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Alteration of intracerebral cytokine production in mice infected with herpes simplex virus types 1 and 2

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Abstract

Previously we reported that a lethal strain of herpes simplex virus type 2 (HSV-2) infects the brain following ocular inoculation of mice. We now demonstrate that HSV-2 mediates an unusual intracellular sequestering of class II major histocompatibility complex (MHC) antigens. With use of an RNase protection assay, we observed a selective inhibition of IFN-γ and IL-6 gene transcription in brains of mice infected with HSV-2. It is likely that the inhibition of cytokine gene expression was mediated through a failure to activate $CD4^+$ lymphocytes. These data suggest that the infecting herpesvirus can influence the profile of intracerebrally produced cytokines, which in turn may determine the outcome of the infection.

1. Introduction

Herpes simplex virus (HSV) has been studied extensively with regard to the establishment of latent infections in neurons (reviewed in Fraser et al., 1991; Ho, 1992), the role of the immune system in eliminating HSV-infected epithelial cells and limiting viral spread in the nervous system (reviewed in Simmons et al., 1992), and the neurotropism and fidelity of transynaptic transport (LaVail et al., 1990; Martin and Dolivo, 1983). Although it is generally accepted that the T cell-mediated immune response is necessary to limit viral spread and to aid in establishing the latent HSV infection in neurons, the mechanism underlying antiviral action in the nervous system remains largely undefined. The T cell-mediated eradication of infectious HSV from neurons is likely to be complex, since neither neurons nor glia express detectable antigens of either major histocompatibility complex (MHC) class I or class II constitutively (Wong et al., 1984; Lampson and Hickey, 1986; Joly and Oldstone, 1991). Consequently, it is unlikely that infectious HSV is eliminated from neurons by immune-mediated cell lysis. Alternatively, production of soluble anti-herpetic mediators acting at the level of the infected neurons has been suggested (Rossol-Voth et al., 1991; Simmons and Tscharke, 1992).

Primary neurotropic viral infections, such as with HSV, elicit a series of interactions between the virus and the highly differentiated cells of the nervous and immune systems. The immune response to viral infection in the nervous system can protect the infected host from death (Nash et al., 1987; Maehlen et al., 1989; Yamaguchi et al., 1991) or, conversely, can cause detrimental neuronal damage (Richt et al., 1989; Doherty et al., 1990; Rodriguez and Lindsley, 1992). Although the causes for these differing response patterns are unknown, studies have indicated that cytokines mediate both immunopathological sequences (Hartung et al., 1992; Huchet et al., 1993) and effective antiviral responses (Klavinskis et al., 1989; Rossol-Voth et al., 1991).

In the brain, pluripotential, pro-inflammatory cytokines including TNF-α, TNF-β, IL-1β, IL-1α, and IL-6, are secreted by non-neuronal brain cells (i.e. astrocytes, oligodendrocytes and microglia) in response to neuronal injury, trauma, and bacterial and viral infections (Liebermann et al., 1986; Chung and Ben-

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veniste, 1990; Giulian and Corpuz, 1993). The T cell-mediated immune response to viral infections in the brain is characterized by the induction of MHC class I and II antigen expression on non-neuronal cells (Olsson et al., 1987; Deschl et al., 1990; Joseph et al., 1990; Weinstein et al., 1990) and recruitment of $CD4^+$ and $CD8^+$ T lymphocytes (Olsson et al., 1987; Chan et al., 1989; Deschl et al., 1990; Williamson et al., 1991). Subsequently, cytokines such as type I and II interferons, IL-6, and IL-2 are also produced by activated T lymphocytes in restricted regions of viral infection. Thus, it is likely that the extent of neuronal damage and/or eradication of viral infection is affected by a complex network of interacting soluble factors produced by both resident brain cells and infiltrating cells.

We have developed a murine model system to study the neural–immune interactions that occur following infection of the central and peripheral nervous systems (CNS and PNS) with either a lethal strain of HSV-2 or non-lethal strain of HSV-1 (Lewandowski et al., 1993). Preliminary characterization of the immune response in the brains of infected mice indicates that the induction of MHC class II gene expression and the recruitment of T lymphocytes to restricted regions of viral infection in the CNS occurs following ocular inoculation with either HSV-1 or HSV-2. However, HSV-2 appears to interfere with MHC class II-restricted viral antigen presentation by inhibiting the expression of MHC class II antigens at the plasma membrane (Lewandowski et al., 1993). Therefore, we postulated that inhibition of MHC class II-restricted viral antigen presentation by HSV-2 would greatly affect the profile of intracerebrally produced cytokines by $CD4^+$ cells and, thus, could significantly influence the outcome of infection. The data reported in this communication demonstrate that the profile of intracerebral cytokine gene transcription is significantly altered in mice infected with HSV-2, and that this phenomenon may result from HSV-2-mediated inhibition of MHC class II-restricted antigen presentation in the brains and trigeminal sensory ganglia.

2. Materials and methods

2.1. Viruses

HSV was propagated according to previously published procedures (Lewandowski et al., 1989). Vero cells were infected with either HSV-1 or HSV-2 at a multiplicity of infection of 0.01 pfu per cell. Following a 1-h adsorption period, the infected cells were incubated until 100% cytopathic effect was observed. Virus yield was determined by titration of serial dilutions of HSV on Vero cells and expressed as pfu/ml. HSV-1 strain F (HSV-1(F)) was a gift from Bernard Roizman, (University of Chicago, IL) and the initial stock of the lethal strain of HSV-2 was a provided by Rachel Schrier, (University of California, San Diego, CA). Subsequent to a previous publication in which the lethal strain of HSV-2 was referred to as HSV-1(KOS) (Lewandowski et al., 1993), this virus was sereotypically identified as a strain of HSV-2. Accordingly, we now refer to this strain of HSV as HSV-2. A large stock of each virus strain was prepared from a first round plaque-purified clone.

2.2. Viral infection of mice

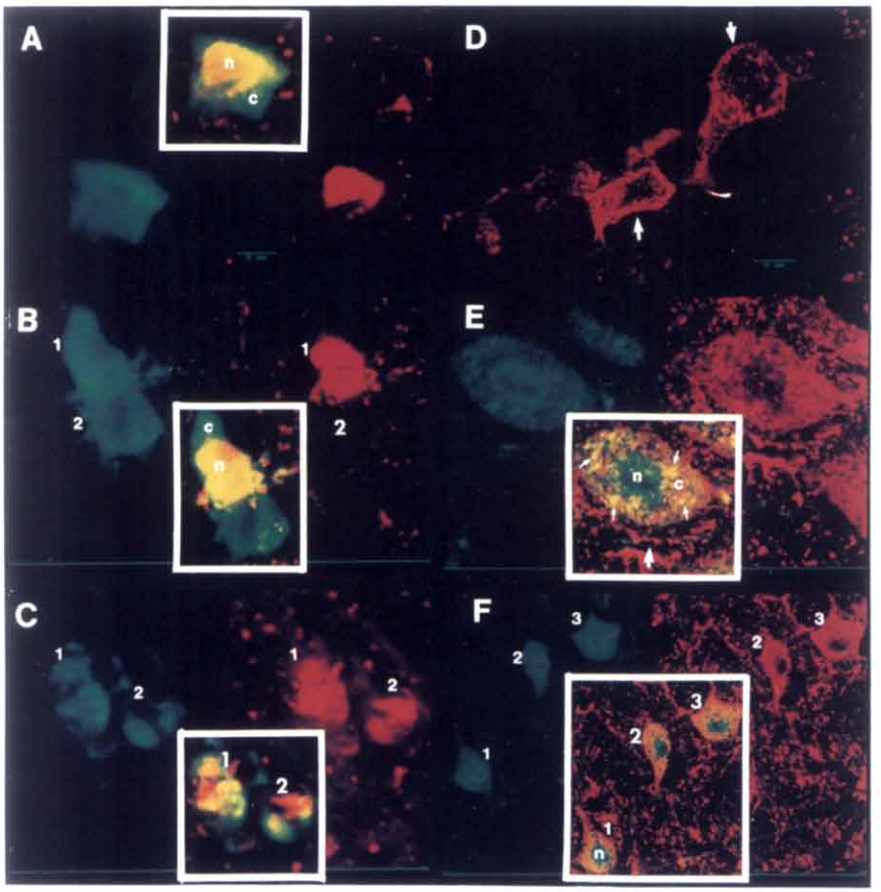
Mice of various backgrounds and haplotypes were ocularly inoculated with either HSV-1 (2×10^5 pfu's per eye) or HSV-2 (1.85×10^4 pfu's per eye) (Lewandowski et al., 1993). All mice were obtained from The Scripps Research Institute rodent breeding colony (La Jolla, CA) and included BALB/cByJ ($H\text{-}2^{d/d}$), BALB/B ($H\text{-}2^{b/b}$), C57BL/6J ($H\text{-}2^{b/b}$), CBA/CAJ ($H\text{-}2^{k/k}$), and B10.D2 ($H\text{-}2^{d/d}$).

