JOURNAL OF VIROLOGY, Jan. 1996, p. 264–271 Vol. 70, No. 1
0022-538X/96/$04.00+0

Inflammatory Infiltration of the Trigeminal Ganglion after Herpes Simplex Virus Type 1 Corneal Infection

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Received 6 June 1995/Accepted 28 September 1995

Following herpes simplex virus type 1 (HSV-1) infection of the cornea, the virus is transmitted to the trigeminal ganglion, where a brief period of virus replication is followed by establishment of a latent infection in neurons. A possible role of the immune system in regulating virus replication and maintaining latency in the sensory neurons has been suggested. We have investigated the phenotype and cytokine pattern of cells that infiltrate the A/J mouse trigeminal ganglion at various times after HSV-1 corneal infection. HSV antigen expression in the trigeminal ganglion (indicative of the viral lytic cycle) increased until day 3 postinfection (p.i.) and then diminished to undetectable levels by day 7 p.i. The period of declining HSV antigen expression was associated with a marked increase in Mac-1$^+$ cells. These cells did not appear to coexpress the F4/80$^+$ (macrophage) or the CD8$^+$ (T cell) markers, and none showed polymorphonuclear leukocyte morphology, suggesting a possible early infiltration of natural killer cells. There was also a significant increase in the trigeminal ganglion of cells expressing the γδ T-cell receptor, and these cells were found almost exclusively in very close association with neurons. This period was also characterized by a rapid and equivalent increase in cells expressing gamma interferon and interleukin-4. The density of the inflammatory infiltrate in the trigeminal ganglion increased until days 12 to 21 p.i., when it was predominated by CD8$^+$, Mac-1$^+$, and tumor necrosis factor-expressing cells, which surrounded many neurons. By day 92 p.i., the inflammatory infiltrate diminished but was heaviest in mice with active periocular skin disease. Our data are consistent with the notion that gamma interferon produced by natural killer cells and/or γδ T cells may play an important role in limiting HSV-1 replication in the trigeminal ganglion during the acute stage of infection. In addition, tumor necrosis factor produced by CD8$^+$ T cells and macrophages may function to maintain the virus in a latent state.

Herpes simplex virus type 1 (HSV-1) corneal infection in the mouse initiates a series of events that culminate within 1 to 2 weeks in a stable latent neuronal infection of the trigeminal ganglion (TG). These events include (i) brief replication in the corneal epithelium, enhancing access to the axonal termini; (ii) retrograde axonal transport to the neuronal cell bodies, where a brief period of replication can occur; and (iii) repression of the lytic cycle and establishment of a latent infection. Latently infected neurons harbor the viral genome, which is transcriptionally silent except for a single family of latency-associated transcripts. Poorly defined stimuli can result in reactivation of HSV from latency in sensory neurons, axonal transport to the periphery, and the establishment of recurrent lesions. The cellular and molecular mechanisms that control HSV replication in the TG during the acute and latent stages of infection remain poorly characterized. Because most HSV pathology is associated with recurrent disease, mechanisms that control the establishment and maintenance of HSV latency in sensory neurons are of prime importance.

Previous studies (19, 30, 35) have established an important role for T lymphocytes (particularly CD8$^+$ T lymphocytes) in controlling HSV replication within the sensory ganglia. In those studies, infection of SCID mice, which are deficient in both T and B lymphocytes (35), or infection of CD8$^+$ T lymphocyte-depleted mice (19, 30) resulted in uncontrolled replication of HSV-1 in the sensory ganglia. These studies established that CD8$^+$ T lymphocytes are necessary for effective control of HSV replication in sensory ganglia. However, there was also some indication that other factors may also contribute to control of the lytic cycle of the virus and establishment of a latent infection. For instance, HSV-1 established a latent infection in some of the sensory neurons of SCID mice (35). This observation suggests either an innate ability of certain neurons to limit HSV replication without exogenous help or a partial ability of nonlymphoid inflammatory cells to limit virus replication in sensory ganglia. Moreover, the mechanism(s) by which T lymphocytes and possibly other inflammatory cells limit virus replication in the neurons of the TG has not been investigated.

Corneal infection of A/J mice with the RE strain of HSV-1 leads to a transient corneal epithelial lesion (2 to 4 days postinfection [p.i.]), followed in a large proportion of animals by corneal inflammation and periocular skin disease beginning 1 to 2 weeks p.i. The corneal inflammation is characterized by a predominantly polymorphonuclear leukocyte (PMN) infiltration of the cornea beginning 8 to 10 days p.i. Most of the T cells in the corneal infiltrate belong to the CD4$^+$ subpopulation (10, 11, 20), and the inflammation is regulated by the Th1 cytokines interleukin (IL-2) and gamma interferon (IFN-γ) (12, 21). Our recent findings established that IFN-γ regulates PMN extravasation from peripheral corneal vessels, whereas IL-2 may regulate their subsequent migration into the central cornea (unpublished data). The PMN cause progressive destruction of the corneal tissue, which appears to be responsible for the blinding complications of HSV corneal infection in humans. The Th2 cytokines IL-4 and IL-10 are not detected in cells that infiltrate the HSV-infected cornea in our model. Moreover, IL-10 injection into the infected cornea was recently shown to inhibit corneal inflammation in a similar model (34).

