Upregulation of Class I Major Histocompatibility Complex Gene Expression in Primary Sensory Neurons, Satellite Cells, and Schwann Cells of Mice in Response to Acute but Not Latent Herpes Simplex Virus Infection In Vivo

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Summary

Major histocompatibility complex (MHC) deficiency is typical of almost all resident cells in normal neural tissue. However, $CD8^+$ T cells, which recognize antigenic peptides in the context of class I MHC molecules, are known to mediate clearance of herpes simplex virus (HSV) from spinal ganglia of experimentally infected mice, leading to the hypothesis that class I expression in the peripheral nervous system must be upregulated in response to HSV infection. In addressing this hypothesis it is shown, in BALB/c ($H\text{-}2^d$) mice, that normally deficient class I transcripts transiently accumulate in peripheral nerve Schwann cells, ganglionic satellite cells, and primary sensory neurons, indicating that in each of these cell types class I expression is regulated at the transcriptional level in vivo. Furthermore, for 3–4 wk after infection, $H\text{-}2K^d/D^d$ antigens are expressed by satellite and Schwann cells but not neurons, suggesting additional posttranscriptional regulation of class I synthesis in neurons. Alternatively, the class I RNAs induced in neurons may not be derived from classical class I genes. Factors regulating H-2 class I expression emanate from within infected ganglia, probably from infected neurons themselves. However, induction of class I molecules was not maintained during latency, when viral gene expression in neurons is restricted to a single region within the virus repeats. These data have implications for the long-term survival of cells in HSV-infected neural tissue.

After cutaneous inoculation, HSV replicates in epidermal cells and concurrently invades the peripheral nervous system (PNS)[1], where primary sensory neurons are the virus' main target (1). Productive infection of sensory neurons generates the potential for lethal spread of virus throughout the nervous system but, in immunocompetent hosts, viral replication is usually terminated rapidly, by timely development of an adaptive immune response (2). After recovery from primary infection, elimination of HSV from the PNS is not complete. Rather, viral DNA sequences persist in a proportion of neurons in a latent, nonreplicating state, forming a reservoir from which infection can periodically reactivate (3). During latency, viral gene expression is repressed, with the exception of a single region within the virus repeats (4). Putative translation products of latency-associated transcripts (LATs) have, to date, been described only in experimental models of latency in vitro (5) and the function of LATs remains poorly understood.

The mechanisms responsible for controlling productive infection and maintaining latency have been studied intensively, mostly in well characterized experimental models (for a review see reference 2). MHC-linked genes influence the severity of herpes simplex (6), in line with the established role of T lymphocytes as mediators of recovery from acute infection. It is interesting to note that the mechanisms responsible for clearance of infectious virus from the skin and the nervous system appear to differ. Resolution of cutaneous lesions depends primarily on $CD4^+$ (MHC class II restricted) CTLs and/or a delayed type hypersensitivity response (7), whereas $CD8^+$ cells play a pivotal role in clearance of HSV from the PNS (8). The mechanisms of action of antiviral $CD8^+$ cells in the peripheral or central nervous systems (CNS) have not been determined, but two lines of evidence suggest that the classic paradigm for control of viral infections—lysis of infected cells by $CD8^+$ CTLs—is not satisfactory for neuronal targets. First, neural tissue is sheltered from the immune system

[1] *Abbreviations used in this paper:* CNS, central nervous system; LAT, latency-associated transcript; TK, thymidine kinase.

in several ways, and prominent among these is deficient expression of MHC molecules by almost all resident cells (for a review see reference 9). Class I MHC gene products are required for presentation of antigenic peptides to $CD8^+$ T lymphocytes, including classical CTLs (10), from which it follows that virus-infected cells must synthesize class I molecules in order to be lysed by CTLs. In the CNS, several neurotropic viruses are known to induce MHC class I antigen synthesis in glial cells, but in this respect, neurons are notably resistant (11). It has been proposed that neurons have evolved a mechanism for escaping CTL attack based on downregulation of class I synthesis, allowing nonlytic antiviral mechanisms to predominate (12, 13). Second, we previously showed that treatment of HSV-infected mice with anti-CD8 enhances neuronal destruction and conversely, survival of viral antigen-positive neurons is dependent on a $CD8^+$ T cell–mediated response (8). The prominent role played by $CD8^+$ cells in clearance of infectious virus from spinal ganglia challenges the view that PNS tissues are entirely MHC deficient, because irrespective of their mechanism of action, $CD8^+$ T cells must be stimulated at the site of infection in a class I–restricted manner. These considerations led to the hypothesis that synthesis of class I molecules by resident cells of the PNS is modulated in response to HSV infection. To address this hypothesis, we examined the effect of HSV on class I RNA and antigen synthesis in spinal ganglia and peripheral nerves of experimentally infected mice.

Two features of the experimental system used in these studies require introduction. First, HSV was introduced into the PNS of mice by retrograde axonal transport along spinal nerves, after inoculation of virus into flank skin (14); the inoculation procedure caused minimal disruption to the physical integrity of spinal ganglia. Second, in some experiments, mice were infected with an HSV thymidine kinase (TK) deletion mutant that cannot replicate its DNA within the PNS and therefore cannot spread from cell to cell. In this way it was possible to strictly confine HSV to neurons directly innervating the site of inoculation and restrict the number of viral lytic genes expressed, in an attempt to determine whether infected neurons participate directly in the afferent arm of the neuroimmunological response.

Northern blot analysis and in situ hybridization showed that class I transcripts were present in low but detectable amounts in PNS tissues of uninfected mice. However, after inoculation of HSV into flank skin, heterogenous and mature class I RNAs transiently increased in abundance in satellite cells, Schwann cells, and primary sensory neurons, suggesting transcriptional regulation of class I expression in each of these cell types in vivo in response to acute ganglionic infection with HSV. Classical class I antigens ($H\text{-}2K^d/D^d$) were detected in satellite and Schwann cells, but not in neurons, suggesting that there may be an additional block in class I synthesis at the posttranscriptional level in neurons. Alternatively, the RNAs detected in neurons may not have been derived from classical class I genes. Factors regulating H-2 class I expression emanated from within infected ganglia, probably from infected neurons. However, latent infection of neurons, during which viral gene expression is restricted to a single region of unknown function within the virus repeats, did not maintain the stimulus for class I expression.

