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Deficiency of 5-Lipoxigenase Abolishes Sex-Related Survival Differences in MRL-\(lpr/lpr\) Mice

Jennifer L. Goulet,*‡ Robert C. Griffiths,* Phillip Ruiz, § Robert F. Spurney,* David S. Pisetsky,‡ Beverly H. Koller,† and Thomas M. Coffman2*

Leukotrienes, the 5-lipoxygenase (5LO) products of arachidonic acid metabolism, have many proinflammatory actions that have been implicated in the pathogenesis of a variety of inflammatory diseases. To investigate the role of LTs in autoimmune disease, we generated an MRL-\(lpr/lpr\) mouse line with a targeted disruption of the \(5\)lo gene. MRL-\(lpr/lpr\) mice spontaneously develop autoimmune disease that has many features resembling human systemic lupus erythematosus, including sex-related survival differences; female MRL-\(lpr/lpr\) mice experience significant early mortality compared with males. Unexpectedly, we found that mortality was accelerated in male 5LO-deficient MRL-\(lpr/lpr\) mice compared with male wild-type MRL-\(lpr/lpr\) animals. In contrast, the \(5\)lo mutation had no effect on survival in females. Mortality was also accelerated in male MRL-\(lpr/lpr\) mice that were treated chronically with a pharmacological inhibitor of LT synthesis. Furthermore, LT-dependent inflammatory responses are enhanced in male MRL-\(lpr/lpr\) mice compared with females, and the \(5\)lo mutation has greater impact on these responses in males. Because immune complex-mediated glomerulonephritis is the major cause of death in MRL-\(lpr/lpr\) mice and has been related to arachidonic acid metabolites, we also assessed kidney function and histopathology. In male MRL-\(lpr/lpr\) mice, renal plasma flow was significantly reduced in the \(5\)lo\(^{-/-}\) group, although there were no differences in the severity of renal histopathology, lymphoid hyperplasia, or arthritis between the groups. These findings suggest that the presence of a functional \(5\)lo gene confers a survival advantage on male MRL-\(lpr/lpr\) mice and that, when 5LO function is inhibited, either genetically or pharmacologically, this advantage is abolished. The Journal of Immunology, 1999, 163: 359–366.
with zymosan showed enhanced production of LTC₄ (30). Leukotrienes may be particularly important in the immune complex-mediated glomerulonephritis of this mouse model (28). Because LT production in normal kidneys is virtually absent, LTs have little role in normal renal physiology. However, in various inflammatory diseases of the kidney, including murine autoimmune nephritis, LT production is markedly enhanced. In vitro production of LTB₄ and LTC₄ by the kidneys of MRL-lpr/lpr mice was significantly increased compared with normal, non-MRL controls (28). In these circumstances, LTs may promote kidney dysfunction and injury through their demonstrated abilities to cause renal vasoconstriction (31–34), to induce mesangial cell contraction (35–37), and to stimulate extracellular matrix protein production (38). We have previously demonstrated that the enhanced renal production of LTs in MRL-lpr/lpr mice correlated with the functional and histopathological severity of renal disease in this mouse model (28), and we found that administration of a specific peptidyl-LT receptor antagonist significantly improved renal hemodynamic function in MRL-lpr/lpr mice (28). The objective of the present study was to use gene targeting to examine the role of LTs in the autoimmune disease of the MRL-lpr/lpr mouse model, under the hypothesis that the absence of LT synthesis would have beneficial effects on the course of disease. Unexpectedly, we found that the absence of a functional 5lo gene had a significant detrimental effect on survival in male MRL-lpr/lpr mice.

Materials and Methods

Mice

All mice were bred and maintained in specific pathogen-free animal barrier facilities at the University of North Carolina and the Durham Veterans Affairs Medical Center. MRL-lpr/lpr mice were purchased from The Jackson Laboratory (Bar Harbor, ME). SLO-deficient MRL-lpr/lpr mice were created by repeated backcrossing mice heterozygous for the targeted 5lo gene mutation (39) to MRL-lpr/lpr mice for 12 generations (N12). Mice were screened for the disrupted 5lo allele by Southern blot analysis of EcoRI-digested tail genomic DNA as previously described (39). Heterozygous intercrosses (N6, N10, and N12) produced MRL-lpr/lpr mice homozygous for the 5lo mutation (lpr-5lo-/-) and homozygous 5lo wild-type MRL-lpr/lpr mice (lpr-5lo+/-). Equal numbers of male and female lpr-5lo-/- and lpr-5lo+/- mice were studied, as described below, or were housed indefinitely for long-term survival assessment.