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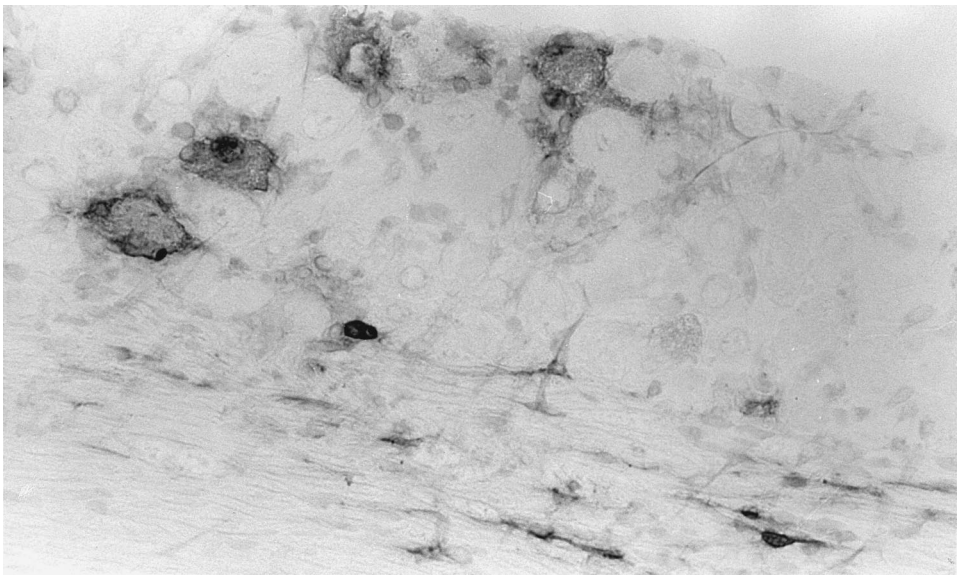

FIG. 1. Representative photomicrograph of a mouse TG obtained 3 days after HSV-1 corneal infection and stained for HSV antigens. Positive staining was observed in neurons and surrounding satellite cells as well as in Schwann cells and/or fibroblasts. Magnification, ×100.

Our current studies demonstrate that corneal infection with the RE strain of HSV-1 results in an infiltrate in the TG markedly different from that in the cornea. In the TG, the infiltrate is virtually devoid of PMN, $CD8^+$ T cells greatly outnumber $CD4^+$ T cells, and a mixture of Th1- and Th2-type cytokines is produced. We hypothesize that IFN-γ and tumor necrosis factor (TNF) may serve to restrict virus replication within the ganglion, while IL-4 and IL-10 may restrict PMN infiltration and irreparable destruction of the neuronal tissue.

MATERIALS AND METHODS

Animals. Female A/J mice (Frederick Cancer Research Center), 6 to 8 weeks old, were anesthetized by intramuscular injection of 2 mg of ketamine hydrochloride (Vetalar; Parke-Davis, Morris Plains, N.J.) and 0.04 mg of acepromazine maleate (Aveco Co., Fort Dodge, Iowa) in 0.1 ml of Hanks' balanced salt solution into the left hind leg.

Virus. The RE strain of HSV-1 was grown in Vero cells, and intact virions were purified on Percoll (Pharmacia) as previously described (8).

Corneal infection. Topical corneal infection of anesthetized mice was achieved by superficially scratching the central cornea 10 times with a 30-gauge needle in a crisscross pattern. A 3-μl virus suspension (10^5 PFU) was applied topically to the scarified cornea and rubbed in with the eyelids. All experimental procedures conformed to the Association for Research in Vision and Ophthalmology resolution on the use of animals in research.

Histologic and immunohistochemical examination of TG. Mice were exsanguinated and sacrificed at various times after unilateral HSV-1 corneal infection. The ipsilateral TG was excised from six mice at each time point. Two TG were processed for paraffin sections, and the remaining TG were processed for frozen sections. For histologic examination, the TG were immediately fixed in 10% neutral buffered formalin, and 5-μm paraffin sections were prepared. The sections were stained with hematoxylin-eosin, mounted with Permount, and covered with a coverslip for microscopic examination.

For immunohistochemical staining, the TG were imbedded in OCT (optimal cryogenic temperature; Tissue Tek; Miles, Naperville, Ill.) and snap-frozen in an isopentane dry ice bath, and 6-μm serial sections were cut at −20°C. The sections were quickly fixed in acetone for 12 s and stored at −20°C. Prior to staining, the sections were fixed in acetone at room temperature for 10 min, air dried, and washed twice for 10 min in phosphate-buffered saline (PBS). The sections were blocked with normal goat serum for 20 to 30 min at 37°C and incubated with primary antibody at 37°C for 1 h (or at 4°C overnight). The sections were washed twice (10 min each time) in PBS and incubated with biotinylated secondary antibody for 30 min at room temperature. After two more washes, the sections were treated with 3% hydrogen peroxide for 10 to 15 min, washed three times, and incubated with ABC reagent (Vectastain ABC kit; Vector Laboratories, Inc., Burlingame, Calif.) at room temperature for 30 min. The sections are treated with diamobenzidine substrate (peroxidase substrate kit DAB SK04100; Vector Laboratories) for 3 min, washed, counterstained with eosin, dehydrated through graded ethanol (95 to 100%), and then cleared in xylene, mounted in Permount, and covered with a coverslip.

Antibodies. The following monoclonal antibodies were used: anti-CD4 (GK1.5, rat immunoglobulin G 2b [rIgG-2b], ATCC TIB207), anti-CD8 (53-6.72, rIgG-2a, ATCC TIB105), antimacrophage (F4/80, rIgG-2b, ATCC HB198), anti-γδ T-cell receptor (TCR) (GL3, hamster IgG; PharMingen), anti-Mac-1 (CD11b, M1/70, rIgG-2b; Hybrid Tech), anti-IFN-γ (R4-6A2, rIgG-1, ATCC HB170), anti-IL-4 (11B11, rIgG-1, ATCC HB188), anti-IL-10 (JES5.2A5, rIgG-1; DNAX Inc., Palo Alto, Calif.), and anti-TNF (2E2, hamster IgG; kindly provided by Edmund Lattime, Thomas Jefferson Medical School, Philadelphia, Pa.). The anti-TNF monoclonal antibody neutralizes mouse TNF-α and TNF-β (16). A human serum with a high titer of anti- HSV-1 antibody was used for detection of HSV-1 antigens.

