Autoimmune Ocular Disease in MRL/Mp-lpr/lpr Mice Is Suppressed by Anti-CD4 Antibody

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MRL/Mp-lpr/lpr (MRL/lpr) mice spontaneously have a systemic autoimmune disease, characterized by vasculitis, lymphadenopathy, glomerulonephritis, and autoantibody formation. Among the many autoimmune lesions present are focal ocular inflammatory infiltrates, involving the choroid and sclera. These lesions appear to be related to the vasculitis seen in MRL/lpr mice and are mediated by L3T4-positive helper T-cells (CD4-positive T-cells). Systemic treatment of MRL/lpr mice with a monoclonal anti-L3T4 antibody (anti-CD4) resulted in a dramatic reduction of both the frequency and severity of the ocular disease, supporting the hypothesis that the CD4-positive T-cells play an essential role in the pathogenesis of the choroiditis and scleritis in this strain. Invest Ophthalmol Vis Sci 32:2718–2722, 1991

Ocular involvement is common in systemic autoimmune diseases in humans. Among the lesions seen in these patients are scleritis, present in patients with rheumatoid arthritis and vasculitis, secondary Sjögren’s syndrome in patients with rheumatoid arthritis and systemic lupus erythematosus (SLE); and uveitis in patients with spondyloarthritis. Choroidal involvement is less frequent, but it was described in patients with SLE and vasculitis. There are several strains of autoimmune mice, including MRL/Mp-lpr/lpr (MRL/lpr), MRL/Mp+/+ (MRL/+), (NZB×NZW) F1 hybrid (NZB/W), B×SB, and Palmerston-North strains. Among the autoimmune lesions seen in MRL/lpr mice are focal ocular inflammatory infiltrates involving the choroid and sclera. These lesions are present in MRL/lpr mice, but not in congenic MRL/+ mice or in NZB/W mice. The choroidal and scleral lesions in these mice appear to be related to the vasculitis commonly seen in MRL/lpr mice, and the lesions consist largely of CD4-positive helper T-cells. We found that treatment of these mice with systemic, monoclonal anti-CD4 antibody results in marked suppression of the ocular autoimmune pathology.

Materials and Methods

Monoclonal Antibodies

Monoclonal antibodies were prepared as previously described. Hybridoma cells secreting a monoclonal antibody (GK 1.5) to the murine CD4 antigen L3T4 were obtained from the American Type Culture Collection (Rockville, MD). One million cells were injected into mice with severe combined immunodeficiency (SCID) obtained from the Jackson Laboratories (Bar Harbor, ME). These mice lack both T- and B-cell function; therefore, all immunoglobulin recovered from the peritoneal cavity of mice with severe combined immunodeficiency (SCID) obtained from the Jackson Laboratories (Bar Harbor, ME). These mice lack both T- and B-cell function; therefore, all immunoglobulin recovered from the peritoneal cavity would be monoclonal antibody secreted by the hybridoma cells. Antibody was harvested from the SCID peritoneal exudate using daily paracentesis starting 8 days after cell transfer. The immunoglobulin was purified partially using ammonium sulfate precipitation and dialysis against phosphate-buffered saline. The partially purified antibody then was standardized for protein content using spectrophotometric absorbance at 280 nm.

Treatment Protocol

The MRL/lpr mice were obtained from the Jackson Laboratories and kept under standard conditions in the animal facilities of the Woods Research Building of the Johns Hopkins Hospital. The animals were treated starting at 1 month of age with one initial intravenous injection and weekly intraperitoneal injections of 2 mg of monoclonal anti-L3T4. Two control groups were used; one was given injections of normal saline, and a second was given injections of normal rat serum (NRS). The normal rat serum used in these experiments underwent ammonium sulfate precipitation and dialysis identical to that used for the prepara-
tion of the monoclonal anti-L3T4. At 5 months of age, the animals were killed by exsanguination, and both their eyes were removed. Both eyes were fixed in 4% buffered formaldehyde, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. These experiments conformed to the ARVO Resolution on the Use of Animals in Research.

Results

The results of treatment are shown in Table 1. In the two control groups, saline and NRS, ocular inflammatory disease was common. Eight of nine saline-treated animals had some type of ocular inflammation, and all nine NRS-treated animals had ocular disease. By contrast, only two of the nine animals treated with monoclonal anti-L3T4 had ocular disease (P < 0.001, by Fisher’s exact test). The ocular inflammation could be divided into three types of lesions. The most common one was an anterior episcleritis; the second, a choroidal infiltrate; and the third, posterior scleritis. Anterior episcleritis was present in six of nine saline-treated and eight of nine NRS-treated animals but only in two of nine anti-L3T4-treated animals (P < 0.001). In general, the episcleral lesions consisted of a mononuclear inflammatory cell infiltrate surrounding small vessels in the episcleral region at the angle of the eye (Fig. 1). Anti-L3T4-treated animals generally had no such lesions (Fig. 2). Similarly, choroiditis was present in five of nine saline-treated and nine of nine NRS-treated mice but only one of nine anti-L3T4-treated mice (P < 0.001). In the control mice, the prominent cell infiltrate was mononuclear in nature (Fig. 3), and anti-L3T4-treated mice had a normal-appearing choroid (Fig. 4) with the single exception noted. Scleritis was seen in four of nine saline-treated, two of nine NRS-treated, and none of the anti-L3T4-treated mice (P = 0.07). The primary lesion in the sclera was centered around small vessels where a mononuclear inflammatory reaction of the same general character was present. Other ocular lesions included orbital vasculitis in one saline-treated mouse.

