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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

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ABSTRACT **Host survival of herpes simplex virus type 1 (HSV-1) infection depends on the establishment of latent infections in both peripheral and central nervous systems. Strains of HSV-1 that are successful in escaping the immune response produce a lethal infection. We now report a possible mechanism of immune response evasion used by HSV-1. After intraocular inoculation of mice, HSV-1 strain F established a latent infection in the brain, whereas strain KOS did not. The immune response to HSV-1 infection (strains KOS and F) in the brain was characterized by induction of major histocompatibility complex class II expression and recruitment of CD4$^+$ and CD8$^+$ cells to highly restricted sites of intracerebral viral infection. Major histocompatibility complex class II antigen expression was primarily intracellular in strain KOS infection centers and at the cell surface in strain F infection centers. We propose that major histocompatibility complex class II-restricted viral-antigen presentation to T cells is interrupted during strain KOS infections, thereby allowing KOS infection to evade T-cell-mediated events that would normally protect the host from a lethal infection. Immunocompromised mice (athymic or irradiated mice) could not survive strain F infections; however, latent F infections were established in irradiated mice reconstituted with naive lymph node and spleen cells. These data suggest that class II-restricted presentation of viral antigens is required for the control of HSV-1 infections in the nervous system.**

Protection of a host from lethal herpes simplex virus type 1 (HSV-1) infections requires a functional immune response. The T-cell-mediated immune response that controls viral spread by clearance of the virus from peripheral lesion sites (1, 2) has also been indicated as a necessary component in the establishment of latent HSV-1 infections (3–6). In most instances, after a primary HSV-1 infection a latent viral infection is established in the peripheral and central nervous systems (7–9). If a latent infection is not established, the uncontrolled HSV-1 infection can spread through the peripheral and central nervous system and cause the death of the host. Recently, some attention has been given to the roles of major histocompatibility complex (MHC) class I-restricted CD8$^+$ and MHC class II-restricted CD4$^+$ T lymphocytes. Although the literature presents mixed results, both CD8$^+$ and CD4$^+$ cells are probably required for complete recovery from HSV-1 infections. Activation of both CD8$^+$ and CD4$^+$ lymphocytes requires viral-antigen presentation in the appropriate MHC complex (10–13). We now report that HSV-1 may cause a lethal infection by escaping the surveillance of the immune system and that a possible evasion mechanism is through interference of MHC class II-restricted antigen presentation.‡

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MATERIALS AND METHODS

Inoculation of Mice with HSV-1. Female BALB/cByJ mice of at least 8 weeks of age were anesthetized by inhalation of Metofane and inoculated in the right eye with 10^5 plaque-forming units (pfu) of either strain KOS or F in a 1-μl vol. Control mice were injected by the same route with saline. At various days postinfection (dpi) the mice were anesthetized with chloral hydrate (250 mg per mouse i.p.) and sacrificed by cardiac perfusion (14). HSV-1 strain F was from Bernard Roizman (University of Chicago) and strain KOS was from Rachael Schrier (University of California San Diego).

Immunohistochemical Detection of HSV-1 and Immune Cells in the Brain. The mice were perfused with 2% (vol/vol) paraformaldehyde for 2 min followed by 10 ml of a 10% sucrose solution. Brains were excised and placed in 18% sucrose on ice. Frozen serial coronal sections of 20 μm through the diencephelon and midbrain of the brain were cut with a cryostat. Sections were incubated overnight at room temperature in a humidified chamber (14) in a phosphate-buffered saline (PBS) solution containing 0.3% Triton X-100 and a rabbit polyclonal antibody to HSV-1 proteins (diluted 1:500 to 1:1000; Dako, Carpinteria, CA). After being washed with PBS, the sections were incubated with goat anti-rabbit IgG conjugated to rhodamine (Boehringer Mannheim). The monoclonal antibody (mAb) for MHC class II (conjugated to biotin) is specific for the α chain of the I-E molecule (clone 14.4.4s) and was used at a dilution of 1:200. The mAb for CD4 was used at a dilution of 1:200, and a mixture of mAbs for the CD8 (a and b) antigens were used at dilutions of 1:50 and 1:100, respectively. All mAbs were purchased from Pharmingen (San Diego). Tissue sections were incubated with the primary antibodies overnight at room temperature, followed by a 45-min incubation with mouse anti-rat IgG conjugated to biotin (Jackson ImmunoResearch). The biotinylated secondary antibody was treated with streptavidin conjugated to peroxidase (Jackson ImmunoResearch) for 30 min at room temperature. The reaction was developed with diaminobenzidine tetrahydrochlorate (Sigma) and hydrogen peroxide for 8–10 min at room temperature. The mAb for MHC class II detection, which is biotinylated, was treated with streptavidin conjugated to Cy3 (a fluorescent marker; Jackson ImmunoResearch) for 2 hr at room temperature.