Fig. 1. Confocal fluorescence microscopic localization of HSV and MHC class II antigens in brain. **(A–C,E–F)** Split-color representations of the cellular localization of HSV antigens (left side, fluorescent green), and MHC class II antigen expression (right side, fluorescent red), the inset in each panel represents a merge of the two fluorescent labels. In each panel corresponding cells are identified by numbers; n indicates the region of the nucleus; and c cytoplasm. **(D–F)** Brain sections from HSV-1-infected mice, 5 days postinfection (dpi), MHC class II antigen expression is evident in the cytoplasm and at the cell perimeter (large white arrows; **D,E**), and HSV-1 antigens can be detected in the nucleus, cytoplasm, and at the cell surface. Additionally, several 'hot-spots' of intense yellow-orange color, in the cytoplasm and at the cell perimeter (small white arrows; **E**), indicate true co-localization of HSV-1 and MHC class II antigens. The scale bar in **(D)** equals 5 μm in **(D,E)**, and 15 μm in **(F)**. **(A–C)** Brain sections from HSV-2-infected mice, 6 dpi. MHC class II expression was observed in the nucleus of the cells as indicated by the red and yellow-red fluorescence and was surrounded by HSV-2 proteins, indicated by the green fluorescence. The scale bar in **A** equals 5 μm in **(A–C)**. Immunohistochemical processing of samples for confocal microscopy is described in Materials and methods.

Fig. 4. Co-localization of MHC class II and Mac-1 antigens in HSV-2-infected brains. In all panels Mac-1 immunoreactivity is represented by the fluorescent green and MHC class II immunoreactivity is represented by the fluorescent red. MHC class $II^+/Mac\text{-}1^+$ cells, indicated by arrows in all panels, are represented by cells containing both fluorescent green and red colors. In some cells an orange fluorescent color created by overlapping green and red fluorescence was observed and indicated the close proximity of both antigens. MHC class $II^+/Mac\text{-}1^-$ cells, indicated by asterisks, are represented by cells that clearly contain only true red fluorescent color. **(A)** A typical field at a lower overview magnification. **(B,C)** Additional fields at higher magnification. Tissue from HSV-2-infected mice, 6 dpi. Scale bars: in **(A)** 20 μm, in **(B)** 5 μm, and 10 μm in **(C)**.

2.3. Immunohistochemistry

The procedure for immunohistochemical detection of antigens in tissues from HSV-infected mice has been previously described (Lewandowski et al., 1993). Mice infected with either HSV-1 or HSV-2 were perfused with 2-4% paraformaldehyde, followed by 5 ml of a 10% sucrose solution. The brains and trigeminal ganglia were removed and further cryoprotected in an 18% sucrose solution. For the simultaneous detection of multiple antigens, frozen cryostat tissue sections were incubated overnight with the primary antibodies



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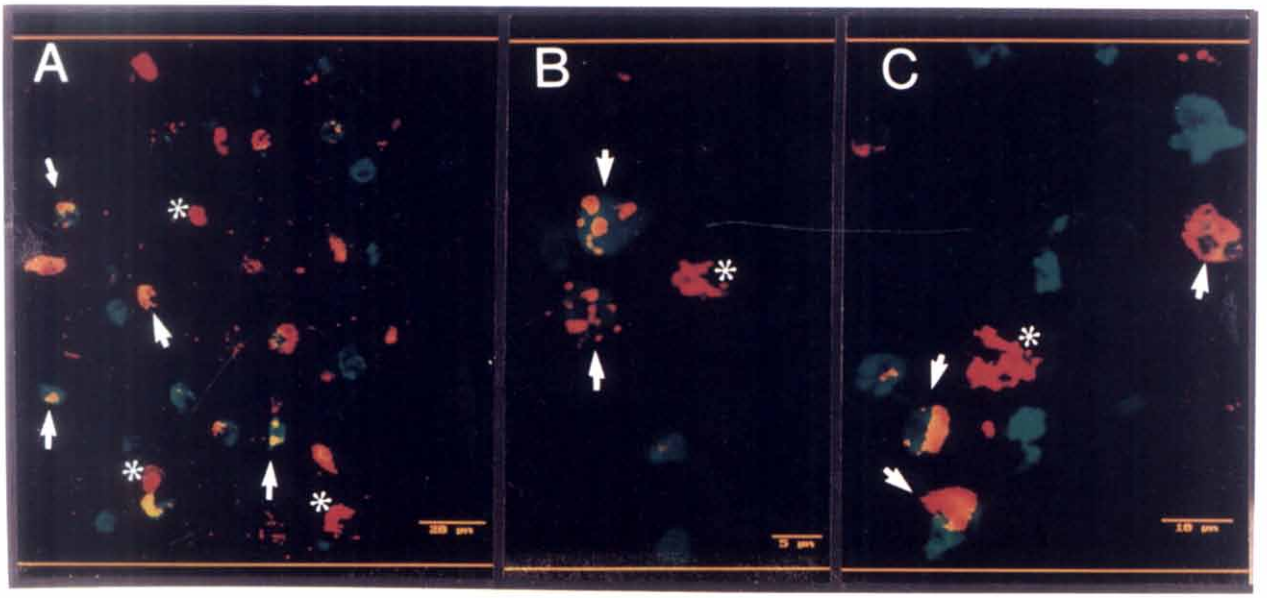

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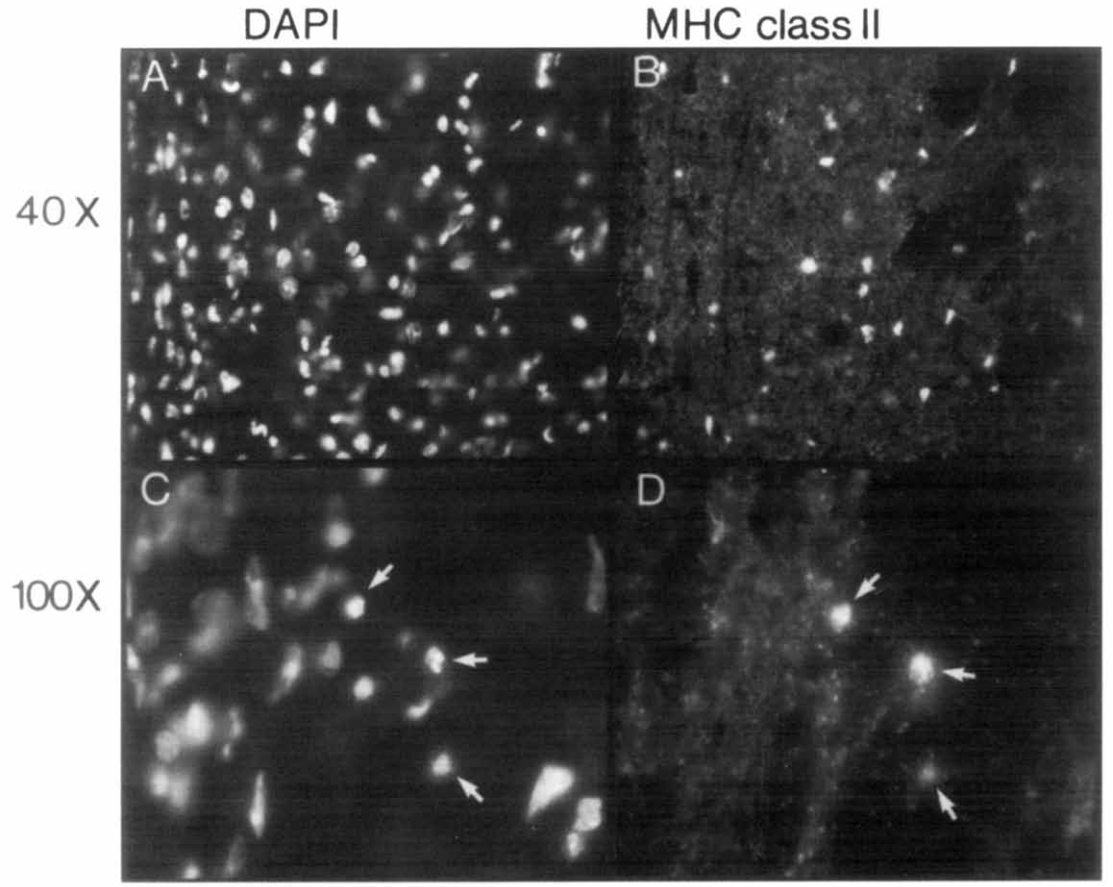