Materials and Methods

Viruses. In most experiments, mice were infected with HSV type 1, strain SC16, a well-characterized isolate that has been shown to be neuroinvasive in several mouse strains (15). Where indicated, mice were infected with HSV type 1, strain TKDM21, which has an 816-bp deletion in the TK gene (16). As a consequence of the TK deletion, TKDM21 cannot replicate in neurons. Strains SC16 and TKDM21 were grown and titrated in Vero cells and stored at −70°C until required. When required, inocula were inactivated by exposure to ultraviolet light (17).

Infection of Mice. Adult female BALB/c ($H\text{-}2^d$) and C3H ($H\text{-}2^k$) mice (Specific Pathogen-Free facility, Animal Resource Centre, Perth, Western Australia) were used at greater than 8 wk of age. Characteristics of the zosteriform model used in these experiments have been described in detail previously (14). Briefly, after depilation with Nair (Carter-Wallace, New South Wales, Australia), a small patch of skin on the left flank, innervated by the eighth through tenth thoracic dorsal root ganglia (T8–T10) was scarified with a 27-gauge needle through a 10 μl drop of virus suspension. In this system, it was shown that infectious virus and viral antigens are cleared from ganglia ~7 d after inoculation with 1.5×10^5 PFU HSV-1 strain SC16 (14, 18).

Immunohistochemistry. H-2 antigens were detected on acetone-fixed frozen sections (6 μm) of pooled ganglia (>30). Biotinylated monoclonal anti-$H\text{-}2K^d/D^d$ primary antibodies were used as follows: HB79 (American Type Culture Collection, Rockville, MD) or 34-7-23S (Cedarlane, Ontario, Canada) at dilutions of 1:20 and 1:10, respectively. Rather than direct detection of avidin-HRP (1:400) binding at this stage it was found that sensitivity could be increased by reacting bound avidin-HRP with rabbit antiavidin (1:50) followed by swine anti–rabbit Igs (1:25) and finally, rabbit peroxidase antiperoxidase complex (1:200) (all reagents from Dakopatts, Glostrup, Denmark, unless stated otherwise). All antibodies were diluted in Tris saline buffer (TSB) (pH 7.4) containing 10% BSA, 5% normal mouse serum, and 5% normal swine serum. Reactions were allowed to proceed for 30 min at 37°C, and sections were washed twice with TSB (pH 7.4) between steps. Bound antibody was detected with 3,3′-diaminobenzidine containing 0.1% H_2O_2. HSV antigens were detected as described previously (18).

Nucleic Acid Probes. pRK1 and pTK were constructed by subcloning the BamHI-EcoRI fragment of pUC-$H\text{-}2K^d$ (19) into Bluescribe $M13^-$ (Stratagene, La Jolla, CA) and pGEM4Z (Promega, Madison, WI), respectively. Transcription of pRK1 and pTK from the T7 promoter generated sense and antisense $H\text{-}2K^d$ mRNA riboprobes, respectively. pSLAT2 (20), containing a 1.6-kb PstI-HpaI subfragment of HSV-I BamHI B (strain SC16), was used to generate riboprobes for detection of LATs. Plasmids were linearized by restriction enzyme digestion and gel purified to remove uncut templates before transcription, which was done according to Promega recommendations with the following modifications: 12 μm-rUTP, 0.085 μm [^{32}P]rUTP, and 250 μm digoxigenin-11-rUTP (Boehringer Mannheim, GmBH, Mannheim, Germany). The following plasmids were used to make DNA probes for Northern blot analyses: pRK1, containing full-length $H\text{-}2K^d$ cDNA (see above); pGEM3β2(A), containing β_2-microglobulin (β_2m) cDNA, derived from pBRcB4 (21) and pAL41 (22), containing murine β actin cDNA. Inserts were purified by gel electrophoresis and labeled with ^{32}P to a specific activity of $1\text{–}2 \times 10^9$ dpm/μg, using a random primed DNA labeling kit (Boehringer Mannheim).

Northern Blot Analysis. Total RNA was extracted from spleens or pooled ganglia and analyzed by Northern blotting using standard protocols (23). Filters were washed at a final stringency of Tm −10°C (i.e., 10°C below the 50% hybrid melting temperature) and bound probe was detected by PhosphorImage analysis (PhosphorImager 400; Molecular Dynamics, Sunnyvale, CA). Bands were quantified relative to uninfected ganglionic RNA, using ImageQuant software (v3.0; Molecular Dynamics). In studies on the kinetics of MHC induction (see Fig. 2), after analysis for H-2K^d, filters were stripped and reprobed for β_2m mRNA and finally, ImageQuant values were normalized after reprobing the filters for transcripts of a cellular housekeeping gene, mouse β-actin.

In situ Hybridization. Nonisotopic detection of class I RNAs and HSV-1 LATs was done on paraformaldehyde-lysine-periodate (PLP) fixed tissue sections (5 μm) as described (20), with the following modifications: (*a*) tissues were refixed in 4% paraformaldehyde in PBS; (*b*) H-2 class I hybridization was done at 55°C (Tm − 30°C), with a stringent posthybridization wash at 53°C (Tm −13°C) for 15 min; and (*c*) LAT probes were hybridized at 65°C (Tm −26.5°C) with a stringent posthybridization wash at 75°C (Tm −5°C). Tissue blocks each contained 30–60 ganglia.

Results

HSV Infection Increases MHC Class I Transcription in the PNS. The effect of HSV on MHC class I transcription was initially investigated by Northern blot analysis of RNA extracted from 60–120 spinal ganglia (left T6-L1) removed from BALB/c mice either before infection, or 9 d after flank inoculation with HSV-1, strain SC16 (Fig. 1). Heterogenous and mature class I RNAs were present at low but detectable levels in uninfected ganglia which, to our knowledge, is the first demonstration of class I RNAs in normal adult neural tissue. 9 d after infection, mRNA levels had increased fivefold compared with uninfected ganglia, without alteration of the ratio between the heterogenous and mature species (~1:10).

