Cyclosporine Therapy Suppresses Ocular and Lacrimal Gland Disease in MRL/Mp-lpr/lpr Mice


Purpose. MRL/Mp-lpr/lpr (MRL/lpr) mice spontaneously develop an autoimmune disease characterized by lymphoproliferation, vasculitis, glomerulonephritis, autoantibody production, and ocular and lacrimal gland inflammation. Lacrimal gland lesions in MRL/lpr mice are a model for the human disorder Sjögren's syndrome. The target organ lesions in MRL/lpr mice, including those in the eye and lacrimal gland, are composed largely of CD4+ T cells, with lesser numbers of CD8+ T cells and B cells. Cyclosporine therapy was evaluated for its effect on the autoimmune disease, particularly in the eye and lacrimal gland.

Methods. MRL/lpr mice were administered cyclosporine intraperitoneally at a dosage of 2 mg daily from age 1 to 5 months. Animals were killed at 5 months and evaluated for the presence of autoimmune disease. Control groups consisted of animals given daily injections with either saline or the cyclosporine diluent.

Results. Cyclosporine therapy was effective in reducing the ocular and lacrimal gland disease. Intraocular inflammation was present in 73% of control animals but in only 15% of cyclosporine-treated animals \((P < 0.003)\). Multifocal lacrimal gland inflammatory infiltrates were present in 100% of controls but in only 23% of cyclosporine-treated animals \((P < 0.0001)\). Mean percent area involved by lacrimal gland inflammation was reduced from 19.7% to 4.7% by cyclosporine therapy \((P = 0.0003)\). Systemic autoimmune disease manifestations, including lymphoproliferation, vasculitis, glomerulonephritis, and serologic abnormalities, also were improved.

Conclusions. Chronic cyclosporine therapy, started at an early age, is effective in controlling the autoimmune disease in MRL/lpr mice, including the ocular and lacrimal gland lesions. Invest Ophthalmol Vis Sci. 1996;37:377-383.

Ocular inflammation, including scleritis and Sjögren's syndrome, are common in patients with systemic autoimmune disorders. MRL/Mp-lpr/lpr (MRL/lpr) mice spontaneously develop an autoimmune disease characterized by lymphadenopathy, vasculitis, glomerulonephritis, serologic abnormalities, and ocular and lacrimal gland inflammatory lesions. MRL/lpr mice are congenic with MRL/Mp—+/+ (MRL/+/) mice and differ only by a single autosomal recessive mutation, the lpr gene. The lpr mutation results in a defective Fas protein and defective lymphocytic apoptosis, causing massive lymphadenopathy. Lymph nodes in MRL/lpr mice are composed largely of CD4—CD8—TCR \(\alpha/\beta^+\) "double negative" T cells, whereas the target organ lesions, including those in the lacrimal gland and eye, are composed largely of CD4+ T cells with lesser numbers of CD8+ T cells and B cells.

Therapies directed at T cells, including neonatal thymectomy, treatment with anti-T cell monoclonal antibody (mAb), and anti-CD4 mAb therapy, have been reported to improve systemic autoimmune and intraocular inflammatory lesions. However, the response of lacrimal gland inflammation to immunosuppressive treatment may be very different from the response seen in systemic autoimmune disease. Treatment of MRL/lpr mice with anti-CD4 mAb largely eliminated the systemic and ocular disease but it did not decrease the lacrimal gland disease; instead anti-CD4 mAb treatment resulted in lacrimal gland...
lesions with an altered morphology and a lymphocytic infiltrate composed largely of CD8+ T cells.20

Although cyclosporine therapy is effective in suppressing the systemic disease in MRL/lpr mice,21 a previous study22 reported that cyclosporine therapy worsened the lacrimal gland disease. In this article, we present evidence to the contrary, namely that early and consistent cyclosporine therapy is effective in suppressing ocular and lacrimal gland lesions in MRL/lpr mice.