Fig. 2. Co-localization of a nuclear stain (DAPI) and MHC class II antigen expression in HSV-2-infected brains. **(A,C)** DAPI staining of brain tissue from HSV-2-infected mice, 6 dpi. **(B,D)** Immunohistochemical detection of MHC class II antigens in the same tissues as **(A,C)**. At a low magnification, a subset of the DAPI-positive cells **(A)** were also positive for MHC class II expression **(B)**. At a higher magnification it was evident that MHC class II antigen expression completely coincided with the DAPI staining **(C,D)**; white arrows indicate cells positive for both DAPI and MHC class II antigen expression.

to ensure complete tissue penetration, without disruption of cellular morphology by detergent. Primary antibodies included biotinylated-anti-I-$E^{k,d,p,r}$ specific for the α-chain (clone 14-4-4s, 1:350, unless otherwise indicated, Pharmingen, San Diego, CA), biotinylated anti-I-$E^{k,r}$ (clone 17-3-3, 1:200, Pharmingen), biotinylated anti-I-A^{d} (clone AMS-32.1, 1:200, Pharmingen), an antibody cocktail containing biotinylated anti-H-$2D^{d}$ (clone 34-2-12, 1:200, Pharmingen) and biotinylated anti-H-$2K^{d}$ (clone SF1-1.1, 1:200, Pharmingen), a rat mAb specific for mouse Mac-1 antigen was used to identify brain microglia and macrophages (1:20, Boehringer Mannheim, Indianapolis, IN), and HSV-1 and -2 antigens were detected with a polyclonal antibody to HSV-1 envelope proteins (DAKO Corporation, Carpinteria, CA). Secondary antibodies and reagents were Cy3-conjugated streptavidin (1:300), DTAF-conjugated mouse anti-rabbit IgG (1:200), and DTAF-conjugated mouse-anti-rat IgG (1:200) (Jackson ImmunoResearch, West Grove PA). Following incubation with secondary antibodies, the tissue sections were washed with TBS, pH 7.6, and mounted with an anti-fade medium (5% *n*-propylgalate in 80% glycerol) or with SlowFade (Molecular Probes, Inc., Eugene, OR).

To evaluate further the subcellular localization of MHC class II antigens, frozen cryostat brain sections were incubated with the mAb specific for MHC class II (14-4-4s) and co-stained with 10 μg/ml 4′,6-diamidino-2-phenylindole hydrochloride (DAPI, Sigma, St. Louis, MO) diluted in TBS, pH 8.0, for 10–30 s, rinsed with water and mounted in anti-fade medium. DAPI is an A–T selective DNA-intercalating agent that emits a fluorescent signal at 455 nm (Sanna et al., 1992). The sections were examined for co-localization of MHC class II antigens with the nuclear DAPI stain by standard light microscopy using a Zeiss Axiophot photomicroscope.

2.4. Confocal Microscopy

Confocal fluorescence microscopy was used to examine the cellular localization of HSV antigens and MHC class II antigen expression in HSV-1- and HSV-2-infected neural tissues, and to determine the nature of

the MHC class II-positive cells in the same tissues. The confocal facility at The Scripps Research Institute is equipped with a Bio Rad MRC 600 system including an inverted Zeiss microscope, an Argon:Krypton laser allowing for dual and triple channel analysis, and all hardware and software necessary for complete 3-dimensional analysis and documentation of data. Samples were prepared as described above, prior to analysis.

2.5. Immunoblot analysis of MHC class II proteins

Extraction of whole-cell proteins from mouse brain and trigeminal ganglia was accomplished as previously described (Laemmli, 1970; Lewandowski et al., 1989). Briefly, following binocular inoculation with either HSV-1 or HSV-2, all mice were sacrificed at 6 dpi by cardiac perfusion with ice-cold PBS, pH 7.5 containing PMSF (0.5 mM). The superior colliculus, trigeminal

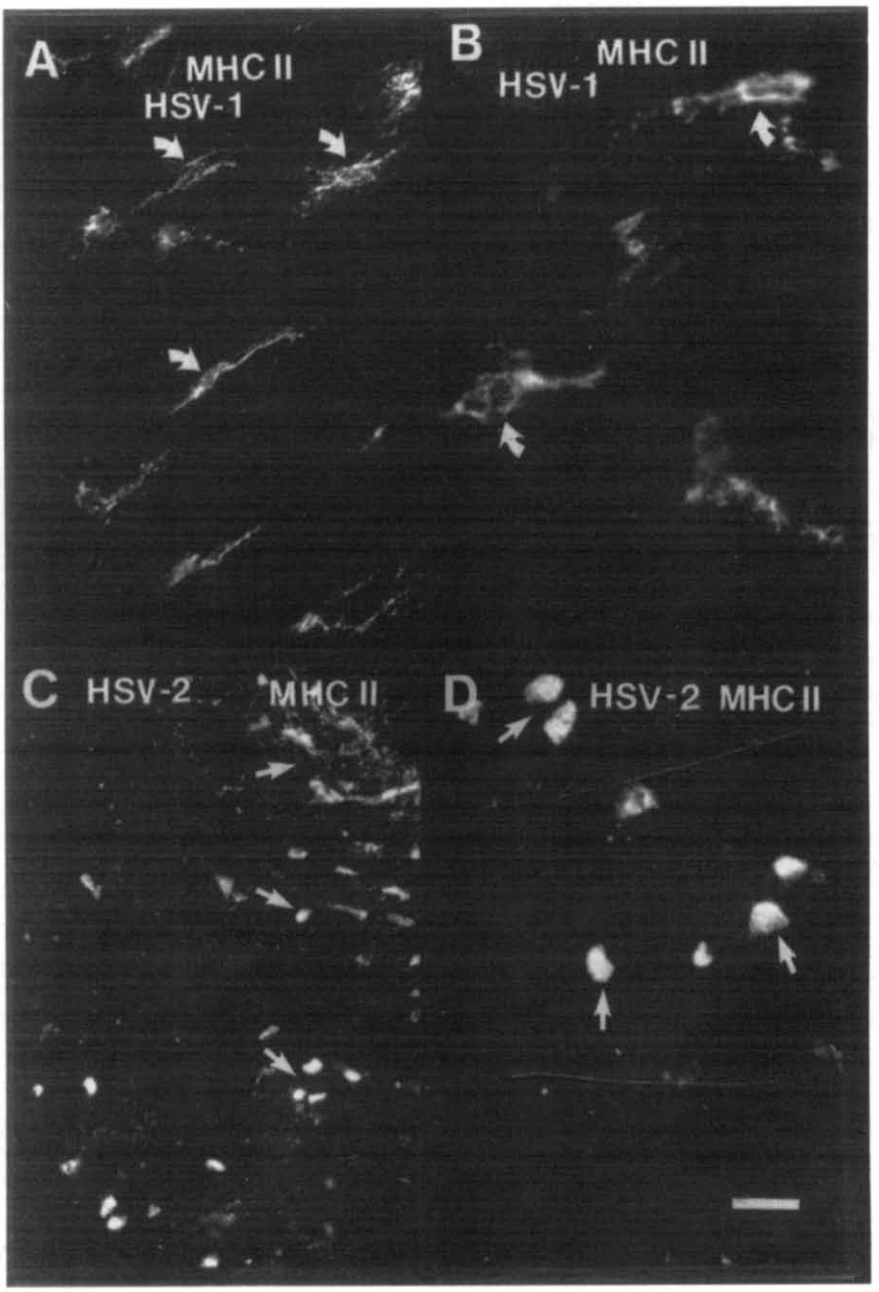

Fig. 3. Cellular localization of MHC class II antigen expression in HSV-infected trigeminal sensory ganglia. MHC class II-positive cells (arrows) are apparent in trigeminal ganglia at 6 dpi with either HSV-1 (**A,B**) or HSV-2 (**C,D**). At higher magnification (**B,D**), the subcellular localization of MHC class II antigen expression can be observed. In HSV-1-infected tissue, localization of the MHC class II antigen expression is cytoplasmic and associated with the cell membrane. In HSV-2-infected tissue, the localization of MHC class II antigen expression is predominantly intracellular. The scale bar in (**D**) represents 23 μm for (**A,C**) and 11.5 μm for (**B,D**).