Pharmacologic studies

We used MK-886 (Merck Frosst, Quebec, Canada) (40), an inhibitor of the 5-lipoxygenase-activating protein (FLAP) (41), to examine the effects of pharmacological inhibition of LT biosynthesis on survival of male MRL-lpr/lpr mice. MK-886 (100 mg/kg) or its vehicle (0.1% methylcellulose) was administered by gavage once daily to male lpr-5lo-/- mice (n = 32). Drug administration was begun when the animals were 12 wk old and was continued for 8 wk.

Renal hemodynamic studies

In separate groups of 16-wk-old mice, clearances of inulin and paraaminohippuric acid (PAH) were measured as described previously (28, 29). Briefly, groups of 3 animals were anesthetized with 0.04 mg/g pentobarbital, and a polyethylene catheter (PE-90) was inserted into the right carotid artery and jugular vein to cannulate with polyethylene catheters (PE-10) for i.v. infusions, to monitor mean arterial pressure and to allow intermittent sampling of arterial blood. Following surgery, normal saline (2% body weight) was infused i.v. over 20 min to replace surgical losses. Priming doses of [carboxyl-14C]inulin and [glycyl-3H]PAH were given, followed by infusion of pentobarbital. [14C]Inulin and [3H]PAH in plasma and urine were measured in a liquid scintillation counter (Nuclear Chicago-TM Analytical, Elk Grove, IL), and clearances were calculated using standard formulas. Inulin clearance is used as a measure of glomerular filtration rate (GFR), and PAH clearance estimates renal plasma flow (RPF).

Histopathologic studies

Following the renal hemodynamic studies, the left kidney and the knee joint of the right hindlimb were removed and fixed in 10% buffered formalin. The joints were decalcified, and both tissues were imbedded in paraffin, sectioned, and stained with hematoxylin and eosin. All of the tissues were examined by a pathologist (P.R.) without knowledge of the genotype of the kidney sections. Histologic sections were microscopically examined for the presence and severity of abnormalities in glomeruli, tubules, vessels, and interstitium. Grading was performed using a semiquantitative scale as described previously (20, 28, 29), where 0 was no abnormality and where 1+, 2+, and 3+ represented mild, moderate, and severe abnormalities, respectively. An overall histologic score for each kidney was obtained by summing the grades for glomeruli, tubules, vessels, and interstitium (28, 29).

In the joints, the presence and severity of histopathologic abnormalities were evaluated based upon the following criteria: proliferation of the synovial lining and stroma cells, fibrosis of the synovial membrane, destruction of pannus and cartilage, presence of intra- and extracellular inflammatory infiltrates, and fibrin exudation. Grading was performed using a semiquantitative scale as described previously (42, 43), where 0 was no abnormality, and where 1+, 2+, and 3+ represented mild, moderate, and severe abnormalities, respectively. An overall histologic score was calculated by summing the values for all parameters examined.

The spleen and left axillary lymph node of each mouse were removed, weighed, and prepared for histologic and flow cytometric analyses.

Flow cytometric analysis

Single cell suspensions were prepared from spleens, thymuses, and lymph nodes of lpr-5lo-/- and lpr-5lo+/- mice (n = 5–7 per group). Cells (1–2 × 10⁶/ml) were washed and resuspended in PBS containing 2% FCS and 0.02% sodium azide. Staining was performed in 100-μl volumes at 4°C using predetermined optimal concentrations of Abs. All Abs and isotype controls were purchased from PharmMingen (San Diego, CA). The Abs used were as follows: FITC-labeled RA3-6B2 (anti-CD45R/B220); FITC-labeled 53-6.7 (anti-mouse CD8α); PE-labeled 5H12 (anti-Thy-1.2); PE-labeled RM4-5 (anti-mouse CD4); and FITC- and PE-labeled RM5-95 (anti-rat IgG2a,κ). After final washing, cells were fixed with PBS containing 2% formalin and analyzed within 72 h. At least 1.5 × 10⁶ cells were analyzed for each Ab combination. Analyses were performed on a FACScan (Becton Dickinson, Mountain View, CA) at the Flow Cytometry Facility at Duke University (Durham, NC).

