Immunopathologic uveitis in the mouse due to lymphocytic choriomeningitis virus

U. Ticho, G. A. Cole, and A. M. Silverstein

Lymphocytic choriomeningitis (LCM) virus induces a fatal immunopathologic process in the central nervous system (CNS) of the adult mouse after intracerebral inoculation without accompanying eye disease. Intracocular injection of virus results in uveitis starting about the seventh day, with little or no CNS involvement. The ocular disease in its mild form is an iridocyclitis, but the more severe form may involve the entire uvea and even take the form of a panophthalmitis. The inflammatory infiltrate, which is almost exclusively composed of lymphocytes and histiocytes, follows the distribution of viral antigen within ocular tissues as determined by fluorescent antibody staining. Immunosuppression with cyclophosphamide prevents the disease, although the virus can, thereafter, be found persisting both within the eye and in extraocular sites. Ocular disease can be evoked in the chronic virus carrier by adoptive transfer of sensitized lymphocytes from virus-immune syngeneic donors. Development of the immune-mediated inflammatory reaction in either the acutely infected animal or the adoptively immunized virus carrier is accompanied by clearance of the viral infection. Thus, the same mechanism responsible for viral clearance apparently mediates the ocular disease.

Key words: uveitis, immunopathology, virus, lymphocytic choriomeningitis.

Immunologic uveitis has been experimentally produced by bacterial antigens, serum proteins, and autoantigens. At present there is no experimental model for an immunopathologic uveitis induced by a viral agent. However, immunopathologic retinopathy has been induced by intracerebral virus inoculation of the newborn rat.

Only a few viruses are known to cause intraocular disease either in humans or animals. Pathogenesis of the disease in most instances has been attributed either to direct cytopathic effect of the virus, to its inhibition of cell multiplication, or to its interference with the metabolic needs of the infected cells.

Lymphocytic choriomeningitis (LCM) virus is known to induce a fatal central nervous system (CNS) disease in adult mice following intracerebral inoculation, but no eye disease. Infection of the fetus in utero (vertical transmission), of the
newborn mouse, or of the immunosuppressed adult results in animals that are persistent virus carriers, but show no CNS pathology. The immunopathologic nature of the CNS disease is illustrated by adoptive immunization of these virus carriers with lymphoid cells from sensitized animals, which results in the induction of fatal CNS disease.

This report describes the development of a severe uveitis and panophthalmitis following intraocular inoculation of LCM virus in the adult mouse. An immunopathologic basis for the eye disease is demonstrated.

Materials and methods

Mice. Adult BALB/c mice (Flow Laboratories, Dublin, Va.) were employed throughout.

Virus. The E-350 strain of LCM virus (American Type Culture Collection VR 134), derived from the original isolate of Armstrong and Lillie, was used in the form of a clarified suspension of infected mouse-brain tissue in phosphate-buffered saline. The titer of the brain suspension used, in terms of intracerebral 50 per cent lethal doses (LD50) in adult mice, was 10⁶ per 0.03 ml.

Intraocular virus injection. The anterior chamber of the mouse eye was inoculated with 0.01 ml of a 1:20 dilution of stock virus suspension (≥ 3,000 LD50). The mice were anesthetized with ether and the inoculation performed with the aid of a surgical microscope. The 30-gauge needle was introduced through the temporal corner of the limbus, avoiding injury to lens or iris.

As a control, 0.01 ml of 20 per cent uninfected mouse-brain suspension in phosphate-buffered saline was injected into the anterior chamber of the mouse eye.

Immunosuppression. Cyclophosphamide (Cytoxan, Mead Johnson Laboratories, Evansville, Ind.) dissolved in sterile water was inoculated intraperitoneally on the third day following eye inoculation, using 0.175 mg. per gram mouse weight.

Immune cell transfer. Donor mice were prepared by intraperitoneal injection of 1.0 ml of a 10⁻² dilution of the stock infected mouse-brain suspension, with two successive injections seven days apart. The animals were killed seven days after the second injection, and their spleens were gently teased through wire mesh, centrifuged at 250 x g, and the intact cells resuspended in Eagle's medium without serum. Recipient mice were inoculated intraperitoneally with 1 ml of spleen-cell suspension containing 2 x 10⁸ viable nucleated cells assessed by the trypan blue exclusion test.

Histology. Mice were killed at various intervals after inoculation, immunosuppression, and immune cell transfer. After perfusion with normal saline through the left ventricle, one eye, half of the brain, and parts of selected organs were fixed in 10 per cent neutral formalin containing 20 per cent ethanol. Paraffin sections were stained with hematoxylin and eosin.

