Ocular Inflammation in Autoimmune MRL/Mp Mice

Douglas A. Jobs,*† Elaine L. Alexander,‡ and W. Richard Green*

Congenic mice of the MRL/Mp strain spontaneously develop an autoimmune connective tissue disease that shares immunologic and histopathologic features with the human disorders systemic lupus erythematosus, rheumatoid arthritis, and systemic vasculitis. The autoimmune disorder in these mice is markedly accelerated by the recessive gene lpr. Older MRL/Mp-lpr/lpr mice develop significant inflammatory ocular disease, including choroiditis, scleritis, and orbital vasculitis. Animals of both the MRL/Mp-+/+ and MRL/Mp-lpr/lpr substrains develop lacrimal gland inflammatory infiltrates. The MRL/Mp mouse provides a potential model for ocular inflammatory disease and for Sjögren’s syndrome. Invest Ophthalmol Vis Sci 26:1223-1229, 1985

A variety of murine models of human autoimmune disease, including systemic lupus erythematosus (SLE), have been described. These models include the New Zealand Black (NZB) mouse, the New Zealand Black/New Zealand White (NZB/NZW) F1 hybrid, and more recently the MRL/Mp mouse.1-3 The MRL mouse was initially described in 1977 and first bred as two lines, the MRL/n and MRL/l. Further breeding has led to the development of two substrains, the MRL/Mp-+/+ and MRL/Mp-lpr/lpr. The lpr gene appears to represent a single autosomal recessive mutation, which leads to massive lymphoproliferation and markedly accelerates the autoimmune disease in the MRL mouse. Both substrains develop autoantibodies and autoimmune disease; but the MRL/Mp-+/+ mouse fails to develop lymphadenopathy and has a mean life span of approximately 2 yr, while the lifespan of the MRL/Mp-lpr/lpr mouse is approximately 6 mo. In addition, 15 to 25% of the MRL/Mp-lpr/lpr mice develop a rheumatoid factor-positive synovitis; and older MRL/Mp-lpr/lpr mice demonstrate an acute polyarteritis with a necrotizing cellular response. Thus, the MRL/Mp-lpr/lpr mouse, initially proposed as a model for SLE, has also been proposed as a possible model for rheumatoid arthritis and for vasculitis.1-3

Previous investigators have suggested that a Sjögren’s-like picture develops in the NZB and NZW mouse.4 More recent investigations have suggested that the MRL/Mp mouse may also be a model for Sjögren’s syndrome.5,6 We have noted the presence of chronic sialadenitis in greater than 90% of aged MRL/Mp-+/+ and MRL/Mp-lpr/lpr mice (Alexander et al, unpublished observations). Because of these data, we undertook an investigation of the ophthalmic abnormalities present in the MRL/Mp mice of both substrains. In this communication, we demonstrate histopathologically that both the MRL/Mp-+/+ and MRL/Mp-lpr/lpr mice develop a mononuclear inflammatory cell infiltrate in the lacrimal gland, and that older MRL/Mp-lpr/lpr mice develop inflammatory infiltrates in the choroid, sclera, and orbit.

Materials and Methods

Animals

All strains of the mice studied were initially obtained from the Jackson Laboratories at Bar Harbor, Maine. Colonies of the MRL/Mp-+/+ and MRL/Mp-lpr/lpr mice have been established at the Johns Hopkins Medical Institutions. Animals were fed a diet of Agway Pro Lab RHM (Agway, Inc.; Syracuse, NY) 1,000 and maintained as previously described.7 Animals were grouped by approximate age and strain. For the histologic studies, MRL/Mp-+/+ mice were examined at either 6 mo 1 yr of age. The 6-mo group consisted of nine animals aged 24 to 27 wk (mean, 26 wk); and the 1-yr group, 10 animals all aged 59 wk. The MRL/Mp-lpr/lpr mice, initially proposed as a model for SLE, have also been proposed as a possible model for rheumatoid arthritis and for vasculitis.1-3

From the Wilmer Ophthalmological Institute, the Department of Ophthalmology,* and the Divisions of Clinical Immunology† and Rheumatology,‡ Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland.

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Reprint requests: Douglas A. Jabs, MD, Wilmer 300, Wilmer Ophthalmological Institute, The Johns Hopkins Hospital, 600 N. Wolfe Street, Baltimore, MD 21205.
4% formaldehyde and imbedded in paraffin. Six to eight micron thick sections were prepared and stained with hematoxylin and eosin, the periodic acid-Schiff (PAS) reaction, and the von Kossa technique. A tissue was judged to be abnormal. In four MRL/Mp-+/+ animals, only ocular and Harderian gland tissues were obtained.