Secondary antibodies were goat anti-rat IgG (heavy and light chains; Jackson ImmunoResearch Laboratories, Inc.), goat anti-hamster IgG (heavy and light chains; Vector Laboratories), and goat anti-human IgG (Fcγ; Jackson Immunoresearch Laboratories).

Statistics. The significance of differences in the number of immunohistochemically stained cells was assessed by a Student's *t* test.

RESULTS

Expression of HSV antigens in the TG. HSV-1 infection of the mouse cornea is followed by transport of the virus to the TG. Within the TG, the virus can briefly enter a lytic cycle that is characterized by the expression of virus glycoproteins on the surface of infected cells (30). In this study, immunohistochemical staining for HSV antigens revealed their expression on neurons and surrounding satellite cells beginning 2 days p.i. Maximal expression of HSV antigens in the TG was observed 3 days p.i., when neurons, surrounding satellite cells, and numerous Schwann cells and/or fibroblasts were positively identified with human antiserum to HSV antigens (Fig. 1). HSV antigen-positive neurons were restricted to the specific location within the ganglion, previously characterized as representing the ophthalmic branch (1, 33). HSV antigen expression diminished in the TG from days 3 to 5 p.i. and was no longer detectable by day 7.

Inflammatory infiltrate during the acute (lytic) stage of infection in the TG. A few scattered $Mac\text{-}1^+$ and $F4/80^+$ cells were detected in the TG of normal (non-HSV-infected) mice (not shown). The number of $Mac\text{-}1^+$ cells increased dramatically from days 3 to 5 p.i. (Fig. 2). Mac-1 is expressed on

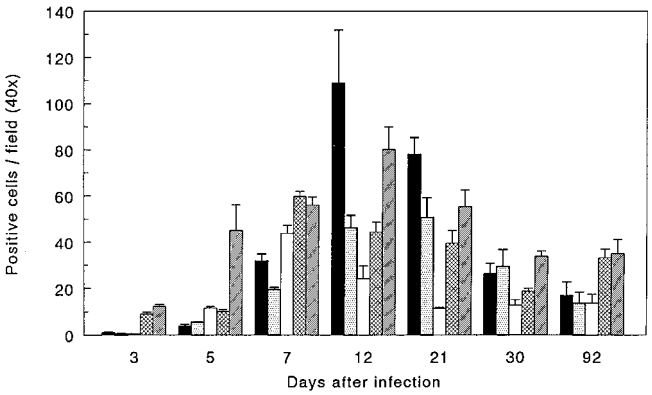

FIG. 2. Composition of the inflammatory infiltrate in the TG after HSV-1 corneal infection. TG were obtained from mice at various times after HSV-1 corneal infection. Various leukocyte populations were identified by immunohistochemical staining of frozen sections. For each leukocyte population and each time point, a total of 72 40× fields were examined (four TG, each obtained from an individual mouse, three sections per TG, six representative 40× fields per section). The data are listed as the mean number ± standard error of the mean of each leukocyte population observed per field. Symbols: ■, CD8; ▨, CD4; □, γδ TCR; ▩, F4/80; ▨, Mac-1.

macrophages, natural killer (NK) cells, some $CD8^+$ cells, and PMN. The number of $F4/80^+$ macrophages and $CD8^+$ T cells was significantly lower than the number of Mac-1^+ cells on day 5 p.i. ($P < 0.01$), and there were no cells with characteristic PMN morphology (assessed in hematoxylin-eosin-stained paraffin sections; not shown) detected at any time in the infected TG. Thus, the Mac-1^+ cells that infiltrated the TG during this period may have been NK cells, although this could not be established because of the lack of a suitable NK marker in this strain of mice.

The number of $F4/80^+$ cells, γδ TCR^+ cells, and $CD8^+$ cells in the TG markedly increased between days 5 and 7 p.i. (Fig. 2). The Mac-1^+, $F4/80^+$, and γδ TCR^+ cells were found surrounding the neurons in the ophthalmic branch of the TG, although the γδ TCR^+ and Mac-1^+ cells tended to be more closely associated with the neuron cell bodies (Fig. 3).

The number of cells producing the cytokines IFN-γ and IL-4 increased steadily and in tandem between days 3 and 7 p.i. (Fig. 4). As expected, the distribution in serial sections of cells producing these two cytokines did not suggest coproduction of IFN-γ and IL-4 by the same cells. The IFN-γ-producing cells were more heavily concentrated in the ophthalmic branch of the ganglion, whereas the IL-4-producing cells were scattered throughout the neuronal body and axonal areas of the ganglion (not shown). Cells producing the cytokines TNF and IL-10 did not appear until day 7 p.i. (Fig. 4).

Inflammatory infiltration of the TG during the latent stage of infection. The period following cessation of HSV antigen expression in the TG was marked by a continued increase in the magnitude but a change in the composition of the inflammatory infiltrate. From days 7 to 12 p.i., there was a significant increase in $CD8^+$ cells ($P < 0.01$), many of which localized to the ophthalmic branch, where they surrounded the neuron cell bodies (Fig. 2 and 5A). Although the $CD8^+$ cells surrounded