Measurement of autoantibody levels

Anti-DNA Ab levels were determined by ELISA as previously described (43). Calf thymus DNA was purchased from Sigma (St. Louis, MO) and further purified by repeated phenol/chloroform extraction followed by ethanolic precipitation. ssDNA Ag was prepared by boiling the DNA solution for 10 min followed by rapid cooling in an ice bath. dsDNA was obtained by treatment with S1 nuclease (Sigma) to remove single-stranded regions, followed by phenol extraction to remove the contaminating enzymes. Mice were injected intraperitoneally with 5×10⁵ plaque-forming units of ss- or dsDNA Ag at 2 μg/ml in SSC (0.87% sodium chloride, 0.44% sodium citrate, pH 8.0) overnight at 4°C. Plates were washed with PBS, and sera, diluted in PBS containing 1% BSA, and 0.05% Tween 20 (BSA-PBS-T) was added to the wells. After 1 h at room temperature, the plates were washed, and 0.01% 3,3',5,5'-tetramethylbenzidine hydrochloride substrate (Sigma) and 0.01% hydrogen peroxide diluted in 0.1 M sodium citrate buffer (pH 4.0) were added to the wells. After 30 min at room temperature, the OD₅₅₀ was read using an automated plate reader (Molecular Devices, Menlo Park, CA).

Induction and measurement of inflammatory responses in mouse ear tissue

The inside of the left ear of each mouse was painted with 20 μl of AA (100 μg/μl in acetone; Sigma), whereas the right ear was treated with 20 μl of acetone alone. At 1 h after treatment, mice were sacrificed, and an 8-mm-diameter disc of tissue was punched from the center of each ear. For analysis of edema and protein extravasation, animals were injected i.v. with 0.5% Evans blue dye solution (10 ml dye solution/kg body weight) dissolved in PBS (pH 7.5) before AA application. Edema was measured by determining the wet weight of the right ear disc. To extract extravasated Evans blue dye, tissue samples were incubated in 1 ml of formamide at 55°C for 48 h. Dye extravasation was quantified by measuring the absorbance of the formamide extracts at 610 nm with a spectrophotometer (44).
mice that lack a functional 5LO enzyme \(lpr-5lo\) renal disease-modifying loci on chromosomes 7 and 12 (19, 20).

able to use a backcross-breeding strategy because the 5lo mice for twelve consecutive generations. We were

lpr/lpr survival analysis of the life span of male MRL-\(lpr/lpr\) mice. Survival of female 5-lo^{-/-} mice (233 ± 24 days) was not significantly different from that of female 5-lo^{+/+} mice (203 ± 19 days; \(p = 0.330\)). In contrast, the absence of a functional 5lo gene had a significant detrimental effect on survival in male MRL-\(lpr/lpr\) mice as depicted in Fig. 1B. Mean survival time was significantly reduced in male 5-lo^{-/-} mice (177 ± 13 days) compared with the male 5-lo^{+/+} animals (229 ± 15 days; \(p = 0.0010\)). Moreover, the enhanced mortality in 5lo-deficient male MRL-\(lpr/lpr\) mice resulted in a survival curve similar to that of female MRL-\(lpr/lpr\) mice.

Statistical analysis

Data are presented as the mean ± SEM. For the hemodynamic studies, data points for each animal represent the mean of the values measured during at least two clearance periods. Statistical significance for comparisons between groups was determined using an unpaired two-sample \(t\) test. Survival analysis was performed using the SAS software package (SAS Institute, Cary, NC), and the significance of differences in survival between 5-lo^{+/+} and 5-lo^{-/-} groups was determined using Wilcoxon rank sum and Kruskal-Wallis tests.

Results

Survival of 5lo-deficient MRL-\(lpr/lpr\) mice

To examine the role of LTs in the pathogenesis of autoimmune disease in the MRL-\(lpr/lpr\) mouse model, we created MRL-\(lpr/lpr\) mice that lack a functional 5LO enzyme [5-lo^{-/-}]. 5LO catalyzes the conversion of AA to LTA\(_4\), the initial step in the synthesis of LTs. The 5lo-deficient mice were generated previously by gene targeting in embryonic stem cells, and we found that calcium ionophore-stimulated peritoneal macrophages isolated from these mice are unable to produce LTs (39). MRL-\(lpr/lpr\) mice deficient in 5LO were created by repeatedly crossing 5-lo^{+/+} mice to MRL-\(lpr/lpr\) mice for twelve consecutive generations. We were able to use a backcross-breeding strategy because the 5lo gene (Alox5), which has been mapped to mouse chromosome 6 (45), is not linked to the lpr locus on mouse chromosome 19 or to the known renal disease-modifying loci on chromosomes 7 and 12 (19, 20).