HSV-1 Infections in Immunodeficient Mice. The course of HSV-1 infection was studied in two immunodeficient mouse models: athymic BALB/c (*nu/nu*) mice and irradiated BALB/c mice. For the irradiation studies, mice were irradiated to 900 rads (1 rad = 0.01 Gy) and infected with either strain KOS or strain F, as described above. In the facility at

Abbreviations: HSV-1, herpes simplex virus type 1; MHC, major histocompatability complex; CNS, central nervous system; dpi, days postinfection; mAb, monoclonal antibody.
‡The results reported in this article were presented at the 16th International Herpesvirus Workshop, August 1–7, 1992, Edinburgh, Scotland, and at the 22nd Annual Society for Neuroscience meeting, October 25–30, 1992, Anaheim, CA, by G.A.L.

The Scripps Research Institute irradiation at this level was found nonlethal to uninfected control mice ($n = 6$). In the reconstitution experiment, ≈4 hr after the mice were irradiated, they were infused with 10^8 cells isolated from the spleens and lymph nodes of normal BALB/cByJ mice (15). After a recuperation period of ≈24 hr the mice (irradiated and reconstituted/irradiated) were infected intraocularly with strain F.

RESULTS

HSV-1 Strain KOS, But Not Strain F, Produces a Lethal Infection in Immunocompetent Mice After Ocular Inoculation. Immunohistochemistry for HSV-1 proteins after either strain KOS or strain F ocular infections in mice indicated that both viruses enter the central nervous system (CNS) at similar rates through classic visual synaptic connections to the lateral geniculate and superior colliculus (16). The viral spread of the KOS infection occurred rapidly between 4 and 6 dpi, and all mice died by 9 dpi (25/25; Figs. 1 and 2). Subsequent to infection with strain F, the mice exhibited mild physical signs of illness, but all mice fully recovered by 7 dpi (Fig. 1). HSV-1-positive cells (presumptively neurons) were readily detected in the lateral geniculate and superior collicus at 4 dpi (35/35; Fig. 2). The number of positive cells steadily decreased until viral proteins could no longer be detected (10 dpi). These observations are consistent with several previous reports demonstrating that HSV-1 strain F is not cleared from the CNS, but, rather, establishes a latent infection (18–21). Accordingly, in this experimental system, two strains of HSV-1 infecting the identical host brain visual circuits differed markedly in their ability to produce lethal infections.

Cellular Localization of MHC Class II Antigen Expression Differs Markedly in F and KOS Infection Centers in the Brain. The immune response during acute HSV-1 (strain KOS or F) infection was characterized by infiltration of macrophages and activation of microglia in regions of virus infection (17, 22, 23) and specific recruitment of $CD4^+$ and $CD8^+$ lymphocytes to the sites of viral infection (Fig. 3). Brains of the control mice were negative for detection of CD4, CD8, and I-E antigens. Although MHC class II (I-E) protein synthesis was induced in presumptive microglia during both strain KOS and F infections, a striking difference in the MHC class II antigen expression was seen. In KOS-infected mice the pattern of MHC class II expression remains largely intracellular, showing both intranuclear and more limited cytoplasmic compartmentalization, whereas in F infections MHC class II appears at the cell perimeter near the plasma membrane (Fig. 4, and EM observations; G.A.L., M. Morales, and F.E.B., unpublished work). One interpretation of these data is that the transport of the MHC class II molecule to the surface of the antigen-presenting cell may be interrupted during KOS infection. In the absence of viral-antigen presentation an effective immune response cannot occur, and KOS can continue to replicate and spread throughout the CNS. By contrast, in strain F-infected mice the cellular staining pattern for MHC class II suggests that efficient antigen presentation does occur, and the immune response controls viral spread in the CNS.

