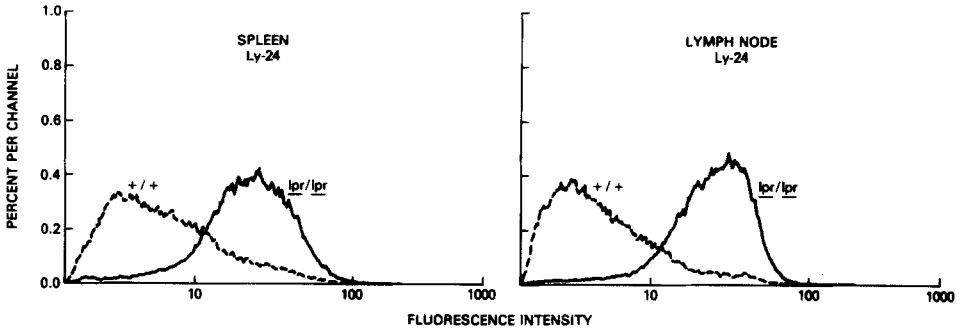

FIGURE 2. FMF analyses of spleen and lymph node cells from SJL-*lpr/lpr* (solid lines) and SJL-+/+ mice (dashed lines) reacted with anti-Ly-24, washed, and then treated with fluorescein isothiocyanate–labeled rabbit anti–rat Ig.

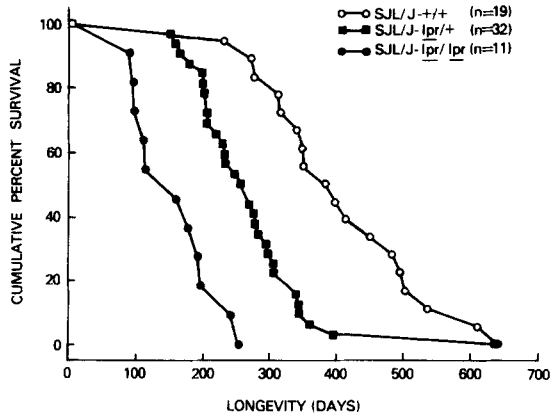

FIGURE 3. Mortality curves for SJL-+/+, SJL-*lpr*/+, and SJL-*lpr/lpr* mice.

respectively, control expression of these cell surface markers (J. B. Roths, E. D. Murphy, unpublished results).

Lifespans and Disease Patterns of SJL-+/+, -lpr/+, and -lpr/lpr Mice. We compared the longevity of mutant homozygous or heterozygous mice, or homozygous wild types (Fig. 3). The lifespan of *lpr* homozygotes was markedly shortened in comparison with that of +/+ mice (mean survival of *lpr/lpr* was 155 ± 18 d vs. 408 ± 28 d for homozygous wild types; $P < 0.001$). By comparison, the survival curve for *lpr*/+ mice was intermediate to that of the other populations (mean survival was 268 ± 16 d, $P < 0.001$ compared with either *lpr/lpr* or +/+).

Gross and microscopic examinations of tissues from 5–6-mo-old SJL-*lpr/lpr* mice revealed marked differences to the pathology described for MRL-*lpr/lpr* mice. Whereas MRL-*lpr/lpr* mice die with severe renal disease (Fig. 4 *D*) (1–3), vasculitis (Fig. 4 *F*), including myocardial infarctions (27) and arthritis (28), SJL-*lpr/lpr* mice exhibited minimal renal lesions (Fig. 4 *C*), rare focal vasculitic lesions (Fig. 4 *E*), and had none of the clinical signs of arthritis shown by MRL-*lpr/lpr* mice (J. B. Roths, personal observations). However, perivascular, peribronchiolar, interstitial and intraalveolar infiltrates of lymphocytes and inflammatory cells