Among 5-lo^{+/+} mice, survival of males (229 ± 15 days) exceeded that of females (203 ± 19 days; \(p = 0.149\)) (Fig. 1). This difference in survival between sexes is consistent with previous reports demonstrating that autoimmune disease in this mouse model is more severe in females and, consequently, that female MRL-\(lpr/lpr\) mice die at earlier ages than males (14, 16, 18). As shown in Fig. 1A, the 5lo mutation had no effect on survival in female MRL-\(lpr/lpr\) mice. Survival of female 5-lo^{-/-} mice (233 ± 24 days) was not significantly different from that of female 5-lo^{+/+} mice (203 ± 19 days; \(p = 0.330\)). In contrast, the absence of a functional 5lo gene had a significant detrimental effect on survival in male MRL-\(lpr/lpr\) mice as depicted in Fig. 1B. Mean survival time was significantly reduced in male 5-lo^{-/-} mice (177 ± 13 days) compared with the male 5-lo^{+/+} animals (229 ± 15 days; \(p = 0.0010\)). Moreover, the enhanced mortality in 5lo-deficient male MRL-\(lpr/lpr\) mice resulted in a survival curve similar to that of female MRL-\(lpr/lpr\) mice.

Survival of male MRL-\(lpr/lpr\) mice treated with a LT inhibitor

To confirm that the decreased survival of male 5-lo^{-/-} mice was due to reduced LT production, we examined the survival of male 5-lo^{-/-} mice that were treated with the FLAP inhibitor, MK-886 (40), a potent inhibitor of LT synthesis (Fig. 2). Between 12 and 20 wk of age, none of the vehicle-treated mice died. In contrast, the mortality rate was 50% by 19 wk of age in the group that received MK-886. At the end of the 8-wk treatment period, 8 of 16 MK-886-treated mice had died, whereas all of the vehicle-treated mice survived (\(p = 0.005\)). These data suggest that reduced LT production is detrimental to survival in male MRL-\(lpr/lpr\) mice, whether inhibition of 5LO is accomplished pharmacologically or genetically.

Renal function in 5lo-deficient MRL-\(lpr/lpr\) mice

Immune complex-mediated glomerulonephritis is considered to be the major cause of death in MRL-\(lpr/lpr\) mice. To determine the effect of the 5lo mutation on kidney function, we measured GFR and RPF in both male and female 16-wk-old 5-lo^{+/+} and 5-lo^{-/-} mice. Because none of the experimental groups exhibited significant mortality before 16 wk of age, we chose to measure renal function and to assess kidney histology in mice at this age to avoid selection biases related to differences in mortality between groups.

GFR, measured by inulin clearance, was modestly reduced in male and female 5-lo^{+/+} mice (8.6 ± 1.1 and 9.2 ± 1.9 ml/min/kg, respectively) compared with the normal range for congenic MRL/MpJ (MRL +/-) mice (10–12 ml/min/kg) (29). MRL +/- mice lack the lpr mutation and develop a late onset,
mild form of autoimmune disease (16). As shown in Fig. 3A, the 5lo mutation had no effect on GFR in either the males (7.2 ± 0.8 ml/min/kg) or the females (7.3 ± 1.5 ml/min/kg), and there were no significant differences in GFR between the experimental groups. In contrast, as depicted in Fig. 3B, RPF was higher in male lpr-5lo+/+ mice (15.3 ± 2.8 ml/min/kg) compared with female lpr-5lo+/+ mice (8.1 ± 1.6 ml/min/kg; p = 0.0247), reflecting the lesser severity of autoimmune disease in male vs female MRL-lpr/lpr mice. However, RPF was significantly reduced in male lpr-5lo−/− mice (7.2 ± 1.4 ml/min/kg) compared with male lpr-5lo+/+ mice (p = 0.0146). Thus, the absence of a functional 5lo gene had a significant detrimental effect on RPF in male MRL-lpr/lpr mice. Moreover, RPF in male lpr-5lo−/− mice was not significantly different from that of female MRL-lpr/lpr animals. In contrast, the 5lo mutation had no effect on RPF in female MRL-lpr/lpr mice, and RPF was virtually identical in female lpr-5lo+/+ and lpr-5lo−/− mice (8.1 ± 1.6 and 7.8 ± 1.5 ml/min/kg, respectively; p = 0.452).

Renal histopathology in 5LO-deficient MRL-lpr/lpr mice

Along with the deterioration of renal function, the severity of renal histopathology also increases with age in MRL-lpr/lpr mice. MRL-lpr/lpr animals typically manifest a proliferative immune complex-mediated glomerulonephritis with pathologic features including scattered crescent formation, basement membrane thickening, perivascular and interstitial inflammation and focal areas of interstitial fibrosis and glomerulosclerosis (14, 16, 17, 28).

