Paradoxic Effect of Anti-CD4 Therapy on Lacrimal Gland Disease in MRL/Mp-lpr/lpr Mice

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Purpose. MRL/Mp-lpr/lpr mice (MRL/lpr) spontaneously develop lacrimal gland inflammatory lesions and are a model for the human disease Sjögren’s syndrome. Therapy with monoclonal antibodies (mAb) to CD4 ameliorates the autoimmune renal, vasculitic, and intraocular inflammatory lesions in MRL/lpr mice. The effect of anti-CD4 mAb therapy on lacrimal gland immunopathology was evaluated.

Methods. From 1 to 5 months of age, MRL/lpr mice were treated with weekly intraperitoneal injections of 2 mg anti-CD4 mAb, after which they were killed and their lacrimal glands were removed for histologic evaluation and immunocytochemistry. Control mice were administered weekly intraperitoneal injections of either saline or normal rat immunoglobulin.

Results. Anti-CD4 mAb treatment produced no reduction in lacrimal gland inflammation but did change its morphology. In control mice, there were multiple sharply delineated foci of inflammatory cells in the lacrimal gland, whereas in anti-CD4 mAb-treated mice, there was a more diffuse inflammation surrounding ill-defined foci that spread throughout the gland. Immunocytochemistry revealed that in control mice, lesions were composed predominantly of CD4+ T cells, but in anti-CD4 mAb-treated mice, CD8+ T cells predominated.

Conclusions. Although anti-CD4 mAb therapy of MRL/lpr mice eliminated autoimmune renal disease, autoantibody formation, and ocular inflammatory disease, it had a paradoxical effect on lacrimal gland lesions. Lacrimal gland lesions in the anti-CD4 mAb-treated mice were not decreased, but they had a different morphology and a different immunocytochemical profile. Invest Ophthalmol Vis Sci. 1996;37:246–250.

MRL/Mp-lpr/lpr (MRL/lpr) mice spontaneously develop an autoimmune disease characterized by vasculitis, lymphadenopathy, glomerulonephritis, and autoantibody production.1 Among the autoimmune lesions are lacrimal gland inflammatory infiltrates, which are a model for the human disorder, Sjögren’s syndrome.2 These lacrimal gland lesions are composed largely of CD4+ T cells, as are the other autoimmune lesions (including ocular and vasculitic), whereas the massively enlarged lymph nodes are composed primarily of Thy 1.2+, CD4−, and CD8− TCR α/β− “double-negative” T cells.2,3 MRL/lpr mice are congenic with MRL/Mp−/+ (MRL/+), which develop a later onset and milder systemic autoimmune disease.1 The lpr mutation results in a defective Fas protein and defective apoptosis of lymphocytes.4 The consequences of this defective apoptosis include the accumulation of double-negative T cells, resultant massive lymphadenopathy, and acceleration of the autoimmune disease inherent in the MRL/Mp strain.1 Previous studies5,6 have demonstrated that treatment of MRL/lpr mice with monoclonal antibodies (mAb) to the CD4 molecule resulted in a marked improvement in systemic autoimmune disease, including reduction in lymphadenopathy and autoantibody formation, and the elimination of renal and ocular disease. In this report, we describe the effect of anti-CD4 mAb therapy on the lacrimal gland lesions in MRL/lpr mice.

MATERIALS AND METHODS. Monoclonal Antibodies. Monoclonal antibodies were prepared as previously described.5,6 Hybridoma cells secreting mAb of rat origin to the murine CD4 antigen L3T4 (GK 1.5) were obtained from ATCC (Rockville, MD).7 One million cells were injected into the peritoneal cavity of severe combined immunodeficiency mice obtained from the Jackson Laboratories (Bar Harbor, ME). Antibody was harvested from the severe combined immunodeficiency peritoneal exudate by paracentesis starting 8 days after cell transfer. Immunoglobulin (Ig) was purified using 50% saturated ammonium sulfate precipitation, followed by solubilization and dialysis against phosphate-buffered saline (PBS). The partially purified mAb was then standardized for protein content using spectrophotometric absorbance at 280 nm.