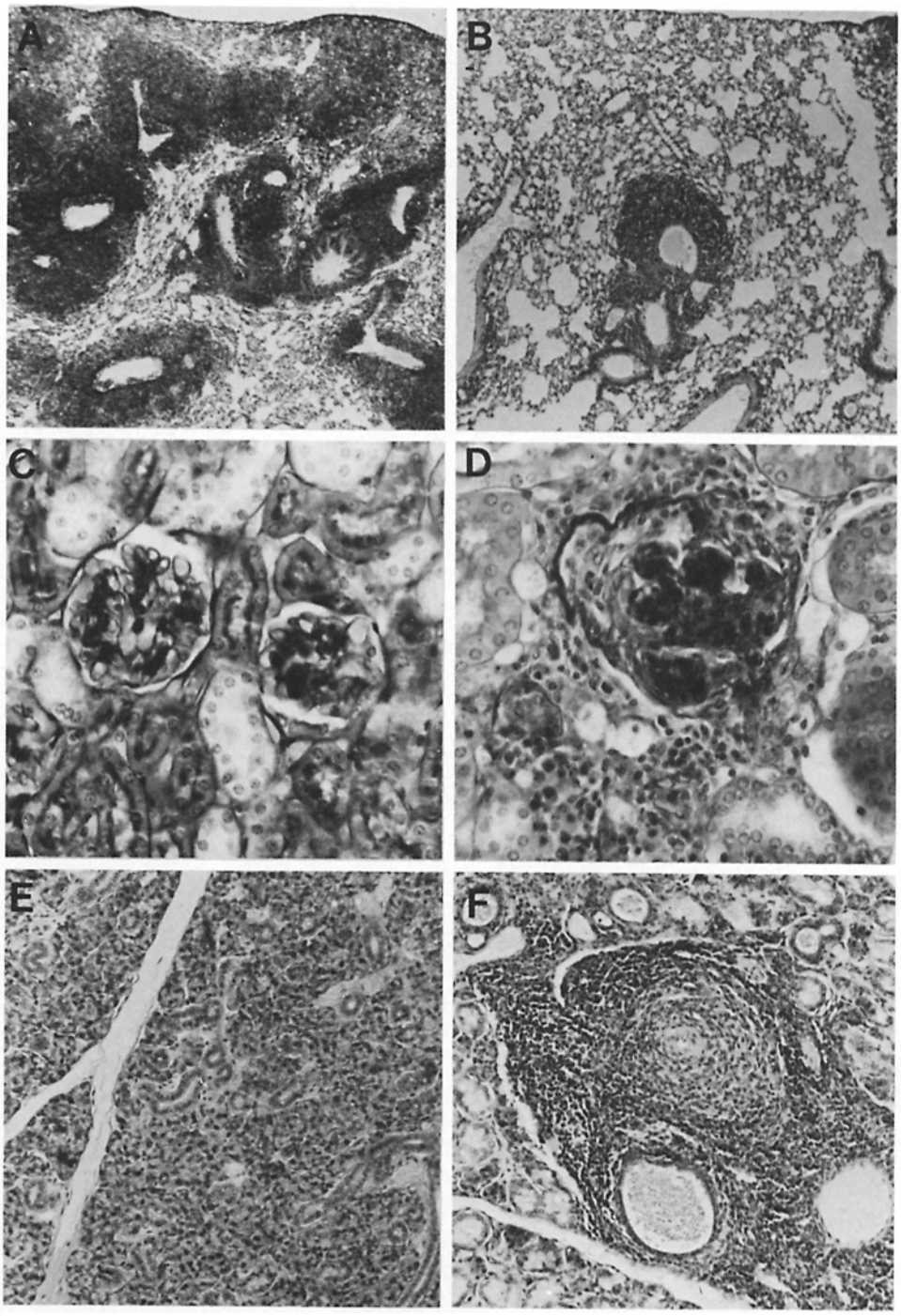

FIGURE 4. Histopathology of 5-mo-old SJL/J-*lpr/lpr* (*A*, *C*, *E*) and MRL/Mp-*lpr/lpr* (*B*, *D*, *F*) mice. (*A* and *B*) Lung, hematoxylin and eosin. × 46. (*A*) Extensive perivascular and peribronchial infiltration of lymphoid cells and chronic interstitial inflammatory disease especially prominent at the periphery. Dense accumulations of lymphoid cells fill alveolar spaces. (*B*) Limited perivascular and peribronchial infiltration without interstitial disease. (*C* and *D*) Kidney, periodic acid/Schiff, × 114. (*C*) Essentially normal glomerular and tubular morphology. (*D*) Extensive glomerular disease with sclerosis, crescent formation, adhesions and periglomerular interstitial lymphoid infiltration. (*E* and *F*) Submandibular gland, hematoxylin and eosin, × 285. (*E*) Normal morphology. (*F*) Extensive perivascular lymphoid infiltration, chronic arteritis, and periarteritis characterized by thickened eosinophilic intima, narrowed lumen, and fibrous tissue in the adventitia.

were striking histologic features of lung sections from SJL-*lpr/lpr* mice (Fig. 4 *A*), whereas pulmonary lesions in MRL-*lpr/lpr* mice were limited to some perivascular and peribronchiolar cuffing without alveolar infiltrates (Fig. 4 *B*). Sections from other tissues of SJL-*lpr/lpr* mice showed venous congestion and capillary stasis. In view of the marked reduction in the volume of the thoracic cavity occasioned by lymph node hypertrophy, the lung lesions of SJL-*lpr/lpr* mice probably result in death due to pulmonary insufficiency combined with right-sided heart failure.

Comparable histopathologic studies of nine moribund SJL-*lpr*/+ mice showed that all the animals died with lymphomas, of apparent B cell origin, which resembled the characteristic neoplasms of SJL-+/+ mice (29). Using a recently defined system that allows comparisons of the histopathology of murine and human lymphomas (30, 31), the lesions of SJL-*lpr*/+ mice included follicular center cell lymphomas, immunoblastic lymphomas, and a plasmacytoid lymphoma.

