Chronic retinitis in rats infected as neonates with lymphocytic choriomeningitis virus: a clinical, histopathologic, and electroretinographic study

Manuel del Cerro, Donald A. Grover, Andrew A. Monjan, Charles J. Pfau, and Jane E. Dematte

The long-term sequelae to infection of neonatal rats with lymphocytic choriomeningitis virus were studied by a variety of approaches, including indirect ophthalmoscopic, electroretinographic, and histopathologic methods. Data from these studies demonstrated that a progressive chronic retinitis develops after the acute, virus-specific, immune-mediated retinopathy. This chronic inflammation eventually leads to a total destruction of the retinal architecture. An autoimmune reaction against normally sequestered retinal antigens, released during the acute stage of necrotizing retinitis, is probably the initiating mechanism of the chronic disease. This experimental disease, triggered by infection with a relatively harmless virus, constitutes a very convenient animal model of chronic retinitis. (INVEST OPHTHALMOL VIS SCI 23:697-714, 1982.)

Key words: lymphocytic choriomeningitis, inflammation, retinitis, autoimmune disease, immunopathology, macrophages

Neonatal infection of rats with lymphocytic choriomeningitis virus (LCMV) induces an acute necrotizing immunoretinopathy that decimates the receptor population while clearing the retina of virus. We have found that after the acute stage, there is a chronic inflammatory process that leads to a severe destruction of remaining retinal tissue. A description of the clinical, electroretinographic, and histopathologic characteristics of this late persistent ocular disease, triggered by perinatal infection, constitutes the substance of this article.

Materials and methods

Animals and virus. Eighty albino (Holtzman Wistar) and 25 Long-Evans hooded rats were inoculated on the day of birth with the E-350 strain of LCMV (ATCC-VR 1271) and given individual numbers for identification. Virus was quantitated by a suspension plaque assay using BHK21/13S cells. Two virus solutions were used for intracranial injections (the vehicle being 30 μl of 0.9% saline). One was a 10-fold dilution of a crude 20% homogenized mouse brain stock titering 2 x 10^6 plaque forming units (PFU)/ml, while the other was partially purified virus from the same source titering 2 x 10^4 PFU/ml. Littermate animals similarly injected with vehicle alone, and uninjected
animals of the same age from intact litters, served as controls. All groups were studied during a period extending from 2 to 18 months after birth.

Ophthalmologic examination. Forty-eight experimental and control animals received one or more ophthalmologic examinations. Each animal was inspected for abnormalities of the cornea, anterior chamber, iris, and lens using the Ams Jena dissecting microscope at a magnification of 6.3X. Pupillary dilation was performed with topical 1% tropicamide and 2.5% phenylephrine ophthalmic drops. The external examination was repeated as well as examination of the lens. Five of the 48 animals of the same age from intact litters, served as controls. All groups were studied during a period extending from 2 to 18 months after birth.

**Table I. Number of animals per group**

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Group</th>
<th>LCMV-infected</th>
<th>Control animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>I</td>
<td>35</td>
<td>8</td>
</tr>
<tr>
<td>3-4</td>
<td>II</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>6-8</td>
<td>III</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>10-18</td>
<td>IV</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>76</td>
<td>30</td>
</tr>
</tbody>
</table>

The distribution of infected and control animals by age is shown in Table I.

Optical microscopic procedures. After predetermined survival times ranging from 2 to 15 months, the animals were anesthetized and their eyes were surgically removed and immediately immersed in a mixture of 2% paraformaldehyde and 2% glutaraldehyde in 0.1M sodium cacodylate with 4.5 mM CaCl$_2$; the pH ranged from 7.5 to 7.8. The osmolarity was approximately 900 mOsm/kg and the solution was used at room temperature. The eyes were slit under fixative and allowed to fix for approximately 48 hr at 4°C. At this point selected eyes were examined and photographed under a dissecting microscope to corroborate the ophthalmologic findings. The specimens were rinsed in 0.1M sodium cacodylate in 5% dextrose, postfixed in 2% osmium tetroxide for 2 hr, stained en bloc in aqueous 2% uranyl acetate, dehydrated with ethanol series, and embedded in Poly-Bed 812 resin (Polysciences, Inc., Warrington, Pa.). Sections for optical microscopy were cut at 1 to 0.4 μm and stained with Stevenel blue. They were surveyed to search for histopathologic changes and to select representative areas for further ultrastructural study.