Immunofluorescent observations. The contralateral eye, the remaining half of each brain, and other organs were frozen at -70° C. for immunofluorescent staining. The sections were prepared in a cryostat, stained with fluorescein-conjugated LCM virus antiserum containing Evans blue as a counterstain, and examined with a fluorescent microscope.

Results

The results are summarized in Table I.

Initial disease. The initial disease developed in all 40 inoculated eyes of 20 adult mice (100 per cent). The clinical signs started about the seventh day as an acute iridocyclitis, and progressed to a panophthalmitis by about the eleventh day. On slit-lamp examination the cornea was cloudy, with flare and keratic precipitates in the anterior chamber and the formation of posterior synechiae. On the eleventh day the cornea was completely opaque, limiting further clinical observation of the intraocular structures. Some of the eyes went on later to develop phthisis bulbi.

Histologically, an inflammatory infiltrate accumulated in the anterior segment of the eye during the first week, invading the iris, ciliary body, and peripheral cornea, and forming clumps of cells on the back of the cornea (Fig. 1). The inflammatory infl-

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Table I. LCM eye disease in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Severe disease (%)</th>
<th>Mild disease (%)</th>
<th>No disease (%)</th>
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<tbody>
<tr>
<td>LCM eye (20 mice)</td>
<td>100</td>
<td>0</td>
<td>0</td>
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<tr>
<td>(Days 7 to 10)</td>
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<tr>
<td>LCM eye (40 mice*)</td>
<td>0</td>
<td>60</td>
<td>40</td>
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<tr>
<td>Cytoxan, Day 3</td>
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<td>(Days 10 to 14)</td>
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<tr>
<td>LCM eye (20 mice)</td>
<td>0</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>Cytoxan, Day 3</td>
<td></td>
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<tr>
<td>Cells, Day 14</td>
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<td>(Days 6 to 10 after cells)</td>
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*Five of these animals died of Cytoxan toxicity.
Immunopathologic uveitis in mouse

Fig. 1. Acute disease seven days following intraocular LCM virus inoculation. Note the intensive inflammatory cell infiltration of the anterior uvea and cornea, with cells and proteinaceous exudate in the anterior chamber (Hematoxylin and eosin, ×100.)

Fig. 2. Posterior segment disease 10 days following LCM virus inoculation. There is extensive folding and disorganization of the outer layers of the retina in the absence of infiltrating inflammatory cells. The choroid, and to a lesser extent, the inner-nuclear layer are infiltrated by inflammatory cells. (Hematoxylin and eosin, ×150.)

inflammatory infiltrate was almost exclusively composed of lymphocytes and histiocytes in the uveal tract, with proteinaceous exudate and fibrin present in the anterior chamber.

On about the eighth to tenth day, the inflammatory infiltrate had extended to the posterior segment of the eye as well, invading the entire uvea, vitreous, and inner-nuclear layer of the retina. The outer layers of the retina did not show any cell infiltration but demonstrated extensive folding and disorganization (Fig. 2). Later than 11 days, all intraocular structures showed extensive destruction, with only the outer layers of the retina still devoid of inflammatory cells (Figs. 3A and B).

By immunofluorescent staining, the viral antigen could be demonstrated in the anterior segment of the eyes, in particular the iris and ciliary body, up to the sixth day. Except for one eye, none of the 40 eyes
Fig. 4. Localization of LCM viral antigen by direct fluorescein-labeled antibody staining in the Cytoxan-immunosuppressed chronic virus carrier. A, The bright areas in the ciliary body show the presence of much viral antigen. B, In the posterior segment, positive staining for viral antigen is seen scattered throughout, but mostly in the inner-nuclear layer, pigment epithelium, and choroid. (Fluorescent antibody stain, x280.)

showed intraocular antigen later than seven days after inoculation when the inflammatory manifestations were apparent.

The 10 control mice injected with normal brain suspension into the anterior chamber did not show any abnormal clinical or histopathologic findings.

**Immunosuppression.** Forty mice were immunosuppressed with intraperitoneal Cytoxan on the third day following intraocular virus inoculation. Five animals died of drug toxicity, the expected mortality rate.15 Fourteen mice (40 per cent of survivors) did not show any clinical or histologic findings, while 20 mice (60 per cent) showed only a mild anterior uveitis between the tenth to fourteenth day following inoculation. None of the suppressed animals presented the acute, violent inflammatory manifestation typical of the unsuppressed animals.

As revealed by immunofluorescent staining, the 14 mice that did not manifest any disease showed persisting viral antigen in the eye, brain, and other organs. In the eye the viral antigen was present throughout the entire uvea, pigment epithelium, and all retinal layers (Figs. 4A and B). Beginning on the sixteenth day, brain sections revealed antigen in the choroid plexus and some cerebellar cells. In addition, the glomeruli of the kidneys and Kupffer cells in the liver showed positive fluorescent staining.