In Vivo and In Vitro Passage of lpr/+ Lymphomas. In earlier studies, (32–34) it was found that SJL-+/+ lymphomas may be difficult to establish as continuous in vivo or in vitro lines, raising the possibility that the SJL-*lpr*/+ and -+/+ lesions may not be truly malignant, in spite of their histologic appearances. To evaluate this question, young adult SJL-+/+ mice were inoculated subcutaneously with fragments of lymph nodes from +/+ and *lpr*/+ mice carrying lymphomas. The transplantation studies demonstrated that both +/+ and *lpr*/+ lymphomas could be passaged in +/+ recipients. The frequency with which the lymphomas were successfully transplanted was higher for *lpr*/+ (78%, $n = 9$) than for +/+ lymphomas (59%, $n = 27$).

Studies of MuLV. Expression of infectious endogenous or exogenous MuLV-related genomes has been shown to influence the development of B lineage lymphomas (31, 35). To determine if the effects of *lpr* on B cell neoplasms in SJL mice could be related to alterations in production of MuLV, tissues from prelymphomatous +/+ and *lpr*/+ mice, as well as *lpr/lpr* mice were tested for expression of infectious ecotropic, xenotropic, and mink cell focus–inducing (MCF) MuLV. Viruses belonging to the latter two classes of MuLV were not produced by thymocytes, lymph node or spleen cells from any of the animals tested (data not shown), but infectious ecotropic MuLV was produced, to a highly variable extent, by lymphocytes from all three types of mice (Table II). The data in this table can be summarized as follows: First, a low proportion ($<10^2$ out of 10^7) of cells from thymus, spleen, or lymph nodes from +/+ mice produced infectious ecotropic MuLV. Second, $>5 \times 10^2$ out of 10^7 cells from all lymphoid tissues of *lpr/lpr* mice 3 mo of age or older produced infectious virus of this class. Finally, the virus phenotype of tissues from *lpr*/+ mice appeared to differ from that of +/+ or *lpr/lpr* mice. The frequency of virus-positive mice at different ages, and the frequency of virus-producing cells from virus-positive animals tended to be higher in *lpr*/+ than in +/+ mice, but lower than in *lpr/lpr* mice. It should be noted that this trend is especially apparent for *lpr*/+ mice no more than 4 mo old. In the older age groups (>4 mo), mice with prelymphomatous lesions or overt lymphomas were excluded to avoid the possibility that expression of MuLV might be altered by neoplastic changes. These results