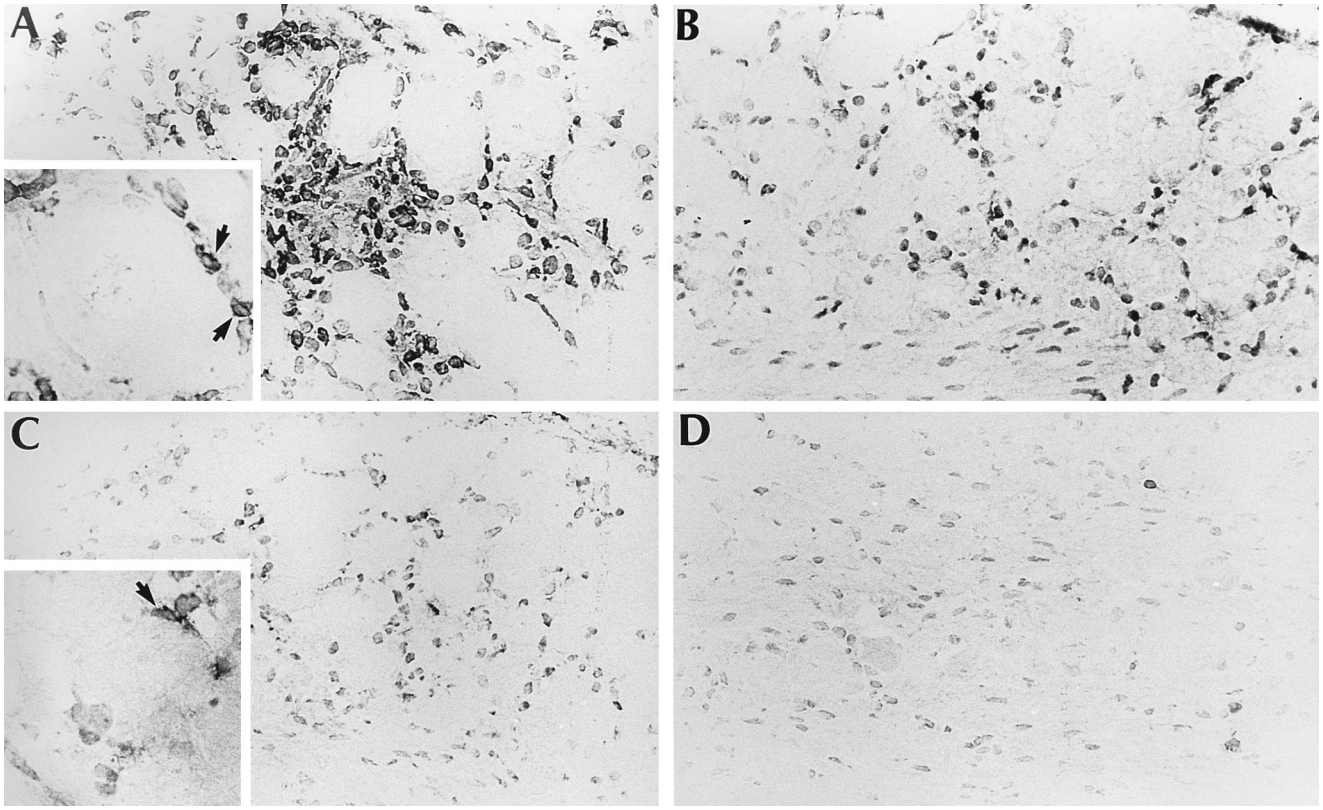

FIG. 3. Representative photomicrographs of a mouse TG obtained 5 days after HSV-1 corneal infection and stained by an ABC immunoperoxidase technique for Mac-1 (A), F4/80 (B), γδ TCR (C), or CD8 (D) (magnification, ×100). Note the accumulation of Mac-1^+, $F4/80^+$, and γδ TCR^+ cells in the regions of the ganglion occupied by the neuron cell bodies. Inserts illustrate the close association of the Mac-1^+ and γδ TCR^+ cells (arrowheads) with the neuron cell bodies (magnification, ×330).

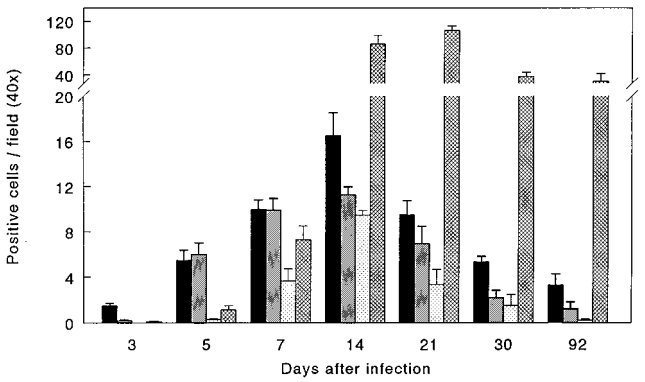

FIG. 4. Cytokine production by inflammatory cells that invaded the TG following HSV-1 corneal infection. TG were obtained from mice at various times after HSV-1 corneal infection. Cells expressing various cytokines were identified by immunohistochemical staining of frozen sections. For each cytokine and each time point, a total of 72 40× fields were examined (four TG, each obtained from an individual mouse, three sections per TG, six representative 40× fields per section). The data are listed as the mean number ± standard error of the mean of cells producing each cytokine observed per field. Symbols: ■, IFN-γ; ▨, IL-4; ▩, IL-10; ▩, TNF.

the neurons, they were not in direct contact with the neuron body but rather tended to associate with satellite cells (Fig. 6). The number of Mac-1^+ cells also increased during this period, whereas the number of F4/80^+ cells declined. The distributions of $CD8^+$ and Mac-1^+ cells in serial sections were quite similar, though it could not be determined from these studies if some of the $CD8^+$ cells coexpressed Mac-1. There was also a modest increase in $CD4^+$ T cells during this period, whereas the number of γδ TCR^+ cells began to decline.

This period (day 7 to 12 p.i.) was also marked by a dramatic increase in the number of TNF^+ cells in the TG (Fig. 4). The TNF^+ cells were localized in areas exhibiting large numbers of $CD8^+$ cells, Mac-1^+ cells, and F4/80^+ cells (Fig. 5). The fact that the number of TNF^+ cells in the TG increased in concert with $CD8^+$ cells and during a period of declining numbers of F4/80^+ cells suggested that $CD8^+$ T lymphocytes may have been a major source of TNF during this period. There was also a modest increase in the number of IFN-γ^+ and IL-10^+ cells during this period, whereas the number of IL-4^+ cells remained constant (Fig. 4 and 7).

