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Evidence for Induction of Interferon-α and Interferon-β in Retinal Glial Cells of Müller

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Studies were performed to determine if retinal glial cells of Müller transcribe the genes for interferon-α (IFNα) or IFNβ upon exposure to virus. Responses to herpes simplex virus type 1 (HSV-1) were tested with cultured murine Müller cells and, *in vivo,* with retinas obtained after bilateral injection of either HSV-1 or buffer into the anterior chamber of the eyes of BALB/c mice. Induction of IFN transcription and relative temporal changes in transcript levels occurred over time after either *in vitro* or *in vivo* exposure to HSV-1. Transcription of both IFN genes was induced in cultured glia within 1 hr after exposure to virus. IFN transcripts were detected in retinas by 24 hr postinfection and these were maximal at 3 days. By *in situ* hybridization (ISH), IFNα_2 mRNA localized to focal areas in the intact retinas of virus-injected eyes and was consistent with our previous report of a transient, focal appearance of viral antigens in those retinas. Uninfected cells and ocular tissues were negative for IFN transcripts. Combined ISH and immunohistochemistry on retinal impression smears confirmed that glial fibrillary acidic protein-positive Müller cells are an intraretinal source of IFNα and IFNβ transcripts after ocular exposure to HSV-1. Our results support a role for Müller cells as participants in intraretinal antiviral or immunomodulatory responses via type 1 IFN production and may have implications for future therapeutic interventions.

The type 1 interferons (IFN), IFNα and IFNβ, provide an early defense against viral infection (Isaacs and Lindemann, 1957; Dorr, 1993; Sen and Ransohoff, 1993; Gutterman, 1994; Muller *et al.,* 1994). These soluble mediators are produced by a wide variety of cells upon exposure to many viruses or double-stranded RNA (Engler *et al.,* 1982; Allen and Shellam, 1985; Morahan *et al.,* 1991; Pyo *et al.,* 1991; Barnhart *et al.,* 1992) and are critical in natural resistance to several herpesviruses including herpes simplex virus type 1 (HSV-1), HSV-2, and murine cytolomegalovirus (CMV) [Gresser *et al.,* 1976; Grundy (Chalmer) *et al.,* 1982; Kapoor *et al.,* 1982; Chong *et al.,* 1983; Allen and Shellam, 1985; Ellermann-Eriksen *et al.,* 1986; Quinnan and Manischewitz, 1987; Kunder *et al.,* 1993]. Type 1 IFN-receptor knockout mice (Muller *et al.,* 1994; Fiette *et al.,* 1995) and mice treated with anti-IFNα/β antibodies (Gresser *et al.,* 1976; Hendricks *et al.,* 1991) are more susceptible to disseminated viral infections, including HSV-1, and to subsequent death.

Herpesvirus-mediated retinal infections are rare except in immunodeficient individuals (Pepose *et al.,* 1984) and may support a role for immune-mediated protection of the retina. An experimental model of herpetic retinitis was characterized by Whittum *et al.* (1984) and others (Atherton and Streilein, 1987; Cousins *et al.,* 1989) in which unilateral inoculation of HSV-1 into the anterior chamber (AC) of BALB/c eyes results in virus-mediated destruction of the retina of contralateral eyes, while retinas of virus-injected eyes are preserved. If HSV-1 is injected into the AC of both eyes, both retinas are preserved indefinitely (Whittum *et al.,* 1984). Though T lymphocytes, presumably in the inflamed anterior segment, constitute an immunologic component of retinal preservation (Whittum *et al.,* 1984; Whittum-Hudson *et al.,* 1985), the contribution of resident retinal cells to protection has not been defined.

Retinal Müller cells are glial cells derived from neuroectoderm. This population participates in retinal development and differentiation and produces neuron survival-promoting growth factors (Manthorpe *et al.,* 1988; LaVail *et al.,* 1992; Wen *et al.,* 1995). Müller cells respond to anterior segment inflammation or infection and to retinal degeneration via the rapid upregulation of glial fibrillary acidic protein (GFAP), an intermediate filament protein (Eng, 1985). Intense GFAP expression is also induced in the majority of murine Müller cells after AC virus injection (Whittum-Hudson, 1992). While the functional significance of this GFAP response is unknown, the *in vivo* levels of GFAP expression correlate with the timing and extent of anterior segment inflammation in the retinitis model (Whittum *et al.,* 1984; Whittum-Hudson, 1992) and are maximal within 3–5 days in virus-injected eyes. We recently demonstrated that transcription of genes for two proinflammatory cytokines (TNFα and IL-6) is upregulated in retinas of virus-injected eyes within this period

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(Drescher and Whittum-Hudson, 1996b). Taken together with the GFAP upregulation, Müller cells appear to respond in multiple ways during ocular viral infection.