To determine whether the loss of 5LO affected the development and progression of glomerulonephritis in MRL-lpr/lpr mice, histopathologic analysis was performed on the kidneys of 16-wk-old lpr-5lo+/+ and lpr-5lo−/− mice. These results are summarized in Table I. Total scores were slightly higher for kidneys from female lpr-5lo+/+ mice compared with the male lpr-5lo+/+ animals, consistent with the more aggressive nature of autoimmune disease in females in this mouse model (14, 16, 18). As shown in Table I, the loss of 5LO had virtually no effect on the severity of histopathologic renal abnormalities. The individual scores for abnormalities in the glomeruli and interstitium, as well as the total scores, were similar between lpr-5lo+/+ and lpr-5lo−/− males and females (p > 0.05). The pattern and accumulation of polymorphonuclear leukocytes in the glomeruli was also similar in males and females of both 5lo genotypes. Tubules of the kidneys from both lpr-5lo+/+ and lpr-5lo−/− males and females were essentially normal. These data indicate that the 5lo mutation had little impact on the histopathologic manifestations of glomerulonephritis in MRL-lpr/lpr mice.

Lymphoproliferation in 5LO-deficient MRL-lpr/lpr mice

We also examined whether the 5lo mutation affected other components of autoimmune disease in MRL-lpr/lpr mice. One of the predominant features of autoimmune disease in the MRL-lpr/lpr strain is massive accumulation of lymphocytes in peripheral lymphoid organs. To determine whether 5LO deficiency affected lymphoid hyperplasia in MRL-lpr/lpr mice, left axillary lymph nodes and spleens of 16-wk-old lpr-5lo+/+ and lpr-5lo−/− mice were removed and weighed. These data are summarized in Table II. All MRL-lpr/lpr mice in our colony displayed some degree of lymphoid hyperplasia. The average lymph node weights for lpr-5lo+/+ and lpr-5lo−/− males and females were similar (p > 0.05), and

| Table I. Histopathologic scores of kidneys from lpr-5lo+/+ and lpr-5lo−/− mice |
|------------------------|------------------------|------------------------|------------------------|------------------------|
| Group                  | Glomeruli              | Interstitium           | Tubules                | Total                  |
| lpr-5lo+/+             | Male 5.6 ± 1.6         | 2.1 ± 0.5              | 0.1 ± 0.1              | 7.9 ± 2.0              |
| lpr-5lo−/−             | Male 4.9 ± 1.5         | 2.9 ± 0.7              | 0.3 ± 0.3              | 8.0 ± 2.1              |
| lpr-5lo+/+             | Female 7.7 ± 2.1       | 4.1 ± 0.7              | 0.6 ± 0.6              | 12.4 ± 3.3             |
| lpr-5lo−/−             | Female 8.8 ± 2.2       | 3.8 ± 0.7              | 0.8 ± 0.5              | 13.3 ± 3.2             |

* Sixteen-week-old lpr-5lo+/+ and lpr-5lo−/− mice (n = 6–8 per group) were euthanized, and formalin-fixed kidney tissue was sectioned and stained with hematoxylin and eosin. Slides were scored for renal injury in a blinded manner. Glomerular scores represent the sum of scores for eight criteria: crescent formation, epithelial cell reactivity, fibrosis, focal hypercellularity, focal segmental mesangial expansion, hyperlobulation, necrosis, and thickened membranes. Interstitium scores represent the sum of scores for three criteria: chronic inflammation, fibrosis, and focal hypercellularity. Scores are based on the following scale: 0 = no abnormality; 1 = mild; 2 = moderate; 3 = moderate-severe; and 4 = severe. Values are means ± SEM.

* The total histopathologic score for each group was obtained by summing the overall grades for the glomeruli, interstitium, and tubules.
splenic enlargement was also evident to a similar degree in all groups of animals \((p > 0.05)\).

In MRL-\(lpr/lpr\) mice, the predominant cell type that accumulates in the lymphoid tissues is thymus-derived DN (CD4+CD8+CD3+) T cells (17). These DN T cells also express B220 (CD45R), a cell surface marker that is usually restricted to B lymphocytes (17). Although the 5lo gene is expressed primarily in cells of myeloid lineage, LTs have been reported to affect the production of cytokines involved in lymphoid activation and function (11–13). Therefore, we evaluated the effects of the 5lo mutation on cell populations in the expanded lymph nodes, spleens, and thymuses of 5lo+/+ and 5lo−/− mice. Two color FACS analysis confirmed that the predominant phenotype of cells from all groups of mice was B220+CD4+CD8− (data not shown). Also, there were no differences in the cellular composition of lymphoid tissues between 5lo+/+ and 5lo−/− mice, suggesting that the absence of LTs does not affect the development or expansion of these populations.