The magnitude of the inflammatory infiltrate declined steadily in the TG between days 21 and 92 p.i., although there was significant variation in the infiltrate in different ganglia on day 92. At that time, the magnitude of the infiltrate appeared to correlate with the presence of viral disease at a peripheral site (Fig. 8). Periocular skin disease consisting of loss of fur, vesicles, and edema was observed in 70% of infected mice approximately 1 week p.i. The vesicles typically healed by 2 weeks p.i., but fur loss and edema persisted in approximately 10% of these animals for up to 6 months (longest observation period) p.i. However, we were not able to detect HSV antigens in frozen sections of periocular skin of any animals more than 2 weeks p.i. We failed to recover replicating HSV-1 from homogenates of periocular skin obtained from mice with persistent disease. Thus, it is not clear if the persistent skin disease reflects a low-grade persistent infection that is below the sensitivity of our assay or perhaps an immunopathological response similar to that seen in the cornea. Full recovery from

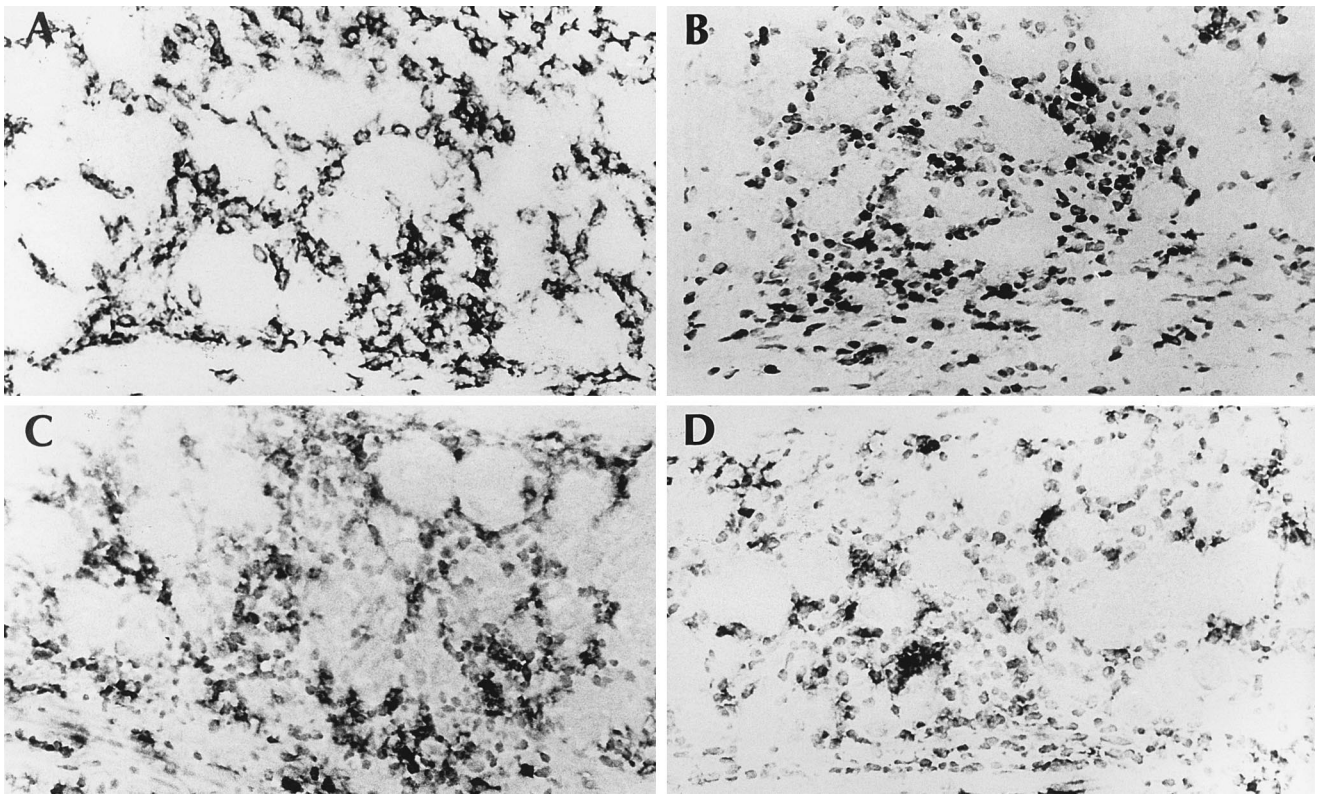

FIG. 5. Representative photomicrographs of a mouse TG obtained 12 days after HSV-1 corneal infection and stained for CD8 (A), TNF (B), Mac-1 (C), and F4/80 (D). Note the distribution of all four cell types surrounding the neuronal cell bodies. Magnification, ×100.

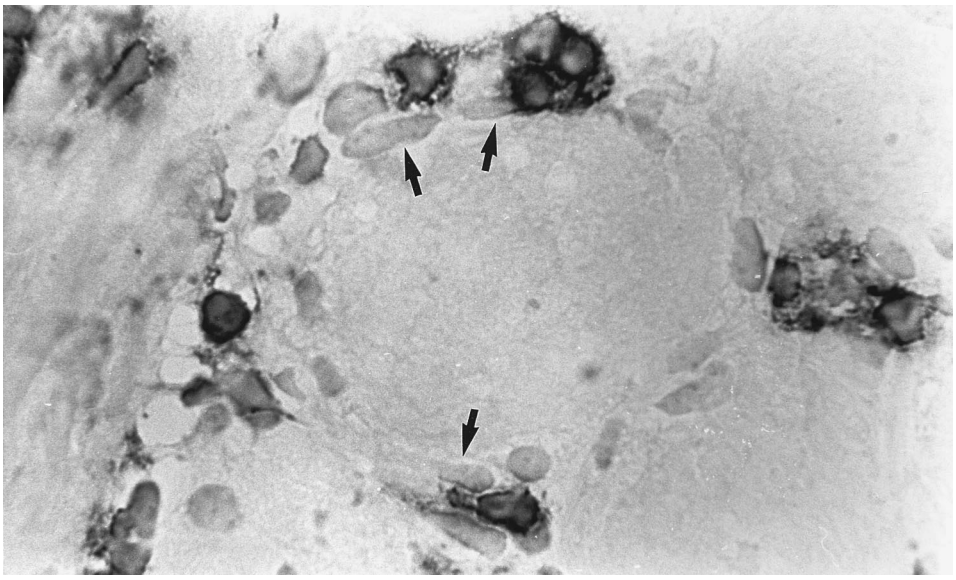

FIG. 6. Distribution of $CD8^+$ cells relative to satellite cells and neurons in the TG. Shown is a photomicrograph of a mouse TG obtained 12 days after HSV-1 corneal infection and stained for CD8. Note the tendency of $CD8^+$ cells to bind to satellite cells (arrows) but not to neurons. Magnification, ×330.