MATERIALS AND METHODS

Treatment Protocol

Female MRL/lpr mice were obtained from the Jackson Laboratories (Bar Harbor, ME) and kept under standard conditions in the animal facilities of the Woods Research Building of the Johns Hopkins Hospital. Treatment was begun at 1 month of age, and animals were given daily intraperitoneal injections (7 days/week) of 2 mg of cyclosporine (Sandoz Pharmaceutical, East Hanover, NJ), in 0.5 ml diluted with 5% Alkamuls (Rhône-Poulenc, Cranbury, NJ), a dose equivalent to 40 mg/kg body weight. Control animals were given either daily intraperitoneal injections of normal saline or diluent. Animals were killed at 5 months of age by exsanguination. Sera were collected for the studies outlined below. Submandibular, axillary, and inguinal lymph nodes were removed and weighed as a measure of lymphadenopathy. Spleens were removed and weighed separately. Eyes, lacrimal glands, and kidneys were removed for histologic processing, fixed in 4% buffered formaldehyde, sectioned at 5 μm, and stained with hematoxylin and eosin. Tissue sections were evaluated for the presence of disease by a masked reader (RAP) unaware of the treatment group for each animal. These experiments conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Cyclosporine Levels

Plasma cyclosporine levels were measured in a subset of animals at 3 months and 5 months of age using high-performance liquid chromatography at Smith Kline Beecham Clinical Laboratories (Owings Mills, MD).

Evaluation of Lacrimal Gland Histology

Lacrimal gland sections were graded using the previously described, modified focus score scale.5-7 Using this system, lacrimal gland histologic sections were scored from 0 to 4 based on the presence or absence of foci of 50 or more mononuclear inflammatory cells; grade 0 = no inflammatory cells; grade 1 = inflammation without any focus; grade 2 = the presence of at least one focus; grade 3 = multiple foci; and grade 4 = multiple foci plus evidence of lacrimal gland destruction (e.g., effacement of lobular architecture by mononuclear inflammatory cells, fibrosis, or other evidence of damage). In addition, the percentage of area in the lacrimal gland involved by inflammatory cells was measured by planimetry, using a Zeiss (Oberkochen, Germany) projection microscope.

Antinuclear Antibodies

Antinuclear antibodies were measured using indirect immunofluorescence as previously described.23 Sera were screened at a 1:10 dilution, using 4-μm frozen sections of rat liver as the substrate and a fluorescein isothiocyanate-conjugated goat anti-mouse mAb as the second antibody. Antinuclear antibodies were read in a masked fashion and scored on a semiquantitative scale score of 0 to 4+, where 0 = negative, 1+ = weak, 2+ = medium, 3+ = strong, and 4+ = very strong staining.

Measurement of Anti-dsDNA Antibodies

Determination of anti-double-stranded DNA (anti-dsDNA) antibodies was performed using the method described by Pisetsky and Peters.24 Briefly, ELISA plates (Corning Laboratory Sciences, Corning, NY) were coated with 200 μl of salmon testes DNA (Sigma, St. Louis, MO) 5 μg/ml in phosphate-buffered saline (PBS) in each well, and incubated at 37°C for 2 to 3 hours. After decanting and washing the plates three times with PBS–TWEEN 20, the remaining sites in each well were blocked by incubation of 200 μl of 1 μg/ml methylated bovine serum albumin at 25°C for 1 hour. Plates were washed three times with PBS–TWEEN 20; then, serum diluted 1:2 with PBS–TWEEN 20 was added, and the plates were incubated at 25°C for 1 hour. At the end of the incubation, plates were washed three times, and 100 μl of 1:1000 dilution of alkaline phosphatase-coupled goat anti-mouse (immunoglobulin G [IgG]) was added to each well. After 1 hour of incubation at 25°C, each well was again washed three times, and then p-phenol phosphate was added as a substrate. Reactions were stopped with the addition of 50 μl of 3 M NaOH in each well for 10 minutes. Serial dilutions were tested, beginning at 1:25. Each serum also was tested on plates without coating with DNA. Plates were read at 410 nm. Results are expressed as dilution (titer) giving optical density of ≤0.10. All determinations were performed in duplicate.

Determination of Total Immunoglobulin G

ELISA plates were coated with 100 μl of 5 μg/ml goat anti-mouse IgG (Capell, Cochravanille, PA) in each well at 4°C overnight. Plates were blocked with 1% bovine serum albumin; after being washed three times with
Cyclosporine Therapy of MRL/lpr Mice

PBS–Tween 20, 100 μl of a 10⁵ serum dilution was added to the individual wells and incubated at 25°C for 1 hour. Plates were read using optical density at 410 nm. All autoantibody determinations and immunoglobulin measurements were performed in a masked fashion.

Statistics

Group proportions were compared using either chi-square analysis or the Fisher’s exact test. Group medians were compared using the Wilcoxon rank sum test.