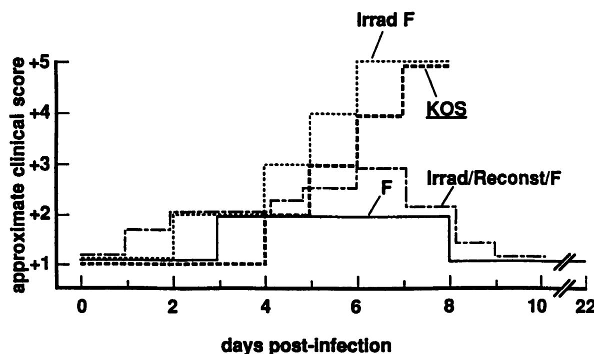

FIG. 1. Clinical course of disease in KOS- and F-infected normal (nonmanipulated), irradiated (Irrad), and reconstituted/irradiated (Irrad/Reconst) mice. The progressive physical manifestations of HSV-1 infections of the CNS in rats have been reported (17) and include ruffled fur as a result of cessation of grooming, lethargy, weight loss, hunched posture, and death. Daily observations of several mice in each treatment groups were made. The figure represents schematically cumulative observations of the course of disease. Clinical scores correspond to the following symptoms: +1, active with no outward symptoms of physical illness; +2, active with ruffled fur; +3, lethargy and weight loss; +4, hunched posture and no activity; and +5, death. ——, Strain F-infected mice; - -, strain KOS-infected mice; - -, strain F-infected irradiated mice; ---, strain F-infected reconstituted/irradiated mice. The reconstituted mice exhibited a longer acute phase of infection before fully recovering, which may reflect delay in reestablishment of the immune system in reconstituted mice.

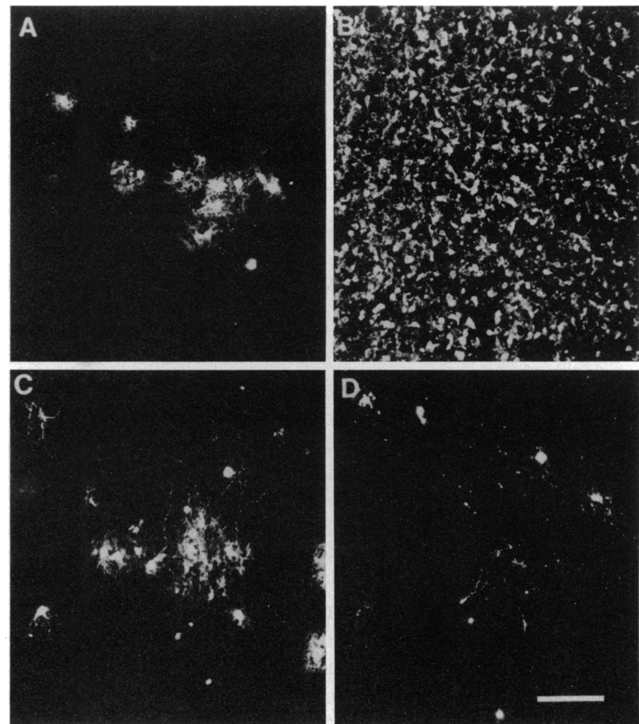

FIG. 2. Detection of HSV-1 viral proteins over time in brain after intraocular inoculation with strain KOS or strain F. Typical immunohistochemical staining for HSV-1 in the region of superior colliculus is shown for each time point and experimental group. (*A* and *B*) Immunohistochemistry for HSV-1 proteins in KOS-infected mice at 4 dpi (*A*) and 6 dpi (*B*). (*C* and *D*) Immunohistochemistry for HSV-1 proteins in F-infected mice at 4 dpi (*C*) and 7 dpi (*D*). (Bar in *D* = 61 μm.)

HSV-1 Strain F Produces a Lethal Infection in Immunocompromised Mice. From the above results we postulated that the immune response was essential to the control of viral spread of HSV-1 infection in neurons of the CNS. The corollary hypothesis, that HSV-1 strain F would produce a lethal infection in immunodeficient mice, was tested by infecting irradiated mice and athymic nude BALB/c mice with strain F. Irradiation of mice (900 rads) effectively ablates the immune system (15) with minimal effects on postmitotic cells (24), as indicated by the absence of $CD4^+$ and $CD8^+$ cells and expression of MHC class II antigens after infection with strain F (data not shown). Immunohistochemistry to HSV-1 proteins in irradiated mice indicated that the intra- and