periocular skin disease was associated with a mild TG inflammatory infiltrate composed primarily of $F4/80^+$ cells and some $CD4^+$ and $CD8^+$ T lymphocytes surrounding the neuronal cell bodies. Mild periocular skin disease (incomplete recovery of fur and mild edema) was associated with an intermediate infiltrate of similar composition. In contrast, periocular skin disease of moderate intensity was associated with retention of a heavy TG inflammatory infiltrate composed of roughly equivalent numbers of $F4/80^+$ macrophages and $CD4^+$ and $CD8^+$ T lymphocytes. At this time, many of the cells surrounding the neurons expressed TNF (not shown). There were a few cells producing IFN-γ, which also localized to the areas of the TG occupied by the neuronal cell bodies. A few $IL\text{-}4^+$ and $IL\text{-}10^+$ cells were scattered throughout the ganglion.

DISCUSSION

It is becoming increasingly evident that the nature of an immune response can be tailored to the needs of a particular tissue. In some cases, antigen-presenting cells (APC) at the site of antigen deposition may influence the nature of the response that is induced in the peripheral organs (6, 7, 13, 24, 26, 27, 31, 37, 38). Conversely, our previous study demonstrated that the depletion of APC from one cornea followed by bilateral HSV-1 corneal infection abrogated the immunologically mediated inflammatory response to HSV-1 in the APC-depleted cornea only (7). This latter observation suggested that local APC can influence which, if any, of the systemically induced immunologic functions will be exercised within a specific tissue.

Corneal infection of A/J mice with the RE strain of HSV-1 results in a chronic immunologically mediated inflammatory response that leads to total destruction of the corneal architecture and corneal perforation. The predominant infiltrating cell is the PMN. $CD4^+$ T cells are also a prominent feature of the inflammatory infiltrate, and their depletion prevents PMN infiltration and abrogates the inflammatory response (10). The cytokines IL-2 and IFN-γ are requisite mediators of this inflammation (12). We have recently shown in this model that IFN-γ regulates PMN extravasation from corneal vessels, while IL-2 is required to maintain a PMN chemotactic gradient within the cornea (unpublished data).

Several studies have demonstrated the plasticity of the immune response to HSV-1 that is generated systemically following corneal infection. For instance, HSV-reactive $CD8^+$ cells are greatly expanded in the lymph nodes following RE HSV-1 corneal infection (9). However, $CD8^+$ cells are very sparse in the corneal infiltrate, and their depletion does not diminish the inflammatory response following RE HSV-1 corneal infection (3, 10). Moreover, although HSV-1 corneal infection induces a predominantly Th1-type response in the regional lymph nodes and spleen, cells expressing IL-4 (our unpublished observation) and IL-10 (21) are also detectable. However, in the RE HSV-1-infected cornea, we and others have failed to detect cells capable of producing IL-4 and IL-10 (12, 21). Thus, not all of the immune functions that are induced in the lymphoid organs are expressed in the infected cornea. It would appear, therefore, that individual tissues may be able to shape an existing immune response to fit their unique needs by influencing the migration and/or activation of functionally distinct populations of leukocytes.

In this study, HSV antigens were detectable in the TG 2 days after corneal infection with RE HSV-1. However, the presence of RE HSV-1 in the ganglion resulted in an inflammatory infiltrate markedly different from that seen in the cornea. The PMN that represented a major component of the corneal infiltrate were virtually absent from the TG infiltrate, despite the fact that IFN-γ, which appears to regulate PMN extravasation into the infected cornea, was prominently produced in the TG. It is notable, therefore, that IL-4, which was absent from the infected cornea, was produced in conjunction with IFN-γ in the TG. IFN-γ possesses antiviral activity and may contribute to the restriction of HSV-1 replication and concomitant establishment of latency in the ganglion. However, IL-4 is not known to possess antiviral activity. We hypothesize, therefore, that the function of IL-4 may be to prevent the infiltration of the ganglion with PMN and their subsequent irreparable destruction of neural tissue. IL-4 and IL-10 may also favor the massive $CD8^+$ cell infiltration of the ganglion, which is not a feature of the corneal infiltrate. Such regulation of leukocyte infiltration could be effected through modulation of adhesion molecules on vascular endothelium of local blood vessels. For instance, IL-4 can up-regulate VCAM-1 and down-regulate