TABLE II

Expression of Infectious Ecotropic MuLV in Lymphoid Tissues of Prelymphomatous SJL Mice

Age	XC Plaques/10^7 Cells (log_{10})								
	Thymus			Spleen			Node		
	+/+	*lpr*/+	*lpr*/*lpr*	+/+	*lpr*/+	*lpr*/*lpr*	+/+	*lpr*/+	*lpr*/*lpr*
mo									
2.5	−*, −	−, 0.0	<1.0, −	0.8, 1.5	1.2, 0.8	<1.0, 2.6	−, −	−, −	<1.0, 2.8
3.0	−, −			1.5, 2.2		3.0, 2.8,3.1			2.6, 2.6, 1.9
3.5	−, −, −	1.4, −, −		1.2, 1.3, 1.0	3.2, 1.2, 2.3		0.8, −, −	1.5, 0.5	
4.0	−, −, −	3.6, 0.3	4.0, 2.9, 2.7	0.8, 0.6, 1.8	2.5, 0.8	4.0, 3.9, 3.4	−, −	3.3, 0.5	4.0, 3.5, 3.9
5.0	−			1.6					
5.5	−, −, −	−, −, −		0.7, 1.9, −	−, 1.7, 0.6		−, 1.2, −	−, 1.1, −	
	−, −	−, 2.4		0.3, 2.0	−, 2.0		−, 1.7	−, 1.6	
6.0					−, −	2.8, 5.1		1.5, 1.0	4.0, 5.5
6.5	−, 1.5, −		4.7	1.4, 2.3, 1.7		≥5.0	1.6, 2.3, 1.0		≥5.0

* − Denotes no plaques/10^7 cells.

TABLE III

Expression of Infectious MuLV in Lymphoid Tissues of Strains Homozygous For lpr

Strain	Ecotropic virus (XC plaques/10^7 cells [log_{10}])					
	Thymus		Spleen		Node	
	+/+	*lpr*/*lpr*	+/+	*lpr*/*lpr*	+/+	*lpr*/*lpr*
C57BL/6J	−*		−, −	−, −	−	−, <0.3
C3H/HeJ			−, 1.3	−, 1.4	−, −	−, −
AKR/J	5.1, 4.3	4.3, 5.0	5.1, 5.0	5.1, 5.1	5.2, 4.9	4.9, 4.8
	Xenotropic virus (S^+L^- foci/10^7 cells [log_{10}])					
NZB/BlNJ	2.4, 2.5	3.0, 3.3	3.9, 3.9	3.7, 3.8	2.6, 3.0	3.3, 3.3

* − Denotes no plaques/10^7 cells.

demonstrate that expression of infectious ecotropic MuLV is markedly increased in lymphoid tissues of SJL-*lpr/lpr* vs. -+/+ mice, and suggest that MuLV expression may be enhanced in *lpr*/+ mice, but to a lesser and variable extent.

We studied the possibility that heightened expression of infectious ecotropic MuLV in SJL-*lpr/lpr* mice might occur in nonlymphoid as well as lymphoid tissues by testing cell-free extracts from tail biopsies of eight *lpr/lpr*, four *lpr*/+, and eight +/+ mice 2–4.5 mo old. Extracts from one *lpr/lpr* and two +/+ mice produced low numbers (2–26) of XC cell line plaques under conditions that would yield $>10^3$ plaques in comparable tests of AKR mouse tissue. These data suggest that expression of infectious ecotropic MuLV is amplified in lymphoid but not in nonlymphoid tissues of SJL-*lpr/lpr* mice.