Transmission electron microscopy (TEM). With the 1 μm thick sections as a guide, mesas were trimmed in the plastic blocks. Ultrathin sections containing the desired area of the retina were cut using a diamond knife; these sections were stained with lead citrate and studied with a Siemens electron microscope.

Scanning electron microscopy (SEM). Eyes fixed with aldehydes as for transmission microscopy were opened to remove the lens, dehydrated, and critically dried under Freon 13. The samples were mounted on a pedestal and coated with gold under high vacuum; observations were made under a Nanometrics HPS 50 field emission scanning electron microscope operating at 20 kV.

**Results**

**Fundus and external examination**

Control animals. Fourteen normal rats ranging in age from 2 to 11 months served as...
controls. In every case the external examination of the cornea, anterior chamber, iris, and lens was normal except for one 2-month-old animal whose right eye was 1.5 times normal size and very hard, presumably from some form of glaucoma. The fundus of this enlarged proptotic eye could not be examined. A second animal had bilateral segmental iridocorneal adhesions and leukomas. The remaining 25 control fundi were normal.

The normal optic nerve varied in color from slightly grayish-white to fairly pink. The retinal arteries and veins radiated from the center of the optic nerve like the spokes of a cartwheel (Fig. 1, A). The retinal vein-to-artery ratio was slightly less than 2:1. The large retinal vessels had a slight translucent halo running parallel to their course, which presumably represented the outermost edge of the vascular wall. The normal retina appeared extremely thick, with the greatest thickness located approximately one disc diameter peripheral to the optic nerve; it became slightly thinner as the far periphery was approached. In the albino rats the pigment epithelium was completely transparent, with a prominent lacework of choroidal vessels showing through.

Experimental animals. Thirty-four LCMV-infected rats between 2 and 10 months old
Fig. 2. ERG recordings. A, Normal and b-waves can be seen in the ERG of a 7-month-old control rat. B, Depressed waves and increased latency in a 6-month-old infected rat. This record represents the maximum response ever obtained from an animal in this age group. C, Lack of response, 8 months after infection. Same animal as in Fig. 1, D. Calibration bars, Perpendicular 0.1 mV, horizontal 0.2 sec.

were available for clinical examination. The animals were divided into two groups of 17 animals each. Animals in one group were examined and sacrificed at 2 months of age; those in the other group were examined and sacrificed at ages between 4 and 10 months. Many of these animals were examined repeatedly at different survival times and all shortly before being sacrificed. The following clinical descriptions deal with the final examination prior to histopathologic examination.

GROUP I (2 MONHTS OLD). The external examination of all eyes was normal, except for three eyes in two animals, which showed corneal leukomas. The fundi of 12 eyes were within normal limits. Ten eyes had varying degrees of a peculiar geographic translucent mottling underneath the retina, probably at the level of the pigment epithelium, as their only clinically apparent change. This mottling was best identified by moving the hand-held indirect lens back and forth so that the light could be seen to glisten off the surface of these translucent areas. In all instances, these retinas appeared to be of normal thickness and no specific abnormalities of the vascular tree or optic nerve could be identified. The remaining eyes showed various combinations of the following abnormalities: six eyes showed the above described translucency defects; six eyes showed varying degrees of vascular attenuation, tortuosity, and sheathing. Four eyes showed evidence of retinal detachment, but only one was totally detached. One of the eyes with a retinal detachment had clinically apparent cysts in the detached retina. Six eyes showed subretinal hemorrhages (Fig. 1, B), with some eyes having a single hemorrhage occupying anywhere from 50% to 75% of the subretinal space, while other eyes showed multiple smaller subretinal hemorrhages. Three eyes had evidence of subretinal exudates varying from brown to white that were thought to represent old blood in