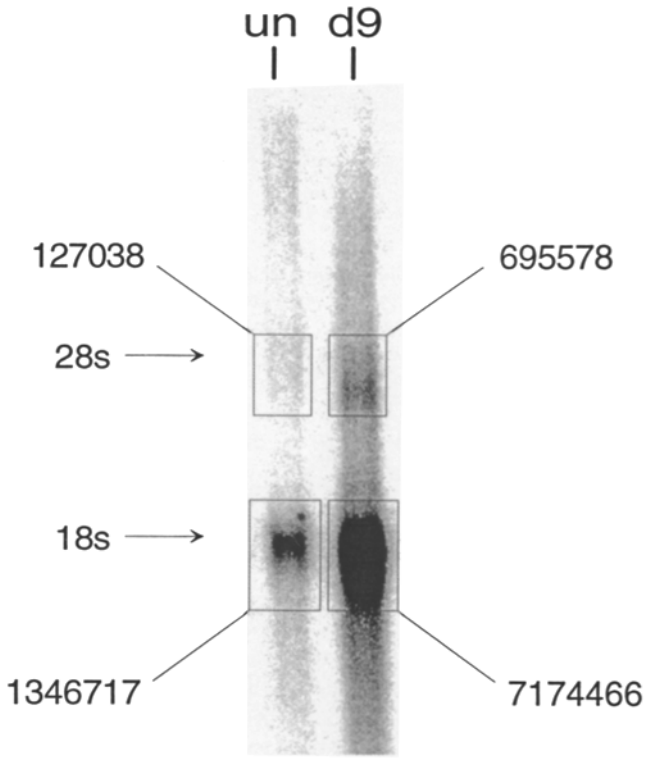

Figure 1. Northern blot comparing class I RNAs in ganglia removed from BALB/c mice before (*un*) or 9 d after (*d9*) cutaneous inoculation. Phosphorimage pixel values (determined using ImageQuant, v3.0) are indicated for the boxed regions containing heterogenous and mature H-2 class I mRNAs. The approximate sizes of heterogenous and mature class I RNAs (5 and 1.9 kb, respectively) were determined by comparison with the migrations of 28S and 18S rRNAs (*arrows*), respectively. Total RNA per lane, 5 μg.

The kinetics of induction of class I heavy and light (β_2m) chain RNA synthesis were determined by studying RNA samples extracted from BALB/c ganglia 5, 11, and 17 d after infection (Fig. 2). As bases for comparison, RNA was also extracted from spinal ganglia and spleens of uninfected animals. Class I heavy chain and β_2m mRNAs accumulated progressively in a coordinated manner for at least 17 d after infection, by which time the levels of these transcripts in ganglia exceeded those present in spleens of uninfected animals. We reasoned that accumulation of class I pre-mRNAs in the PNS after infection with HSV, together with the stable pre-mRNA/mRNA ratio, might indicate enhanced transcription of class I genes in resident neural cells, infiltrating cells, or both. This issue was addressed by studying the cellular location of MHC transcripts by in situ hybridization.

Neurons Synthesize Class I RNA Molecules. Spinal ganglia (left T6-L1) were removed from groups of uninfected, acutely infected, and latently infected BALB/c mice and PLP fixed sections (5 μm) were hybridized with a strand-specific digoxigenin-labeled riboprobe generated from full-length H-2K^d cDNA, under conditions designed to detect class I RNA transcripts in situ. Class I molecules were generally not detected in uninfected ganglia (Fig. 3 *a*), though in some reactions very weak hybridization was observed in occasional Schwann cell nuclei. Schwann cells may therefore be the main source of the low level of class I transcripts detected in uninfected PNS tissue by Northern hybridization. As early as 3 d after flank inoculation with 1.5 × 10^5 PFU HSV-1 strain

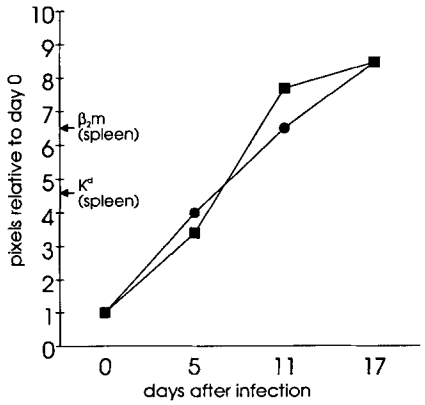

Figure 2. Kinetics of induction of class I heavy chain (●) and β_2m (■) mRNA synthesis. Values represented, which were generated by Northern blot analysis of RNA extracted from ganglia before (day 0) and various days after infection, indicate phosphorimage pixels relative to day 0. Also shown are the levels of class I heavy chain and β_2m mRNAs extracted from normal spleen, relative to the levels of corresponding transcripts from uninfected (day 0) ganglia.