ganglia, and spleens were removed and snap-frozen in liquid nitrogen. Proteins were extracted from the tissues by homogenizing and boiling in extraction buffer (10 mM Tris, pH 7.5, 1.5 mM $MgCl_2$, 3 mM DTT, 50 mM PMSF). Extracts were mixed 1:1 with 2 × sample buffer (50 mM Tris · HCl, pH 6.8, 1% SDS, 0.7 M 2β-mercaptoethanol, 20% glycerol and 0.1% Bromophenol blue, 0.05 mg tissue per ml of buffer) and were boiled for 10 min. Proteins were separated by SDS-PAGE (10%) in a mini-gel apparatus (Idea Scientific, Minneapolis, MN) and were transferred to nitrocellulose paper with a Genie Blotter apparatus (Idea Scientific). The filter was incubated overnight at room temperature in a solution containing the biotinylated-mAb to MHC class II (14-4-4s, diluted 1:200) and 3% non-fat dry milk (w/v) in TBS. Primary antibody binding was detected by incubation with streptavidin-conjugated alkaline phosphatase, with subsequent incubation with the NBT/BCIP substrate system (Bio-Rad, Richmond, CA).

2.6. Detection of cytokine transcripts in brain

The major cytokine transcripts produced in the brain during HSV-1 and HSV-2 infections were detected by using an RNase protection assay exactly as described (Hobbs et al., 1993), with anti-sense RNA probes generated from a template set including mIL-1α (B), mIL-1β (A), mIL-2 (A), mIL-3 (B), mIL-4 (B), mIL-5 (C), mIL-6 (B), mIFN-γ (B), mTNF-α (A), mTNF-β (A), and L32 (A). Prior to removal of tissue mice were perfused with ice-cold PBS (treated with DEPC). Total RNA was isolated from the superior colliculus by the method of Chomczynski and Sacchi (1987).

3. Results

3.1. HSV-2 infection mediates intracellular sequestering of MHC class II antigens in neural tissues

With use of confocal fluorescence microscopy we observed a strikingly different subcellular localization of MHC class II antigens in brain tissue from HSV-1- versus that from HSV-2-infected mice (Fig. 1). In brain tissue from HSV-1-infected mice, MHC class II antigen expression was evident in the cytoplasm and at the cell perimeter, while HSV-1 antigens were detected in the nucleus, cytoplasm, and at the perimeter of these same cells (Fig. 1D–F). In brain tissue from HSV-2-infected mice, the expression of MHC class II antigens was never observed at the perimeter of cells containing immunoreactive HSV-2 proteins. Unexpectedly, the profile of MHC class II immunoreactivity in cells that also contained viral proteins was consistent with nuclear localization (Fig. 1A–C). The nuclear localization of MHC class II antigen expression in tissue from HSV-2-infected mice was further corroborated by the apparent co-localization of MHC class II immunoreactivity with the DNA-specific stain, DAPI (Fig. 2).

The prominent intracellular confinement of MHC class II immunoreactivity was not limited to visual pathways of mouse brains infected with HSV-2, but was also observed in cells in the corresponding trigeminal sensory ganglia. By use of conventional fluorescence microscopy, we observed MHC class II antigen expression in the cytoplasm and at the perimeter of cells in HSV-1-infected trigeminal ganglia (Fig. 3A,B). In contrast, MHC class II immunoreactivity was predominantly intracellular and was not present at the

Table 1
Haplotype specificity of MHC class II antigen expression in HSV-2-infected mice

Background and haplotype [a]	14-4-4s I-$E^{k,d,p,r}$ [b]	17-3-3 I-$E^{k,r}$	AMS-32.1 *I-A*^{d}
BALB.C (d/d)	Strongly positive Intracellullar sequestering	Negative	Positive Intracellular sequestering
BALB.B (b/b)	A few positive cells	Negative	Negative
C57BL.6J (b/b)	A few positive cells	Negative	Negative
CBA/CAJ (k/k)	Strongly positive Intracellular sequestering	Strongly positive (fewer cells than with 14-4-4s) Intracellular sequestering	Negative
B10.D2 (d/d)	Strongly positive Intracellular sequestering	Negative	Strongly positive Intracellular sequestering

[a] The background of each group of mice is indicated in capital letters, and the H-2 haplotype is indicated in parentheses. Mice were intra-ocularly inoculated with 1 μl of HSV-2. Viral spread through the brain was determined at 6 days postinfection by immunohistochemical detection of HSV-2 antigens, and was found to be extensive in all groups of mice inoculated.

[b] MHC class II-specific mAbs (clone designations, MHC class II molecule and haplotype specificities). MHC class II antigens were detected immunohistochemically by conventional light microscopy in brain tissues from HSV-2-infected mice. All mAbs were biotinylated and were used at a dilution of 1:200. MHC class II immunoreactivity was rated as negative, positive or strongly positive. Profiles of MHC class II immunoreactivity obtained with the various mAbs that were consistent with the intracellular sequestering of MHC class II molecules detected with clone 14-4-4s in BALB.cByJ mice were designated as 'intracellular sequestering'.

perimeter of cells in HSV-2-infected trigeminal ganglia (Fig. 3C,D).

Because of the unusual nuclear localization of the MHC class II immunoreactivity, the specificity of immunohistochemical detection of MHC class II antigens was rigorously verified in HSV-2-infected mice of various haplotypes by using a panel of mAbs specific for MHC class II antigens. The results of this study are shown in Table 1. HSV-2 spread extensively through the contralateral and ipsilaterateral brain visual system in all mice inoculated regardless of background or haplotype (data not shown). Immunohistochemical detection of MHC class II antigens in brain tissue from these mice with all three mAbs was haplotype-specific, with clone 14-4-4s showing a low level of cross-reactivity. By conventional light microscopy the profile of cellular localization of MHC class II immunoreactivity with each mAb was consistent with that observed with clone 14-4-4s in BALB/cByJ mice, specifically, intracellular, non-plasma-membrane-associated. Altogether, these data suggest that the observed nuclear detection of MHC class II immunoreactivity in HSV-2-infected neural tissues represents specific binding of mAbs to MHC class II antigens.

3.2. MHC class II-positive cells in HSV-2-infected brains are predominantly Mac-1 positive

Several reports have demonstrated that expression of MHC class I and II antigens is frequently induced on brain microglia during conditions of inflammation (Frei et al., 1988; Hickey and Kimura, 1988; Streit et al., 1989). To determine whether microglia and macrophages were the source of MHC class II expres-