RESULTS

Cyclosporine Levels

Plasma cyclosporine levels were measured in two saline-treated and two Alkamuls-treated (cyclosporine diluent) mice at 3 months and another two from each control group at 5 months of age. In all eight control mice, plasma levels were below the lower limit of detection (<30 μg/l), that is, zero. Cyclosporine levels were measured in three cyclosporine-treated mice at 3 months and three other mice at 5 months of age. The mean level in the 3-month-old treated mice was 875 μg/l (range, 334 to 1590 μg/l), and in the 5-month-old treated mice, it was >1020 μg/l (range, 904 to >1000 μg/l). Despite the plasma levels achieved, there was no evidence of wasting or cyclosporine toxicity in the cyclosporine-treated group.

Lymphoproliferation

Lymphoproliferation was markedly improved by treatment with cyclosporine (Table 1). Median lymph node weight was reduced 82%, and median spleen weight was reduced 50% by cyclosporine treatment (P = 0.0001 and P = 0.0002, respectively).

Ocular Pathology

Ocular disease was markedly suppressed by cyclosporine therapy (Table 2, Figure 1). The presence of any ocular inflammation was decreased from 73% in control animals to 15% in cyclosporine-treated animals (P < 0.003). This improvement was particularly evident with the more severe ocular lesions, such as choroiditis and scleritis, which collectively were present in 34% of control animals but in none of the cyclosporine-treated animals (P = 0.04). Other ocular inflammatory lesions seen in controls but not cyclosporine-treated animals included anterior uveitis (one mouse) and orbital vasculitis (one mouse).

Lacrimal Gland Pathology

Lacrimal gland inflammation was markedly suppressed by chronic cyclosporine therapy (Table 2). One hundred percent of control animals had severe lacrimal gland disease, grade 3 or 4, using the modified focus score scale (Fig. 2). Only 23% of cyclosporine-treated animals had grade 3 or greater lacrimal gland disease (P < 0.0001). Furthermore, the evaluation of percentage area involved by inflammation in the lacrimal gland revealed a 76% reduction in the mean amount of inflammation present, from 19.7% in control animals to 4.7% in cyclosporine-treated animals (P = 0.0003).

Renal Pathology

Renal disease was decreased by cyclosporine therapy (Table 1). Glomerulonephritis was present in 100% of controls, and the median grade was 3+ using our previously described 0 to 4+ scoring system. Glomerulonephritis was detected in only 15% of cyclosporine-treated animals (P < 0.00001). Vasculitis and interstitial nephritis were detected in 47% and 60% of control animals, respectively, but vasculitis was absent and interstitial nephritis was present in only 15% of cyclosporine-treated animals, respectively (P < 0.01 and P = 0.02, respectively).

Autoantibodies and Immunoglobulin Levels

Serologic abnormalities were decreased by cyclosporine therapy (Table 1). The median titer of anti-dsDNA was reduced from 1:600 in controls to 1:37.5 in cyclosporine-treated animals (P = 0.004). Total IgG levels were reduced from a median optical density of 0.80 to 0.50 by cyclosporine therapy (P = 0.008), and the median antinuclear antibody grade was decreased from 3+ in controls to 1+ in cyclosporine-treated mice (P = 0.002).

DISCUSSION

MRL/lpr mice differ from the congenic MRL/+ mice only by a single autosomal recessive gene, the lpr mutation. The lpr mutation results in a defective Fas antigen and thereby defective lymphocytic apoptosis, which leads to the accumulation of double negative T cells in the lymph nodes and hastens the development of autoreactive T cells. These autoreactive T cells appear to drive the autoimmune disease in these animals. Both MRL/lpr and MRL/+ mice develop lacrimal gland lesions and serologic abnormalities, but MRL/lpr mice develop a much more aggressive and rapidly fatal autoimmune disease than do MRL/+ mice. Furthermore, the lacrimal gland disease in MRL/lpr mice has a different immunocytochemical profile than that in MRL/+ mice. Both mice have a predominance of CD4+ T cells with few double negative T cells present in the lacrimal glands, but MRL/lpr mice have a significantly greater proportion of...
### TABLE 1. Systemic Autoimmune Disease in Control and Cyclosporine-treated MRL/lpr Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline</th>
<th>Alkamuls*</th>
<th>Controls†</th>
<th>Cyclosporine</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>7</td>
<td>8</td>
<td>15</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Lymphoproliferation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (mean ± SD) lymph node weight (g)</td>
<td>2.5 (2.8 ± 1.6)</td>
<td>1.6 (2.1 ± 1.2)</td>
<td>2.2 (2.4 ± 1.4)</td>
<td>0.4 (0.5 ± 0.6)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Median (mean ± SD) spleen weight (g)</td>
<td>0.6 (0.7 ± 0.4)</td>
<td>0.6 (0.6 ± 0.2)</td>
<td>0.6 (0.6 ± 0.3)</td>
<td>0.3 (0.3 ± 0.1)</td>
<td>0.0002</td>
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<tr>
<td>Renal disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis (%)§</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Median grade</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Vasculitis (%)§</td>
<td>86</td>
<td>12</td>
<td>47</td>
<td>0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Interstitial nephritis (%)§</td>
<td>86</td>
<td>38</td>
<td>60</td>
<td>15</td>
<td>0.02</td>
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<tr>
<td>Serology</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td></td>
<td></td>
<td>450</td>
<td>600</td>
<td>600</td>
</tr>
<tr>
<td>Total IgU</td>
<td>3.5</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>0.002</td>
</tr>
</tbody>
</table>