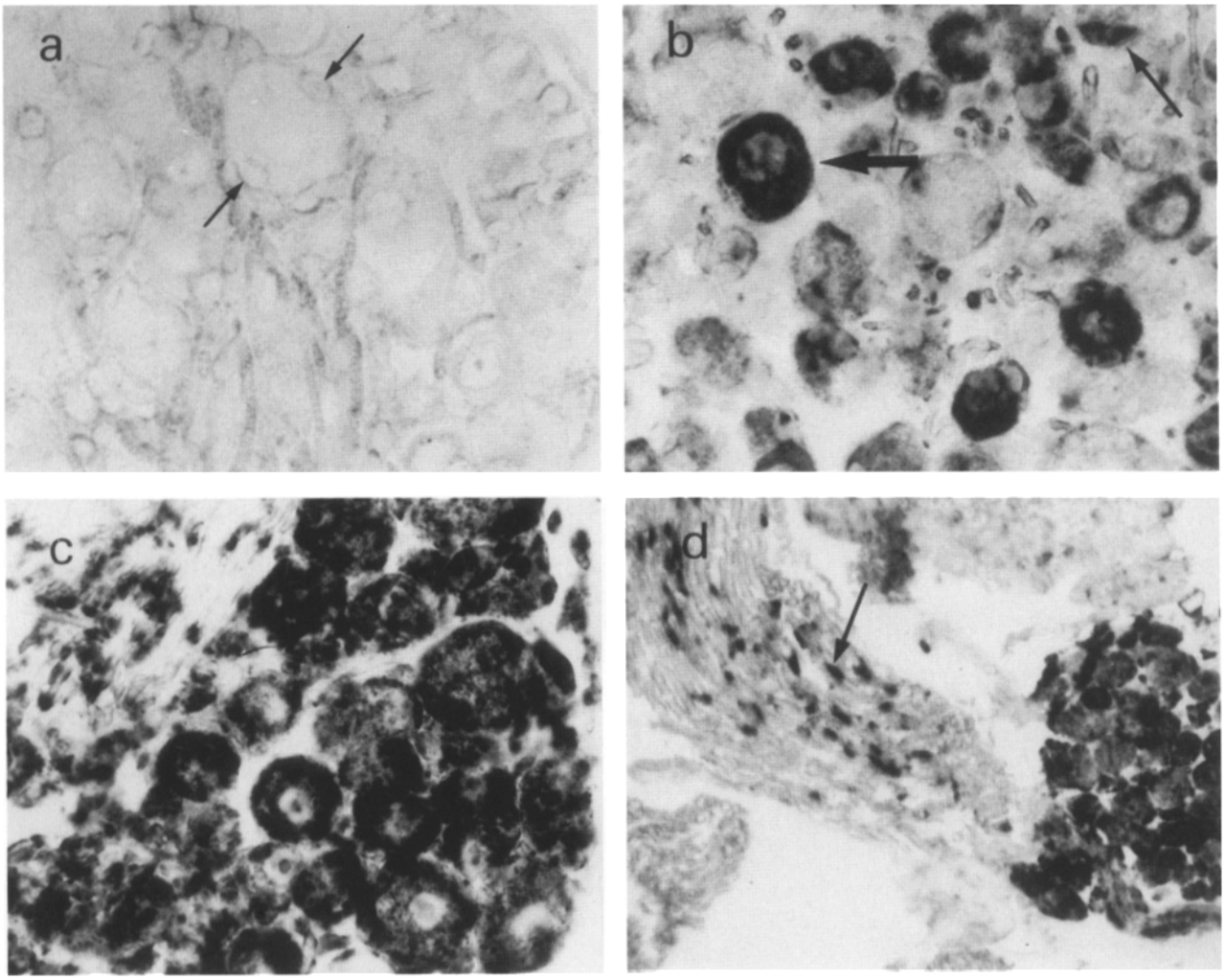

Figure 3. Detection of MHC class I RNA transcripts in BALB/c mice by in situ hybridization, using a digoxigenin-labeled probe. (*a*) Typical section from an uninfected mouse showing ganglionic architecture, in particular the location of satellite cell nuclei (e.g., *arrows*) in relation to somas of primary sensory neurons. (*b*) Strong staining (*black areas*) in satellite cells (e.g., *small arrow*) and neurons (e.g., *large arrow*) 3 d after infection. (*c*) Confluent staining 8 d after infection. (*d*) Abundant class I^+ Schwann cells (e.g., *arrow*) in spinal nerve 6 d after infection. (*a–c*, ×390; *d*, ×156).

SC16, strong hybridization was detected in the nuclei and cytoplasms of numerous satellite cells, Schwann cells, and primary sensory neurons (Fig. 3 *b*). On days 5–8, confluent staining of neurons and satellite cells was observed in many ganglia (e.g., Fig. 3 *c*) and staining of Schwann cell nuclei was widespread (e.g., Fig. 3 *d*). The extent of HSV infection 6 and 8 d after infection was estimated by staining ganglionic sections for viral antigens and, in concordance with previous studies (18), <10% of neurons were viral antigen positive on day 6 and acute infection had resolved by day 8 (data not shown).

In the model system described here, HSV is known to spread, via neural connections, from the inoculated neurodermatomes (T9/T10) to adjacent ipsilateral areas of the PNS (e.g., T11-T13) but, in immunocompetent animals, contralateral spread of virus is minimal (24). These characteristics of the model were used to address the issue of whether MHC induction is regulated by local or other factors. First, in situ hybridization was used to compare ipsilateral and contralateral ganglia from a group of mice infected 7 d earlier with SC16. Despite extensive induction of class I RNAs in mid-thoracic ganglia on the side of inoculation (Fig. 4 *a*), in contralateral ganglia the H-$2K^d$ probe hybridized weakly and to only a minority of cells (Fig. 4 *b*), suggesting localized rather than systemic MHC regulation. Second, ganglia directly innervating the inoculation site (T9) were compared with neighboring ipsilateral ganglia (T13), which receive virus via neural pathways. 30 ganglia from each spinal level were removed 5 and 8 d after infection, pooled, and tested for the presence of class I transcripts in situ. Temporally, MHC induction at T13 lagged T9 (Fig. 4, *c–f*), presumably reflecting the time taken for transneuronal transfer of virus within the PNS (25). Nonetheless, widespread appearance of class I transcripts on day 8 in satellite cells and neurons at T13 (Fig. 4 *f*) strongly implied local MHC regulation in response to spread of virus to this site, rather than retrograde axonal trans-