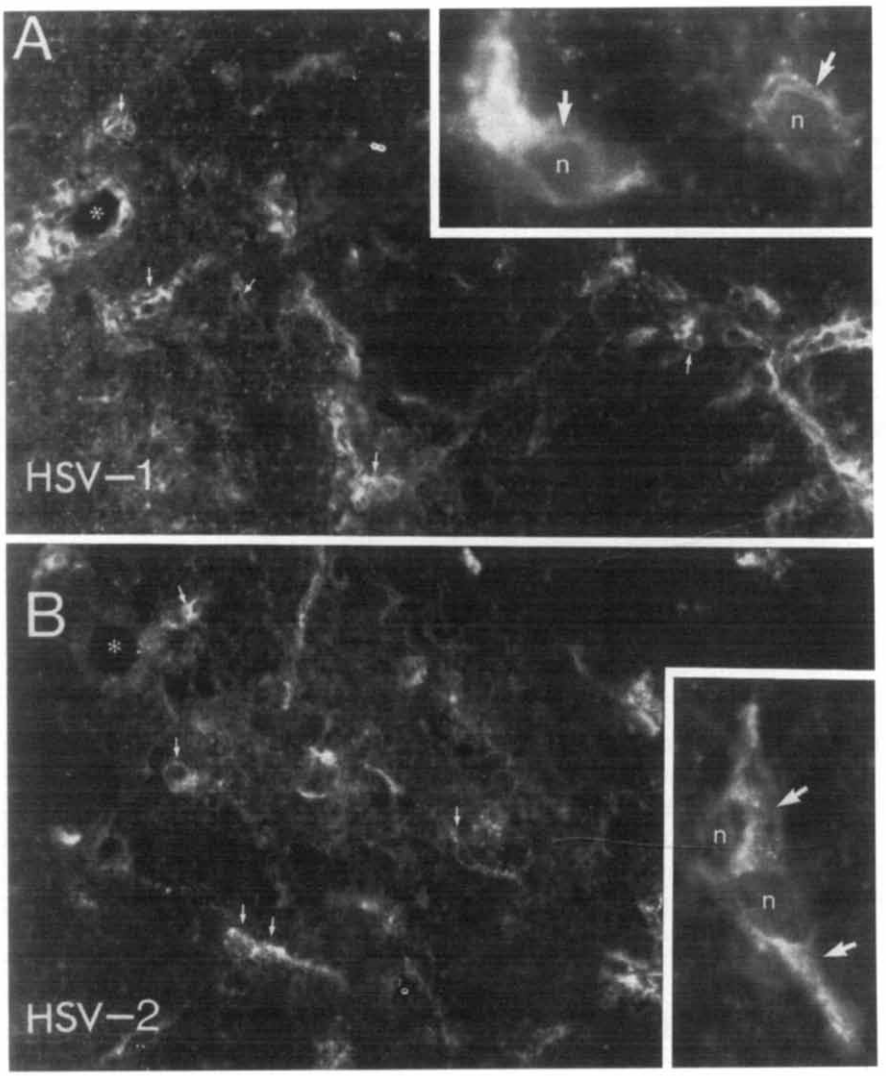

Fig. 5. Cellular localization of MHC class I antigen expression in HSV-1 and HSV-2-infected brains. MHC class I-positive cells (arrows) are apparent in HSV-1-infected brains, 5 dpi (**A**), and in HSV-2-infected brains, 6 dpi (**B**). The cellular localization of MHC class I antigen expression was cytoplasmic and associated with the cell perimeter in brain sections from both groups of mice. In the high magnification inset panels, thick arrows indicate localization of MHC class I antigen expression and n, the nucleus. The asterisks indicates blood vessels in (**A**) and (**B**).

sion in our system, tissue sections from HSV-2-infected brains were simultaneously incubated with mAbs to MHC class II (14-4-4s) and Mac-1 antigen. Confocal microscopy was used to evaluate the distribution of the immunoreactivities. In any given field, cells positive for intracellular MHC class II immunoreactivity were predominantly also positive for Mac-1 (Fig. 4), indicating, in agreement with the literature, that MHC class II antigen expression was induced in microglia and brain macrophages during HSV-2 infection. However, a second population of MHC class II-positive, Mac-1-negative cells was also observed. Two possible cell types exist for this phenotype: astrocytes or neurons. Previously we observed that HSV-2-infected regions of brain are negative for the astrocyte marker, GFAP (data not shown). Thus, if this smaller population of MHC class II-positive cells are astrocytes, then they have become GFAP-negative. Currently it is widely accepted that neuronal cells cannot be induced to express MHC antigens (Wong et al., 1984; Mauerhoff et al., 1988; Joly and Oldstone, 1991); however, preliminary ultrastructural data from this laboratory suggest that these MHC class II-positive cells may be HSV-2-infected neurons (M. Morales, Department of Neuropharmacology, The Scripps Research Institute, manuscript in preparation).

3.3. MHC class I immunoreactivity is detected at the cell surface in HSV-1 and HSV-2-infected brains

The intracellular sequestering of MHC molecules appeared to be selective for class II molecules, as MHC class I antigen expression was immunohistochemically detected at the perimeter and in the cytoplasm of cells in brain tissue from mice infected with either HSV-1 or HSV-2 (Fig. 5).

3.4. HSV-2 does not interfere with the post-translational processing of MHC class II molecules in neural tissues

Considering the abundant MHC class II immunoreactivity observed in HSV-2-infected neural tissues, it was unlikely that the apparent inhibition of cell surface expression of MHC class II antigens resulted from reduced synthesis of the protein. Alternatively, HSV-2 may disrupt transport of MHC class II molecules to the cell surface by interfering with post-translational processing. Immunoblot analysis was used to determine the relative molecular masses of MHC class II molecules in brain, trigeminal ganglia, and spleen tissue from HSV-1- and HSV-2-infected mice (Fig. 6). Using the MHC class II mAb (clone 14-4-4s), the major protein band detected in all samples was of the expected molecular mass for the mature a chain of MHC class II (34 000 daltons). These data suggest that HSV-2 does not interfere with the post-translational processing of MHC class II I-E molecule, since incompletely processed MHC class II molecules would not migrate to the same molecular mass as the mature molecule.

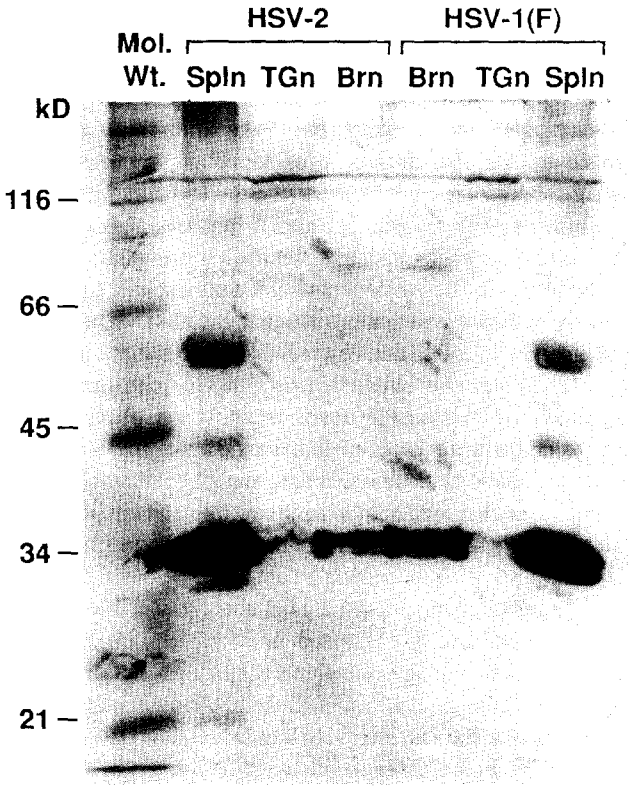

Fig. 6. Immunoblot analysis of MHC class II molecules in HSV-1 and HSV-2-infected neural tissues. Preparation of and immunoblot analysis of protein extracts from brains, trigeminal ganglia, and spleens of HSV-1 and HSV-2-infected mice is described in Materials and methods. A major protein band of 34 kDa was detected in all samples. Additionally, the heterodimer form of MHC class II (α and β chains) was detected in the spleen samples. Abbreviations: Spln (spleen), Brn (brain), TGn (trigeminal ganglion nerve).

3.5. Intracerebral cytokine gene transcription is altered in brains of HSV-2-infected mice

Based on the observed intracellular confinement of MHC class II immunoreactivity in HSV-2-infected neural tissue, it is unlikely that MHC class II-restricted presentation of viral antigen would occur. We postulated that in the absence of MHC class II-restricted antigen presentation, the local activation of $CD4^+$ lymphocytes would be impaired and result in a reduced expression of T cell-related cytokines. Using an RNase protection assay, we determined the induction profile of a series of cytokine transcripts in the brains of mice infected with either HSV-1 or HSV-2. Using an extensive probe set, six major cytokine transcripts were detected: TNF-α, TNF-β, IFN-γ, IL-6, IL-1α and IL-1β (Fig. 7). All of these cytokine transcripts were abundantly expressed and detected by 5 days postinfection

