Immunohistopathologic Findings in Herpes Simplex Virus Chorioretinitis in the Von Szily Model

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After anterior chamber inoculation of herpes simplex virus type 1 (HSV-1), some mice have a characteristic pattern of ocular disease, including ipsilateral anterior uveitis, relative sparing of the ipsilateral retina, and necrotizing contralateral chorioretinitis. It was reported previously that susceptibility to the contralateral chorioretinitis is associated with the Igh-1 locus; congenic mice differing at this locus have different rates of contralateral disease. The immunohistopathologic findings of this model in Igh-1-disparate congenic mice are reported after examining immune cell populations (CD4, CD8, Thy 1.2, Ia, Mac, and immunoglobulin G cells) in both ipsilateral and contralateral eyes and the recruitment kinetics of these cell types in various ocular tissues. In both HSV-susceptible BALB/c and HSV-resistant C.B-17 mice, the ipsilateral eye undergoes early cellular infiltration, and the contralateral retina is devoid of cells until day 10 postinoculation. In BALB/c mice, a late dramatic rise develops in Mac and Ia cells in the ipsilateral and contralateral choroid (P < 0.005) compared with C.B-17 mice. The C.B-17 mice have an earlier, mild cellular infiltration of the uveal tract in the ipsilateral eye, abrogation of the late Mac and Ia cellular recruitment in the ipsilateral choroid, and an absent contralateral response. These strain-specific immunohistopathologic differences help to explain Igh-1-linked HSV retinitis patterns in this model. Invest Ophthalmol Vis Sci 33:68–77, 1992

The importance of the host immune response in controlling ocular herpetic disease is shown by the high incidence of herpes keratitis, uveitis, and retinitis in immunosuppressed patients (eg, patients with acquired immune deficiency syndrome and transplant recipients).1–3 The von Szily model of herpes chorioretinitis affords an opportunity to define the role of host immunologic parameters in herpetic retinal infection. In this model, inoculation of herpes simplex virus type 1 (HSV-1) into the anterior chamber (AC) of one eye results in ipsilateral anterior uveitis, relative sparing of the ipsilateral retina, and necrotizing chorioretinitis of the contralateral eye.4,5 Studies with immunodeficient mice found that T-cell-mediated immune responses probably play a role in the protection of the ipsilateral retina.6 In addition, ipsilateral protection is lost when the virus is injected into the vitreous cavity rather than into the AC, suggesting that the route of viral inoculation is important in immunologic priming and the subsequent generation of ipsilateral retinitis.7,8

A clue to the immunopathogenesis of the contralateral chorioretinitis may be the Igh-1 gene locus; congenic animals disparate at this locus have different patterns of contralateral disease.9,10 These congenic mice are genetically identical except for a limited region of diversity on chromosome 12 in the region encoding for immunoglobulin heavy chain synthesis (Igh-1).11 Because these Igh-1-disparate congenic animals show equivalent permissivity to HSV-1 in various ocular tissues (retinal pigment epithelial cells and keratocytes), host permissivity for HSV replication differences cannot account for their variable disease susceptibility patterns.12,13 Some authors believe that the development of contralateral retinitis in this model correlates with changes in systemic immunity they call AC-associated immune deviation (AC-AID).5,8,14–16 This disorder is characterized by suppression of delayed-type hypersensitivity (DTH) responses to the injected antigen (HSV) with an intact HSV-1 neutralizing antibody response. However, we recently showed that the Igh-1-congenic animals have equivalent suppression of DTH after intracameral inoculation.17 Similar observations were reported in various inbred murine strains with a dissociation between contralateral HSV chorioretinitis and the development of ACAID.17 We describe the immunopathologic characteristics of the ipsilateral and contralateral eyes of Igh-1-disparate

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congenic mice after AC injection of HSV into one eye and analyze the dynamics of cell-type traffic kinetics during the evolution of the von Szily model.

Materials and Methods

Virus

The HSV-1 (strain KOS) was obtained from Dr. David Knipe (Harvard Medical School, Boston, MA) and passed twice in Vero cells (CCL 81; American Type Culture Collection, Rockville, MD). The virus for all experiments was produced from infected Vero cell monolayers as described previously.18

Animals

The BALB/cByJ (Igh-1b) breeding pairs were obtained from Jackson Laboratories (Bar Harbor, ME). The C.B-17 (Igh-1b) breeding pairs were provided by Dr. Charles Sidman (Jackson). All herpes-infected mice were housed in a VR-1 isolator (Lab Products, Inc., Maywood, NJ) in a professionally staffed animal care facility. Animals were handled in accordance with the ARVO Resolution on the Use of Animals in Research.