Autoantibody production in SLO-deficient MRL-\(lpr/lpr\) mice

Autoimmune disease in the MRL-\(lpr/lpr\) mouse strain is also associated with increased production of a variety of autoantibodies, including those directed against nucleic acids (14, 16, 17). Therefore, to further investigate a possible mechanism for the increased mortality of SLO-deficient MRL-\(lpr/lpr\) mice, we determined the effect of SLO-deficiency on autoantibody activity by measuring levels of IgG anti-ssDNA and anti-dsDNA Abs in serum from 5lo+/+ and 5lo−/− mice. As shown in Fig. 4A, serum levels of Abs to ssDNA did not differ between 5lo+/+ males and females \((p > 0.05)\). We also found that the loss of SLO did not alter the production of anti-ssDNA Abs in MRL-\(lpr/lpr\) animals (Fig. 4A; \(p > 0.05\)). However, anti-ssDNA Ab activity was slightly higher for 5lo−/− females compared with 5lo−/− males at all of the serum dilutions tested (Fig. 4A; \(p < 0.05\)). As can be seen in Fig. 4B, no significant differences in the production of Abs reactive with dsDNA were detected between the experimental groups \((p > 0.05)\).

Arthritis in SLO-deficient MRL-\(lpr/lpr\) mice

Another feature of autoimmune disease in MRL-\(lpr/lpr\) mice is the spontaneous development of arthritis, which has features similar to those of rheumatoid arthritis in humans (15, 16). Initially, this arthritis is characterized by proliferation of the synovial lining cells, followed by infiltration of the synovium by lymphocytes, plasma cells, histiocytes, neutrophils, and eosinophils. The most pronounced changes are typically observed in the hindpaw, forepaw, and knee joints. Pannus occurs with low frequency and is associated only with minor destruction of the cartilage (15, 16).

To determine whether the loss of SLO affects the severity of arthritis in this mouse model, histopathologic evaluation of knee joints from 16-wk-old wild-type and SLO-deficient MRL-\(lpr/lpr\) mice was performed. These results are summarized in Table III. The overall histologic scores for the knee joints of 5lo+/+ and 5lo−/− mice were low, indicating a mild, early stage of joint disease. The mean scores were slightly higher in females than in males of both 5lo genotypes, suggesting that the arthritis may be more severe in female than male MRL-\(lpr/lpr\) mice. However, these sex differences were not significant \((p > 0.05)\). Although the absence of SLO had no effect on the aggregate severity of arthritic lesions in male or female MRL-\(lpr/lpr\) mice, there were significant differences in the prevalence of joint pathology. Only three of eight (38%) 5lo+/+ males had significant joint pathology, including histological abnormalities in the synovium and/or evidence of sub-synovial or tendon/muscle inflammation, compared with seven of seven (100%) 5lo−/− females at 16 wk of age \((p = 0.0376)\).

In contrast, histological abnormalities were also present in the joints of all of the 5lo−/− males examined \((7 of 7)\). The difference in the prevalence of arthritis between 5lo+/+ and 5lo−/− males was significant \((p = 0.0376)\). Moreover, the prevalence of arthritis in the 5lo−/− males was not different from that of 5lo−/− \((7 of 8 or 87.5%; p = 0.626)\) and 5lo+/+ \((100%)\).

Acute inflammatory responses in male and female MRL-\(lpr/lpr\) mice

To evaluate whether there might be significant differences in other inflammatory responses between male and female MRL-\(lpr/lpr\)

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### Table II. Lymphadenopathy and splenomegaly in 1pr-5lo+/+ and 1pr-5lo−/− mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Node Weight (mg)</th>
<th>Spleen Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1pr-5lo+/+</td>
<td>Male 286 ± 46</td>
<td>506 ± 120</td>
</tr>
<tr>
<td>1pr-5lo−/−</td>
<td>Male 294 ± 22</td>
<td>376 ± 18</td>
</tr>
<tr>
<td>1pr-5lo+/+</td>
<td>Female 200 ± 36</td>
<td>424 ± 56</td>
</tr>
<tr>
<td>1pr-5lo−/−</td>
<td>Female 286 ± 36</td>
<td>503 ± 29</td>
</tr>
</tbody>
</table>