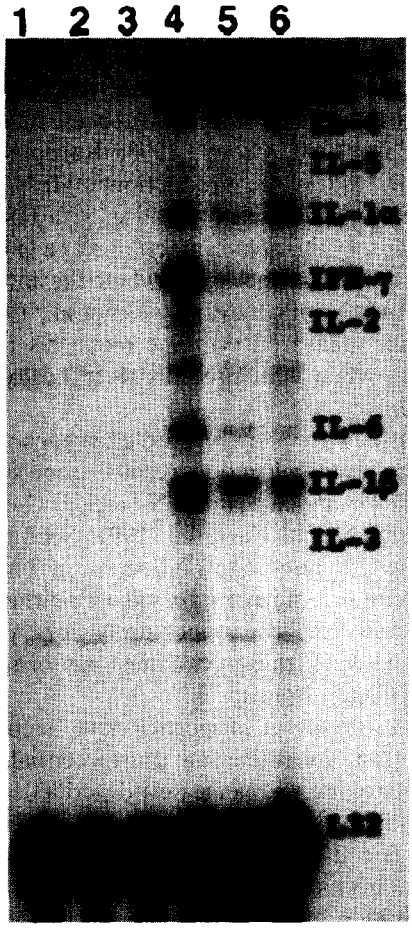

Fig. 7. Production of IFN-γ and IL-6 is significantly inhibited in HSV-2-infected brains. Cytokine transcription was not detected in brains from mock-infected mice (lane 1), HSV-1- (lane 2) or HSV-2- (lane 3) infected mice at 3 dpi. The positions of all possible cytokine transcripts obtainable with the probe set used are indicated to the right of lane 6. Induction of six major transcripts was detected in the brains of mice infected with either HSV-1 (lane 4) or HSV-2 (lane 5) by 5 dpi: TNF-β, TNF-α, IL-1α, IFN-γ, IL-6, IL-1β. There was a significant inhibition of IFN-γ and IL-6 gene transcription in HSV-2-infected brains (lane 5), whilst TNF-α, TNF-β, IL-1α and IL-1β gene transcription was similar to that seen in HSV-1-infected brains. Only the levels of IFN-γ and IL-6 return to near baseline in HSV-1-infected brains (7 dpi, lane 6). Total RNA was isolated and pool from the superior colliculus region of five mice per group at each time point. Cytokine transcripts were detected with an RNase protection assay as previously published.

(dpi) in neural tissue from HSV-1-infected mice (Fig. 7, lane 4). In HSV-2- infected mice, all six major transcripts were also detected; however, in striking contrast to HSV-1-infected tissues, transcripts specific for IFN-γ and IL-6 were nearly undetectable at the same post-inoculation interval (Fig. 7, lane 5). This observation was not the result of an overall decrease in cytokine induction, as the levels of TNF-α, TNF-β, IL-1α and IL-1β transcription after inoculation with HSV-2 were similar to those seen in HSV-1-infected mice.

4. Discussion

Confocal microscopic analysis of HSV-infected murine neural tissues indicates that a lethal strain of HSV-2 mediates inhibition of cell surface expression of MHC class II antigens. These data are consistent with the supposition that viruses can regulate the expression of MHC molecules leading to either immune evasion or autoimmunity (Maudsley and Pound, 1991). Virus-induced suppression of MHC class I antigen expression has now been demonstrated for adenovirus (Burgert et al., 1987), vaccinia virus (Kohonen-Corish et al., 1989), HIV (Scheppler et al., 1989) pseudorabies virus (Mellencamp et al., 1991), CMV (murine) (Campbell et al., 1992; Del Val et al., 1992), mouse hepatitis virus (Ceman et al., 1992), and HSV types 1 and 2 (Jennings et al., 1985). The suppression of MHC class II surface expression by human CMV has also been demonstrated (Buchmeier and Cooper, 1989). Our results extend current information regarding viral regulation of MHC antigen expression, which has been primarily derived from virus-infected cell culture systems, by providing evidence that HSV-2 mediates alteration of MHC class II expression in the intact host.

Currently, we do not understand how HSV-2 mediates the inhibition of cell surface expression of MHC class II. However, the abundance of MHC class II immunoreactivity and immunoblot analysis of the form of MHC class II molecules in HSV-2-infected neural tissues indicates that HSV-2 does not exert an action at the level of MHC class II protein induction or protein processing. We are now investigating the influence of HSV-2 on the process of MHC class II transport.

Although microglia/macorphages appear to be the primary MHC class II-positive cell type in HSV-2-infected neural tissue, a second population of MHC class II-positive cells also are present. However, the sequestering of MHC class II molecules away from the plasma membrane occurs in all MHC class II-positive cells containing HSV-2. Accordingly, it is unlikely that microglia, astrocytes or neurons are efficiently presenting viral antigen in complexes with MHC class II molecules.

Activation of T lymphocytes requires recognition of viral antigen in complexes with glycoproteins of class I and II MHC at the surface of antigen-presenting cells (Brodsky and Guagliardi, 1991). In the probable absence of MHC class II-restricted antigen presentation in HSV-2-infected neural tissues, it is unlikely that the recruited $CD4^+$ cells would be activated, greatly influencing the production of T-cell-related cytokines. We have demonstrated that the profile of cytokine gene transcription in HSV-2-infected brains is significantly modified. Based on the profile of the major cytokines induced in HSV-1-infected brains, there is a notable suppression of IFN-γ and IL-6 production. Failure to activate $CD4^+$ cells could decrease IFN-γ production

through both direct and indirect mechanisms. First, production of IFN-γ by activated $CD4^+$ cells (T_H1 subtype) (Mosmann et al., 1986), a primary source of IFN-γ, would be greatly reduced. Second, the production of IFN-γ by $CD8^+$ T cells and natural killer cells would also be significantly diminished, since IFN-γ production from natural killer and $CD8^+$ cells is enhanced by IL-2 produced by activated $CD4^+$ cells (reviewed in Swain et al., 1991). However, as is evident in Fig. 7, the induction of IFN-γ was not completely blocked, suggesting that the inhibition of $CD4^+$ T cell activation is not 100%, and/or that IFN-γ is being produced by IL-2-independent $CD8^+$ T cells. We have immunohistochemically detected comparable numbers of $CD4^+$-, $CD8^+$-, and natural killer cells in brain tissues from mice infected with either HSV-1 or HSV-2 (Lewandowski et al., 1993; and Lewandowski, unpublished data). In context of the cellular localization of MHC class I and MHC class II immunoreactivity in HSV-2-infected brain tissue, the selective impairment of IFN-γ and IL-6 transcription strongly suggests a functional inhibition of MHC class II-restricted antigen presentation in brains of HSV-2-infected mice. The inhibition of $CD4^+$ activation should also be evidenced by significant reductions in IL-2, IL-4, and IL-5 production. With our current RNAse protection assay we have not yet detected significant production of IL-2, IL-4 or IL-5 in HSV-2-infected brains. However, since a very extensive probe set was used, it is possible that less abundant cytokine transcripts were masked by the highly expressed cytokine transcripts migrating near them. Currently we are investigating the possible production of other less abundant cytokine gene transcripts, including IL-2, IL-4, IL-5, IL-10, and IL-12, by using more limited cytokine probe sets.

We postulate that alteration in CNS cytokine production in HSV-2-infected mice contributes to the ineffective antiviral immune response that allows continual replication and spread of HSV-2. This postulation is in agreement with the notion that a soluble antiviral factor may be required to eliminate infectious HSV from neurons. Tscharke and Simmons have proposed that this soluble factor is a T cell-related cytokine (Simmons and Tscharke, 1992). Antiviral properties have been reported for TNF-α in HSV-1-infected mice (Rossol-Voth et al., 1991) and for TNF-α and -β in virally infected cells (Wong and Goeddel, 1986). In our system, both TNF-α and TNF-β are produced in HSV-2-infected brains; however, it is apparent that the presence of these cytokines alone is not enough to eliminate HSV-2.

We suggest that the near absence of IFN-γ in HSV-2-infected brains profoundly influences the outcome of the infection. This suggestion is supported by previous reports that IFN promotes survival and viral clearance in herpesvirus-infected mice (Kumano et al., 1987; Yamada et al., 1988; Kunder et al., 1993) and inhibition of viral replication in cell culture systems (Svennerholm et al., 1989). Most recently the antiviral actions of IFN-induced proteins have been investigated (Sokawa et al., 1980; Croen, 1993; Karupiah et al., 1993). In our studies the production of IFN-γ corresponds to the time-course of infection with HSV-1: the levels peak during the acute infection stage (5 dpi) and decrease to near baseline levels when the infection is largely resolved (7 dpi) (Lewandowski et al., 1993). The levels of other measured cytokine messages remained elevated even when viral proteins were no longer detectable (7 dpi).

Additionally, a number of studies suggest that the most effective antiviral action results from synergism between TNF-(α and β) and IFN-(α,β,γ) (Feduchi et al., 1989; Feduchi and Carrasco, 1991; Schmitt et al., 1992). We have demonstrated induction of TNF-α and TNF-β in the brains of mice infected with either HSV-1 or HSV-2; however, the opportunity for synergistic interactions between these cytokines may exist only in the HSV-1-infected brains.

