Immunopathogenesis of LCM virus-induced uveitis: the role of T lymphocytes.

Uriel Ticho,* Arthur M. Silverstein, and Gerald A. Cole.

The immunopathologic uveitis and panophthalmitis produced by intraocular injection of lymphocytic choriomeningitis virus in mice can be abrogated by immunosuppression with Cytoxan. Ocular inflammation is triggered in the resulting chronic virus carriers following adoptive transfer of immune spleen cells. This report demonstrates that destruction of the thymus-dependent (T cell) class of lymphocytes in the transferred cell population, by treatment with anti-δ antibody and complement, prevents the induction of ocular pathology. The residual cells do form humoral antibody and mediate a degree of virus clearance in the host, although without evidence of disease. Thus, this model of virus-induced immunopathology is clearly based upon a mechanism of cellular immunity.

We showed in an earlier communication that immunopathologic uveitis and panophthalmitis result from intraocular inoculation of adult BALB/c mice with lymphocytic choriomeningitis (LCM) virus. The immunologic basis of this disease is supported by the observation that infected mice adequately immunosuppressed with cyclophosphamide failed to manifest any disease, but rather become persistent virus carriers. However, adoptive immunization of immunosuppressed virus carriers with sensitized lymphocytes from immunized syngeneic donors did result in the development of uveitis. Each of these observations on LCM virus-induced ocular disease has its counterpart in the well-established immunopathologic acute choriomeningitis of the mouse initiated by intracerebral injection of virus.1-3

The existence of subpopulations of lymphocytes subserving different roles in the immune response is now well established.4 Cell-mediated immunity, associated with such immunologic reactions as delayed hypersensitivity and allograft rejection, is a property of the thymus-dependent population of lymphocytes (T cells). On the other hand, bone marrow-derived lymphocytes (B cells) are responsible for the production of immunoglobulins and humoral immunity. With the demonstration that choriomeningitis in the mouse is mediated by T cells characterized by their possession of a surface membrane antigen designated as δ; it appeared desirable to show that the analogous LCM virus-induced ocular disease was also based upon a mechanism of cellular immunity.

Adult BALB/c mice were inoculated in the anterior chamber with 3,000 intracerebral LD₅₀ of E 350 strain of LCM virus in 0.01 ml, as previously described.1 Three days after injection, the mice were immunosuppressed by intraperitoneal administration of 175 mg. of cyclophosphamide (Cytoxan, Mead Johnson, Evansville, Ind.) per kilogram of body weight. The mice were observed daily for two weeks after infection, and only those mice showing no signs of ocular disease (i.e., those animals in which immunosuppression had been adequate) were employed in subsequent experiments. Some 40 to 50 per cent of these mice became persistent virus carriers.

Immune spleen cells for adoptive transfer were obtained from donor BALB/c mice ten days after the last of two weekly intraperitoneal injections of 0.5 ml. of a 1:100 dilution of LCM virus-infected mouse brain suspension (10⁴LD₅₀). Cells were teased from the pooled spleens of a group of immunized animals, and red cells in the resulting suspension hemolyzed by treatment with 0.83 per cent NH₄Cl. One-half of the resulting washed suspension, adjusted to contain 10⁸ cells per milliliter, was then treated with heterologous anti-δ serum and complement, under conditions described by Cole, Nathanson, and Prendergast.5 Anti-δ serum was prepared in rabbits by immunization with BALB/c mouse brain suspension according to the method of Golub.6 This treatment was found to be 98 to 100 per cent cytotoxic for thymocytes and 30 to 35 per cent cytotoxic for spleen cells. Substitution of similarly prepared normal rabbit serum for the anti-δ serum resulted in only 5 to 10 per cent toxicity for both splenic and thymic lymphocytes.

Two groups of 10 immunosuppressed virus-carrier mice were employed as recipients for the adoptive immunization. The control group (A) received, intraperitoneally, 1 ml. of immune spleen cells treated with normal rabbit serum, while the test group (B) were given 1 ml. of spleen cell suspension depleted of T cells by anti-δ treatment. Passive transfer of lymphocytes was done in both groups on the fourteenth day after intraocular inoculation with virus.