### Table II. Summary of clinically identifiable lesions

<table>
<thead>
<tr>
<th>Time after infection</th>
<th>No.</th>
<th>% of Eyes</th>
<th>No.</th>
<th>% of Eyes</th>
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<tr>
<td>2 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corneal leukomas</td>
<td>3</td>
<td>8.8</td>
<td>4</td>
<td>11.8</td>
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<tr>
<td>Iridocorneal adhesions</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>8.8</td>
</tr>
<tr>
<td>Iris vascular loops</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Normal</td>
<td>31</td>
<td>91.2</td>
<td>29</td>
<td>85.3</td>
</tr>
<tr>
<td>Fundus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mottling of PE</td>
<td>10</td>
<td>29.4</td>
<td>1</td>
<td>2.9</td>
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<tr>
<td>Vascular attenuation, tortuosity, or sheathing</td>
<td>6</td>
<td>17.6</td>
<td>17</td>
<td>50.0</td>
</tr>
<tr>
<td>Retinal detachment</td>
<td>4</td>
<td>11.8</td>
<td>3</td>
<td>8.8</td>
</tr>
<tr>
<td>Subretinal hemorrhage</td>
<td>6</td>
<td>17.6</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Subretinal exudates</td>
<td>3</td>
<td>8.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cysts</td>
<td>1</td>
<td>2.9</td>
<td>2</td>
<td>5.9</td>
</tr>
<tr>
<td>Thinning of retina</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>20.6</td>
</tr>
<tr>
<td>Normal</td>
<td>12</td>
<td>35.3</td>
<td>16</td>
<td>47.1</td>
</tr>
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</table>
various stages of reabsorption; at least two eyes showed unequivocal intravitreal hemorrhages (Fig. 1, C).

Group II (4 to 10 months old). The external examination was normal, except for five eyes. Three eyes showed iridocorneal adhesions with associated corneal leukomas. A fourth eye showed only a corneal leukoma, and the fifth showed iris vascular loops at the pupillary border. Sixteen of the retinas appeared normal, with one having nasal dragging of the vessels. This was thought to be a congenital anomaly, since it was also present shortly after birth and remained unchanged throughout the follow-up. Of the 18 remaining eyes, 14 showed vascular attenuation, while only one showed vascular tortuosity and two had vascular sheathing. The next most common finding was thinning of the retina in seven eyes. Three eyes had retinal detachments, two of which had clinically visible cystic degeneration (Fig. 1, D). Only one eye had any residual hemorrhage and only one eye had evidence of the translucency defects so readily identified in the 2-month-old animals. No subretinal exudates were seen in this group; however, one animal had some fluffy white retrolental exudates that limited the view of the posterior pole.

The data from both groups are summarized in Table II.

Electroretinographic observations. Comparison of the ERGs obtained from LCMV-infected and normal rats demonstrated a marked difference in retinal electrical activity, which became more pronounced as the animals aged. In the control animals there was a full response showing distinct a- and b-waves (Fig. 2, A). In contrast, response in the infected rats was reduced or entirely absent (Fig. 2, B and C). In 2-month-old animals most eyes were still responsive, although none had a totally normal response. Some animals had one totally unresponsive eye, while small a- and b-waves were still present in the contralateral eye. In these cases the latency between the a- and b-waves, as well as their amplitudes, were abnormal. The number of unresponsive eyes increased with time, and no response could be elicited from the eyes of animals older than 6 months.

Histopathologic findings. Specimens from 106 rats were studied microscopically; the animals were divided in groups by age, as described in Table I.

Group I (2 months old). The retinas from control animals (Fig. 3) showed the normal characteristics of the mature mammalian retina. In sharp contrast to this, the retinas of LCMV-inoculated rats presented exten-
Fig. 5. Prominent peroxidase-positive granules populate the cytoplasm of pigment epithelial cells (P) and macrophages (M) of this 6-month-old animal, which exhibited an extensive retinal detachment and had three layers of PE. Prepared by the method of del Cerro et al.\textsuperscript{13}, Choroid. (Bar = 10 \textmu m.)

Degenerative but unevenly distributed lesions. The degree of damage varied to some extent from animal to animal; differences in the severity of lesions were also commonly present between the eyes of any given animal. A description of the more common abnormalities seen in different layers follows.

Pigment epithelium (PE) cells were found to be hypertrophic and pale staining, atrophic and dark, or near normal; sometimes these three situations coexisted. Many PE cells had lucent vacuoles (Fig. 4) or large granules that stained heavily with Stevenel blue; the latter correlated with the residual bodies observed with the electron microscope in this material. Peroxidase-positive granules, demonstrated by histochemical reactions,\textsuperscript{13} were prominent in the PE cell at all stages of the disease (Fig. 5). The basal aspect of the PE cells often became irregular, with invaginations filled with material from the basal lamina. Irregularly enlarged intercellular spaces occurred between PE cells; these spaces were filled with material with the same tinctorial properties of the basal lamina, of which they seem to be an abnormal extension.