Inoculation

The mice were inoculated with 1.5 X 10^4 plaque-forming units of HSV-1 strain KOS into the ACs of their right eyes. The animals were anesthetized with ether and topical proparacaine, and the injection was done under an operating microscope. Aqueous drainage through a paracentesis with a glass needle was followed by injection of 5 μl of the appropriately diluted virus into the AC through the beveled corneal paracentesis wound using a 33-gauge needle connected to a 50-μl Hamilton (Reno, NV) syringe.

Immunohistopathologic Findings

The eyes were snap frozen and embedded in Tissue-Tek OCT Compound (Miles, Elkhart, IN) immediately after removal. Using an IEC Minotome (Needham Heights, MA) cryostat, the eyes were sectioned serially at a thickness of 4 μm. Whole-eye cross-sections were fixed to gelatin-coated 12-well microscope slides and stored at −70°C until analysis. The slides were then air dried, fixed for 10 min in acetone, and incubated for 20 min in 1% bovine serum albumin. Then the sections were incubated with 45 min with the primary antibody at various dilutions. Optimal dilutions of the primary antibodies were obtained in our laboratory by staining sections of mouse spleen and lymph node with the immunoperoxidase procedure. Primary antibodies and their working dilutions included: anti-Thy 1.2 (T-lymphocytes) 1:299, anti-Lyt-1 (CD4, helper/inducer T-lymphocytes) 1:100, and anti-Lyt-2 (CD8, suppressor/cytotoxic T-lymphocytes) 1:10 (Becton Dickinson, Mountain View, CA); anti-mouse Ia (antigen-presenting cells) 1:100, and anti-mouse anti-human Mac-1 antigen (macrophages, natural-killer cells, and polymorphonuclear neutrophils) 1:25 (Hybritech, San Diego, CA); and horse anti-mouse IgG (Vector, Burlingame CA).

After incubating with the primary antibodies, sections were washed three times with 0.01 M phosphate-buffered saline (PBS) and blocked for endogenous peroxidase using 0.3% H2O2 in PBS for 30 min. Then the sections were incubated with a 1:500 dilution of biotin-conjugated affinity-purified mouse anti-rat IgG (Jackson Immunoresearch, Avondale, PA) for 45 min. After three rinses in PBS, the tissue was incubated for 45 min with a 1:1000 dilution of peroxidase-conjugated streptavidin (Jackson Immunoresearch). The tissue was rinsed in PBS and then overlaid with peroxidase substrate containing 3-amin-9-ethyl-carbazole and hydrogen peroxide in 0.1 M sodium acetate buffer. The sections were fixed in 4% formalin, counterstained with Gill’s #3 hema-toxylin, rinsed, and cover slipped with Vinol 205 (Air Products and Chemicals, Allentown, PA). Experimental controls included tissue sections without the addition of the primary and/or secondary antibody; positive controls included murine spleen sections.

Experimental Design

Three representative BALB/cByJ and C.B-17 mice were killed by anesthesia overdose on days 2, 4, 6, 8, and 10 postinoculation. Two animals from each strain receiving an injection without virus served as control animals. Inoculated (ipsilateral) and uninoculated (contralateral) eyes from a total of 34 animals were removed and processed as described.

Using a Zeiss (Oberkochen, Germany) photomicroscope III, positively staining cells were counted in the uveal tract, vitreous, and retina. Three high-power fields (HPF, with 100 mm^2 = 1 HPF) from two representative stained sections were examined for each of the three animals for a given day postinjection, creating the potential for 18 data points for each tissue per murine strain and time. The results were tabulated for each day and each mouse strain. Group means and standard errors of the means were calculated. Data from the two congenic strains were compared using Student’s t-test to assess significant differences between means. In the eyes stained for IgG, individual cell counts were not possible. The eyes were scored as + if there was retinal staining and − if no retinal staining was present. All cell counts and evaluations were done in a masked fashion.
Results

Ipsilateral (Inoculated) Eyes

Ciliary body: In BALB/c mice, injected eyes revealed progressive recruitment of each cell type in the ciliary body (Fig. 1). Cellular infiltration began on day 4 and peaked on day 10 postinjection. Thy 1.2-positive cells were prominent with CD4 (Lyt 1) cells being slightly more numerous than CD8 (Lyt 2) cells (CD4:CD8 = 1.5:1). The most frequent cell types identified were Mac and la cells with numbers in the ciliary body approaching 200 ± 19 cells/HPF for Mac+ cells by day 10 (Figs. 2A–B).