Treatment Protocol. Female 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. Animals were treated as previously described5,6 with one initial intravenous and one intraperitoneal injection of 2 mg each, starting at 1 month of age, followed by weekly intraperitoneal injections of 2 mg of anti-CD4 mAb. Two control groups were used: The first was given injections of normal saline, and the second was given injections of normal rat Ig. The rat Ig
used in these experiments was produced by subjecting normal rat serum to an ammonium sulfate precipitation and dialysis identical to that used from the preparation of the anti-CD4 mAb.

Animals were killed by exsanguination at 5 months of age. One lacrimal gland was removed, fixed in 4% buffered formaldehyde, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Histopathologic evaluation was performed by a masked reader (RAP) unaware of the treatment for each animal. In 10 control and 9 anti-CD4 mAb-treated mice, the other lacrimal gland was removed, embedded in OCT compound (Miles, Elkhart, IN), frozen in liquid nitrogen, sectioned at 6 μm on a cryostat, and stained as outlined below. These experiments conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Immunocytochemistry.** Analysis of frozen sections of lacrimal glands was performed using a panel of monoclonal antibodies to cell surface markers and the avidin-biotin-peroxidase complex (ABC) technique. Briefly, frozen sections were fixed in chilled (4°C) acetone, air-dried, rehydrated in PBS, and incubated with the appropriate blocking agent (Vector Laboratories, Burlingame, CA) for 20 minutes. The primary antibody was applied, and the slides were incubated for 60 minutes. Slides were washed in PBS, incubated with a biotinylated secondary antibody for 30 minutes, rinsed in PBS, incubated with the ABC agent for 45 minutes, washed again in PBS, developed with a 0.1% nitrogen peroxide and 3-amino-9-ethyl-carbazole containing acetate buffer, and counterstained with Harris’ hematoxylin (Sigma, St. Louis, MO). The percentage of positive mononuclear inflammatory cell staining acetate buffer, and counterstained with Harris’ hematoxylin (Sigma, St. Louis, MO). The percentage of positive mononuclear inflammatory cell staining of lacrimal glands was performed using a panel of monoclonal antibodies to cell surface markers and the avidin-biotin-peroxidase complex (ABC) technique.

**RESULTS. Lacrimal Gland Histology.** Lacrimal glands were obtained from nine saline-treated and nine rat Ig-treated control mice for histologic evaluation. All lacrimal glands from control mice developed the typical multifocal, well-demarcated inflammatory lesions previously described in MRL/lpr mice and graded either grade 3 or 4 using the previously described modified focus score scale. In contrast, the lacrimal glands from the nine anti-CD4 mAb-treated mice presented a distinct histopathology (Fig. 1), in which there was a more diffuse inflammatory infiltrate surrounding ill-defined foci that spread throughout the lacrimal gland parenchyma. Thus, there was no diminution in the amount of inflammation after anti-CD4 mAb treatment, but there was a change in the morphology of the inflammation.

**Lacrimal Gland Immunocytochemistry.** Lacrimal glands from five saline-treated, five rat Ig-treated, and nine anti-CD4 mAb-treated mice were stained for cell surface markers (Table 1). Results of the saline-treated and rat Ig-treated control groups were similar, and both control groups were combined for an analysis compared to anti-CD4 mAb-treated mice. In both control groups, the infiltrate was predominately a CD4+ T cell infiltrate (mean, 55%), with lesser numbers of CD8+ T cells (mean, 19%) and B cells (mean, 15%). In contrast, although the infiltrate in anti-CD4 mAb-treated mice was largely a T-cell infiltrate, it was composed primarily of CD8+ T cells (Fig. 2); 11% of the infiltrating cells in lacrimal glands of anti-CD4 mAb-treated mice were CD4+ T cells (P = 0.003 versus controls), whereas 63% were CD8+ T cells (P = 0.003 versus controls). The percentage of B cells in the lacrimal glands of anti-CD4 mAb-treated mice also was decreased when compared to control animals (mean, 4% versus 15%; P = 0.007).

**Lacrimal Gland Virology.** Because a viral infection that required CD4+ T cells for control might produce lacrimal gland damage in anti-CD4 mAb-treated mice, lacrimal gland sections were evaluated for the presence of murine cytomegalovirus, the virus most likely to infect the salivary and lacrimal glands. Histology of
FIGURE 1. Lacrimal gland histology of MRL/lpr mice. (A, top) Saline-treated mouse, showing typical well-demarcated multifocal inflammatory cell infiltrate. (B, bottom) Anti-CD4 mAb-treated mouse, showing ill-defined infiltrate with diffuse extension into the parenchyma. Hematoxylin and eosin; magnification, ×160.