In the present studies, we investigated whether local production of IFNα and β by activated Müller cells contributes to the antiviral responses which account for retinal preservation in a murine model of herpetic retinitis. Intraretinal type 1 IFN production could directly inhibit HSV-1 replication or enhance intraocular T-cell responses (e.g., Belardelli and Gresser, 1996) to thereby protect this relatively immunologically privileged tissue with limited regenerative capacity. If evidence for intraretinal IFN production were obtained, this might guide new therapeutic strategies for retinal viral infections.

MATERIALS AND METHODS

Mice

Young adult breeding pairs or pregnant BALB/c mice were obtained from Harlan Sprague–Dawley (Indianapolis, IN). Adult BALB/c female mice used for *in vivo* studies were obtained from the Charles River Breeding Facility (Wilmington, MA). Mice were maintained in the animal facilities of the Wilmer Institute in the Johns Hopkins Medical Institutions and were treated in accordance with the NIH Guidelines for the Care of Laboratory Animals and the ARVO resolution on the Use of Animals in Research.

Retinal glial cell cultures

Primary cultures of retinal glia (flat cells) were established from eyes 24–48 hr postnatally using our published techniques (Merges and Whittum-Hudson, 1990; Drescher and Whittum-Hudson, 1996a,b). Glial cells from passage numbers of less than 20 to over 100 were compared to primary cultures in these studies. Cells were seeded into tissue culture flasks or 60-mm^2 petri dishes and grown in complete Dulbecco's minimal essential medium (D-MEM; Gibco, Grand Island, NY) containing 10% fetal calf serum (FCS; Gibco), penicillin [100 U/ml (Gibco)], streptomycin sulfate [100 μg/ml (Gibco)], 2 m*M* L-glutamine (Gibco), and Fungizone (0.5 mg/ml; Gibco). Primary or passaged retinal glia were adsorbed with HSV (m.o.i. = 0.1) or NDV (m.o.i. = 30) and then cultured for up to 24 hr in complete D-MEM (total volume of 10 ml), after which total cellular nucleic acids were extracted.

In vivo virus infection

Anesthetized (0.66 mg ketamine hydrochloride; Vetalar, Parke, Davis, & Co., Detroit, MI) mice were inoculated into the anterior chamber of both eyes with live HSV (2×10^4 pfu), UV-inactivated HSV, or Hanks' balanced salt solution (HBSS) in a total volume of 4 μl as previously described (Whittum *et al.,* 1984). Virus dilutions were made in HBSS. HSV (KOS strain) was propagated in Vero cells as previously described (Whittum *et al.,* 1984). Mice were sacrificed 1–7 days postinoculation by cervical dislocation. The eyes were immediately removed and sterilely dissected into anterior segment or isolated retina. Total RNA was isolated as described below. Ocular tissues from normal mice were prepared in parallel.

In some experiments, posterior segment impression smears (PSS) from retinas were prepared by a modified impression cytology method. Eyes were transected at the limbus to remove the anterior segment, lens, and vitreous, and the retinal tissue was sequentially smeared onto multiple poly-L-lysine-coated (Sigma, St. Louis, MO) slides. This process disrupted the retinal architecture, but left most individual cells intact and morphologically distinguishable as neurons, photoreceptors, or Müller cells. Slides containing impression smears (approximately 10 slides per retina) were air-dried and fixed in chilled acetone for 5 sec for immunohistochemistry or in 4% paraformaldehyde for *in situ* hybridization (ISH).