The C.B-17 mice showed trends similar to those seen in the BALB/c mice with all cells seen in the ciliary body by day 4. Unlike BALB/c mice, however, by day 10, there was a significantly abrogated Mac response in the ciliary body of C.B-17 mice (80 ± 16 cells/HPF) compared with the ciliary body of BALB/c mice (200 ± cells/HPF, \( P < 0.005 \)). This contrasted with early observations in C.B-17 mice where there tended to be larger numbers of Mac mononuclear cells and minimal staining for la markers compared with BALB/c mice (Figs. 2C–D).

Vitreous: The vitreous of inoculated BALB/c eyes showed progressive infiltration of all cell types. In general, cell numbers increased until day 8 and diminished thereafter. T-cells first appeared on day 5; the CD4:CD8 (Lyt 1:Lyt 2) ratio on day 8 was 2:1. Mac cells appeared in parallel with an la cell population.

The C.B-17 mice had a less prominent cellular reaction in the ipsilateral vitreous. On day 8, fewer Mac cells were seen in the C.B-17 mice (7 ± cells/HPF) compared with the BALB/c mice (21 ± 3 cells/HPF, \( P < 0.005 \)).

Inner and outer retina: In the retina of ipsilateral eyes, significant numbers of inflammatory cells infiltrated the intact retinal architecture (Fig. 3). Cell populations paralleled those found in the vitreous, and inflammation appeared to be greater in the inner retina compared with the outer retina. Although cells of all phenotypes were seen, Thy 1.2 staining was not interpretable in the inner retina because ganglion cells and inner nuclear layers normally stain for Thy 1.2. The IgG staining was not seen in control eyes but was present in both BALB/c and C.B-17 ipsilateral retinas on days 2–10.

The inner retina of BALB/c mice showed a peak in CD4 and CD8 cells on day 10 and a CD4:CD8 ratio of 1:1 (Fig. 3). Mac cells were the most frequent cells detected at each time with 61 ± 13 cells/HPF seen on day 8. Ia cells also were found on serial sections.

By contrast, C.B-17 mice tended to have fewer CD8 cells in the ipsilateral retina, causing the CD4:CD8 ratio to be greater in C.B-17 mice (2:1) than that in the BALB/c mice. The C.B-17 mice also showed an early peak of Mac cells without Ia staining on day 4 whereas
Fig. 2. Photomicrographs of the ipsilateral ciliary body (×160). (a) and (b) show a BALB/c ciliary body stained for Ia and Mac, respectively (day 10 p.i.). (c) and (d) show a C.B-17 ciliary body stained for Ia and Mac, respectively (day 4 p.i.).

Fig. 3. Graph of cell types found in the ipsilateral inner retina.
both populations were detected in BALB/c mice (Figs. 4A-B). By day 8, C.B-17 mice had significantly fewer Mac cells in the inner retina than did BALB/c mice ($P < 0.005$).

There were few inflammatory cells in the outer retina of either BALB/c or C.B-17 animals. The general patterns of these cell types, however, followed those found in the inner retina (Fig. 4).

Choroid: In the BALB/c choroid, Thy 1.2, CD4, and CD8 cells appeared on day 6 and decreased after day 8; the CD4:CD8 ratio (day 8) was 1:1 (Fig. 5). The Ia and Mac cells were the most numerous cell type in this tissue with the greatest numbers seen on day 9.

C.B-17 mice contrasted with the BALB/c mice trend to an earlier infiltration with Thy 1.2 and Mac cells, but not Ia staining cells in the choroid on day 4. On day 8, there were significantly fewer Mac+ cells in C.B-17 mice (12 ± 3 cells/HPF) compared with BALB/c mice (77 ± 16 cells/HPF, $P < 0.005$).

Contralateral (Uninoculated) Eyes

Ciliary body: T-cells appeared in the contralateral ciliary body of BALB/c mice between days 8 and 10, with a CD4:CD8 ratio of 3:1 by day 10. The Mac and Ia cells appeared simultaneously and in similar numbers to those of T-cells (45 ± 13 cells/HPF). The ciliary body of C.B-17 mice was devoid of all inflammatory cells.