FIGURE 2. Immunohistology of anti-CD4 mAb-treated MRL/lpr mice. (A, top) Staining for CD4+ cells, showing relative paucity of CD4+ T cells. (B, bottom) Staining for CD8+ cells, showing predominance of CD8+ T cells. Magnification, ×250.

The lacrimal glands showed no evidence of cytomegalic inclusions, and both culture and polymerase chain reaction were negative for cytomegalovirus. Hence, there was no evidence that the lacrimal gland disease in the anti-CD4 mAb-treated mice was caused by cytomegalovirus infection.

**DISCUSSION.** Lacrimal gland inflammatory lesions are characteristic of several autoimmune mouse strains, including MRL/lpr mice, congenic MRL/+ mice, and (NZBxNZW) F1 hybrid (NZB/W) mice. The lacrimal gland lesions in MRL/lpr and MRL/+ are composed largely of CD4+ T cells, although there is a greater percentage of CD8+ T cells in MRL/+ mice than in MRL/lpr mice.23 In both MRL/lpr and MRL/+ mice, B cells constitute a small percentage of the infiltrate, although there is a late accumulation of B cells (up to 28% of the total) at 18 months of age in MRL/+ mice. Double-negative T cells, if present in the lacrimal gland lesions of MRL/lpr mice, constitute only a small percentage of the infiltrate.23

The target organ lesions in the eye and kidney in MRL/lpr mice are, like lacrimal gland lesions, composed largely of CD4+ T cells.23 We have reported2,6 that anti-CD4 mAb treatment reduces the percentage of CD4+ T cells in the spleen by 97% and largely eliminates ocular and renal disease and autoantibody formation in MRL/lpr mice. Therefore, we initially hypothesized that such treatment would eliminate lacrimal gland lesions. However, the response seen was unanticipated. Instead of improving the lacrimal gland lesions, anti-CD4 mAb that treatment resulted in persistent inflammation with a different morphologic picture than seen in controls. In addition, the
The immunocytochemical profile in the lacrimal glands was altered markedly by anti-CD4 mAb treatment. CD4+ T cells largely were absent, and the infiltrate was composed largely of CD8+ T cells. Although immunocytochemistry evaluates the percentage of cells staining positively and not the absolute number of cells per unit of tissue, the fact that the overall infiltrate was not diminished suggests that there was an absolute increase in the number of CD8+ T cells in the lacrimal glands of anti-CD4 mAb-treated mice compared to control mice.

The explanation for the paradoxical response of the lacrimal gland inflammation after anti-CD4 mAb treatment remains uncertain. There was no evidence of a viral infection that might have been worsened by immunosuppression after anti-CD4 mAb treatment. However, the striking increase in CD8+ T cells in anti-CD4 mAb-treated mice indicates that CD8+ effector cells can be generated without CD4+ helper T cell participation, a result similar to that seen after the genetic deletion of CD4 by Rahemtulla et al. These investigators demonstrated that CD8+ T cells were increased in mice genetically incapable of making CD4+ T cells. Because CD8+ T cells do not require CD4+ T cells for normal development, our data suggest that the lacrimal gland disease intrinsic to the MRL/Mp strain is mediated by both CD4+ and CD8+ T cells and that one effect of the \( lpr \) mutation is a shift to a more aggressive CD4+ T cell-mediated autoimmune disease as seen in MRL/\( lpr \) mice. Elimination of CD4+ T cells by anti-CD4 mAb treatment does not eliminate lacrimal gland disease but does permit the CD8+ T cell response to predominate. Because ocular and vasculitic lesions are seen primarily in MRL/\( lpr \) mice and not in MRL/+ mice1,2,5,6 and because these lesions are mediated primarily by CD4+ T cells, they are suppressed by anti-CD4 mAb therapy.