Two major questions are raised by these observations. First, is the alteration in MuLV expression detected in SJL-*lpr/lpr* mice restricted to this strain, or is virus expression also changed in other *lpr* congenics? This question was examined by testing lymphoid tissues from 3–5-mo-old C57BL/6J, C3H/HeJ, AKR/J, and NZB/BlNJ *lpr* homozygotes and +/+ mice for expression of infectious MuLV (Table III). The proportions of thymocytes, spleen cells, and lymph node cells

producing ecotropic MuLV were not significantly altered by the *lpr* mutation in the ecotropic MuLV-positive strains C57BL/6, AKR, or C3H, and neither xenotropic nor MCF MuLV were detected in any of the cultures of cells from these mice. Tests for infectious xenotropic MuLV in ecotropic MuLV-negative NZB mice showed no significant differences between +/+ and *lpr/lpr* for the proportions of virus-producing cells from spleens or lymph nodes. The difference between normal and *lpr* mice for expression of infectious xenotropic MuLV by thymocytes (Table III) may reflect contamination of *lpr/lpr* thymus preparations with parathymic lymph nodes or abnormal cells infiltrating the thymuses of these mice. Taken together, the analyses of MuLV in different strains indicate that the effect of *lpr* on virus expression is limited to ecotropic loci in SJL mice.

Second, since the *lpr* gene introduced into SJL came from MRL, a strain derived from crosses between AKR, C57BL/6, C3H/Di, and LG (1), does the altered expression of ecotropic MuLV in SJL-*lpr/lpr* mice reflect inadvertent introduction of an ecotropic locus from one of these strains to SJL in linkage with or unlinked to *lpr*, or was *lpr* introduced into linkage with one of the SJL ecotropic loci? To evaluate these possibilities, Southern blots of restricted cellular DNA from SJL-+/+ and *lpr/lpr* mice were hybridized with a probe specific for the *env* gene of ecotropic MuLV (data not shown). The results demonstrated that mutant mice had two ecotropic MuLV-related sequences identical in size to those in SJL-+/+ DNA, indicating that *lpr* alters the expression of one or both of the endogenous MuLV sequences found in normal SJL mice (36).

To determine if altered MuLV expression might reflect linkage between one of the SJL ecotropic MuLV loci and *lpr*, virus-negative NFS/N female mice were bred to SJL-*lpr/lpr* male mice, and F_1 males were then backcrossed to NFS/N females. Backcross females were tested for *lpr* by matings with B6-*lpr/lpr* mice, and for SJL ecotropic MuLV loci by Southern blot hybridization of their splenic DNA restricted with Xba I with an ecotropic *env*-specific probe, pEc-B4. The results of these analyses (Table IV) showed that one of two mice inheriting the 6.3 kb pEc-B4–reactive Xba I fragment, two out of five mice inheriting the 11.5

TABLE IV

Relations Between Ecotropic MuLV Loci and lpr in SJL-lpr/lpr Mice

Mouse*	pEc-B4-Reactive Xba I Fragment		*lpr*	*lpr/lpr* in progeny test
	6.3 kb	11.5 kb		
6106	−	−	−	0/7
6107	+	−	+	4/8
6108	−	+	−	0/9
6109	−	+	+	4/10
6110	−	+	−	0/11
6111	+	−	−	0/7
6112	−	+	−	0/8
6113	−	−	−	0/9
6114	−	+	+	2/6

* Backcross (NFS × SJL-*lpr/lpr*) × NFS mice (6106–6114) outcrossed to C57BL/6-*lpr/lpr* to test for presence of *lpr*. DNA prepared from spleens was restricted with Xba I, and tested for pEc-B4-reactive sequences.

kb fragment, and none of two mice that inherited neither fragment were *lpr*/+. The results indicated that *lpr* was not linked to either of the SJL ecotropic MuLV loci.