Additional Schirmer tests were performed on 10 1-year-old MRL/Mp-+/+ and nine 6-month-old MRL/Mp-+/+ animals. The 1-year-old MRL/Mp-+/+ group consisted of five male and five female mice aged 56–65 wk (mean, 60 wk); and the MRL/Mp-+/+ group consisted of five female and four male mice aged 19–32 wk (mean, 24 wk). Controls consisted of four male and four female C3H/HeJ mice aged 35–38 wk (mean, 36 wk). These investigations conformed to the ARVO Resolution on the Use of Animals in Research.

<table>
<thead>
<tr>
<th>Group</th>
<th>6 mo</th>
<th>1 yr</th>
<th>4–5 mo</th>
<th>6–7 mo</th>
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<tr>
<td>Number</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Male/female</td>
<td>4/5</td>
<td>5/5</td>
<td>4/7</td>
<td>10/5</td>
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<tr>
<td>Band</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Keratopathy</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Vascularization</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3 (20%)</td>
</tr>
<tr>
<td>Choroiditis</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6 (40%)</td>
</tr>
<tr>
<td>EOM myositis</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Orbital vasculitis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Optic perineuritis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Other</td>
<td>panniculitis (1)</td>
<td>endophthalmitis (2)</td>
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<td></td>
</tr>
</tbody>
</table>

Histopathology

 Appropriately aged animals were randomly selected from available stocks. Animals were anesthetized with methoxyflurane and killed by either exsanguination or cervical dislocation. Bilateral orbital exenterations were performed. Tissues were fixed immediately in 4% formaldehyde and imbedded in paraffin. Six to eight micron thick sections were prepared and stained with hematoxylin and eosin, the periodic acid-Schiff (PAS) reaction, and the von Kossa technique. A minimum of 20 to 30 sections per eye and per gland was studied.

Lacrimal and Harderian gland inflammatory cell infiltration was scored using a modified focus score scale. In this system, the intensity of mononuclear cell infiltration was scored as 0 to IV based upon the number of foci of 50 or more inflammatory cells per gland per section: 0 = no inflammation; I = any inflammation, but no foci; II = at least one focus of 50 mononuclear cells per gland; III = two or more foci per gland; and IV = grade III plus glandular destruction, extensive replacement of glandular tissue by mononuclear cells, or fibrosis. All sections were read independently by two observers (DAJ and WRG); one observer (WRG) was masked as to the specimen source. Agreement between both observers was excellent, and the scale score was found to be highly reproducible. Because all lacrimal glands from control animals had an inflammatory cell infiltrate of grade I or less, a score of grade II or greater was selected as being abnormal for the experimental groups. Similarly, because no control animal had inflammation in the Harderian gland, a score of grade I or greater for the Harderian glands in the experimental groups was judged to be abnormal. In four MRL/Mp-+/+ animals, only ocular and Harderian gland tissues were obtained.

Schirmer Tests

Mice were anesthetized with methoxyflurane. A 0.5 X 3.0 mm strip of Whatman #1 filter paper was then inserted under the lower lid near the medial canthus. After 2 min the strip of filter paper was removed, and the amount of wetting was marked. The length of wetting was then measured using a micrometer and a dissecting microscope. Schirmer tests were measured on both eyes of all animals.

Statistical analysis of differences between the experimental groups was performed using Student’s t-test for unpaired observations.

Results

Ocular Histopathology

The ocular histopathologic features are recorded in Table 1. Band keratopathy was found in one (5%) MRL/Mp-+/+ animal and four (15%) MRL/Mp-lpr/lpr animals. Of these, all but one animal had concomitant corneal vascularization. One additional MRL/Mp-+/+ animal had corneal vascularization without band keratopathy. A mononuclear inflammatory cell infiltrate was found in the choroid of three (20%) of the older MRL/Mp-lpr/lpr animals (Fig. 1) but not in the younger, 4- to 5-month-old, MRL/Mp-lpr/lpr animals. No choroidal infiltrates were found in any of the MRL/Mp-+/+ animals examined. Six (40%) of the older and one of the younger MRL/Mp-lpr/lpr animals had a mononuclear inflammatory cell infiltrate in the posterior sclera (Fig. 2). This infiltrate was most often perivascular and was often located near the junction of the sclera and optic nerve. One of the older MRL/Mp-lpr/lpr mice had an anterior scleritis. Inflammatory orbital lesions were present in older animals of both substrains, particularly the 6- to 7-month-old MRL/Mp-lpr/lpr mice. Myositis of the extraocular muscles was seen in two MRL/Mp-lpr/lpr mice and one older MRL/Mp-+/+ animal (Fig. 3). Perineural mononuclear inflammatory cell infiltrates located between the nerve bundles and dura were present in two older
MRL/Mp-\textit{Ipr}/\textit{Ipr} animals, and orbital vasculitis in two older MRL/Mp-\textit{Ipr}/\textit{Ipr} mice (Fig. 4). Additionally, two older MRL/Mp-\textit{Ipr}/\textit{Ipr} mice were found to have an acute monocular endophthalmitis.