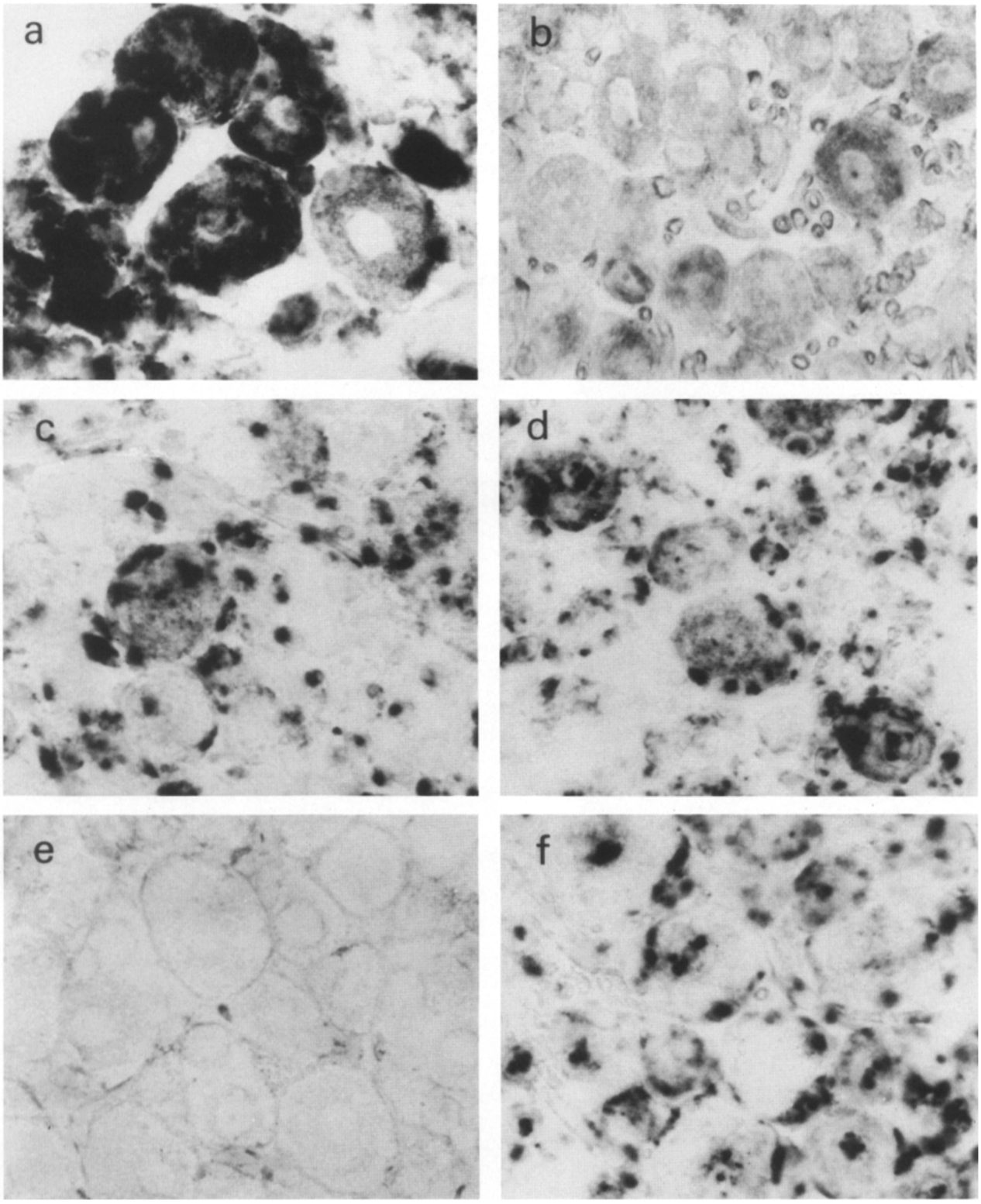

Figure 4. Photomicrographs of in situ hybridization, showing anatomical restriction of class I RNA induction. Typical widespread staining (*black areas*), 7 d after infection, in spinal ganglia (T6-L1) ipsilateral to the site of inoculation (*a*), is compared with an example of the sparse, weak class I staining seen in a minority of contralateral ganglia (*b*). Staining of ipsilateral ganglia from T9 (*c* and *d*) and T13 (*e* and *f*) either 5 (*c* and *e*) or 8 (*d* and *f*) d after infection showed delayed MHC induction at T13. ×1,000.

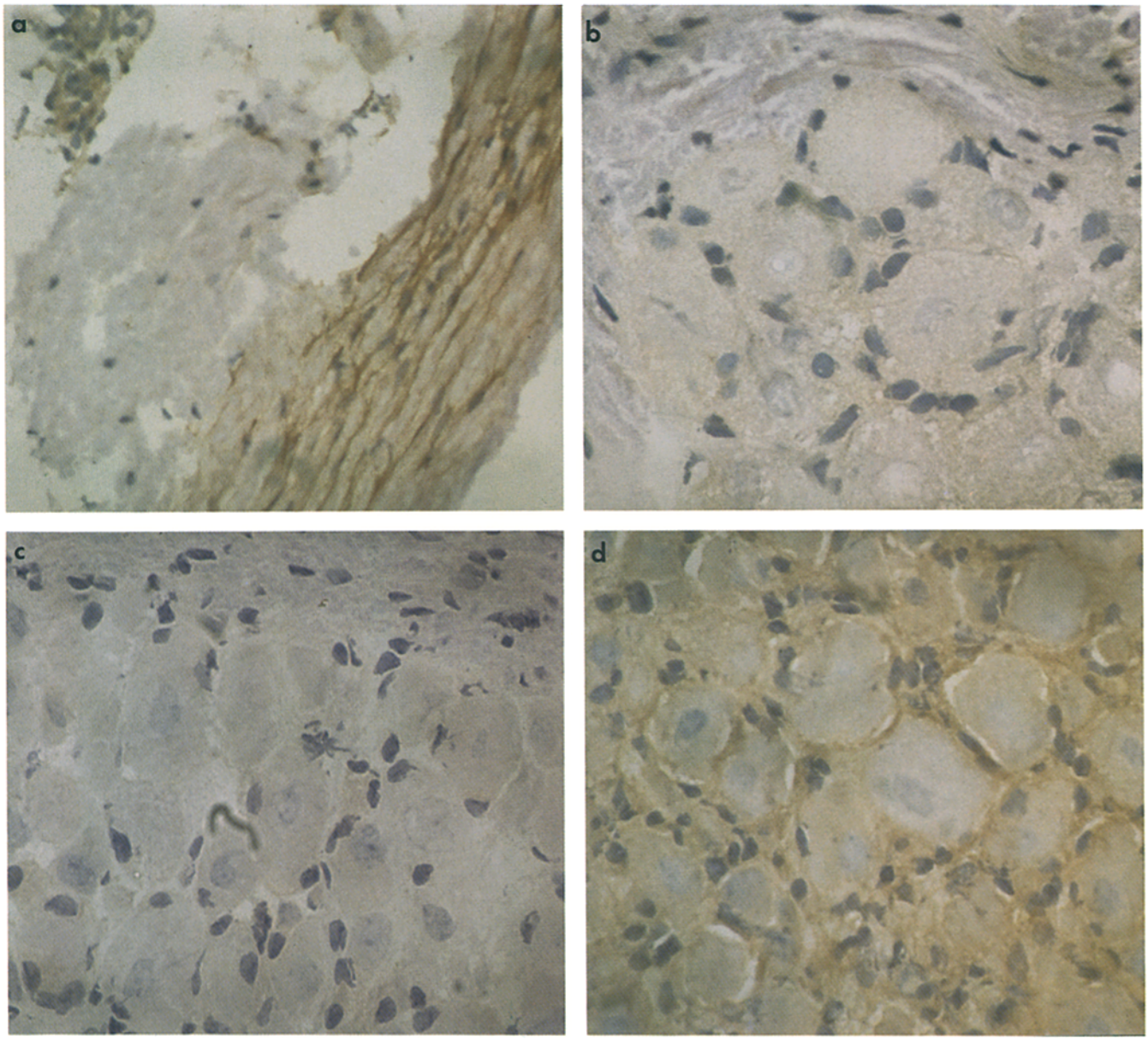

Figure 5. Immunohistochemical detection of H-2 K^d/D^d with mAb HB79 (*brown areas*). (*a*) Typical staining in nuclei and cytoplasms of Schwann cells in spinal nerve, 10 d after inoculation of HSV-1, strain SC16, into flanks of BALB/c mice. At the same time point, no staining was detected in nerves or ganglia of C3H (H-2^k) mice (*b*), or animals infected with UV inactivated SC16 (*c*). In contrast, widespread, strong staining was observed in ganglia of TKDM21-infected BALB/c mice (*d*). Sections were lightly counterstained with hematoxylin.

port of cytokines from infected skin. Acute ganglionitis (histologically discernable infiltration of leukocytes) was detected in ganglia at T9 but not T13.