Isolation of RNA and analysis of interferon-α/β transcripts

Total cellular RNA was extracted from cultured cells; reverse transcription (RT) and subsequent polymerase chain reactions (PCRs) were performed as previously described (Drescher and Whittum-Hudson, 1996b). Primer pairs for IFNα_4 and IFNβ were gifts from Dr. Paula Pitha-Rowe (Johns Hopkins Medical Institutions, Baltimore, MD). Southern slot blotting of serial twofold dilutions of the PCR products was performed with DIG-labeled internal sequences using published standard methods (Schatz *et al.,* 1992; Drescher and Whittum-Hudson, 1996b). Sequence-specific oligonucleotides for IFNα (bases 523–540 in the coding sequence) and IFNβ (bases 1752–1767) were derived from published GenBank DNA sequences with Gene Runner software (Hastings Software, Inc., Hastings, NY) and synthesized in the Johns Hopkins Core facility. Oligonucleotides were end-labeled with DIG and used for Southern blotting as reported previously (Drescher and Whittum-Hudson, 1996b). The use of random hexamer priming in the RT step reduced intersample variability since aliquots of the same resulting cDNA were then amplified with the different primer sets; cDNA obtained from normal retinal or glial cell RNA was included as negative control. Samples from all time points of each time course experiment were reverse transcribed in the same RT; PCR for IFNα or β, and hypoxanthine phosphoribosyl transferase (HPRT), were then performed with the various cDNA in the linear portion of the amplification curves as previously described (Schatz *et al.,* 1992; Drescher and Whittum-Hudson, 1996b). Sizes of PCR products were IFNα, 672 bp; IFNβ, 629 bp; and HPRT, 162 bp (Murray and Martens, 1990).

Blots were analyzed by densitometry and signals were

normalized to those obtained for HPRT. Values are expressed as the mean relative signal (±SEM) obtained from two dilutions for duplicate slots (four densitometric values per time point). As no IFNα or IFNβ mRNA was detected under standard culture conditions without exposure to virus, and none was detected in uninfected retinas, the fold increase in transcript levels was calculated from the lowest signal detected, which was arbitrarily assigned a value of 1. These signals were those detected at 1 hr (cells) and 1 day (retinas) after exposure to HSV-1; signal for both IFNs was detected over several twofold dilutions at those times. Two independent time course experiments were performed, each with duplicate samples of extracted retinal tissue and cultured retinal glia.

Probe preparation and *in situ* hybridization

Plasmids containing cDNA specific for IFNα_2 were a gift from Dr. Paula M. Pitha-Rowe. cDNA coding for IFNα_4 and IFNβ was cloned into pGEM to generate sense and antisense RNA probes (Promega). Antisense RNA probes were DIG-labeled for ISH with a commercially available kit using the SP6 promoter; negative control, sense probes were prepared using the T7 promoter (Boehringer-Mannheim, Indianapolis, IN).

Cultured retinal glia were trypsinized and cytospun onto poly-L-lysine-coated (Sigma) slides (3×10^5 cells/ml). Frozen sections of BALB/c eyes (8–10 μm) were placed on Vectabond-coated slides (Vector Laboratories; Burlingame, CA). All slides for ISH were paraformaldehyde-fixed (4%), dehydrated in 90% methanol, and stored at −80° until use. At the time of ISH assay, slides were rehydrated in a methanol series (75, 50, and 25%) and acetylated [300 ml 0.1% diethylpyrocarbonate (depc) water, 3.5 ml of 0.1 M triethanolamine, 750 μl acetic anhydride]. Tissues were proteinase K-treated (1 μg/ml) prior to acetylation, but this step was not required for cells. Unless otherwise indicated, ISH reagents were obtained from the same source (Boehringer-Mannheim). Slides were prehybridized for 1 hr at 55° prior to addition of the probe (Li *et al.,* 1994). Following overnight hybridization (55°), slides were washed sequentially in 50% deionized formamide/2× sodium citrate (SSC) (90 min), and treated with 4 μg/ml RNase A and 20 U/ml RNase T1 (30 min at 37°). After incubation with 2% blocking reagent (1 hr), slides were incubated at room temperature with the alkaline phosphatase-conjugated anti-DIG antibody (1:500) for 2 hr. Development with NBT and BCIP followed, and slides were mounted with coverslips for viewing.