All control animals had entirely normal ocular histologic findings bilaterally.

**Lacrimal Gland Histopathology**

The results of the lacrimal gland histopathologic examinations are recorded in Fig. 5. Extensive lacrimal gland inflammation was found in both MRL substrains regardless of age, with 75–100% of each group

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**Fig. 1.** Choroiditis in a 26-week-old MRL/Mp-\textit{Ipr}/\textit{Ipr} mouse (hematoxylin and eosin, \textit{x}350).

**Fig. 2.** Posterior scleral inflammation in a 25-week-old MRL/Mp-\textit{Ipr}/\textit{Ipr} mouse (hematoxylin and eosin, \textit{x}310).
having grade II or greater inflammatory changes. The majority had grade III or IV changes (Fig. 6). Within the MRL/Mp~lpr/lpr substrain, there was a significant difference between male and female mice in the intensity of lacrimal gland inflammation. The mean grade for the female mice was 3.6, whereas that for the male mice was 2.1 (P < 0.0005). In the younger MRL/Mp~+/+ group, the mean grade of inflamma-

Fig. 3. Myositis of the extraocular muscles in a 40-week-old MRL/Mp~lpr/lpr mouse (hematoxylin and eosin, ×225).

Fig. 4. Orbital vasculitis in a 29-week-old MRL/Mp~lpr/lpr mouse (hematoxylin and eosin, ×285).
tion for the female mice was 3.6 versus 1.8 for the males, but the number of animals studied (nine) was small.

Of the 10 control animals, eight had no inflammation, and two had only grade I changes.

**Harderian Gland Histopathology**

The results of the Harderian gland histopathologic examinations are recorded in Fig. 7. The majority of younger MRL/Mp-+/+ mice and MRL/Mp-lpr/lpr mice had normal Harderian glands; eight of nine 6-mo MRL/Mp-+/+ mice and 10 of 11 4–5-mo MRL/Mp-lpr/lpr mice had grade 0 findings. Mild inflammatory infiltrates were found in the majority of 1-year-old MRL/Mp-+/+ mice (eight of 10 (80%) grade I or greater) and in the majority of 6- to 7-month-old MRL/Mp-lpr/lpr mice (nine of 15 (60%) grade I or greater).
No control animal had inflammation of the Harderian glands.

Schirmer Test Results

The number of eyes, mean amount of wetting in millimeters, and standard error of the mean for each of the three groups were as follows: MRL/Mp-+/+(n = 20), 3.3 ± 0.3; MRL/Mp-lpr/lpr(n = 18), 2.5 ± 0.2; and controls(n = 16), 2.5 ± 0.2. There were no significant differences between the results in either MRL/Mp substrain and the control animals.

Discussion

The most striking findings were the presence of a posterior scleral mononuclear inflammatory cell infiltrate (scleritis) in the eyes of 40% of aged MRL/Mp-lpr/lpr mice and vasculitis and related inflammatory infiltrates in the orbital tissues of these animals. To the best of our knowledge, scleritis, orbital vasculitis, and extraocular muscle myositis have not been described previously in this or any other murine model of autoimmune disease. Choroiditis, seen in 20% of our older MRL/Mp-lpr/lpr mice, has been described in up to 35% of MRL/l mice. The findings of a posterior perivascular "scleritis," choroidal infiltrate, and orbital vasculitis are reminiscent of those lesions seen in patients with a systemic vasculitis, such as Wegener's granulomatosis or polyarteritis nodosa. Indeed, MRL/Mp mice of both substrains develop a systemic vasculitis, which is accelerated by the presence of the lpr gene.5 Thus, MRL/Mp mice may represent a model for the ocular complications of systemic vasculitis. Furthermore, because of the difficulties in completely examining orbital tissue, our findings of orbital vasculitis and extraocular muscle myositis represent a minimum estimate of the prevalence of these abnormalities in MRL/Mp-lpr/lpr mice.