Macrophages invaded all retinal layers and unquestionably were the most abundant inflammatory cell. In normal animals macrophages are rarely found at the PE-photoreceptor interface; large numbers of them appeared at this location in infected animals, particularly when the retina was detached. As judged by morphologic criteria, retinal macrophages were very active at this stage of the disease. The cells exhibited numerous pseudopods\textsuperscript{*} and abundant cytoplasm loaded with debris, e.g., cell nuclei and red cells (Fig. 4), which were often present in the subretinal space as remnants of early hemorrhages. Peroxidase-positive granules, similar to those seen in the PE, are also found in the cytoplasm of macrophages (Fig. 5).

Photoreceptor cells were absent over extended areas; other areas contained a few rod cells as the only remnant of the outer nuclear layer (Fig. 6). Initially, the outer segments of affected rod cells became misshapen and then disappeared. Degeneration continued, with the inner segments becoming thick and deformed (Fig. 6), and ended with the obliteration of the cell somas. The latter occurred either by necrosis in situ (Figs. 7 and 9) or by extrusion of the perikaryon into the subretinal space (Figs. 7 and 8). This phenomenon of cell extrusion, first observed in normal animals by Lai,\textsuperscript{15} is a very prominent feature in the LCMV-induced retinopathy; in fact, it appeared to be the key factor in the destruction of the outer retina. In those areas where the outer nuclear layer remained thick, abnormalities such as dysplasia of rod cells, folds, and rosette formation (Fig. 7) were seen. The rosettes usually contained macrophages with ingested fragments of outer segments. The inner nuclear layer of LCMV-infected animals appeared thinner than that of control animals; pyknosis and cytoplasmic swelling were visible in many cells within this layer. Streams of dysplastic rod cells invaded the inner nuclear layer (Fig. 7) as well as the inner plexiform layer, which showed some wandering macrophages, occasional plasmacytes, and often perivascular cuffing. The ganglion cell layer presented a similar cellular infiltration; the density of ganglion cells appeared di-

\textsuperscript{*}We used the word "pseudopod" to refer to all cytoplasmic projections observed under optical and transmission electron microscopes. SEM permits differentiation of these projections into various types, as described by Craft et al.\textsuperscript{14}
Fig. 6. TEM photograph of outer layers of near peripheral retina (70-day-old rat). The outer nuclear layer is limited to a single discontinuous row of cell nuclei (RN); these lay parallel to, rather than perpendicular to, the PE (P). The receptor layer is reduced to a few degenerating elements (IS); in the intervening areas proliferated Müller cell processes (MU) confront pigment epithelial microvilli (PD). Portions of an attenuated outer plexiform layer (OP) and of the inner nuclear layer (IN) can be seen. (Bar = 1 μm.)
Fig. 7. Detached and very dysplastic retina (69-day-old rat). The photoreceptor layer is absent, except for a few surviving elements (arrowheads). Degenerating outer and inner segments do occur within rosettes (R). Rod nuclei are being extruded at the right side of the picture (RN). There is extensive cell degeneration and a cyst (asterisk); also shown is the extensive mixing of the inner and outer nuclear layers. IP, Inner plexiform layer; V, vitreal surface. (Bar = 20 μm.)

minished, but at this stage the change was too subtle to be accepted without formal quantification.

The surface of the inner limiting membrane was often populated by macrophages (Fig. 10). Since these cells are particularly scarce at this location in normal animals, the presence of even low numbers of macrophages at this locus becomes a sensitive indicator of inflammation. In addition to macrophages, extensive deposits of fibrillar material, probably fibrin, covered the inner limiting membrane of the LCMV-infected animals (Fig. 10). These deposits may be a substratum for the formation of the vitreal membranes. Small cysts appeared in the retina at this stage in all the layers, but predominantly at the outer nuclear and ganglion cell layers (Figs. 9, 11, 12, and 14); they formed after the lysis of cell somas.

The intensity of extraretinal reaction was modest. Minimal choroiditis occurred in many rats. Mild iridocyclitis was found often, while iridocorneal synechia were a sporadic finding.