*Sixteen-week-old 1pr-5lo+/+ and 1pr-5lo−/− mice \((n = 7–8 per group)\) were euthanized, and the spleen and left axillary lymph node from each mouse were removed and weighed. Values are mean ± SEM.*
mice, we examined ear inflammation in mice treated with AA. This response is highly dependent on 5LO products (39, 46). As can be seen in Fig. 5, edema and vasopermeability, as measured by alterations in ear weight and dye extravasation, respectively, were significantly higher in male lpr-5lo+/+ mice compared with female lpr-5lo+/+ mice (p = 0.0037 and 0.0130, respectively), suggesting that male MRL-lpr/lpr mice are more responsive than females to this inflammatory stimulus. AA-induced changes in ear weight and protein extravasation were significantly reduced in male lpr-5lo−/− mice compared with male lpr-5lo+/+ mice (p = 0.0317 and 0.0368, respectively). In contrast, the 5lo mutation had very little impact on edema in female MRL-lpr/lpr mice, as measured by changes in the weight of ear tissue biopsies (Fig. 5A, p = 0.191). Moreover, only a slight decrease in serum protein extravasation, as determined by the amount of Evans blue dye in the ear tissue, was observed in female lpr-5lo−/− mice compared with lpr-5lo+/+ females (Fig. 5B, p = 0.0005).

**Discussion**

In this study, we examined the role of LTs in the pathogenesis of autoimmune disease in the MRL-lpr/lpr mouse strain by generating MRL-lpr/lpr mice that are genetically deficient in the 5LO enzyme. Previous reports have suggested that LTs are involved in the disease, particularly the glomerulonephritis, of the MRL-lpr/lpr mouse model (28, 30). Based on these findings and on the well-characterized proinflammatory activities of LTs, we expected that the 5lo mutation might affect the autoimmune disease of the MRL-lpr/lpr mice by slowing the onset and/or lessening the severity of the disease. However, we have shown here that the absence of a functional gene for 5LO results in a marked decrease in the survival of MRL-lpr/lpr mice, particularly males, whereas other manifestations of the autoimmune disease remain unchanged.

In the MRL-lpr/lpr mouse strain, the autoimmune disease in females is more severe, and females succumb to the autoimmune disease earlier than males (14, 16, 18). A similar sex-related expression of disease is known to occur in several other animal models of autoimmune disease (14, 16, 18), as well as in humans with SLE (23–25). Studies have established that the underlying basis for this sex-related susceptibility, in both the human and animal models, can be related to specific sex hormones (16, 23, 24). For example, administration of estrogen to MRL-lpr/lpr mice increases mortality, lymphoproliferation, antihistone activity, autoantibody formation, and the severity of glomerulonephritis (47–49). In contrast, androgen therapy of MRL-lpr/lpr mice leads to reduced autoantibody levels, improved renal function, and prolonged survival, without significant effects on lymphoproliferation (50, 51). Taken together, these studies indicate that male sex hormones consistently had protective effects, whereas, in general, estrogen treatment accelerated the disease in the MRL-lpr/lpr mouse model. However, the mechanisms of action of sex hormones in these settings are not precisely understood.

The present study identifies another difference between male and female MRL-lpr/lpr mice: the role of 5LO in the pathogenesis

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**Table III. Histopathologic scores of knee joints from lpr-5lo+/+ and lpr-5lo−/− mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>Synovial Surface</th>
<th>Subsynovial Inflammation</th>
<th>Tendon/Muscle Inflammation</th>
<th>Totala</th>
</tr>
</thead>
<tbody>
<tr>
<td>lpr-5lo+/+</td>
<td>Male</td>
<td>0.25 ± 0.2</td>
<td>0.44 ± 0.3</td>
<td>0.25 ± 0.3</td>
</tr>
<tr>
<td>lpr-5lo−/−</td>
<td>Male</td>
<td>0.79 ± 0.4</td>
<td>0.93 ± 0.3</td>
<td>0.14 ± 0.1</td>
</tr>
<tr>
<td>lpr-5lo+/+</td>
<td>Female</td>
<td>1.29 ± 0.4</td>
<td>1.21 ± 0.2</td>
<td>0.36 ± 0.2</td>
</tr>
<tr>
<td>lpr-5lo−/−</td>
<td>Female</td>
<td>1.00 ± 0.3</td>
<td>1.00 ± 0.3</td>
<td>0.25 ± 0.2</td>
</tr>
</tbody>
</table>

*a Sixteen-week-old lpr-5lo+/+ and lpr-5lo−/− mice (n = 7–10 per group) were euthanized, and formalin-fixed knee joints were decalcified, sectioned, and stained with hematoxylin and eosin. Histopathologic joint abnormalities were blindly graded according to the following scale: 0 = no abnormality; 1 = mild; 2 = moderate; and 3 = severe. Values are means ± SEM.

a An overall histopathologic score for each group was obtained by summing the grades for the synovium, subsynovium, and tendon and muscle.