RESULTS AND DISCUSSION

Primary or passaged retinal glia were adsorbed with HSV-1 (m.o.i. = 0.1) or NDV (m.o.i. = 30) and then cultured in a total volume of 10 ml for 1–24 hr in complete DMEM. Preliminary nonquantitative RT-PCR (35 cycles) demonstrated that neither IFNα_4 nor IFNβ was transcribed by retinal glia cultured under standard conditions (Merges and Whittum-Hudson, 1990; Drescher and Whittum-Hudson, 1996a,b). However, within 4 hr after exposure to Newcastle disease virus or HSV-1, both IFN genes were activated in cultured Müller cells (data not shown). To test whether intraretinally derived type 1 IFNs could contribute to early defenses against HSV-1 retinal infection, we investigated the temporal sequence of transcriptional activation of the genes for IFNα_4 and IFNβ in Müller cells after *in vitro* exposure to HSV-1. A more quantitative assay of RT-PCR followed by Southern slot blotting was performed using a modification of the method of Wynn *et al.* (1993) as we have reported previously (Drescher and Whittum-Hudson, 1996b). IFN-specific cDNA was amplified for a total of 27 cycles for Southern slot blot analyses; HPRT cDNA was amplified for 25 cycles. Transcription of both IFNα_4 and IFNβ was induced rapidly by HSV-1 and was easily detected by slot blot analyses of several dilutions of each cDNA within 1 hr of exposure to virus (Figs. 1A and 1B). Earlier times were not tested. IFNα_4 transcript levels increased by more than eightfold within 4 hr of exposure to HSV-1. IFNβ transcription appeared to follow delayed kinetics compared to IFNα: maximal levels of IFNβ transcripts (approximately threefold increases) were detected at 8 hr. *In vitro* HSV-1 infection causes a lytic infection in Müller cells (Merges and Whittum-Hudson, 1990), and times after 24 hr were not tested. Representative results for IFNα_4 and IFNβ from one of two independent time course experiments are shown in Figs. 1A and 1B. In each experiment, all comparisons were made to results with RNA obtained from the same pool of uninfected glial cells after normalization to HPRT cDNA obtained at each time point. Since this assay does not allow quantitative comparisons between the two cytokines, our results do not reveal whether more IFNα_4 or β is produced by HSV-1-stimulated glia. However, ISH performed on cultured glia revealed that essentially 100% of the cells were transcribing both IFNs 2–24 hr after exposure to HSV-1 (not shown).

We wished to test whether the IFN responses to HSV-1 by cultured retinal glia extended to those of Müller cells in intact retinas of eyes infected via the anterior chamber with HSV-1. Retinas were obtained from eyes of young adult BALB/c mice. Eyes of anesthetized mice were inoculated via the anterior chamber with live HSV-1 (2×10^4 PFU), UV-inactivated HSV-1, or diluent (HBSS) in a total volume of 4 μl as previously described (Whittum *et al.,* 1984). Mice were sacrificed 1–7 days postinoculation (p.i.) by cervical dislocation. The eyes were immediately removed and either embedded in OCT for preparation of frozen sections or dissected into the anterior segment or isolated retina for isolation of RNA. Ocular tissues from normal mice were prepared in parallel.

We showed previously that viral antigens are detected only transiently as small foci in retinas between Days 1

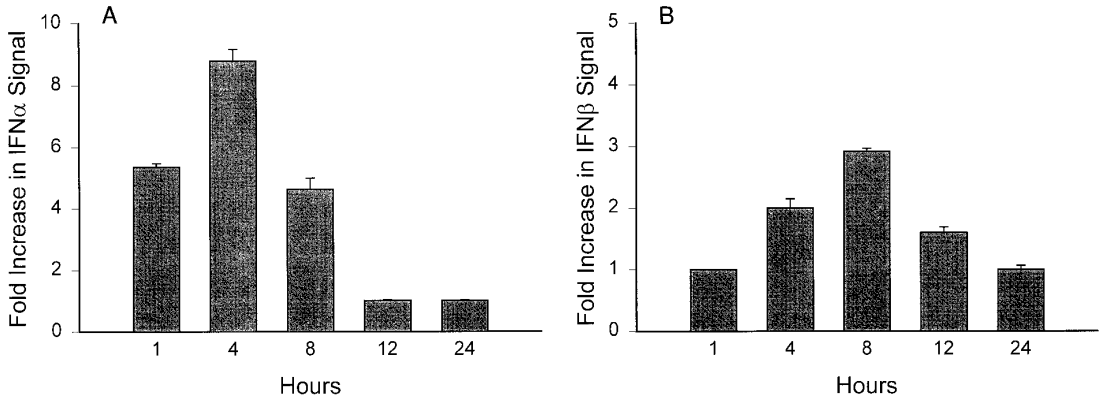

FIG. 1. Temporal changes in IFNα_4 and IFNβ transcript levels in cultured retinal glia after exposure to HSV-1. Using RT-PCR (27 cycles for IFNs; 25 cycles for HPRT) and quantitation by Southern slot blotting, relative transcript levels of IFNα_4 (A) and IFNβ (B) in cultured retinal glia were followed over a 24-hr period after initial exposure to HSV-1. Relative changes in transcript levels were determined as previously described (Drescher and Whittum-Hudson, 1996b). Results are expressed as mean fold increase in transcript signal (±SEM) relative to the lowest positive signal, which was arbitrarily assigned a value of 1. IFN transcript signals were normalized to HPRT signal for the same RNA pool. See Materials and Methods for details.