Clinical and histologic examinations for ocular disease in these animals were performed as previously described,1 as was immunofluorescent staining for the presence of viral antigen in ocular tissues. Circulating virus-specific antibodies were assayed by the indirect immunofluorescent technique using blood obtained from the retrobulbar venous plexus 30 days after the adoptive transfer of lymphoid cells. The source of viral antigen was
uveitis with histologic evidence of retinal disorganization, as was described previously. In contrast, carrier mice adoptively transferred with immune spleen cells which had been depleted of the T-cell component (group B) showed neither clinical nor histologic signs of disease. However, transfer of immune cells depleted of the T-lymphocyte component was not completely without effect, since 30 days later only three of five eyes and one of six brains examined contained demonstrable amounts of viral antigen by direct immunofluorescent staining. Since these lymphocyte preparations still contained normal numbers of B cells, this relative decrease of viral antigen might be related to active formation of circulating antibodies in the recipient host. Indeed, earlier studies of similar adoptive transfer experiments in cytotoxic-induced virus carriers infected intracerebrally showed all animals to have complement-fixing antibody at about two weeks after adoptive transfer. In the present study, two out of eight animals were found to have circulating antibody 30 days after transfer, as assessed by indirect immunofluorescent staining.

In summary, these experiments demonstrate that while adoptive transfer of immune spleen cells to mice that are persistent "ocular" carriers of LCM virus leads to the development of immunopathologic eye disease, the thymus-dependent class of lymphocytes is a necessary component of the reaction. Ablation of T cells in the transferred cell population, using anti-θ serum plus complement, prevents the induction of eye pathology. The transferred B lymphocytes which remain after anti-θ treatment do produce antibody and a degree of viral clearance in the recipient host, although without evidence of disease. Thus, lymphocytic choriomeningitis virus-induced eye disease is clearly based upon a mechanism of cellular immunity.

From the Wilmer Institute and School of Hygiene and Public Health, Johns Hopkins Medical Institutions, Baltimore, Md. This work was supported in part by United States Public Health Service Research Grants EY 00279 and AI 09401, Research Career Development Award NS 46242 (GAC), and an Independent Order of Odd Fellows Research Professorship (AMS). Submitted for publication Nov. 26, 1973. Reprint requests: Dr. A. M. Silverstein, The Wilmer Institute, Johns Hopkins Medical School, 601 N. Broadway, Baltimore, Md. 21205. *Dr. Ticho's present address: Hadassah University Hospital, Jerusalem, Israel.

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

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Table I. Production of ocular disease after adoptive transfer of intact or T-cell-depleted immune lymphocytes to LCM virus carrier mice

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<th>Eye disease</th>
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<td>10⁶ immune spleen cells treated with normal rabbit serum</td>
<td>10/10 8 to 10 days following cell transfer</td>
<td>Negative</td>
<td>Not done</td>
</tr>
<tr>
<td>B</td>
<td>10⁶ immune spleen cells treated with anti-θ + complement</td>
<td>0/91 followed for 30 days after cell transfer</td>
<td>Eye +% Brain +4%</td>
<td>2/8</td>
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*Recipient mice were infected intraocularly with LCM virus, immunosuppressed with Cytoxan on Day 3, and adoptively immunized on Day 14.

†One animal died of nonspecific causes.

methanol-fixed virus-infected L cells, previously grown on microscope slides. Twofold dilutions of serum were placed on the methanol-fixed infected cells and incubated in a humid chamber for 45 minutes using uninfected L-cell cultures as controls. After washing with normal saline, the slides were overlaid for 30 minutes with a fluorescein-labeled goat antimouse immunoglobulin antiserum (Meloy Laboratories), using Evans blue as a counterstain. The slides were then washed in saline and mounted in buffered glycerine.

The results of these experiments are summarized in Table I. All of the mice in control group A, which received immune spleen cells treated with normal rabbit serum, developed clinically apparent uveitis with histologic evidence of retinal disorganization, as described previously. In contrast, carrier mice adoptively transferred with immune spleen cells which had been depleted of the T-cell component (group B) showed neither clinical nor histologic signs of disease. However, transfer of immune cells depleted of the T-lymphocyte component was not completely without effect, since 30 days later only three of five eyes and one of six brains examined contained demonstrable amounts of viral antigen by direct immunofluorescent staining. Since these lymphocyte preparations still contained normal numbers of B cells, this relative decrease of viral antigen might be related to active formation of circulating antibodies in the recipient host. Indeed, earlier studies of similar adoptive transfer experiments in cytotoxic-induced virus carriers infected intracerebrally showed all animals to have complement-fixing antibody at about two weeks after adoptive transfer. In the present study, two out of eight animals were found to have circulating antibody 30 days after transfer, as assessed by indirect immunofluorescent staining.