HSV Infection Induces Classical MHC Class I Antigen Expression in Satellite and Schwann Cells but Not Neurons. Numerous immunohistochemical studies have indicated that CNS neurons lack the ability to express MHC class I genes in vivo (for a review see reference 9), even in response to viral infections, including herpes simplex (26). In view of the readily detectable HSV-induced synthesis of class I RNAs in primary sensory neurons and other resident cells of the PNS (see above), induction of classical H-$2K^d/D^d$ antigens was studied immunohistochemically in dorsal root ganglia and proximal spinal nerve trunks (left T8-T13), using two unrelated primary antibodies. In uninfected mice, H-$2K^d/D^d$ antigens could not be detected, despite the presence of low levels of class I mRNAs in the PNS (see above). However, widespread class I antigen induction was detected, by two anti-H-$2K^d/D^d$ antibodies (HB79 and 34-7-23S), in ganglia and nerves of mice infected 10 d earlier with strain SC16 (Fig. 5). Staining was prominent in satellite cells which surround primary sensory neurons and peripheral nerve Schwann cells (e.g., HB79, Fig. 5 *a*), but no staining was observed in neurons. Staining was H-2^d specific; primary antibodies did not react with

ganglia from infected C3H (H-2^k) mice (e.g., HB79, Fig. 5 *b*). No staining was seen after inoculation with 10^7 PFU UV-inactivated SC16 (Fig. 5 *c*), from which we concluded that viral infection, rather than the trauma of cutaneous inoculation, was the stimulus for class I expression. Thus, classical MHC class I proteins are expressed in satellite and Schwann cells of the PNS in response to HSV infection but, like their CNS counterparts, PNS neurons are resistant to MHC antigen induction.

A characteristic feature of the experimental model used in these experiments is delivery of virus to ganglia by retrograde axonal transport, which initially restricts viral access to neurons alone. Infectious virus and viral antigen positive neurons can first be detected in this system 3 d after inoculation of the flank (18), at which time induction of MHC class I mRNAs could be demonstrated readily (Fig. 3 *b*). These data implicate infected sensory neurons as the initial source of the local stimulus for MHC class I induction and this hypothesis was addressed by infecting mice with a mutant strain of HSV-1, TKDM21 (16). This virus has an 816-bp deletion in the TK region of the genome, which selectively eliminates viral DNA replication in neurons compared with epidermal cells (16), thereby preventing cell to cell spread of HSV in the PNS. In ganglia removed from BALB/c mice 10 d after flank inoculation with 10^7 PFU of strain TKDM21, immunohistochemical staining with anti-H-$2K^d/D^d$ (HB79) disclosed widespread expression of MHC class I genes in satellite cells (Fig. 5 *d*) and Schwann cells, despite the restricted nature of the infection.

Class I Synthesis Is Transient and Not Stimulated by Latent Infection. Avoidance of effective immune surveillance is an implicit feature of virus persistence and several strategies used by viruses to evade the immune response have been described. Prominent among these are (*a*) downregulation of normal antigen presentation mechanisms (27) and (*b*) infection of cells, especially neurons, that appear to be deficient in constitutive MHC class I expression (e.g., 28, and this report). The function of HSV LATs is unknown, and we were interested to determine whether this restricted form of viral gene expression was sufficient to maintain the stimulus for enhanced MHC class I expression in the PNS. Spinal ganglia from a group of BALB/c mice killed 400 d after infection (tissue PS320, kindly provided by P. Speck, Cambridge University, Cambridge, UK) were tested for the presence of class I RNAs and HSV-1 LATs by in situ hybridization. Class I transcripts were not detected whereas, in the same tissue, a substantial proportion (>10%) of neurons were LAT^+ (data not shown).

To characterize the transient nature of class I antigen induction, H-$2K^d/D^d$ antigen synthesis was studied in BALB/c ganglia removed at various times after cutaneous inoculation with 3×10^7 PFU HSV-1, strain TKDM21. In thoracic ganglia pooled from groups of six mice killed 10, 17, 21, and 27 d after midflank inoculation, immunohistochemical staining (using HB79) of satellite and Schwann cells increased in intensity up to and including day 17, declined by day 21, and was undetectable by day 27. On day 27, PLP-fixed ganglia from an additional group of concomitantly infected mice were paraffin embedded and sections (5 μm) were examined for HSV-1 LATs by in situ hybridization (Fig. 6). LAT^+ neurons were readily detected, demonstrating the presence of TKDM21 latent infection at this time point. Overall, these data indicate that (*a*) MHC class I antigens are detectable for at least 15 d after viral antigen synthesis ceases; (*b*) the PNS returns to a state of relative MHC deficiency; and (*c*) the restricted viral transcriptional activity associated with established latency does not maintain the stimulus for H-2 antigen expression.

Discussion

In previous studies, MHC-linked genes and $CD8^+$ T cells were shown to strongly influence the severity of HSV infec-

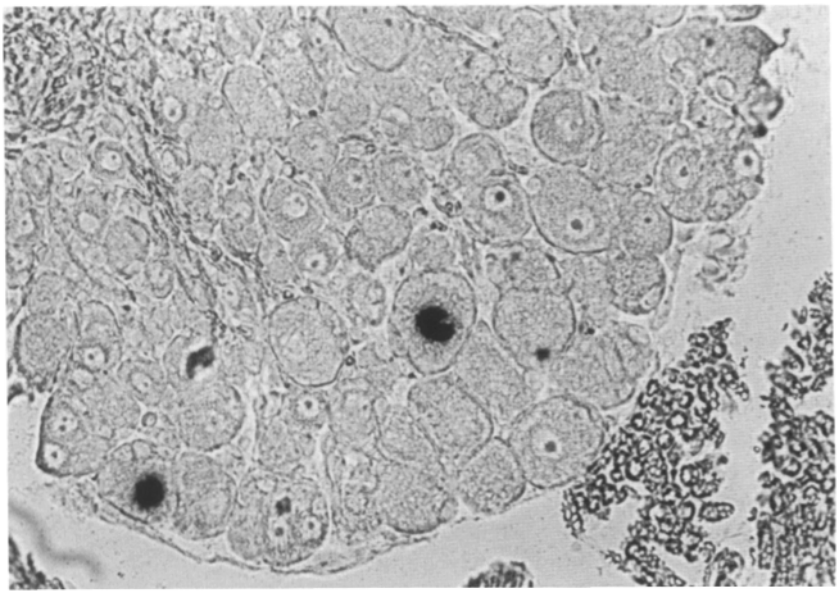

Figure 6. Photomicrograph of HSV-1 LATs (*black areas*) detected in neuronal nuclei by in situ hybridization, in spinal ganglia removed 27 d after infection. ×390.