Although no antiviral properties have been demonstrated for IL-6, its role as a pluripotential, pro-inflammatory cytokine has been well described. IL-6 has multiple actions (reviewed in Van Snick, 1990) including roles in B cell differentiation, in T cell activation, and as an inducer of acute phase proteins. It is likely that the inhibition of IL-6 production in the HSV-2-infected brains greatly disrupts regulation of the intricate network of cytokines produced during viral infections.

In summary, we postulate that neurotropic viruses that cause neuronal damage, but are able to evade CNS immune surveillance, can evoke a general inflammation in the CNS. In the absence of T-cell activation, cytokines with putative antiviral actions may not be produced. However, cytokines and monokines produced by resident brain cells in response to neuronal damage with pro-inflammatory, catabolic properties, e.g. TNF-α, TNF-β, IL-6, IL-1α, and IL-1β (Arai et al., 1990; Hartung et al., 1992; Merrill et al., 1992; Dickson et al., 1993) may further neuronal damage without abating the viral infection. In these studies we demonstrated that a lethal strain of HSV-2 evades immune surveillance in both the peripheral and central nervous systems through inhibition of antigen presentation, and propose that this inhibition of antigen presentation significantly influences the profile of cytokines produced. Since IL-2 and IFN-γ are not inducible in glial cells, the cytokine profile observed in the HSV-2-infected brains may reflect primarily the glial cell response to the viral infection in the brain. Our data suggest that the combination of intracerebrally produced cytokines (of both lymphocyte and glial origin) may determine the outcome of viral infections in the brain. An extensive examination of the transcrip-

tional profiles of several cytokines in HSV-1 and HSV-2-infected brains and trigeminal ganglion nerves is now underway to determine if transcription of other $CD4^+$ cell-dependent cytokines is similarily altered.

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References

Arai, K.-I., Lee, F., Miyajima, A., Miyatake, S., Arai, N. and Yokata, T. (1990) Cytokines: Coordinators of immune and inflammatory responses. Annu. Rev. Biochem. 59, 783–836.

Brodsky, F.M. and Guagliardi, L.E. (1991) The cell biology of antigen processing and presentation. Annu. Rev. Immunol. 9, 707–744.

Buchmeier, N.A. and Cooper, N.R. (1989) Suppression of monocyte functions by human cytomegalovirus. Immunology 66, 278–283.

Burgert, H.G., Maryanski, J.L. and Kvist, S. (1987) 'E3/19k' protein of adenovirus type 2 inhibits lysis of cytolytic T lymphocytes by blocking cell-surface expression of histocompatibility class I antigens. Proc. Natl. Acad. Sci. USA 84, 1356–1360.

Campbell, A., Slater, J., Cavanaugh, V. and Stenberg, R. (1992) An early event in murine cytomegalovirus replication inhibits presentation of cellular antigens to cytotoxic T lymphocytes. J. Virol. 66(5), 3011–3017.

Ceman, S., Rudersdorf, R., Long, E.O. and Demars, R. (1992) MHC class II deletion mutant expresses normal levels of transgene encoded class II molecules that have abnormal conformation and impaired antigen presentation ability. J. Immunol. 149, 754–761.

Chan, W.L., Javanovic, T. and Lukic, M.L. (1989) Infiltration of immune T cells in the brain of mice with herpes simplex virus-induced encephalitis. J. Neuroimmunol. 23, 195–201.

Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156–159.

Chung, I.Y. and Benveniste, E.N. (1990) Tumor necrosis factor alpha production by astrocytes. J. Immunol. 144, 2999.

Croen, K.D. (1993) Evidence for an antiviral effect of nitric oxide. Inhibition of herpes simplex virus type 1 replication. J. Clin. Invest. 91, 2446–2452.

Del Val, M., Hengel, H., Häcker, H., Hartlaub, U., Ruppert, T., Lucin, P. and Koszinowski, U.H. (1992) Cytomegalovirus prevents antigen presentation by blocking the transport of peptide-loaded major histocompatibility complex class I molecules into the medial-Golgi compartment. J. Exp. Med. 176, 729–738.

Deschl, U., Stitz, L., Herzog, S., Frese, K. and Rott, R. (1990) Determination of immune cells and expression of major histocompatibility complex class II antigen in encephalitic lesions of experimental Borna disease. Acta Neuropathol. 81, 41–50.

Dickson, D.W., Lee, S.C., Mattiace, L.A., Yen, S.-H.C. and Brosnan, C. (1993) Microglia and cytokines in neurological disease, with special reference to AIDS and Alzheimer's disease. Glia 7, 75–83.

Doherty, P.C., Allan, J.E., Lynch, F. and Ceredig, R. (1990) Dissection of an inflammatory process induced by CD8+ T cells. Immunol. Today 11, 55–59.

Feduchi, E. and Carrasco, L. (1991) Mechanism of inhibition of HSV-1 replication by tumor necrosis factor and interferon-gamma. Virology 180, 822–825.

Feduchi, E., Alonso, M.A. and Carrasco, L. (1989) Human gamma interferon and tumor necrosis factor exert a synergistic blockade on the replication of herpes simplex virus. J. Virol. 63, 1354–1359.

Fraser, N.W., Spivack, J.G., Wroblewska, Z., Block, T., Deshmane, S.L., Valyi-Nagy, T., Natarajan, R. and Gesser, R.M. (1991) A review of the molecular mechanism of HSV-1 latency. Current Eye Research 10, 1–13.

Frei, K., Siepl, C., Groscurth, P., Bodner, S. and Fontana, A. (1988) Immunobiology of microglial cells. Ann. NY. Acad. Sci. 540, 218.

Giulian, D. and Corpuz, M. (1993) Microglial secretion products and their impact on the nervous system. Adv. Neurol. 59, 315–320.

Hartung, H.-P., Jung, S., Stoll, G., Zielasek, J., Schmidt, B., Archelos, J.J. and Toyka, K.V. (1992) Inflammatory mediators in demyelinating disorders of the CNS and PNS. J. Neuroimmunol. 40, 197–210.

Hickey, W.F. and Kimura, H. (1988) Perivascular microglial cells of the CNS are bone marrow derived and present antigen in vivo. Science 239, 290–293.

Ho, D.Y. (1992) Herpes simplex virus latency: Molecular Aspects. Prog. Med. Virol. 39, 76–115.

Hobbs, M., Weigle, W.O., Noonan, D.J., Torbett, B.R., McEvilly, R.J., Koch, R.J., Cardenas, G.J. and Ernst, D.N. (1993) Pattern of cytokine gene expression by CD4+ T cells from young and old mice. J. Immunol. 150, 3602–3614.

Huchet, R., Bruley-Rosset, M., Mathiot, C., Grandjon, D. and Halle-Pannenko, O. (1993) Involvement of IFN-gamma and transforming growth factor-beta in graft-vs-host reaction-associated immunosuppression. J. Immunol. 150, 2517–2524.

Jennings, S.R., Rice, P.L., Kloszewski, E.D., Anderson, R.W., Thompson, D.L. and Tevethia, S.S. (1985) Effect of herpes simplex virus types 1 and 2 on surface expression of class I major histocompatibility complex antigens on infected cells. J. Virol. 56, 757–766.

Joly, E. and Oldstone, M.B.A. (1991) Viral persistence in neurons explained by lack of major histocompatibility class I expression. Science 253, 1283–1285.

Joseph, J., Knobler, R.L., Lublin, F.D. and Hart, M.N. (1990) In: Cavanagh, D. and Brown, T.D.K. (Eds.), Coronaviruses and Their Diseases. Plenum Press, New York, NY, pp. 579–591.

Karupiah, G., Xie, Q.-W., Buller, R.M.L., Nathan, C., Duarte, C. and MacMicking, J.D. (1993) Inhibition of viral replication by interferon-gamma-induced nitric oxide synthase. Science 261, 1445–1448.

Klavinskis, L.S., Geckeler, R. and Oldstone, M.B.A. (1989) Cytotoxic T lymphocyte control of acute lymphocytic choriomening itis virus infection: Interferon gamma, but not tumor necrosis factor alpha, displays antiviral activity in vivo. J. Gen. Virol. 70, 3317–3325.

Kohonen-Corish, M.R., Blanden, R.V. and King, N.J. (1989) Induction of cell surface expression of HLA antigens by human INF-gamma encoded by recombinant vaccinia virus. J. Immunol. 143, 623–627.