and 6 after AC inoculation of HSV-1; by Day 7 p.i., HSV-1 antigens are no longer detected in retina despite their continuing expression in the anterior segment of the same eyes (Whittum-Hudson and Pepose, 1987). Using semiquantitative RT-PCR with Southern slot blot analyses (Drescher and Whittum-Hudson, 1996b), we tested whether the temporal patterns of IFN transcription in retinas isolated from HBSS and virus-injected eyes corresponded to the transient presence of HSV-1 in the latter. Low levels of IFNα_4 (Fig. 2A) and IFNβ (Fig. 2B) transcripts were detected as early as Day 1 p.i. after intraocular infection with live HSV-1. Transcript signals increased rapidly and significantly between 2 and 4 days p.i. for both IFNs. High transcript levels of both IFNs were observed in retinas removed on Day 3 (a 3.9-fold increase in IFNα and an 8.8-fold increase in IFNβ signal compared to Day 1 levels). Transcript levels remained high through Day 4 and began to decline by Day 5. By Day 7, IFNα_4 levels remained only slightly higher than Day 1 values, while IFNβ levels remained approximately 4-fold higher than those detected at Day 1. No evidence of IFNα_4 or IFNβ transcription was detected in retinas of HBSS-injected or uninjected eyes at any time. As expected, the induction of IFN genes in retina was detected only after exposure to virus.

Evidence of IFNα and IFNβ transcriptional activity in whole retinal extracts, though congruent with results for cultured retinal glia, did not prove that Müller cells were an intraretinal source of type 1 interferon transcripts. To better localize the cellular source(s) of these IFNs, ISH

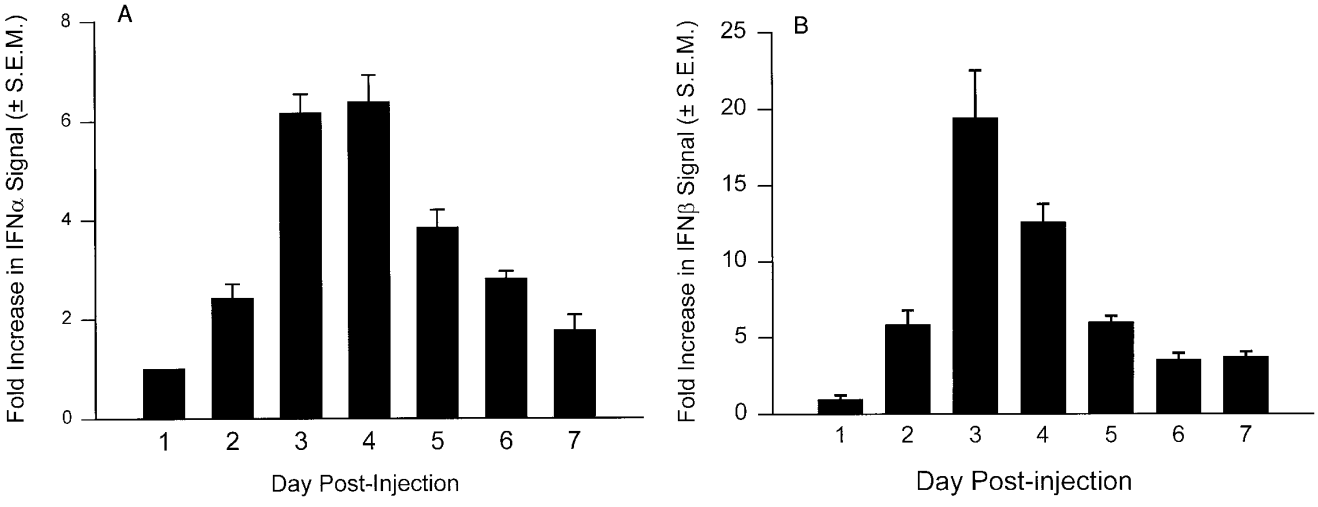

FIG. 2. Time-dependent modulation of IFNα_4 and IFNβ transcription in BALB/c retinas after ocular HSV-1 infection. RT-PCR followed by Southern slot blotting was used to detect changes in relative transcript levels of IFNα_4 (A) and IFNβ (B) over 7 days after their induction by intraocular injection of HSV-1 (2×10^4 PFU/eye). Results are expressed as mean IFN transcript signal relative to controls as measured by densitometry as described in the legend to Fig. 1, except the lowest level of IFN signal was at Day 1. Similar results were obtained in two independent time course experiments.