Group II (4 to 6 months old). The eyes of control rats in this group did not differ from those in group I. In contrast, the damage to retinal structure in the LCMV-inoculated rats was definitely more pronounced than that in animals of group I. The PE cells showed irregularities in thickness, shape, and accumulation of phagosomes. Areas lacking outer nuclear layer were very extensive; in those places where rod cells were still present, any vestiges of outer and inner segments were rare. The inner nuclear layer had become thinner, with an apparent indiscriminate destruction of all the various cell types in it. Macrophages loaded with phagosomes and having a considerable number of pseudopods were visible throughout the entire thickness of the affected neuroretina. The ganglion cell layer contained infiltrating plasmacytes and macrophages; vascular cuffing was common. Macrophages on the vitreal surface and in the subretinal space remained numerous, and apparently active as judged by morphologic criteria. SEM most clearly revealed the extent and distribution of macrophagic infiltration on
the vitreal surface (Fig. 10) or on the exposed PE and neural surface of detached retinas. Intraretinal cysts, such as those observed in the rats of group I, persisted in this stage and indeed were a feature of all ages studied. No signs of fresh hemorrhages were found in this group. Areas loaded with brownish dustlike pigments remained as sequelae of old hemorrhages; some of this pigment appeared in the cytoplasm of macrophages.

Group III (6 to 8 months old). The degree of retinal damage continued to increase in the LCMV animals of this age group. The PE cells were clearly abnormal in many areas; they had numerous phagosomes and hypertrophic apical microvilli, as well as microvilli on their lateral borders (Fig. 14). Duplication of the layer was not uncommon, neither was vascularization by capillaries (Fig. 13). No intact photoreceptor cells have been seen in preparations from these animals. Only a narrow acellular band separated the PE from the inner nuclear layer. This zone can be recognized under the electron microscope as a reminder of the outer plexiform layer; it was populated by a reduced number of neurites and abundant Müller cell processes (Fig. 11); apparently both the Müller cells and the PE villi overdevelop to compensate, in part, for the loss of substance undergone by the neural retina. The inner nuclear layer was mostly reduced to a two-cell thickness; it moved into the space vacated by the outer layers, thus confronting the PE. Cysts were present in variable numbers (Figs. 12 and 14), sometimes forming small clusters, with each cyst separated from the next by a narrow septum. The profile of the small cysts was more or less rounded, but the bigger ones tended to be irregular as if they had partially collapsed. There were plasmacytes infiltrating the retina, with a clear preference for perivascular...
Fig. 9. Extensive intermingling of the outer and inner nuclear layers (N) results in obliteration of the outer plexiform layer; there is total degeneration of photoreceptors, so that the rod cell somas contact the PE. A cyst (asterisk) is present in the outer neural retina (69-day-old rat). C, Choroid; P, PE; IP, inner plexiform layer. (Bar = 20 μm.)

location. The basal laminae of arterioles, capillaries, and venules showed considerable thickening (Fig. 13), which was unrelated to perivascular cuffing. The ganglion cell population was reduced. Macrophages also occurred on the inner limiting membrane and in the subretinal spaces of the eyes of this group. The nature of the macrophage population, however, differed from that seen in younger infected animals. There were some "active" cells, as judged by the presence of pseudopods on their surface, abundant cytoplasm, and phagocytic particles, but there were also "quiescent" macrophages that were avoid, lacking pseudopods, and containing limited amounts of cytoplasm. Collagen-containing membranes populated by mesenchymal elements, pigment epithelial cells, and capillaries covered vast portions of the vitreal surface of the retina (Figs. 12 and 14, inset).

Group IV (10 to 18 months old). The retinas of control rats in this group remained within normal limits, except for a small accumulation of lipofuchsin in the ganglion cells. In dramatic contrast, the retinas of LCMV-inoculated rats studied at this late state had practically nothing left of the normal histoarchitecture. The PE exhibited a number of pathologic features, including vacuolization, capillary invasion, and doubling (Figs. 13 and 14). It appeared that some PE cells detached from their neighbors and became free macrophages (Fig. 15). At this stage, the neural retina was a thin layer of inflammatory tissue with a few scattered surviving neurons, probably ganglion cells, and debris-laden macrophages.

Cysts, large and small, appeared to be in larger numbers than before, probably the result of the considerable reduction in volume of the retina. The vitreal surface of the retina was covered by vascularized membranes (Fig. 14, inset). Macrophages exhibiting various degrees of activity were still present, indicating ongoing inflammation. Collagen and amorphous material accumulated between Bruch's membrane and the basal aspect of the PE.