Vitreous: In the contralateral BALB/c vitreous, CD4, Thy 1.2, and Mac cells appeared on day 10.
There were no la staining cells, and the Mac population was negative for this activation marker. The most numerous cells were Mac staining with 15 ± 3 cell/HPF. The C.B-17 mice had no vitreal cells.

**Inner and outer retina:** The cellular infiltration in the contralateral retina of BALB/c mice was similar to the phenotypic pattern in the vitreous (Fig. 6). Like the ipsilateral eye, the retinitis was more severe in the inner retinal layers. On day 10, BALB/c mice showed few CD4 (2 ± 0.6 cells/HPF and CD8 (1 ± 0.2 cells/HPF) cells and a large infiltration of Mac cells (125 ± 19 cells/HPF) without the la cells or la activation marker on the macrophages (Fig. 7). There was no IgG staining in the contralateral retina on days 2–8, but it appeared on day 10 (Fig. 8) in BALB/c mice. The C.B-17 mice had no inflammatory cells or IgG in their contralateral retinas.

**Choroid:** The choroid of BALB/c mice showed a distinct cell profile different from that of the vitreous and retina (Fig. 9). T-cells were more numerous in the choroid, with a CD4:CD8 ratio of 3:1 on day 10. Large numbers of Mac and la cells were seen, in contrast to the absence of la cells in the contralateral vitreous and retina (Figs. 7B–C). All cell populations appeared on day 10. The C.B-17 mice did not have chorioiditis, and inflammatory cells were not seen.

**Discussion**

We previously reported that, after intracameral inoculation of HSV-1, Igh-1 congenic animals have different rates of contralateral chorioretinitis; 75% in BALB/c, 30% in C.AL-20, and 5% in C.B-17 mice have contralateral disease. These disease patterns do not correlate with systemic DTH response or host permissivity to HSV-1, suggesting that local ocular immune phenomena may play a role in the development of the characteristic pathologic findings. Others described the general histopathologic features of this model, namely ipsilateral iridocyclitis and retinal sparing with contralateral chorioretinitis. In this study, we describe both the inflammatory cell subpopulations involved in the von Szily model using immunohistochemistry and distinct cellular recruitment kinetics in inoculated and uninoculated eyes of BALB/c and C.B-17 mice.

A vigorous T-cell response was seen in the anterior structures of the ipsilateral eye early after inoculation with HSV. Further analysis found that both susceptible BALB/c and resistant C.B-17 mice had TH/I (CD4) responses greater than TC/S (CD8) responses in the ipsilateral ciliary body. This T-cell response preceded the later peak in Mac and la cell recruit-
ment. These kinetics suggest a helper T-cell-mediated recruitment of activated macrophages or antigen-presenting cells in response to viral challenge but do not account for differences in congenic disease patterns.

Although the architecture of the ipsilateral retina is preserved, the posterior structures of the ipsilateral eye also appear to participate in the inflammatory process. It was noted that ipsilateral “retinal sparing” is a relative term, with the presence of mononuclear cells and retinal folds differentiating inoculated eyes from normal ones. Our findings support this observation. A large number of T-cells and macrophages were found in the ipsilateral retina and choroid, even though retinal architecture and cell layers were preserved. The pattern of inflammatory cells found in the retina paralleled those found in the vitreous and ciliary body. It would appear that the inflammatory cells involved in the ipsilateral retinitis come from either retinal blood vessels or the ciliary body and not from the underlying choroid because most of the cells were found in the inner retina with few in the outer retinal layers. The IgG staining also was detected in the ipsilateral retina beginning on day 2 postinjection, indicating an early breakdown in the blood–retina barrier and/or a rapid antibody response in the ipsilateral eye. These observations document the involvement of the ipsilateral retina in the inflammatory process and highlight the possibility of local antigen processing in these tissues.

Coinciding with the development of contralateral chorioretinitis, there is a significant immunohisto-
chemical difference in ipsilateral eyes of the congenic mouse strains. BALB/c mice recruit large numbers of Mac and Ia cells in the vitreous, retina, and choroid through day 10 versus the abrogated cellular infiltration in C.B-17 mice. This may represent downregulation of the local ocular immune response or more efficient control of the replicating pathogen by HSV-resistant C.B-17 mice.