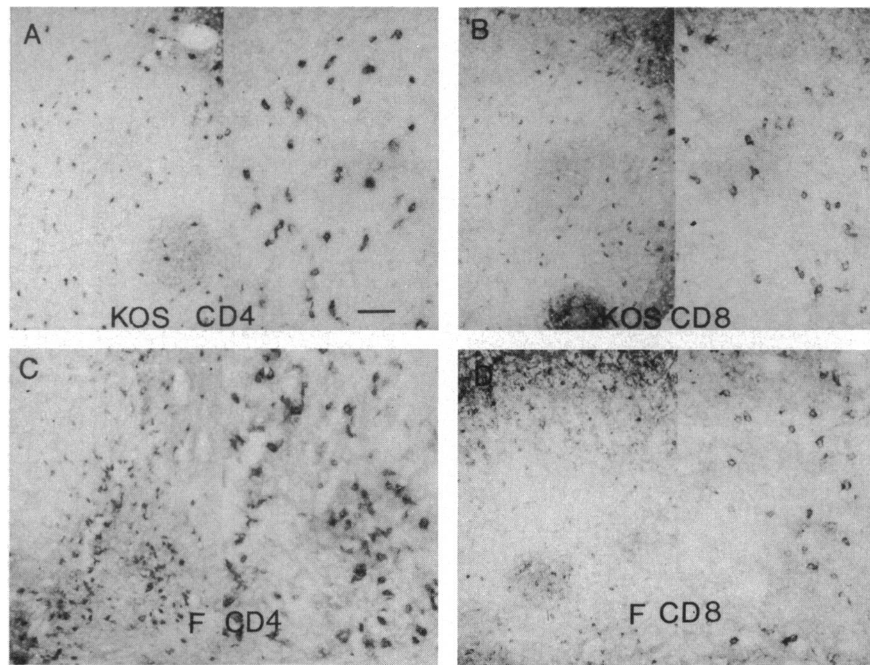

FIG. 3. Recruitment of $CD4^+$ and $CD8^+$ lymphocytes to sites of HSV-1 infection in brain. $CD4^+$ and $CD8^+$ cells were detected in mice brains after intraocular inoculation with either HSV-1 strain KOS or F with mAbs for CD4 and CD8 antigens. Detection of $CD4^+$ (*A*) and $CD8^+$ (*B*) cells in the lateral geniculate region of strain KOS-infected mice, 6 dpi. Detection of $CD4^+$ cells in the oculomotor region of strain F-infected mice, 6 dpi (*C*). Detection of $CD8^+$ cells in the superior colliculus region of strain F-infected mice, 6 dpi (*D*). ***A–D Right*** shows a higher magnification of recruited lymphocytes. Bar in *A* = 93.7 μm for *A–D Left* and 46.9 μm for *A–D Right*.

interneuronal spread of KOS proceeded as in unirradiated mice (10/10; Fig. 5*A*). However, in irradiated mice F infections no longer progressed to a latent phase, and all mice died by 7 dpi, strongly suggesting that the elimination of the immune response prevented the control of viral spread (22/22; Figs. 5*C* and 1). To confirm that the extensive spread of strain F was not due to nonspecific irradiation effects on brain cells, irradiated mice were reconstituted with lymph node and spleen cells from *normal immunologically naive* mice, preceding infection with strain F. These fully reconstituted mice could survive infection with strain F; the amount of HSV-1 protein detected in the brain decreased significantly by 10 dpi (Fig. 5*D*), indicating that viral infection was being resolved. The immunohistological detection of recruited $CD4^+$ and $CD8^+$ cells and the MHC class II antigen expression were consistent with strain F-infected nonirradiated mice (data not shown).

Similarly, HSV-1 strain F also produced a lethal infection in athymic nude mice, which are deficient only in T lymphocytes (8/8; Fig. 5*B*). After strain F infection, MHC class II antigen expression is induced in the brain, but no T lymphocytes are recruited. Thus, in this model the inhibition of the immune response cascade occurs at the T-cell recruitment step. These results suggest that the early immune response events leading to HSV-1 strain F latency are T-lymphocyte-dependent because the presence of B lymphocytes alone is not sufficient for the establishment of a latent HSV-1 infection or in limiting the initial viral spread through the CNS. These data are supported by the demonstration that passive administration of antibody to HSV-1-infected nude mice does not eliminate the acute phase of HSV-1 infection (25).