A corresponding Sjögren's-like clinical syndrome exists in humans. Patients infected with human immune deficiency virus (HIV) have an absolute loss of CD4+ T cells as a consequence of HIV-mediated damage to this T-cell subset. A syndrome known as diffuse infiltrative lymphocytosis syndrome may develop in these patients; lacrimal gland and salivary gland infiltration are primary components of the disorder.10 A clinical syndrome similar to Sjögren's syndrome, including dry eyes and dry mouth, develops in patients with diffuse infiltrative lymphocytosis syndrome, but histologic evaluation of minor salivary gland biopsies in these patients has demonstrated that they are largely composed of CD8+ T cells, not CD4+ T cells. Conversely, minor salivary gland biopsies from patients with Sjögren's syndrome have revealed that the lesions are composed largely of CD4+ T cells. Hence, patients with diffuse infiltrative lymphocytosis, in whom CD4+ T cells are depleted, contract Sjögren's-like syndrome with CD8+ T-cell mediated disease, a situation analogous to that seen in our anti-CD4 mAb-treated MRL/\( lpr \) mice.

**Key Words**

autoimmune response, immunopathology, lacrimal gland, monoclonal antibody therapy, Sjögren's syndrome

**References**

Genetic Predisposition to Coronavirus-Induced Retinal Disease

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Purpose. Retinal inflammatory and degenerative processes in humans and animals frequently are associated with genetic factors. The murine coronavirus, mouse hepatitis virus (MHV), JHM strain, induces a biphasic retinal disease in adult BALB/c mice. The genetic constitution of the host and the virus serotype can be critical factors in determining the outcome of a virus infection. The purpose of this study was to evaluate the possible role of host genetics in murine coronavirus-induced retinal disease.

Methods. JHM virus was inoculated by the intravitreal route into BALB/c, CD-1, and A/J mice. At varying times after inoculation, eye tissues were evaluated histologically. Antibody responses to the virus were evaluated by neutralization assays.

Results. JHM virus induces a biphasic retinal disease in BALB/c mice. In the early phase, 1 to 7 days after inoculation, retinal vasculitis is observed. The second phase, characterized by retinal degeneration in the absence of inflammation, is seen by day 10 and progresses for several months. There is a similar biphasic disease process in JHM virus-infected A/J mice. However, retinal changes are less severe than those seen in BALB/c mice. Retinal tissue damage induced by JHM virus in CD-1 mice is different. Only the early phase of the disease, consisting of retinal vasculitis, was observed. These CD-1 mice do not develop the retinal degenerative disease. In fact, after day 10, the retina has a normal appearance. These differences in retinal tissue damage are seen over a wide range of infectivity of the virus inocula. Virus concentrations ranging from $10^{4.4}$ to $10^{1.4}$ TCID$_{50}$/5 µl were capable of inducing both inflammation and degeneration in BALB/c mice, whereas, the highest concentration of virus ($10^{4.4}$ TCID$_{50}$/5 µl) in CD-1 mice resulted in only the early inflammatory changes.

Conclusions. The authors show that the genetics of the host can profoundly affect the nature of retinal tissue damage. These studies substantiate the concept that a virus can indeed trigger retinal degenerative processes in genetically susceptible hosts. Invest Ophthalmol Vis Sci. 1996;37:250–254.

Coronaviruses induce a number of intriguing pathologic processes. The mouse hepatitis virus (MHV), JHM strain, induces central nervous system disease characterized by acute infections and chronic demyelinating diseases. JHM virus also can induce a biphasic retinal disease in BALB/c mice. In the early phase, 1 to 7 days after inoculation, mild retinal vasculitis is observed. This initial phase is associated with the presence of viral proteins and the detection of infectious virus within the retina. The second stage in seen by day 10 and progresses for several months. This stage is characterized by a retinal degeneration in the absence of vasculitis or inflammation. This degenerative process is associated with a reduction of the photoreceptor layer, loss of interphotoreceptor binding protein, abnormality in the retinal pigment epithelium, and retinal detachment. In this later phase, infectious virus and viral proteins are not detected. However, autoantibodies directed against the retinal pigment epithelium (RPE) cell and the retina are observed.

Murine coronavirus infections can be modified or regulated by various factors, such as virus serotype, genetic constitution of the host, age or developmental stage of the host, and route of inoculation. Genetic factors of the host can be a critical factor in determining the outcome of a viral infection. In fact, the genetic background can influence the type and intensity of the host immune response, the presence of cell membrane surface molecules used for viral adsorption.