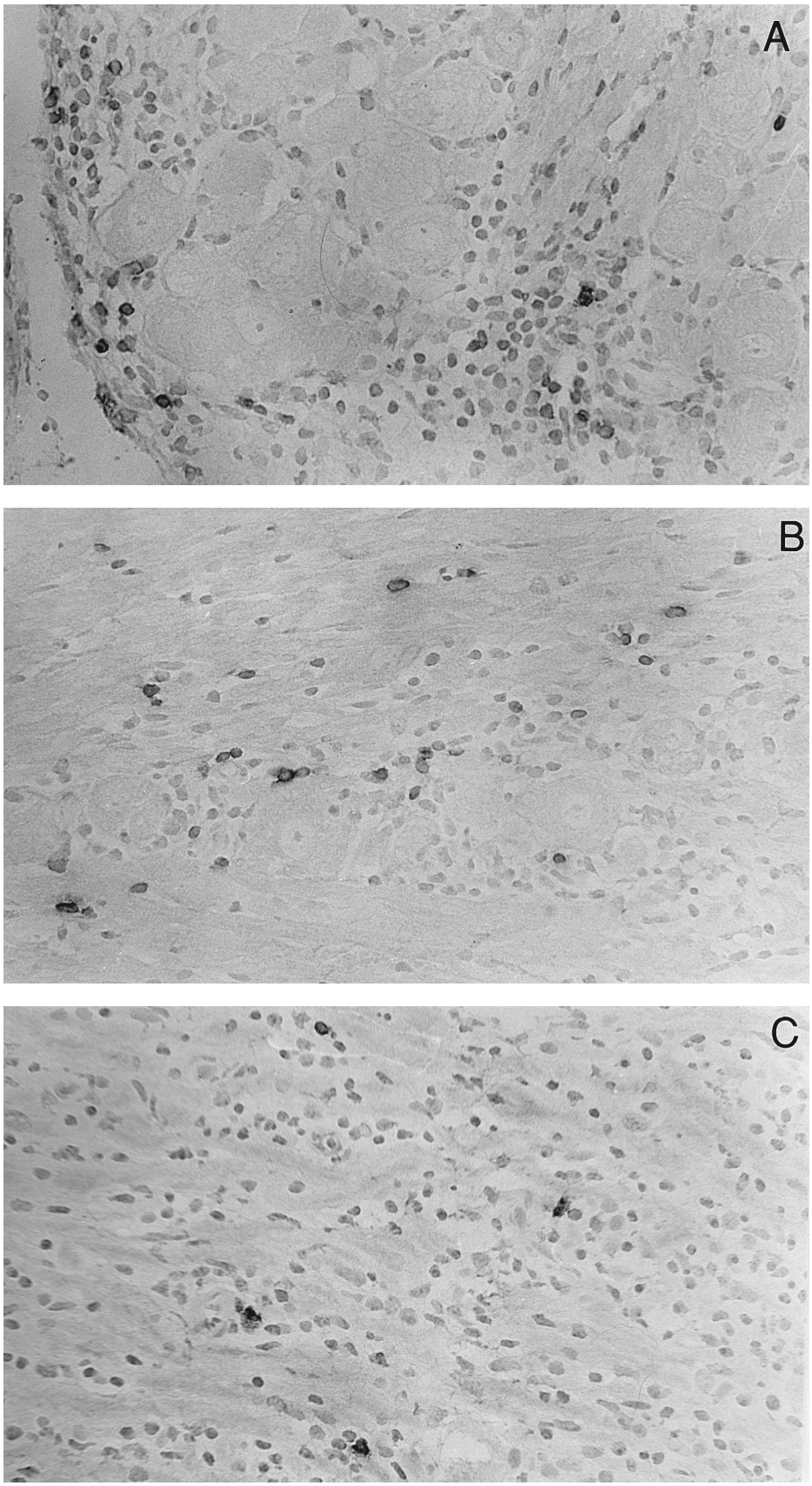

FIG. 7. Photomicrographs showing representative sections of TG obtained 14 days after HSV-1 corneal infection and stained for IFN-γ (A), IL-4 (B), or IL-10 (C). Magnification, ×100.

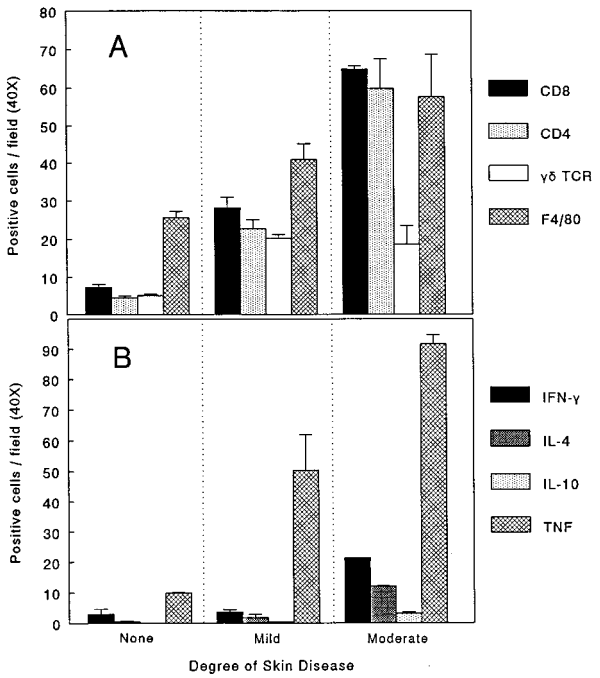

FIG. 8. Composition of the inflammatory infiltrate in the TG 92 days after HSV-1 corneal infection. TG were obtained from mice exhibiting different degrees of periocular skin disease 92 days after HSV-1 corneal infection. Various leukocyte populations (A) or cytokine-producing cells (B) were identified by immunohistochemical staining of frozen sections. Six representative 40× fields were examined from two sections of each TG. The data are listed as the mean number ± standard error of the mean of positive cells observed per field.

ICAM-1 and ELAM-1 expression on vascular endothelial cells, an effect that may discourage PMN extravasation and favor T-cell infiltration (2, 22, 25). Moreover, IL-10 was shown to be chemotactic for $CD8^+$ cells while inhibiting the migration of $CD4^+$ cells (15). IL-10 was also recently shown to inhibit the CD4 T-cell-regulated PMN migration into HSV-1-infected mouse corneas (34). Thus, the observed differences in the compositions of the inflammatory infiltrates in HSV-1-infected corneas and TG can be explained by known effects of IL-4 and IL-10.