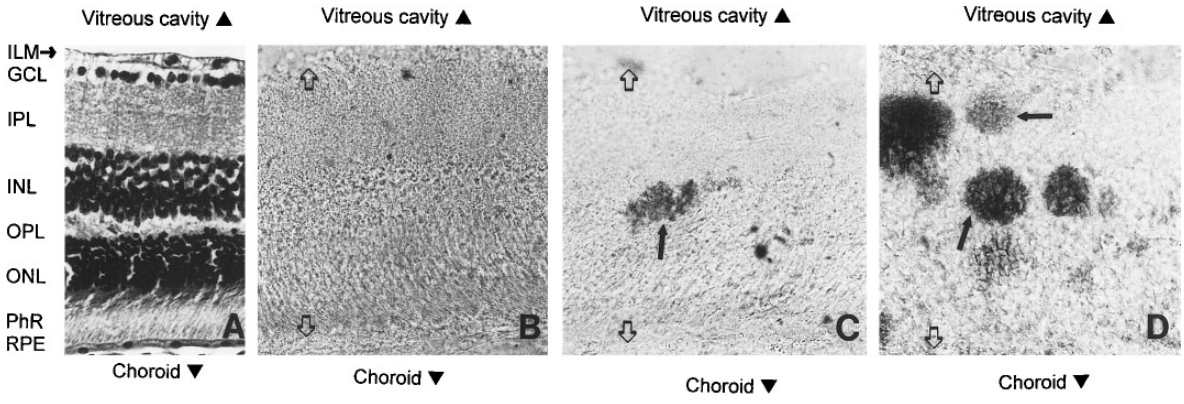

FIG. 3. *In situ* hybridization for IFNα_2 mRNA in intact retina after ocular HSV-1 infection. Using a digoxigenin (DIG)-labeled RNA probe for IFNα_2, frozen sections of BALB/c eyes were tested by ISH to localize IFN transcripts within retina at various times after intraocular HSV-1 infection. (A) Normal retina, hematoxylin and eosin stained. B–D are aligned to A with the vitreous above the retina and choroid underlying the retina. ILM, inner limiting membrane (arrowhead); GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; PhR, photoreceptor cells (rods and cones); RPE, retinal pigment epithelium. (B) Retina of an uninfected eye contains no hybridization signal. (C) Three days after HSV-1 injection. Positive signal is seen in a single, small focus (approximately 50 cells in the plane of view) in the inner nuclear layer (INL) of the retina. (D) Retina at Day 7 p.i. Multiple, larger foci of $>$100 cells/focus were detected throughout the retina in each section. Open arrows indicate the limits of the retina; solid arrows indicate positive signal. Tissues in (B–D) were not counterstained and ISH signal reproduces as gray-black; uninfected controls and samples from each time point were run in the same assay. See Materials and Methods for details. Original magnifications of all figures, 250×.

for IFNα_2 was performed on eyes obtained from normal mice or mice which had been injected via the anterior chamber with either HBSS or HSV-1. Retinal localization of IFNα and IFNβ was consistent with their production by Müller cells, but positive signal was more circumscribed than GFAP staining which had encompassed the entire retina (Whittum-Hudson, 1992). Representative changes in ISH localization in eyes removed at Days 3 and 7 p.i. are shown for IFNα_2 in Fig. 3. IFNα_2 was never detected in the retinas of normal or HBSS-injected eyes (Fig. 3B). By 3 days after injection of HSV-1 into the anterior chamber, a single, small focus ($<$50 cells) of hybridization signal for IFNα localized to the inner nuclear layer (INL) of the retina and was consistent with the location of Müller cell bodies (compare Figs. 3A and 3C). By Day 7, multiple, larger foci were detected throughout the retina (Fig. 3D). Positive foci were similarly distributed for both the Day 3 and the Day 7 samples in duplicate serial sections and in additional eyes taken from the same time points.