Table III summarizes the histopathologic findings.

Discussion

Infection of newborn rats with LCMV at birth consistently produces acute retinal lesions. Histologic indications of inflammation appear as soon as 8 to 10 days after inoculation, with the disease rapidly progressing into an immune-mediated acute retinitis characterized by viral proliferation, subretinal hemorrhages, and extended degeneration of photoreceptors.1-4 This condition slowly evolves into the chronic stage described in this article; since the transition is gradual, the chronologic separation of one stage from the other requires the selection of a somewhat arbitrary point. We have chosen the end of the eighth postnatal (postinfection) week as such a point based on two facts: first, no new hemorrhages appear in the infected eyes after this time, and second, histoimmunologic data indicate that at this age demonstrable viral antigen has cleared from the retina.1

All the converging approaches used in this study confirm this evolution from an acute retinitis to a chronic state. Clinically apparent is the onset of a chorioretinoviritis with vitreous clumps and opacities, serous retinal detachment, vascular attenuation, and vitreal,
intraretinal, and subretinal hemorrhages as well as subretinal exudates. With time, these anomalies tend to clear and the fundus may resume either a rather normal appearance, an atrophic appearance, or show a chronic traction detachment.

The ERG recordings also indicate a progressive deterioration of function. Although no infected animal gave a perfect ERG response at any time during this study, it was clear that as time elapsed, a larger number of animals showed greatly diminished or absent response. The response was absent in all of the infected animals from the oldest age groups. Comparison of data showed that histologic and functional damage progress on parallel courses.

The chronic period is characterized by a relentless deterioration of the retinal histarchitectue. A clear outside-in progression of tissue destruction from photoreceptors to ganglion cells is evident throughout the entire process. Various mechanisms may interplay to produce the tissue damage, and their relative contribution is likely to be different at different times and locations. In the case of the photoreceptor cells, for example, extrusion of the cell bodies into the subretinal space accounts for the destruction of as many or even more cells than in situ cytolysis. On the other hand, in situ cytolysis appears to be responsible for the protracted but steady decimation of cells in the inner nuclear and ganglion cell layers. Although the causes for cell lysis re-
Fig. 11. TEM photograph of a retina in a group III animal (8 months old). There are no traces of the photoreceptors, outer limitans, or outer nuclear layers. The PE cells (P) contain large numbers of inclusion bodies; their apical microvilli (PD) interdigitate with Müller cell processes (MU). The outer plexiform layer (OP) is rudimentary. The margins of some cysts (asterisks) can be observed at the top of the figure. (Bar = 1 μm.)
main unknown, it is reasonable to assume that it may be initiated or exacerbated by the invasion of the retina by macrophages.

The presence of high numbers of macrophages constitutes one of the most constant histopathologic features of all stages of the LCMV retinal disease. Parenthetically, it should be mentioned that "exuberant" macrophage infiltration has been seen by electron microscopy in a human eye suffering from a postsurgical immune reaction. These macrophages are probably playing a dual role; that is, they may be both killer elements and scavengers. The lytic capabilities of macrophages are well known and have been the object of several recent reviews. The fact that retinal invasion by macrophages precedes the appearance of extensive cell necrosis and that their continuous presence in large numbers throughout the inflammatory process provide circumstantial evidence of their lytic role. If this turns out to be the case, chronic LCMV retinitis would be a macrophage-mediated, or assisted, retinopathy. The attraction of this hypothesis lies in the fact that it can be tested experimentally by procedures that interfere with macrophage activity. A scavenger function of the macrophages is indicated by the presence, within their cytoplasm, of ingested debris such as red cells and pigment granules. They also ingest rod outer segments, a fact particularly noticeable in those macrophages located at the center of photoreceptor rosettes. The ciliary body appears to be the main source of macrophages in the chronic LCMV-induced retinopathy. From there, macrophages follow a migration pattern that takes them through the vitreous body and then progressively deeper into the retina, a pattern similar to that described for the phagocytes that invade the light-damaged retina. The extracellular virus spread is limited by the secretion of specific anti-LCMV antibody by these cells, leading to clearance of virus. The mononuclear cells observed early in the disease are presumably T lymphocytes that have become sensitized to viral antigens and then enlist the macrophages as the agents of cellular destruction. The role of T cells has been previously demonstrated by neonatal thymectomy or treatment with antilymphocyte serum, which halts the necrotizing progression of acute infection.