Following HSV-1 infection of the mouse, the virus is transported to the sensory ganglia, where it enters a brief lytic cycle during which HSV antigens are expressed on the surface of infected cells. The factors that contribute to restriction of the lytic cycle and establishment of latency are just beginning to be elucidated. A recent study demonstrated that depletion of $CD8^+$ T cells prior to infection or infection of SCID mice that are congenitally deficient in both B and T cells resulted in increased HSV replication in the sensory ganglia and a lethal encephalitis. These findings suggest that $CD8^+$ T cells play an important role in restricting virus replication in the neurons. This is consistent with our observation that the TG is heavily infiltrated with $CD8^+$ T cells, which encircle neuron cell bodies primarily in the ophthalmic branch of the ganglion. We also noted a concurrent increase in cells that produce TNF. Our studies could not distinguish between TNF-α and TNF-β, since the monoclonal antibody used recognizes both forms (16). However, activated $CD8^+$ cells have been shown to produce both TNF-α and TNF-β (32, 36). Moreover, although production of TNF-α and production of TNF-β are regulated differently, the two molecules use the same receptor and have overlapping functions (32, 36). Although not definitive, these findings suggest that $CD8^+$ cells may inhibit HSV-1 replication in neurons at least in part through production of TNF. Such a mechanism would be consistent with the known capacity of TNF to inhibit replication of viruses including HSV-1 (4, 5, 14, 17, 18, 28).

Our studies revealed an early (days 3 to 7 p.i.) infiltration of the TG and encircling of neurons with Mac-1^+ cells, most of which did not appear to coexpress the F4/80 macrophage marker. Cells that are known to express Mac-1 include macrophages, NK cells, PMN, and some $CD8^+$ cells. Since $CD8^+$ cells were only sparsely present and PMN were absent from the TG during this period, the Mac-1^+ cells that infiltrated at this time may have been NK cells. We also noted infiltration of γδ TCR^+ T cells during this period. The γδ TCR^+ T cells were restricted to areas of the ganglion that were occupied by neuron cell bodies and appeared to be closely adherent to those cells. Recognition of HSV antigens on major histocompatibility complex (MHC)-negative neurons would be consistent with the functional properties of a recently described γδ TCR^+ T-cell clone that recognizes an unprocessed HSV-1 glycoprotein in the absence of MHC molecules (29). In addition, many IFN-γ^+ cells were observed in the same areas occupied by the γδ TCR^+ T cells and Mac-1^+ F4/80^- $CD8^-$ cells, and this infiltration corresponded temporally with reduced HSV antigen expression in the ganglion. Taken together, these observations are consistent with the notion that NK cells and γδ TCR^+ T cells may play an important early role in restricting HSV replication in the neurons of the TG. This notion was further supported by our recent studies with mice that are genetically deficient in or depleted of these cell types (29a).

It is noteworthy that $CD8^+$ cells did not infiltrate the TG in large numbers until 7 to 12 days p.i., when viral antigens were no longer detectable by immunohistochemical staining. This observation, combined with the kinetics of virus replication in the TG (2 to 7 days p.i.), suggests that $CD8^+$ cells may be more actively involved in maintaining the virus in a latent state than in inhibiting the acute viral replication in the TG. Moreover, while $CD8^+$ cells surrounded neurons, they did not appear to be directly adherent to the neuronal cell bodies but rather tended to associate with satellite cells. In contrast, the γδ TCR^+ T cells and Mac-1^+ cells often appeared to be in direct contact with neuronal cell bodies. This observation is compatible with the hypothesis of Pereira et al. (23) that $CD8^+$ cells may interact with satellite cells, which up-regulate expression of MHC class I molecules after HSV-1 infection, rather than with neurons that do not exhibit classical MHC class I. Activation of $CD8^+$ cells by HSV antigenic peptides in conjunction with MHC class I on satellite cells may maintain their cytokine production. Cytokines such as TNF and IFN-γ may contribute to an environment that is hostile to HSV-1 replication, thus maintaining the virus in a latent state.

In our model, HSV-1 replication in the TG appears to be maximal around 3 to 5 days p.i. and is no longer detectable by 7 days p.i. HSV-1 replication in the periocular skin often does not become apparent until 8 to 10 days after corneal infection and is no longer detectable by 14 to 16 days p.i. Periocular skin disease is characterized by transient vesicles, fur loss, and edema. In general, these signs are no longer observable by 30 days p.i., but in about 10% of mice, the fur loss and edema persist for 6 months or more. It is not clear if the inflammation is induced by a persistent low-level virus infection, since we have not been able to culture virus from extracts of the periocular skin of these mice. Interestingly, persistent inflammation in the skin seems to correlate with persistent inflammation in the TG. While the nature of the link between these tissues is not clear, it is possible that a low level of virus replication at one site causes continuous axonal transport to the other tissue,

resulting in persistent inflammation at both sites. Additional studies incorporating more sensitive methods of detecting replicating virus may resolve this issue.

Our observations are consistent with the notion that the immune system may play a pivotal role in controlling HSV-1 replication in sensory ganglia. Since most of the pathology resulting from HSV infections is associated with recrudescent rather than primary disease, the key to controlling HSV pathology may lie in appropriate regulation of the immune response. Moreover, immune regulation may need to be targeted to specific tissues. For instance, $CD4^+$ T cells appear to be important for controlling HSV-1 replication in the periocular skin but are also responsible for HSV-induced pathology in the cornea (11, 12). Moreover, $CD8^+$ T cells appear to be more important than $CD4^+$ cells in regulating HSV replication in the sensory ganglia (19, 30). Thus, appropriate immune regulation may depend on the stage (active or latent) and the site of HSV disease.

ACKNOWLEDGMENTS

This work was supported by NIH grants EY05945 and EY10359, and Core Grant EY01792; by an unrestricted research grant from Research to Prevent Blindness, Inc., New York; and by the Lions of Illinois Foundation, Maywood, Ill. Robert L. Hendricks is a Research to Prevent Blindness Senior Scientific Investigator.

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