To conclusively demonstrate that type 1 IFNs are transcribed by Müller cells, a combination of ISH for IFN RNA with immunohistochemistry for GFAP was used on cell impression smears prepared from retinas of HBSS- and HSV-1-injected eyes. ISH for IFNα_2 and IFNβ on duplicate retinal smears obtained on Days 1 through 7 p.i. after HSV-1 injection demonstrated that both Müller cells and neurons transcribed the IFN genes. A representative ISH result with the IFNβ probe of a retinal smear obtained at Day 6 p.i. is shown in Fig. 4A. Several cell morphologies expressed IFNβ transcripts. Only Müller cells exhibited positive immunohistochemical staining for GFAP (Fig. 4B); Müller cells were increasingly GFAP-positive in smears from buffer- or virus-injected eyes from Day 1 to Day 7 p.i. Retinal impression smears from normal, uninjected eyes were negative for GFAP (not shown). Combining the two techniques demonstrates unequivocally that GFAP-positive Müller cells are one source of intraretinal IFNβ, although some GFAP-negative cells (neurons) are also ISH signal positive for IFNβ (Fig. 4C). Similar results were obtained for IFNα_2 and IFNα_4 (not shown). Retinas from HBSS-injected or normal eyes were included in each run and were all negative by ISH for IFNs (not shown).

Retinas of HSV-infected eyes remained totally free of inflammation, making it unlikely that macrophages or T lymphocytes accounted for intraretinal viral clearance. Significant IFNα/β responses appeared to occur 1–2 days earlier than those observed previously for TNFα and IL-6 (Drescher and Whittum-Hudson, 1996a,b) and may explain the resolution of a very limited viral infection without inflammation in these retinas. The timing of intraretinal IFN transcription is consistent with its role as a first defense against progressive retinal infection. Since contralateral retinas are not protected, the protective local responses in virus-injected eyes may be enhanced by T-cell-derived cytokines (or additional type 1 IFNs) released in the infected anterior chamber. Intraocular IFNα/β may enhance the latter T-cell responses (Belardelli and Gresser, 1996), and perhaps explain the rapid mononuclear inflammatory response in the anterior segment following virus infection (Whittum-Hudson *et al.*, 1985; Whittum-Hudson and Pepose, 1987). One or more soluble mediators produced locally in response to (a) the arrival of small amounts of HSV-1, (b) retina-wide stimulation via neuropeptide or cytokine release after