Discussion

The data presented in this report demonstrate a number of important points regarding abnormalities related to the *lpr* mutation. From information presented here and elsewhere, it is clear that the *lpr* mutation consistently induces certain abnormalities, regardless of strain background, but that other abnormalities associated with the mutation are highly strain-dependent. Thus, for all strains tested, *lpr* induces lymphadenopathy and splenomegaly, although there is strain-to-strain variation in the extent of proliferation (Fig. 1) (13). Lymph nodes and spleens of *lpr* mice from all strains are expanded with cells that coexpress cell surface antigens usually restricted to T cells (Thy-1) or B cells [Ly-5(B220)] (Table I) (6–9). These cells are definitely not of B cell origin, as (*a*) their expansion is limited in neonatally thymectomized mice (37, 38), and (*b*) most express Ly-1 in addition to Thy-1 (Table I) (4–9). In addition, *lpr* homozygotes of all strains produce antinuclear antibodies (13 and J. B. Roths, unpublished data).

In contrast, the effects of the *lpr* mutation on the development of autoimmune disease and expression of MuLV are markedly strain-dependent. MRL-*lpr*/*lpr* mice develop accelerated immune-complex glomerulonephritis, arthritis, and vasculitis (1–3, 27, 28), whereas C3H-*lpr*/*lpr* and SJL-*lpr*/*lpr* mice develop little or no renal, joint, or vascular disease (Fig. 4) (12). Instead, SJL-*lpr*/*lpr* mice developed extensive lymphocytic and inflammatory cell pulmonary infiltrates, and appeared to die with combined pulmonary insufficiency and right-sided heart failure.

Among strains homozygous for *lpr* and tested for expression of infectious MuLV, the mutation appears to affect only ecotropic MuLV in SJL mice (Tables II and III). The mechanisms responsible for enhanced MuLV expression in this strain are unknown, but linkage studies clearly demonstrated that this phenomenon did not reflect linkage of the *lpr* gene to one of the two endogenous SJL ecotropic MuLV loci (Table IV). Furthermore, no evidence of additional ecotropic MuLV loci was found.

Our results also indicated that the *lpr* gene may be expressed to some extent in the heterozygous state on the SJL background, but not on other backgrounds. This view is based most firmly on the observation of a significant difference in survival between SJL-+/+ and -*lpr*/+ mice (Fig. 3), and less certainly on enhanced expression of ecotropic MuLV in SJL-*lpr*/+ mice <4 mo old (Table II). Similar effects have not been observed with B6, AKR, or MRL *lpr* heterozygotes. It is noteworthy that the apparent effects of this gene in the heterozygous state only partially resemble its effects in the homozygous state. Thus, although SJL-*lpr*/+ mice exhibited a shortened life span and enhanced expression of MuLV, they died with lymphomas rather than pulmonary disease, they did not produce antinuclear antibodies (J. B. Roths, unpublished observation), and their spleens and nodes did not contain cells phenotypically characteristic of *lpr* homozygotes.

The origins and characteristics of the cells responsible for lymphomas of SJL-

+/+ mice have been studied since the initial description of these lesions as reticulum cell sarcomas in 1963 (29). No consensus of opinion has been developed regarding this disease, as some cell lines obtained from old SJL mice were shown to have characteristics of cells in the B lymphocyte lineage (32–34), whereas others had characteristics of macrophages or histiocytes (39). The observation that these neoplasms do not develop in SJL mice rendered deficient of B cells by treatment from birth with anti-IgM (40), is one of the strongest lines of evidence for the B cell nature of these lymphomas. In the current study, we demonstrated that SJL-*lpr*/+ mice died with lymphomas that resembled neoplasms of SJL-+/+ mice. Using recently developed criteria for classification of murine lymphomas (30, 31), the neoplasms of both +/+ (data not shown) and *lpr*/+ mice had characteristics of follicular center–cell and immunoblastic lymphomas. The malignant nature of some of the *lpr*/+ lymphomas was documented by their growth after transplantation. Continuous in vitro cell lines were established from some of the *lpr*/+ neoplasms, but their relationship to the primary neoplasms is uncertain.