There was a trend in resistant animals toward an earlier peak of inflammatory cells, specifically, Mac cells without Ia staining cells on day 4 in the ipsilateral ciliary body, inner retina, and choroid of the C.B-17 mice compared with BALB/c mice. Cell numbers at this early time were too small for statistical significance. Preliminary attempts to characterize this cell population further suggest that these cells stain positively with Asialo GM1 (Wako, Dallas, TX), a marker for natural-killer cells, certain T-cells, and macrophages (unpublished results). Because the T-cell population found in the C.B-17 mice at this time did not account for all of these Mac+ Ia− cells, they may be macrophages that lack Ia activation markers or natural-killer cells. Two other early trends were observed in the ipsilateral eye. The retina of C.B-17 mice had a greater CD4:CD8 ratio than BALB/c mice (2:1 versus 1:1), and the choroid of C.B-17 mice showed an earlier T-cell response than BALB/c mice. These trends suggest that C.B-17 mice may have an earlier helper T-cell-mediated recruitment of other inflammatory cell populations (Mac cells). This more efficient response may assist in modulating the development of contralateral disease.

As previously reported, a striking feature of the model is the lack of clinical and histologic evidence for inflammation in the contralateral eye of C.B-17 mice. Immunohistochemical data in this report confirm these observations. The contralateral retina, vitreous, and choroid were normal in C.B-17 mice despite the presence of HSV-1 at day 10 in these structures. Other investigators showed that virus was present in the contralateral eye of BALB/c mice after infection with HSV-2, even though the contralateral eye was microscopically normal. The absence of immune cells in the contralateral eye despite the presence of live HSV suggests that immunologic events in the ipsilateral eye, regional lymph node, or spleen are critical to prevent contralateral chorioretinitis through unique cellular or humoral mechanisms.

By contrast, the contralateral retina and choroid of BALB/c mice characteristically were destroyed. In our study, there was an absence of inflammatory cells until day 8. All cell populations were detected in the choroid by day 8 and intensify by day 10. Mac cells paralleled Ia cells as the most frequent cell type. In the T-cell population, CD4 cells were more common than CD8 cells. This cellular infiltration coincided with the second wave of virus described in the contralateral eye on days 7–10 in another study. By contrast to the choroid, where all cell types were identified, only Mac cells without markers for Ia antigens
were found in the contralateral retina of BALB/c mice, supporting the observation that the inflammatory cells in the retina appear to originate from the retinal vasculature or the ciliary body and vitreous cavity and not from the choroid. With contiguous infiltration from the underlying choroid, a similar distribution of inflammatory cell subpopulations would be expected; this was not found.

The significance of this distinct mononuclear cell pattern is not understood. Although lymphocyte-depletion studies, nude mouse experiments, and immunohistopathologic findings establish a critical role for T-cells in the generation of HSV-mediated disease, our study supports a role for Mac cells and/or natural-killer cells as the effector cells mediating the retinal destruction.21,22 We did not find CD4 and CD8 cells in the contralateral retina. The marker Thy 1.2 could not be assayed in the retina, and it is possible that T-cells without markers for helper or suppressor subpopulations were present but undetected. Nevertheless, most of the mononuclear cells stained for Mac antigens. It is possible that the Mac mononuclear cells without Ia markers found in the retina represent either inactivated macrophages or natural-killer cells. Both cells stain positively for Mac-1. Retinal sections stained with Asialo GM1 antibody were positive for this natural-killer cell marker and support their possible role. The appearance of Mac+, Asialo GM-1+, and Ia− cells in the retina on day 10 postinjection follows closely the second wave of virus in the contralateral eye and represents an appropriate time sequence for activated natural-killer cells.23,24 Other investigators reported that these cells are effective in killing virus-infected target cells in both in vitro and in vivo studies and found similar murine strain-specific differences in natural-killer cell activity.25,26

Although we cannot define exact pathophysiologic mechanisms on the basis of histopathologic findings alone, our study suggests some interesting hypotheses in the generation of herpetic ocular disease after AC inoculation. First, the marked cellularity of the uveal tract and the ipsilateral retina early after virus inoculation compared with the paucity of immune cells in the contralateral eye supports a role for ipsilateral local ocular antigen processing and subsequent immunoregulatory events that modulate the generation of contralateral disease. Second, the predominance of Mac cells without Ia markers in the contralateral vitreous and retina of susceptible BALB/c mice on day 10 implicates this mononuclear cell subpopulation as an important mediator of the necrotizing contralateral retinitis. Third, gene products linked to the Igh-1 phenotype on experimental herpes simplex virus type 1-infected retinitis. Invest Ophthalmol Vis Sci 26:1524, 1985.

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