SD = standard deviation.
* Alkamuls = diluent for cyclosporine.
† Control group = saline- and alkamuls-treated groups combined.
‡ P value for comparison of control vs. cyclosporine-treated groups. Group proportions were compared using chi-square or Fisher’s exact test, and group medians using the Wilcoxon rank sum test.
§ Percent of animals with disease.
|| Median titer (range 1:25 to 1:3200).
#Antinuclear antibodies, expressed as median grade (scale 0 to 4+).

CD4+ T cells and lower proportion of CD8+ T cells than do MRL/+ mice.6

Treatment directed toward T cells, such as neonatal thymectomy14,15 or anti-T cell mAb therapy,16 results in marked improvement in the systemic autoimmune disease in MRL/lpr mice. However, although treatment with anti-CD4 mAb resulted in marked improvement in the systemic autoimmune disease and intraocular disease,17-19 it did not improve the lacrimal gland disease.20 Instead, the morphology of inflammatory lesions and immunocytochemical profile were altered, but the extent of disease was not diminished. These results demonstrate that the lacrimal gland and systemic autoimmune disease in MRL/lpr mice may respond differently to treatment.

Cyclosporine selectively inhibits T cells by the inhibition of IL-2–IL-2 receptor (IL-2R) autocrine pathway.25,26 Because T cells are affected by cyclosporone, one might hypothesize that cyclosporine therapy would be effective for autoimmune disease in MRL/...

### TABLE 2. Autoimmune Ocular and Lacrimal Gland Disease in Control and Cyclosporine-treated MRL/lpr Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Saline</th>
<th>Alkamuls*</th>
<th>Controls†</th>
<th>Cyclosporine</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>7</td>
<td>8</td>
<td>15</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Ocular disease</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Any (%)§</td>
<td>71</td>
<td>75</td>
<td>73</td>
<td>15</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>Episcleritis (%)</td>
<td>29</td>
<td>38</td>
<td>33</td>
<td>15</td>
<td>0.26</td>
</tr>
<tr>
<td>Choroiditis (%)</td>
<td>14</td>
<td>38</td>
<td>27</td>
<td>0</td>
<td>0.07</td>
</tr>
<tr>
<td>Posterior scleritis (%)</td>
<td>14</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>0.54</td>
</tr>
<tr>
<td>Lacrimal gland disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percent grade ≥3+§</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean ± SD (median) percent area</td>
<td>22.5 ± 12.5 (20)</td>
<td>17.3 ± 17.3 (11.3)</td>
<td>19.7 ± 15.0 (15.9)</td>
<td>4.7 ± 5.5 (2.7)</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

* Alkamuls = diluent for cyclosporine.
† Control group = saline- and alkamuls-treated groups combined.
‡ P value for comparison of control vs. cyclosporine-treated groups. Group proportions were compared using chi-square or Fisher’s exact test, and group medians using the Wilcoxon rank sum test.
§ Percent of animals with disease.
|| Percent lacrimal gland area replaced by inflammatory cells.
Cyclosporine Therapy of MRL/lpr Mice

FIGURE 1. Choroidal lesions in MRL/lpr mice. (A) Choroidal inflammatory infiltrate in a saline-treated MRL/lpr mouse. (B) Choroid of cyclosporine-treated MRL/lpr mouse showing absence of disease (hematoxylin and eosin; original magnification, ×250).

lpr mice, a disease in which T-cell abnormalities appear to be primary. However, adult MRL/lpr mice are deficient in both the production of and the response to IL-2; hence, cyclosporine therapy theoretically could accelerate autoimmune disease in MRL/lpr mice. A previous study by Mountz et al reported that high-dose cyclosporine (40 mg/kg per day) was effective in controlling the systemic autoimmune disease in MRL/lpr mice, including the lymphadenopathy, but that autoantibody formation was not reduced. In that study, lacrimal glands were not evaluated. In these experiments low-dose cyclosporine therapy (10 mg/kg every other day) was considerably less effective than high-dose therapy.