The lesions occurring in the chronic LCMV-induced retinopathy have a number of features in common with those seen in other eye diseases. For instance, the membranes formed in LCMV retinitis mimic closely the morphology of those seen in massive periretinal proliferation, which follows retinal detachment in the human. Cytomegalic inclusion disease is associated with hemorrhages, vitreous precipitates, and whitish granular patches, which suggest massive retinal necrosis with ultimate zones of retinal atrophy. Influenza epidemics have been accompanied by outbreaks of uveitis. These are usually self-limited bilateral iridocyclitis or neuroretinitis occurring during convalescence, but they can become chronic with exacerbations and remissions. In such instances, the virus may have combined with extracellular virus spread.
Fig. 13. Vascularization of the PE occurs in the later phases of the retinitis (6-month-old rat). A capillary (CA) is lined by endothelial cells having some fenestrae (arrowheads), many pinocytotic vesicles, and two erythrocytes (E) within the lumen. The basal lamina (BL) is thick and contains cytoplasmic processes and amorphous deposits. The cytoplasm of the pigment epithelial cell (P) is densely packed with organelles. The apical microvilli (PD) float freely in the subretinal space, since the retina is detached. (Bar = 1 μm.)
Fig. 14. TEM photograph of a triple layer of PE (P) in a nondetached retina (548-day-old rat). The cells have digitations, microvilli (PD), both on their apical and lateral aspects. The cytoplasm of a Müller cell (MU) borders a microcyst (asterisk). (Bar = 2 μm.) Inset (14A), Double and triple layers of PE cells (P), macrophages (white arrows), and cysts (asterisk) are all that is left of this retina (560-day-old rat). Pools of amorphous material (black arrow) separate clusters of PE cells. A preretinal membrane (PM) covers what is left of retinal tissue. C, Choroid. (Bar = 20 μm.)
Fig. 15. Detached pigment epithelial cell (FP) projects elaborate cytoplasmic digitations into the subretinal space (SS) (300-day-old rat). The free cell has similar electron density as the underlying normally located, epithelial cell; both contain premelanosomes (arrowheads). (Bar = 1 µm.)
the uveal tissue, rendered the tissue antigenic to itself, setting the stage for an autoimmune type uveitis.

The cause of the relentless chronic retinitis in rats neonatally infected with LCMV deserves speculation. It occurs in the apparent absence of viral antigens, as indicated by negative immunohistofluorescence tests. This could indicate two possible situations; either there is still virus in the retina but at such a low concentration that the fluorescent antibody test cannot reveal it, or the inflammation is sustained by the action of other antigen(s) after the virus has been cleared by the acute immune response. At present, the first alternative has no experimental support; the second can be entertained on the basis of comparative findings in other forms of ocular disease. In presumed ocular histoplasmosis, for example, chorioretinal lesions occur in individuals who exhibit evidence of previous infection with histoplasma capsulatum, but identification of the fungus in the retinal lesion has not been documented. Immune involvement has been shown in several diseases where there is photoreceptor degeneration and it is also known that uveoretinitis can be elicited by injection of retinal preparations into experimental animals. Thus it is conceivable that the release of normally sequestered antigens, including the highly immunogenic S antigen, must occur during the destructive acute inflammatory phase and may autosensitize the animal and perpetuate the inflammatory status of the eye. A similar mechanism has been postulated as a cause of some forms of human uveitis by Nussenblatt et al.

In the present study, we have delineated an experimental model of chronic inflammatory retinitis initiated by a neonatal viral infection and detailed its pathogenesis by utilizing clinical, electroretinographic, and histopathologic observations. In contrast to the acute LCMV-induced disease, in which the virus-infected cells are the targets, the chronic stage could be an autoimmune disease with a predominantly macrophagic effector arm responding to the normally sequestered antigens exposed during the destruction of cells carrying the LCMV-induced neoantigens.

<table>
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<th>Table III. Histopathologic findings</th>
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This is, then, an example of an acute intraocular viral infection with a strong immunopathogenic component followed by a chronic, and very likely, autoimmune process. This experimental disease, originating from an aberrant immune response to a relatively harmless virus, offers a most convenient model for clinicopathologic studies of chronic retinitis.

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REFERENCES
4. Monjan AA and Hogan RN: Virus-induced retinal