Kumano, Y., Yamamoto, M. and Mori, R. (1987) Protection against herpes simplex virus infection in mice by recombinant murine interferon-gamma in combination with antibody. Antiviral Res. 7, 289–301.

Kunder, S.C., Kelly, K.M. and Morahan, P.S. (1993) Biological response modifier-mediated resistance to herpesvirus infections requires induction of alpha/beta interferon. Antiviral Res. 21, 129–139.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Lampson, L.A. and Hickey, W.F. (1986) Monoclonal antibody analysis of MHC expression in human brain biopsies: tissue ranging from 'histologically normal' to that showing different levels of glial tumor involvement. J. Immunol. 136, 4054–4062.

LaVail, J.H., Zhan, J. and Margolis, T.P. (1990) HSV (type 1) infection of the trigeminal complex. Brain Res. 514, 181–188.

Lewandowski, G.A., Grill, S.P., Fisher, M.H., Dutschman, G.E., Efange, S.M., Bardos, T.J. and Cheng, Y.-C. (1989) Anti-herpes simplex virus activity of 5-substituted 2-pyrimidinone nucleosides. Antimicrob. Agents. Chemother. 33, 340–345.

Lewandowski, G.A., Lo, D. and Bloom, F.E. (1993) Interference with major histocompatibility complex class II-restricted antigen presentation in the brain by herpes simplex virus type 1: A possible mechanism of evasion of the immune response. Proc. Natl. Acad. Sci. USA 90, 2005–2009.

Liebermann, A.P., Pitha, P.M., Shin, H.S. and Shin, M.L. (1986) Production of tumor necrosis factor and other cytokines by astrocytes stimulated with lipopolysaccharide or a neurotropic virus. Proc. Natl. Acad. Sci. USA 86, 6348.

Maehlen, J., Olsson, T., Love, A., Klareskog, L., Norrby, E. and Kristensson, K. (1989) Persistence of measles virus in rat brain neurons is promoted by depletion of CD8+ T cells. J. Neuroimmunol. 21, 149–155.

Martin, X. and Dolivo, M. (1983) Neuronal and transneuronal tracing in the trigeminal system of the rat using the herpes virus suis. Brain Res. 273, 253–276.

Maudsley, D.J. and Pound, J.D. (1991) Modulation of MHC antigen expression by viruses and oncogenes. Immunol. Today 12, 429–431.

Mauerhoff, T., Pujol-Borrell, R., Mirakian, R. and Bottazzo, G.F. (1988) Differential expression and regulation of major histocompatibility complex (MHC) products in neural and glial cells of the human fetal brain. J. Neuroimmunol. 18, 271–289.

Mellencamp, M.W., O'Brien, P.C.M. and Stevenson, J.R. (1991) Pseudorabies virus-induced suppression of major histocompatibility complex class I antigen expression. J. Virol. 65, 3365–3368.

Merrill, J.E., Kono, D.H., Clayton, J., Ando, D.G. and Hinton, D.R. (1992) Inflammatory leukocytes and cytokines in the peptide-induced disease of experimental allergic encephalomyelitis in SJL and B10.PL mice. Proc. Natl. Acad. Sci. USA 89, 574–578.

Mosmann, T.R., Cherwinski, H.M., Bond, M.W., Giedlin, M.A. and Coffman, R.L. (1986) Two types of murine helper T cell clone. J. Immunol. 136, 2348–2357.

Nash, A.A., Jayasuriya, A., Phelan, J., Cobbold, S.P., Waldmann, H. and Prospero, T. (1987) Differential roles for L3T4+ and Lyt 2+ T cell subsets in the control of an acute herpes simplex virus infection of the skin and nervous system. J. Gen. Virol. 68, 825–833.

Olsson, T., Maehlen, J., Love, A., Klareskog, L., Norrby, E. and Kristensson, K. (1987) Induction of class I and class II transplantation antigens in rat brain during fatal and non-fatal measles virus infection. J. Neuroimmunol. 16, 215–224.

Richt, J.A., Stitz, L., Wekerle, H. and Rott, R. (1989) Borna disease, a progressive meningoencephalomyelitis as a model for CD4+ T cell-mediated immunopathology in the brain. J. Exp. Med. 170, 1045–1050.

Rodriguez, M. and Lindsley, M.D. (1992) Immunosuppression promotes CNS remyelination in chronic virus-induced demyelinating disease. Neurology 42, 348–357.

Rossol-Voth, R., Rossol, S., Schutt, K.H,, Corridori, S., de Cian, W. and Falke, D. (1991) In vivo protective effect of tumour necrosis factor alpha against experimental infection with herpes simplex virus type 1. J. Gen. Virol. 72, 143–147.

Sanna, P.P., Jirikowski, G.F., Lewandowski, G.A. and Bloom, F.E. (1992) Applications of DAPI cytochemistry to neurobiology. Biotechnol. Histochem. 67, 346–350.

Scheppler, J.A., Nicholson, J.K., Swan, D.C., Ahmed-Ansari, A. and McDougal, J.S. (1989) Down-modulation of MHC-I in a CD4+ T cell line, CEM-E5, after HIV-1 infection. J. Immunol. 143, 2858–2866.

Schmitt, D.A., Sasaki, H., Pollard, R.B. and Suzuki, F. (1992) Antiviral effects of recombinant human tumor necrosis factor-alpha in combination with natural interferon-beta in mice infected with herpes simplex virus type-1. Antiviral Res. 19, 347–352.

Simmons, A. and Tscharke, D.C. (1992) Anti-CD8 impairs clearance of herpes simplex virus from the nervous system: Implications for the fate of virally infected neurons. J. Exp. Med. 175, 1337–1344.

Simmons, A., Tscharke, D. and Speck, P. (1992) The role of immune mechanisms in control of herpes simplex virus infection of the peripheral nervous system. Curr. Top. Microbiol. Immunol. 179, 31-56.

Sokawa, Y., Ando, T. and Ishihara, Y. (1980) Induction of 2',5'-oligoadenylate synthetase and interferon in mouse trigeminal ganglia infected with herpes simplex virus. Infect. Immun. 28, 719–723.

Streit, W.J., Graeber, M.B. and Kreutzberg, G.W. (1989) Expression of Ia antigen on perivascular and microglial cells after sublethal and lethal motor neuron injury. Exp. Neurol. 105, 115–126.

Svennerholm, B., Ziegler, R. and Lycke, E. (1989) Herpes simplex virus infection of the rat sensory neuron effects of interferon on cultured cells. Arch. Virol. 104, 153–156.

Swain, S.L., Bradley, L.M., Croft, M., Tonkonogy, S., Atkins, G., Weinberg, A.D., Duncan, D.D., Hedrick, S.M., Dutton, R.W. and Huston, G. (1991) Helper T-cell subsets: Phenotype, function and the role of lymphokines in regulating their development. Immunol. Rev. 123, 115–144.

Van Snick, J. (1990) Interleukin-6: An overview. Annu. Rev. Immunol. 8, 253–278.

Weinstein, D.L., Walker, D.G., Akiyama, H. and McGeer, P.L. (1990) Herpes simplex virus type 1 infection of the CNS induces major histocompatibility complex antigen expression on rat microglia. J. Neurosci. Res. 26, 55–65.

Williamson, J.S.P., Sykes, K.C. and Stohlman, S.A. (1991) Characterization of brain-infiltrating mononuclear cells during infection with mouse hepatitis virus strain JHM. J. Neuroimmunol. 32, 199–207.

Wong, G.H.W. and Goeddel, D.V. (1986) Tumour necrosis factors alpha and beta inhibit virus replication and synergize with interferons. Nature 323, 819–822.

Wong, G.H.W., Bartlett, P.F., Clark-Lewis, I., Battye, F. and Schrader, J.W. (1984) Inducible expression of H-2 and Ia antigens on brain cells. Nature 310, 688.

Yamada, M., Arao, Y., Hantano, A., Uno, F. and Nii, S. (1988) Effect of recombinant mouse interferon-beta on acute and latent herpes simplex infection in mice. Arch. Virol. 99, 101–109.

Yamaguchi, K., Goto, N., Kyuwa, S., Hayami, M. and Toyoda, Y. (1991) Protection of mice from a lethal coronavirus infection in the central nervous system by adoptive transfer of virus-specific T cell clones. J. Neuroimmunol. 32, 1–9.