Table 1. Treatment of MRL/lpr mice

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Any ocular inflammation*</th>
<th>Anterior episcleritis</th>
<th>Choroiditis</th>
<th>Scleritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>8/9</td>
<td>6/9</td>
<td>5/9</td>
<td>4/9</td>
</tr>
<tr>
<td>Normal rat serum</td>
<td>9/9</td>
<td>8/9</td>
<td>9/9</td>
<td>2/9</td>
</tr>
<tr>
<td>Anti-L3T4</td>
<td>2/9</td>
<td>2/9</td>
<td>1/9</td>
<td>0/9</td>
</tr>
</tbody>
</table>

* Number of animals with lesions/number of animals analyzed.
and optic perineuritis in one NRS-treated mouse. No anti-L3T4-treated mice had other ocular lesions. These results show a significant reduction in both the frequency and severity of the ocular lesions by treatment with anti-L3T4. Two additional animals were killed at 3 months of age for fluorescein-activated cell sorted (FACS) analysis of the lymphocyte populations of their spleens. This analysis showed virtually complete elimination of the L3T4-positive cell population from the spleens of the anti-L3T4-treated MRL/lpr mice, with a reduction of the L3T4-positive T-cells from 18.1% in the saline-treated group and 20.8% in the NRS-treated group to 0.6% in the anti-L3T4-treated group.

In addition to the beneficial effect of treatment with monoclonal anti-L3T4 on the ocular lesions, such
treatment also had a similar effect on the systemic disease. The adenopathy markedly improved, and lymph node weights were reduced 60%. Double-negative T-cells were still present in the lymph nodes, but the size of nodes markedly improved. The vasculitis similarly was eliminated; renal vasculitis was detected in six saline-treated animals, seven NRS-treated animals, and no anti-L3T4-treated animals. Anti-nDNA antibodies were eliminated by treatment and the elevated immunoglobulin levels seen in control mice were not present in anti-L3T4-treated animals (manuscript in preparation). Thus, anti-L3T4 therapy had a beneficial effect on the systemic autoimmune disease, in particular the vasculitis, compared with the control groups in this study.

Discussion

MRL/lpr mice are congenic with MRL/+ mice and differ from them only by the presence of the autosomal recessive gene, the Ipr gene. This gene accelerates the autoimmune disease in MRL mice and causes a massive lymphadenopathy. The primary immunologic disorder in MRL/lpr mice appears to be T-cell mediated. Neonatal thymectomy of MRL/lpr mice or treatment with monoclonal anti-T-cell antibody results in the amelioration of the autoimmune disease in this strain. Similarly, the polyclonal B-cell activation, hypergammaglobulinemia, and autoantibody formation in MRL/lpr mice appear to be T-cell driven because lymphocytes of MRL/lpr mice spontaneously secrete a B-cell differentiation factor in vitro. Among the many systemic lesions seen in MRL/lpr mice is vasculitis. This vasculitis appears to be largely T-cell mediated with most cells being L3T4-positive helper T-cells. L3T4 is the murine CD4 antigen. Because the ocular lesions in MRL/lpr mice appear to be related to the vasculitis and because this process is largely L3T4-positive helper T-cell mediated, we hypothesized that treatment with anti-L3T4 would result in amelioration of the disease in MRL/lpr mice. A dramatic reduction in both the incidence and severity of the ocular lesions was seen in the group treated with anti-L3T4; this was consistent with our hypothesis that the ocular disease was mediated by CD4-positive (L3T4-positive) helper T-cells.

MRL/lpr mice also develop massive lymphadenopathy not seen in the congenic MRL/+ strain. These massively enlarged lymph nodes are composed primarily of double-negative T-cells. These cells are Thy 1.2 positive but L3T4 and Lyt 2 negative. The exact nature of these double-negative T-cells and their relationship to the other autoimmune lesions seen in MRL/lpr mice are unknown. However, the predominance of the L3T4-positive helper T-cells in the target organ lesions of MRL/lpr mice and the elimination of the ocular inflammatory lesions by anti-L3T4 therapy suggest that these double-negative T-cells are not involved directly in the autoimmune process in the target organ lesions.

In the past, many of the ocular inflammatory lesions in humans, such as scleritis and uveitis, were
believed to be caused by circulating immune complexes and immune complex deposition disease. More recently there has been evidence that ocular inflammatory lesions may be T-cell mediated. Experimental allergic uveitis retinitis (EAU) can be transferred by T-cells,\textsuperscript{23} and others\textsuperscript{24} showed that treatment of EAU with monoclonal anti-CD4 antibodies can ameliorate this disease in rats. Our results in treating scleritis and choroiditis in the MRL/lpr animal model suggest that these lesions are also helper T-cell mediated. The successful treatment of one patient with scleritis with cyclosporine,\textsuperscript{25} a drug that is selectively active against T-cells,\textsuperscript{26,27} suggests that some cases of scleritis in humans also may be helper T-cell mediated.

**Key words:** autoimmune disease, choroiditis, monoclonal antibody therapy, MRL/Mp-lpr/lpr mice, scleritis, vasculitis

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**References**