The "breakthrough" disease which developed in 60 per cent of the Cytoxan-suppressed animals on about the tenth to sixteenth day was similar to, but milder than, that seen in unsuppressed control eyes. There was some cloudiness of the cornea, congestion of the iris vessels, flare, and development of posterior synechiae. Histologically, a mild round cell infiltration of the anterior uvea was seen, with cell aggregates attached to the endothelial side of the cornea and proteinaceous material in the anterior chamber. In the posterior segment, the vitreous and internal nuclear layer of the retina showed inflammatory cell infiltration, whereas the external nuclear layers showed intensive folding and disorganization with no cell infiltration. Immunofluorescent staining revealed virus in the eye up to the fourteenth day. Disappearance of virus coincided with the onset of inflammation.

**Adoptive immunization.** Twenty of the Cytoxan-induced persistently infected mice were adoptively immunized with sensitized spleen cells from 20 virus-immune syngeneic donors. The immune cells were transferred on the fourteenth day after the initial intraocular inoculation.

About six to eight days following cell transfer, 16 mice (80 per cent) developed a mild uveitis, which clinically and histologically could not be differentiated from the breakthrough disease in the immunosuppressed animals. Four mice (20 per
percent) of the chronic virus carriers did not manifest clinically apparent disease following adoptive immunization.

Discussion

The fatal choriomeningitis induced by intracerebral injection of LCM virus in adult mice is widely accepted as resulting from an immunopathologic process.\textsuperscript{15, 17, 18} This is supported by the observation that immunosuppression with cyclophosphamide protects the adult mouse against the pathologic consequences of LCM infection.\textsuperscript{15} In addition, intracerebral inoculation of the same virus into the newborn mouse, or vertical transmission of the virus from infected mother to fetus, confers on the animal the ability to sustain an essentially asymptomatic lifelong infection. The chronically infected carrier mouse is found to carry high levels of virus in the brain, blood, eye, and other tissues, but shows no detectable histopathologic changes in the central nervous system.\textsuperscript{16}

While no ocular involvement accompanies the CNS disease induced by intracerebral inoculation, direct introduction of the virus into the anterior chamber of the mouse eye leads to severe uveitis or panophthalmitis without CNS involvement. When the infected mouse is immunosuppressed with cyclophosphamide, the eye supports virus multiplication, resulting in a similar "carrier" state and no clinical or histopathologic findings.

Both the brain and the eye support virus infection and develop pathology due to the immune response, although each of the organs presents local pathology without involvement of the other organ. Thus, development of the disease seems to be related to the amount of virus present in each organ. It is of some interest that, following LCM virus infection of eye or brain and subsequent immunosuppression with cyclophosphamide, the incidence of "breakthrough" disease (i.e., escape from suppression) differs. Some 60 per cent of eye-infected animals go on to develop delayed uveitis, as compared with delayed choriomeningitis in only about 15 per cent of intracerebrally infected animals.\textsuperscript{15} This is presumably related to differences in the rate of systemic dissemination of the virus from these two organs.

Unlike the brain disease, the eye disease can be followed by clinical observation without the death of the host, and the mild iridocyclitis can be observed by routine eye examination.

An unexplained riddle in both the mild and severe forms of immunopathologic eye disease is the severe disorganization and distortion of the outer nuclear layers of the retina which occurs. These changes are not accompanied by the abundant inflammatory cell infiltrate of these tissues usually associated with an immunopathologic process. This finding is analogous to earlier observations in the newborn rat following intracerebral LCM virus inoculation.\textsuperscript{6} During the disease process of the rat eye, the virus has been demonstrated in the inner-nuclear layer of the retina, while it is the outer-nuclear layer which showed severe disorganization. Similarly, the cerebellar hypoplasia which also develops in these rats is accompanied by massive amounts of viral antigen in the affected tissues, with but minimal round-cell infiltration.\textsuperscript{19}

Further studies need to be done on the "adoptive immune" disease. The fourteenth day was selected for immune-cell transfer in order to eliminate false positive results due to the "breakthrough" disease which occurs spontaneously between the tenth to fourteenth day. It is possible that earlier adoptive cell transfer would cause a more severe disease, similar to the initial process, and that the incidence of this immune-mediated ocular pathology would approach 100 per cent. The nature of the cells which mediate this immunopathologic process can also be studied by transferring selected lymphoid cell (T- or B-cell) populations.

The complete clearance of the virus that accompanies the inflammatory disease in the eye is most striking. It would appear that the same mechanism which clears this otherwise nonpathogenic virus from in-
Infected tissues may also mediate their destruction. In this instance, the cure is the disease. Thus, LCM virus infection may provide an excellent model of the many forms of uveitis in which the etiologic agent cannot be detected once the inflammatory process is underway.

REFERENCES