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Pathology of hemeralopia in the Alaskan malamute dog. G. D. AGUIRRE AND L. F. RUBIN.

A strain of Alaskan malamute dogs has been found to have day blindness (hemeralopia), a genetic anomaly transmitted as a simple autosomal recessive trait. The diagnosis was based on the absence of ophthalmoscopically visible abnormalities and the presence of complete blindness during daylight with return of visual function in dim light. These findings remained unchanged throughout the animal’s life.

In hemeralopic dogs, the cone branch of the flicker fusion response curve was absent. Flicker electroretinography (ERG) with high-intensity stimuli failed to elicit responses when the frequency of stimulation was over 10 Hz. In contrast, nonaffected Alaskan malamutes responded to stimulus frequencies above 75 Hz. The single-flash ERG appeared normal in affected dogs.

Hemeralopia was often present by eight weeks of age. In affected dogs, no abnormalities were found in routine histologic examination, probably because of the difficulty in identifying individual rod and cone photoreceptors in conventionally prepared material. However, a statistically significant reduction in the number of cone nuclei has been found in the retina of adult hemeralopic dogs.

The purpose of this paper is to report the structural abnormalities present in the retina of hemeralopic dogs.

Methods. Five hemeralopic Alaskan malamute dogs, the progeny of affected to affected matings, were studied. Hemeralopia was determined on the basis of complete day blindness and normal vision in dim light. In all dogs, the ERG demonstrated complete absence of cone function and normal rod function.

One dog was seven weeks old, one dog was six months old, one dog was two years old, and two dogs were four-year-old littermates. Sections of age-matched nonhemeralopic control dogs were taken for comparison with the five weeks, six months, and adult hemeralopic dogs. Neither control nor hemeralopic dogs had evidence of concurrent ocular abnormalities.

The dogs were anesthetized with pentobarbital given intravenously and the globes were surgically enucleated. The eyecup was isolated by a frontal cut at the equator with a razor blade and immersed in cold fixative. In four dogs, the eyecup was fixed in 2 per cent osmium tetroxide buffered to pH 7.4 with Veronal acetate. In the seven-week-old dog, the eyecup was fixed in 2.5 per cent glutaraldehyde in 0.0645 M cacodylate buffer and postfixed in osmium tetroxide. All retinas were then dehydrated through ascending concentrations of ethanol and embedded in an epoxy resin.

Results. No retinal abnormalities were found in one micron thick sections of the seven-week-old pup. However, electronmicroscopic examination revealed lesions limited to the cones. Although the majority of the cone outer segments were normal, some had disorganization of the lamellar discs (Fig. 1, A). In some cone inner segments there were accumulations of bundles containing nonbranching filaments. These were found primarily in the cytoplasm vitread to the ellipsoid region (Figs. 1, B and C). In a few cells, filaments were seen in the perinuclear cytoplasm, although this finding was relatively rare (Fig. 1, D). The filaments were approximately 100 Å in diameter and had alternating dark and light bands with a 75 Å periodicity between dark bands (Fig. 1, C). The filaments, though tightly packed, did not appear to be arranged in parallel fashion, and occasionally intertwined with each other. In no instances were the cytoplasmic filaments associated with the ciliary rootlet of the cone inner segment. The filament-containing bundles occupied only a small part of the cone cytoplasm. There was an increased density of polyribosomal aggregates usually surrounding the bundles. No other abnormality was found in the cones. The rods were normal and, with the exception of the ciliary rootlet, lacked the prominent filament bundles present in cones. All other retinal structures were normal.

In the age-matched control dogs, no abnormality was found.

In the six-month-old hemeralopic Alaskan malamute, abnormalities were again limited to the cones. There appeared to be no diminution in the number of cones, but all cones examined were abnormal. Cone outer-segment material was sparse, and when recognizable, was composed of highly disoriented lamellae.

A few inner segments were reduced to ½ normal size; the remainder appeared normal. Occasionally cone nuclei were displaced into the inner segment. In many of these nuclei a bundle of filaments was attached to the outer nuclear envelope. Bundles of filaments were found primarily in the perinuclear cytoplasm rather than in the cone inner segment, and were considerably larger both in the size of the bundle and in the number of filaments contained in a bundle. The number of aggregates of polyribosomes around the bundles were greater than those seen in the seven-week-