tion of the PNS (6, 8), despite the MHC-deficient nature of resident cells in normal neural tissue. We hypothesized that class I expression in the PNS must be upregulated in response to HSV infection and, in the present study, this was shown to be the case. HSV transiently stimulated MHC class I gene expression in satellite and Schwann cells by a regulatory mechanism that acted at the transcriptional level, as determined by Northern blot analysis and in situ hybridization. Satellite cells, which are the most prominent cell type in spinal ganglia, are counterparts of CNS macroglia, which include oligodendrocytes and astrocytes; astrocytes are known to be able to stimulate $CD8^+$ T cells in vitro (29). Satellite cells are not permissive for HSV replication, but abortive infection, based on electron microscopy studies, has been described in vivo (30, 31). Together, the properties of satellite and Schwann cells and their relationship to CNS glia, suggest they may have a role in presenting HSV antigens to $CD8^+$ T cells in the PNS. A study of their immunological properties in vitro may shed further light on this issue.

Primary sensory neurons, like neurons in the CNS, appear to be resistant to induction of MHC antigens in vivo. This conclusion is based on failure to detect classical class I antigens in neurons in HSV-infected spinal ganglia, despite widespread H-$2K^d$/D^d antigen expression in neighboring satellite cells, with the caveat that the sensitivity of class I antigen detection in this and previous studies is unknown. The identity of the transcripts detected in neurons is a focus of future work. It is possible that they are classical class I mRNAs, induced in neurons by the same mechanisms responsible for MHC class I induction in satellite cells and that a posttranscriptional block in synthesis, transport, or processing of class I proteins is responsible for deficient H-$2K^d$/D^d expression. In line with this hypothesis, a defect in peptide transport, preventing efficient assembly of mature class I molecules and transport to the cell surface, has been described in a neuronal cell line in vitro (32). However, it has been suggested that MHC class I gene expression in the nervous system is controlled differently among different cell types (33). Two juxtaposed *cis*-acting regulatory elements upstream of H-2 class I coding sequences have been identified, one of which is an IFN response consensus sequence (34, 35). In the presence of γ-IFN, the regulatory sequences have been shown to enhance MHC class I promoter activity in transfected primary cultures of oligodendrocytes and astrocytes, but not CNS neurons; in neurons, γ-IFN failed to induce a DNA binding factor associated with enhancement of transcription (33). Consequently, the class I RNAs detected in neurons may not be derived from classical class I genes. Several nonclassical class I gene products, which lack the polymorphism characteristic of classical MHC antigens, have been identified in mice, rats, and humans, and a study of the rat MHC locus suggests that many more nonclassical molecules await discovery (36). The role of nonclassical MHC class I molecules is poorly understood, though it has been suggested that these molecules might act as restriction elements in association with invariant antigenic determinants on ubiquitously encountered foreign proteins (37). DNA sequence analysis of several murine nonclassical class I genes has revealed a high degree of similarity with classical genes, with very long segments of identity, particularly in exon 3. By design, the cDNA probe used in this study could not distinguish classical from nonclassical MHC class I transcripts. Whereas probes could be constructed to distinguish, in situ, between classical H-2K/D transcripts and nucleic acid sequences unique to those nonclassical genes for which DNA sequence information is available, novel approaches will be required to determine whether neurons respond to infection by upregulating the synthesis of as yet unidentified nonclassical class I molecules.

The source of the stimulus for class I mRNA and antigen synthesis merits discussion. Class I expression was anatomically restricted to ganglia reached by virus, either directly, by retrograde axonal transfer from skin and or indirectly, via neural connections. Thus, the stimulus for upregulation of class I synthesis most probably emanates from within the infected ganglion. Class I molecules were present in ganglia (e.g., T13) free of histologically evident infiltrating leukocytes, suggesting that MHC regulation is independent of inflammation. Although the number of viral genes expressed by TK^- strains of HSV in the PNS is severely restricted (38), H-$2K^d$/D^d antigen expression was stimulated by peripheral inoculation with TKDM21, which cannot replicate on reaching the PNS and is unable to escape from neurons. These data implicate neurons as the initial source of the stimulus for upregulation of MHC class I, and the previous suggestion that neurons may synthesize γ-IFN or related molecules may be relevant in this respect (39).

MHC class I transcripts were detected in neurons for up to 2 wk after recovery from acute infection. It is not known whether prolonged MHC induction is a general feature of acute viral infections of the nervous system but it is intriguing to speculate that this pattern may be a unique feature of the transition between the productive and latent phases of herpes simplex. MHC class I induction was not maintained during latency, when, judged by the most sensitive methods available, viral gene expression is restricted to a single region within the virus repeats. Putative translation products of LATs have been described only in vitro (5) and the function of LATs remains unknown. Further advances in our understanding of the latency-associated transcription unit may help to determine why latent gene expression does not stimulate class I MHC expression. This issue has broad implications because viruses, including HSV, have long been suspects in the etiology of demyelinating diseases (40) and dysmyelination is a known consequence of prolonged, aberrant expression of class I molecules in the nervous system (41). It is tempting to speculate that neurological damage might be caused by inappropriate class I induction in response to latent viral gene expression, particularly in view of the fact that HSV can persist in the CNS, as well as the PNS (42). The experimental system described in this report may be useful in addressing this hypothesis, because HSV is itself, a useful neurotropic vector (43) into which expression cassettes containing, for instance, MHC or other immune response genes, could be inserted under the control of the LAT promoter.