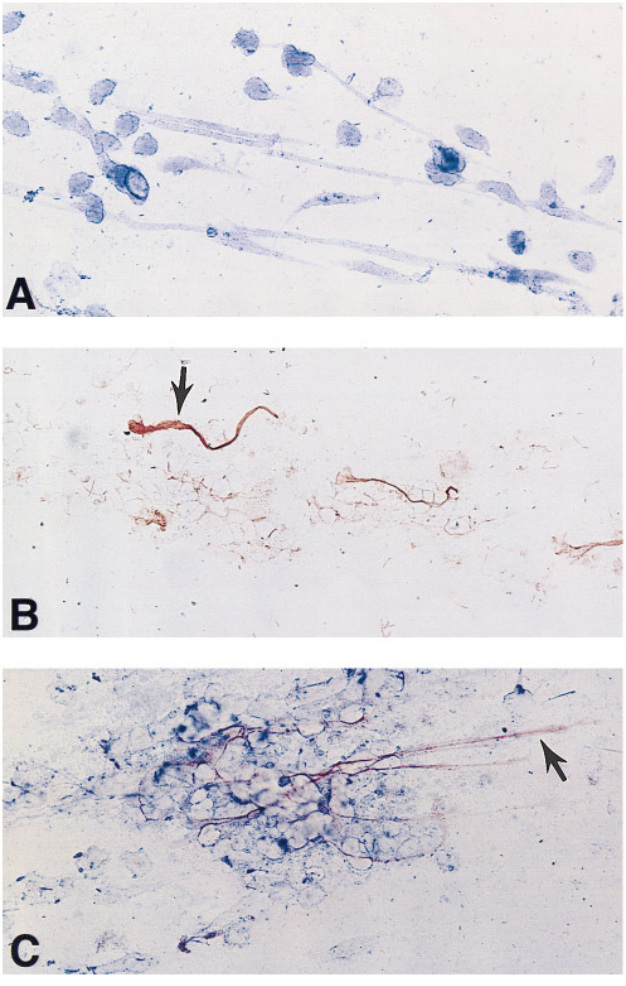

FIG. 4. Localization of IFNβ mRNA to retinal Müller cells by combined ISH and immunohistochemistry for GFAP on impression smears of retina. (A) *In situ* hybridization (ISH) was used to probe for IFNβ mRNA (blue) in posterior segment impression smears from a virus-injected eye at Day 6 p.i. No signal was detected in smears from either uninjected or HBSS-injected eyes (not shown). (B) Duplicate slide which was stained for GFAP (red). Müller cells are the only cells expressing GFAP. (C) A combination of ISH for IFNβ and ABC immunoperoxidase staining for GFAP to detect Müller cells. IFNβ mRNA (blue) and GFAP (red) were colocalized in some cells (arrow) in a duplicate of the impression smears shown in A and B, indicating that Müller cells were one intraretinal source of IFNβ. No counterstain was used in these figures. Similar results were obtained for IFNβ and IFNα on smears from Days 1 to 7 p.i. except for variations in the intensity of GFAP staining which increased between Days 1 and 3 p.i. Original magnifications, 400×. See Whittum-Hudson and Pepose (1987), Merges and Whittum-Hudson (1990), and Drescher and Whittum-Hudson (1996b) for further details of the methods used.

anterior segment infection, or (c) a combination of these two could explain successful clearance of HSV-1 from retinas of virus-injected eyes. Type 1 IFN production in contralateral, uninjected eyes was not investigated in the present studies because our focus was on retinal-protective mechanisms in virus-injected eyes. Moreover, the tempo of virus appearance in the latter eyes is very different from that in ipsilateral, injected eyes — beginning at Day 7 after anterograde spread from the optic nerve (Whittum-Hudson and Pepose, 1987) — and Müller cells upregulate GFAP only in areas of necrotic retina which also correspond to the areas with HSV-infected cells (Whittum *et al.,* 1992). In other ocular and nonocular infection models, secreted type 1 interferons have been demonstrated after HSV-1 or HSV-2 infection (Lopez, 1975; Ellermann-Eriksen *et al.,* 1986; Taylor and O'Brien, 1987; Su *et al.,* 1990; Hendricks *et al.,* 1991; Lausch *et al.,* 1991) and correlated with some resistance to infection (Lausch *et al.,* 1991).

Protection from viral infections would require only minimal IFN transcription (and translation) since type 1 IFNs are among the most potent antivirals known, with only a few IFN molecules required to make cells refractory to infection (Dulbecco, 1988). Given that we were able to detect transcription of IFNα and β in Müller cells by *in situ* hybridization, the levels of IFN produced were presumably above the theoretical lower limit required for protection. While the present studies do not demonstrate production of IFNα and β protein, translation would be assumed to occur as these genes are transcriptionally regulated. In support of this, biologically active IFNα/β was detected by bioassay of whole eye homogenates obtained 4 days after HSV-1 injection into the anterior chamber of BALB/c eyes (J. L. Taylor and J. A. Whittum-Hudson, unpublished results). Intraretinal transcription of the type 1 IFN genes occurred in virus-injected eyes within the time frame (1–3 days) in which retinal Müller cells upregulated and maximally expressed GFAP (Whittum-Hudson, 1992). In addition to the directly antiviral functions of the type 1 interferons, IFNα/β induction has been shown to have immunomodulatory effects such as upregulation of major histocompatibility antigens in both animals and humans (i.e. Migita *et al.,* 1991; Dhib-Jalbut *et al.,* 1995; Belardelli and Gresser, 1996). Chen *et al.* demonstrated *in vitro* that human corneal fibroblast-derived TNFα synergized with relatively low levels of IFNγ (5 U/ml) to induce an anti-HSV-1 response via induction of IFNβ (Chen *et al.,* 1993). The relationship of these cytokines to anti-HSV-1 responses in the intact eye has not been shown. Our previous studies demonstrated that Müller cells produce TNFα and IL-6 (Drescher and Whittum-Hudson, 1996a,b) and, taken together with the present studies, suggest that the Müller cells may participate in *in vivo* antiviral responses via several mechanisms involving immunologic and/or antiviral cytokines. Local cytokine responses may explain why virus-mediated retinopathy is relatively rare except in immunodeficient individuals. Our studies show for the first time that Müller cells produce IFNα and IFNβ and further support the potential importance of Müller cell-derived soluble mediators during ocular infection.

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