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**FIGURE 5.** AA-induced edema and serum protein extravasation. Before the application of 2 mg of AA in acetone to the left ear and acetone alone to the right ear, mice received an i.v. injection of 0.5% Evans blue dye solution. After 1 h, mice were sacrificed, 8-mm-diameter discs were taken from each ear, and the difference in weight between the right ear and left ear (A). The extravasation of dye into the tissue was then quantitated by extraction of dye with formamide and measurement of absorbance at a wavelength of 610 nm (ΔA610). For each animal the ΔA610 obtained for the right ear was subtracted from that obtained for the left ear (B). Results are representative of two experiments. Error bars indicate SEM. *, p < 0.05 vs lpr-5lo+/+ males and **, p < 0.01 vs lpr-5lo−/− females. lpr-5lo+/+ males, n = 11; lpr-5lo−/− males, n = 12; lpr-5lo+/+ females, n = 9; lpr-5lo−/− females, n = 9.
of disease. We found that functional SLO exerts a significant beneficial effect on survival only in male MRL-lpr/lpr mice. When 5LO function is inhibited, genetically or pharmacologically, this sex-related survival advantage is lost. One potential explanation for this difference is that the metabolism or actions of LTs may vary between male and female MRL-lpr/lpr mice. To test this possibility, we examined the responses of male and female MRL-lpr/lpr mice to an acute inflammatory stimulus that is known to be highly dependent on LTs. We show that AA-induced inflammation is markedly enhanced in lpr-5lo−/− males compared with females, as measured by increases in ear weight and serum protein extravasation. Furthermore, AA-induced ear inflammation is significantly reduced in 5LO-deficient MRL-lpr/lpr males compared with wild-type MRL-lpr/lpr males, but the 5lo mutation had very little impact on the low level of AA-stimulated inflammation in female MRL-lpr/lpr mice. These data demonstrate significant differences in the contribution of 5LO metabolites to inflammation in male vs female MRL-lpr/lpr mice. However, it is not clear from these studies whether this difference may be due to enhanced synthesis of 5LO products or increased sensitivity to 5LO metabolites in males. Nonetheless, we suggest that the amplified responses in male mice are responsible for the differential effects of the 5lo mutation in males compared with female MRL-lpr/lpr mice.

There are several potential explanations for the detrimental effects of the loss of a functional 5lo gene on the autoimmune disease process in MRL-lpr/lpr mice. For example, the loss of 5LO may enhance the synthesis and activities of other eicosanoids, particularly the cyclooxygenase products of AA metabolism, which may enhance the synthesis and activities of other eicosanoids, particularly the cyclooxygenase products of AA metabolism, which can adversely contribute to end organ injury. We have previously considered the possibility that the reduced survival of lpr/lpr males may be attributed, in part, to more severe renal dysfunction and/or pathology. In support of this hypothesis, we found that RPF was reduced in male lpr-5lo−/− mice to levels similar to those of female lpr-5lo+/+ mice. Despite this 50% reduction in renal plasma flow, renal histopathology was similar in male lpr-5lo−/− mice compared with male lpr-5lo+/+ animals. These data suggest that the difference in renal function between the groups may be related to hemodynamic changes, rather than marked alterations in renal structure. We speculate that the detrimental effects of the 5lo mutation on renal function in male MRL-lpr/lpr mice may contribute to the enhanced early mortality that is seen in that group. Consistent with these findings, we found that the prevalence of arthritis in male lpr-5lo−/− mice was significantly higher than that in lpr-5lo+/+ males and was similar to that in both female groups. However, the overall severity of arthritis was relatively mild and not different between the groups, and we found no differences in other features of the autoimmune disease, including lymphadenopathy, splenomegaly, and autoantibody activity.

In summary, this study has shown that inhibition of 5LO abrogates the survival advantage seen in male MRL-lpr/lpr mice. These findings may be related to enhanced 5LO-associated inflammatory responses in male vs female MRL-lpr/lpr mice. Further study is needed to determine the extent to which sex-related differential effects of LTs and other inflammatory mediators may affect the course of SLE and other autoimmune diseases.

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References