DISCUSSION

The concept that the immune system plays a role in the control of viral infections is not new. Adoptive transfer of T lymphocytes specific for HSV-1 antigens can confer protection from lethal HSV-1 infections in immunocompetent and immunosuppressed mice (26–30). In this report we have demonstrated further that the immune response to HSV-1 is also critical for control of viral spread in the brain. Irradiated mice and athymic nude mice succumb to a lethal infection with the avirulent strain F with extensive viral spread through the brain. Additional studies indicated that protection from a lethal F infection in irradiated mice does not require HSV-1-specific T lymphocytes; protection can be conferred by passive transfer of *naive, nonspecific lymph node and spleen cells*. To our best awareness, the protection of immunocompromised mice from a lethal HSV-1 infection with immune cells from a naive donor has not previously been demonstrated.

The inability of the athymic mice to survive a HSV-1 (strain F) infection in the CNS is consistent with previous demonstrations of the involvement of the T-cell-mediated response to viral infections. Similar to the T-cell-mediated response in the periphery, $CD4^+$ and $CD8^+$ T cells are recruited to the site of viral infection in the brains of immunocompetent, naive mice infected with either a virulent strain (KOS) or a non-virulent strain (F) of HSV-1. Consequentially, these results indicate that the recruitment of T lymphocytes to the site of viral infection is not enough to protect mice from lethal infection.

In contrast to the peripheral immune response, the nature of host immune responses to HSV-1 infections of the CNS is a unique situation. Typically, the initiation of the immune response to viral infection requires antigen presentation in molecular complexes with MHC molecules. However, differentiated neurons cannot express either MHC class I or II markers (17, 31, 32). Thus, the neuronal cell in which the primary HSV-1 infection and subsequent latency occur is not capable of antigen presentation. Consequently, regardless of subsequent immune events, it should be impossible to clear the virus from neurons by cell lysis. The morphology of the $I\text{-}E^+$ cells suggests that the MHC class II expression was most likely induced in either microglial cells or infiltrating macrophages in lieu of neurons. Indeed, MHC class II

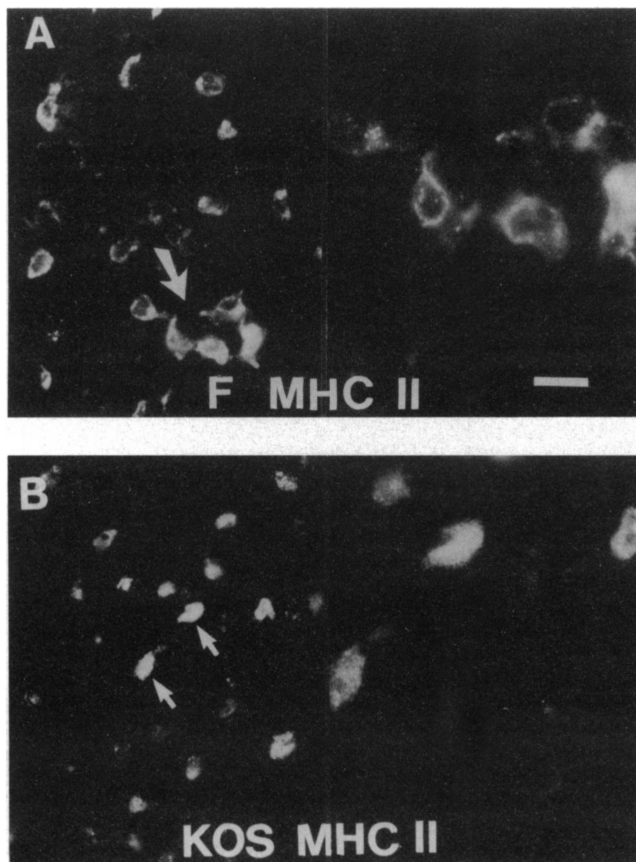

FIG. 4. Induction of MHC class II antigen expression in brains of HSV-1-infected mice. Expression of MHC class II antigens in viral-infection centers after ocular inoculation with HSV-1 strains KOS or F was detected with a mAb specific for α chain of the I-E molecule. The secondary antibody used was conjugated to the fluorescent marker Cy3 (Jackson ImmunoResearch). Arrows in *A* and *B* indicate cells shown at higher magnification on right. Notice the striking difference in localization of MHC class II antigen expression between strains KOS (*B*) and F (*A*). In the F viral-infection centers localization of MHC class II antigen expression is clearly cytoplasmic, associated with cell membrane. Based on a combination of dark- and bright-field microscopy, we interpret the cellular localization of MHC class II antigen expression in the KOS viral-infection center as a nuclear-staining profile. (Bar in A = 23.5 μm for *A* and *B Left* and 9.4 μm for *A* and *B Right*.)