The relationship between MuLV and the development of B-lineage lymphomas has recently been clarified to some extent. Two findings demonstrate that ecotropic MuLV are not required for the development of spontaneous or induced B-lineage neoplasms, and thus suggest, indirectly, that MCF MuLV are also not required. First, NFS mice, which lack the genetic information for ecotropic viruses, develop a low frequency of spontaneous B cell lymphomas (31, 35). Second, BALB/c mice congenic for the *Rmcf*^{r} locus of DBA/2 (41), and lacking ecotropic MuLV, develop plasmacytomas (after treatment with pristane) at a frequency equivalent to that of normal BALB/c mice (42). However, NFS mice congenic for ecotropic MuLV induction loci from AKR or C58 have a much higher frequency of B-lineage lymphomas than normal NFS mice, indicting that ecotropic MuLV can amplify the tendency of this strain to develop B cell neoplasms (31, 35). Enhanced expression of ecotropic MuLV early in the life of SJL-*lpr*/+ mice (Table II) may also act to accelerate the development of neoplasms. The mechanism of such amplification is unknown, but ecotropic virus–induced generation of some type of recombinant MuLV could be involved. Second, although MCF MuLV have been recovered from nonthymic lymphomas of NFS (15) and SJL-*lpr*/+ mice (data not shown), these viruses have not been shown to accelerate the development of similar neoplasms in mice inoculated as newborns.

Taken together with data from earlier studies, these results indicate the marked complexities involved in studies of autoimmune disease, even when "single gene models" are studied. The *lpr* mutation was initially considered as a disease-inducing alteration of the genome (1, 2), but has subsequently been viewed as a defect accelerating the autoimmune disease of MRL/Mp mice (3, 11, 27). Other strains homozygous for this mutation develop little or no evidence of autoimmunity, aside from autoantibodies (9, 13, 43), and die with undetermined causes (9) or, in the case of SJL/J, pulmonary disease (as described herein).

These strain differences may reflect the fact that this single mutation alters the expression of many cellular genes, including those governing expression of cell surface antigens (5–9), protooncogenes (44), and endogenous MuLV (Table

II). In some cases, expression of these genes is enhanced; in other instances expression is depressed. This variation appears to be, in part, strain-dependent. Although elucidation of these strain-related effects is clearly important, it is unlikely that a fundamental understanding of the abnormalities caused by the *lpr* mutation will be obtained until the primary defect, probably enzymatic, has been described. In this regard, it has been suggested that the abnormalities induced by *lpr* and the nonallelic mutation, *gld* (45) may reflect defects common to a metabolic pathway of importance to T cell differentiation and function (46).

Summary

SJL/J mice heterozygous or homozygous for the *lpr* mutation were compared with SJL/J-+/+ mice for longevity, histopathology, antigenic characteristics of lymphocytes and expression of murine leukemia viruses (MuLV). In comparison to +/+ mice, *lpr* homozygotes had a markedly shortened life span, died with infiltrative pulmonary disease, but little or no renal disease, and expressed high levels of infectious ecotropic MuLV in lymphoid tissues. SJL-*lpr*/+ mice had a life span intermediate between SJL-+/+ and -*lpr*/*lpr* mice, died with lymphomas that histologically resembled the neoplasms of +/+ mice, and sometimes expressed high levels of ecotropic MuLV. The lymphomas of *lpr*/+ could be transplanted to +/+ recipients in 78% of cases, and continuous in vitro lines were established from some of them. Similar effects on virus expression or lymphoma development were not observed in other strains homozygous or heterozygous for the *lpr* mutation. These results indicate that the diseases expressed by mice homozygous for the *lpr* mutation are highly strain-dependent, and that this gene can have an effect in the heterozygous state in SJL mice.

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