The dose of cyclosporine used in our experiments was the high dose (40 mg/kg), and our results also demonstrate that cyclosporine therapy was effective in controlling systemic autoimmune disease, including glomerulonephritis and vasculitis. In contrast to the results of Mountz et al, in our study both serologic abnormalities and total IgG were improved when compared to control animals.

In our study, ocular and lacrimal gland diseases were improved markedly by cyclosporine therapy. The success of cyclosporine therapy in suppressing the lacrimal gland disease contrasts with the failure of anti-CD4 mAb therapy. Because IL-2 is produced by CD4+ T cells, the effects of cyclosporine and anti-CD4 mAb might have been predicted to be similar. However, because cyclosporine inhibits the generation of cytotoxic T cells in vitro, it could affect both CD4+ and CD8+ T cells and, hence, it could be effective for the treatment of lacrimal gland disease in MRL/lpr mice, even though anti-CD4 mAb was not. Taken together, these two sets of results suggest that both CD4+ and CD8+ T cells may be important in the pathogenesis of lacrimal gland disease in MRL/lpr mice.

FIGURE 2. Lacrimal glands in MRL/lpr mice. (a) Lacrimal gland in a saline-treated MRL/lpr mouse showing extensive lacrimal gland inflammation. (b) Cyclosporine-treated MRL/lpr mouse showing absence of lacrimal gland disease (hematoxylin and eosin; original magnification, ×160).
Our results stand in marked contrast to those of Sato and Sullivan, who reported that cyclosporine therapy worsened lacrimal gland disease in MRL/lpr mice. Several experimental differences may explain the different results. Sato and Sullivan administered cyclosporine to animals beginning at approximately 5.5 months of age, a time at which autoimmune disease already has begun, whereas in our experiments, cyclosporine therapy was begun at 1 month of age, before the onset of overt autoimmune disease. Previous studies by us using anti-CD4 mAb therapy have demonstrated that treatment begun “late” can have different effects on the autoimmune disease in MRL/lpr mice than treatment begun “early.” Furthermore, 1-month-old MRL/lpr mice are relatively normal in terms of the production of IL-2, whereas aged MRL/lpr mice have defects in both the production of and response to IL-2, at least when lymph node lymphocytes are evaluated. Hence, cyclosporine may have differential effects on MRL/lpr mice depending on the timing of the onset of therapy.

Furthermore, Sato and Sullivan used slow-release pellets implanted subcutaneously instead of daily intraperitoneal injections, and cyclosporine was administered only for a 21-day interval, after which animals were evaluated. In our experiments, cyclosporine therapy was given as daily intraperitoneal injections of cyclosporine, which probably resulted in a much higher exposure to cyclosporine. Although the plasma levels of cyclosporine were not measured in Sato and Sullivan’s experiments, the levels in our experiments were substantial, generally in the 800 to 1000 μg/g/1 levels of cyclosporine were not measured in Sato and Sullivan’s experiments, 22 which probably resulted in a much higher exposure to cyclosporine. Although the plasma levels of cyclosporine were not measured in Sato and Sullivan’s experiments, the levels in our experiments were substantial, generally in the 800 to 1000 μg/g/1 range, and well above the therapeutic range for organ transplantation. Different doses of cyclosporine may have different effects on the immune system. In bone marrow transplants, low-dose cyclosporine enhances the development of autologous graft-versus-host disease, whereas high-dose cyclosporine is effective in preventing and treating allogenic graft-versus-host disease. Our data demonstrate that chronic daily, high-dose cyclosporine therapy begun at 1 month of age suppresses lacrimal gland inflammation in MRL/lpr mice. Further exploration of the differential effects of the dose and timing of onset of therapy using the intraperitoneal route may explain the differences between our results and those of Sato and Sullivan.

In conclusion, our study demonstrates that chronic cyclosporine therapy of MRL/lpr mice is effective in controlling the systemic autoimmune disease, autoantibody formation, intraocular inflammatory lesions, and the lacrimal gland autoimmune disease seen in this strain.

Key Words

autoimmune response, cyclosporine, immunopathology, lacrimal gland, Sjögren’s syndrome

Acknowledgments

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References

16. Wofsy D, Lederker JA, Hendler PL, Seaman WE.
Cyclosporine Therapy of MRL/lpr Mice

383