induction in microglial cells after viral infection has been well established (17, 33, 34). Moreover, microglia and possibly astrocytes have been suggested to present antigen in the CNS (35–37). MHC class II-restricted T lymphocytes ($CD4^+$) have been shown in several studies to be essential in the response to HSV-1 infection (38–41). It is probable that during infection of the central and peripheral nervous systems HSV-1 may "leak" into the surrounding space from the infected neuron and be phagocytized, processed, and presented to the recruited lymphocytes by the nearby microglia. In this scenario, the exogenous pathway of antigen processing would be indicated, activating class II-restricted T cells. Accordingly, our data demonstrate a significant induction of MHC class II expression in the nonneuronal cells in the viral-infection centers in the brain, after ocular infection by either strain of HSV-1. However, the cellular localization of MHC class II expression is vastly different between the two strains. For antigen presentation to be effective the MHC class II–viral-antigen complex must be transported to the surface of the antigen-presenting cell. In strain F viral-infection centers, MHC class II antigens are clearly localized at the cell surface, indicating normal viral-antigen presentation. In contrast, during the lethal infection by strain KOS the expression of MHC class II antigen is largely confined to intracellular compartments, almost entirely intranuclear. In this location, viral-antigen presentation *cannot* occur. We propose that HSV-1 strain KOS evades the immune system by interference with MHC class II-restricted viral-antigen presentation in the CNS, thereby averting the effective antiviral T-cell-mediated response that normally protects the host from a lethal HSV-1 infection.

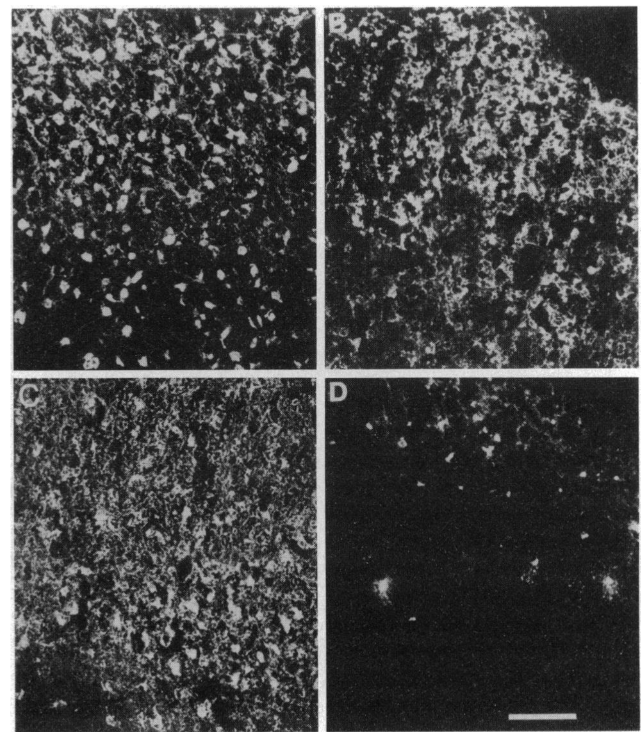

FIG. 5. Viral spread of HSV-1 strains KOS and F in immunodeficient mice. Immunohistochemical detection of HSV-1 proteins in the region of superior colliculus in strain KOS-infected irradiated mice at 6 dpi (*A*), strain F-infected athymic mice (*B*), strain F-infected irradiated mice (*C*), and strain F-infected irradiated mice reconstituted with spleen and lymph node cells (from naive donors) (*D*). The viral spread of F virus could not be controlled in either athymic or irradiated mice; however, reconstitution of the irradiated mice with immune cells from naive donor mice resulted in a stark reduction of viral proteins and recovery of the irradiated mice. (Bar = 61 μm.)

Our data indicate that the rapid cellular immune reactions and the alterations in antigen presentation between virulent and latent strains of virus may provide another approach to study these initial neuroimmune events that are apparently critical for controlling viral infections in the nervous system.

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