Previous investigations have suggested that a Sjögren's-like picture may develop in some strains of "autoimmune" mice.4,6 In 1971, Kessler et al found that both NZB and NZB/NZW F1 hybrid mice develop perivascular and periductal mononuclear cell infiltrates in the lacrimal gland.4 More recently, Hoffman et al have published their analysis of a variety of mouse strains including MRL/l and MRL/n substrains, NZB and NZB/NZW strains, and the Palmerston-North (PN) strain.5,11 These investigators documented decreased Schirmer test results in the NZB/NZW and PN strain compared to the control strains, but normal results in both 6-month-old MRL/l and 9-month-old MRL/n substrains. Nevertheless, they found inflammatory infiltrates in the lacrimal gland in 100% of MRL/l and 95% of MRL/n mice. While lacrimal gland inflammation was found in 85% of NZB, 57% of NZB/NZW and 75% of PN mice, the inflammation in these animals was less severe than that in MRL mice. Kessler et al4 observed corneal changes in seven of ten NZB/NZW mice including corneal epithelial irregularity, desquamation, and basal cell death. They attributed these changes to the effects of decreased tearing. Hoffman et al11 observed conjunctival mononuclear inflammatory cell infiltrates in MRL, NZB/NZW, and PN mice but did not find corneal or conjunctival epithelial disruption or keratinization.
We found significant lacrimal gland inflammation in both substrains of MRL/Mp mouse (89% of MRL/Mp-+/+ mice and 86% of MRL/Mp-lpr/lpr mice). Furthermore, we found lacrimal gland inflammation in 77% of MRL/Mp-+/+ mice even at 6 mo of age, an age at which these animals have been felt not to have significant autoimmune disease. Although we found band keratopathy and corneal stromal vascularization in a small number of animals, we generally did not find corneal disruption or keratinization. This absence of corneal changes is consistent with the normal results in Schirmer testing found by ourselves and Hoffman et al. The short life span of the MRL/Mp-lpr/lpr mouse may explain this apparent normal functional abnormalities as well as histopathologic changes.

In the present investigation, band keratopathy was present histopathologically in only one MRL/Mp-+/+ mouse (5%) and four MRL/Mp-lpr/lpr mice (15%). Furthermore, in all but one case this was associated with corneal vascularization, suggesting that the band keratopathy might be secondary to local inflammatory corneal abnormalities. In contrast to the present study, Hoffman et al10 have described band keratopathy in a significant percentage of MRL/l and MRL/n mice. By biomicroscopy, these investigators detected corneal deposits in 86% of MRL/l and 89% of MRL/n mice. Histologically, however, they found band keratopathy in only 30% of MRL/l and 71% of MRL/n mice; the differences between the clinical and histologic prevalences were unexplained. Hoffman et al11 proposed that the band keratopathy was secondary to hypercalcemia associated with hyperparathyroidism. While we have also found mild elevations of serum calcium in our animals, the differences are small and average less than one milligram per deciliter over those levels found in control mice (Alexander et al, personal communication). Thus, we believe that the band keratopathy seen in a few of our mice is due to local corneal inflammation rather than to hypercalcemia.

The Harderian gland is an orbital glandular structure which secretes an oily component of the tears in mice. Inflammatory infiltrates were present in the majority (80%) of 1-year-old MRL/Mp-+/+ mice and aged MRL/Mp-lpr/lpr mice (60%); these findings are consistent with the inflammatory process present in other orbital structures of these mice. To the best of our knowledge, Harderian gland histopathology has not been previously described in these animals.

In conclusion, the MRL/Mp-lpr/lpr mouse develops scleritis, choroiditis, and an orbital vasculitis and thus provides a murine model for the development of inflammatory ocular disorders. Furthermore, both substrains of the MRL/Mp mouse develop lacrimal gland inflammation which, coupled with the findings of sialedenitis present in aged animals of both substrains, suggests that the animal may provide a model for Sjögren’s syndrome. Further investigations of this experimental model may provide information on the immunopathogenesis of inflammatory ocular disease.

Key words: MRL/Mp mouse, Sjögren’s syndrome, scleritis, uveitis, vasculitis

References