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References

1. Wildy, P., H.J. Field, and A.A. Nash. 1992. Classical herpes latency revisited. Symposium 33, Society for General Microbiology, Cambridge University Press, Cambridge. 133–167.
2. Simmons, A., D.C. Tscharke, and P.G. Speck. 1991. Role of immune mechanisms in control of HSV infection of the peripheral nervous system. *Curr. Top. in Microbiol. and Immunol.* 179:31.
3. Cook, M.L., V.B. Bastone, and J.G. Stevens. 1974. Evidence that neurons harbour latent herpes simplex virus. *Infect. Immun.* 9:946.
4. Stevens, J.G. 1989. Human herpesviruses: a consideration of the latent state. *Microbial. Rev.* 53:318.
5. Doerig, C., L.I. Pizer, and C.L. Wilcox. 1991. An antigen encoded by the latency associated transcript in neuronal cell cultures latently infected with herpes simplex virus type-1. *J. Virol.* 65:2724.
6. Simmons, A. 1989. H-2-linked genes influence the severity of herpes simplex virus infection of the peripheral nervous system. *J. Exp. Med.* 169:1503.
7. Nash, A.A., A. Jayasuriya, J. Phelan, S.P. Cobbold, H. Waldmann, and T. Prospero. 1987. Different 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.
8. Simmons, A., and D.C. Tscharke. 1992. Anti-CD8 impairs clearance of herpes simplex virus from the peripheral nervous system: implications for the fate of virally infected neurons. *J. Exp. Med.* 175:1337.
9. Wong, G.H., P.F. Bartlett, I. Clark-Lewis, J.L. Mckimm-Breschkin, and J.W. Schrader. 1985. Inteferon-γ induces the expression of H-2 and Ia antigens on brain cells. *J. Neuroimmunol.* 7:255.
10. Marrack, P., and J. Kappler. 1987. The T-cell receptor. *Science (Wash. DC).* 238:1073.
11. Specter, S., M. Bendinelli, and H. Friedman. 1992. Neuropathogenic Viruses and Immunity. Infectious Agents and Pathogenesis. Plenum Press, New York. 353 pp.
12. Levine, B., J.M. Hardwick, B.D. Trapp, T.O. Crawford, R.C. Bollinger, and D.E. Griffin. 1991. Antibody-mediated clearance of alphavirus infection from neurons. *Science (Wash. DC).* 254:856.
13. Joly, E., L. Mucke, and M.B.A. Oldstone. 1991. Viral persistence in neurons explained by lack of major histocompatibility Class I expression. *Science (Wash. DC).* 253:1283.
14. Simmons, A., and A.A. Nash. 1984. Zosteriform spread of herpes simplex virus as a model of recrudescence and its use to investigate the role of immune cells in prevention of recurrent disease. *J. Virol.* 52:816.
15. Simmons, A., and A.B. La Vista, 1989. Neural infection in mice after cutaneous inoculation with HSV-1 is under complex host genetic control. *Virus Res.* 13:263.
16. Efstathiou, S., S. Kemp. G. Darby, and A.C. Minson. 1989. The role of herpes simplex virus type 1 thymidine kinase in pathogenesis. *J. Gen. Virol.* 70:869.
17. Leung, K.N., A.A. Nash, D.Y. Sia, and P. Wildy. 1984. Clonal analysis of T cell responses to herpes simplex virus: isolation, characterisation and antiviral properties of an antigen-specific helper T-cell clone. *Immunology.* 53:623.
18. Speck, P.G., and A. Simmons. 1992. Synchronous appearance of antigen positive and latently infected neurons in spinal ganglia of mice infected with a virulent strain of herpes simplex virus. *J. Gen. Virol.* 73:1281.
19. Coupar, B.E.H., M.E. Andrew, D.B. Boyle, and R.V. Blanden. 1986. Immune response to H-2K^d antigen expressed by recombinant vaccinia virus. *Proc. Natl. Acad. Sci. USA.* 83:7879.
20. Arthur, J., S. Efstathiou, and A. Simmons. 1993. Intranuclear foci containing low abundance herpes simplex virus latency-associated transcripts visualized by non-isotopic in situ hybridisation. *J. Gen. Virol.* 74:1363.
21. Parnes, J.R., and J.G. Seidman. 1982. Structure of wild-type and mutant beta 2-microglobulin genes. *Cell.* 29:661.
22. Minty, A.J., M. Caravatti, B. Robert, A. Cohen, P. Daubas, A. Weydert, F. Gros, and M.E. Buckingham. 1981. Mouse actin mRNAs. Construction and characterization of a recombinant plasmid molecule containing a complementary DNA transcript of mouse α-actin mRNA. *J. Biol. Chem.* 256:1008.
23. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Book 1, 7.39–7.52.
24. Simmons, A., and A.A. Nash. 1987. B cell suppression on primary infection and reinfection of mice with herpes simplex virus. *J. Infect. Dis.* 155:649.
25. Ugolini, G., H.G.J.M. Kuypers, and A. Simmons. 1987. Retrograde transneuronal transfer of herpes simplex virus type 1 (HSV 1) from motoneurones. *Brain Res.* 422:242.
26. Weinstein, D.L., D.G. Walker, H. Akiyama, and P.L. McGeer. 1990. Herpes simplex virus type 1 infection of the CNS induces major histocompatibility complex antigen expression on rat microglia. *J. Neurosci. Res.* 26:55.
27. Schrier, P.I., R. Bernards, R.T. Vaessen, A. Houweling, and A.J. van der Eb. 1983. Expression of class I major histocompatibility antigen switched off by highly oncogenic adenovirus

12 in transformed rat cells. *Nature (Lond.).* 305:771.
28. Oldstone, M.B.A. 1991. Molecular anatomy of viral persistence. *J. Virol.* 65:6381.
29. Sedgwick, J.D., R. Mößner, S. Schwender, and V. ter Meulen. 1991. Major histocompatibility complex–expressing non-hematopoietic astroglial cells prime only $CD8^+$ T lymphocytes: astroglial cells as perpetuators but not initiators of $CD4^+$ T cell responses in the central nervous system. *J. Exp. Med.* 173:1235.
30. Dillard, S.H., W.J. Cheatham, and H.L. Moses. 1972. Electron microscopy of zosteriform herpes simplex infection in the mouse. *Lab. Invest.* 26:391.
31. Cook, M.L., and J.G. Stevens. 1973. Pathogenesis of herpetic neuritis and ganglionitis in mice: evidence for intra-axonal transport of infection. *Infect. Immun.* 7:272.
32. Joly, E., and M.B.A. Oldstone. 1992. Neuronal cells are deficient in loading peptides onto MHC class I molecules. *Neuron.* 8:1185.
33. Massa, P.T., K. Ozato, and D.E. McFarlin. 1993. Cell type-specific regulation of major histocompatibility complex (MHC) class I gene expression in astrocytes, oligodendrocytes, and neurons. *Glia.* 8:201.
34. Kimura, A., A. Israel, O. Le Bail, and P. Kourilsky. 1986. Detailed analysis of the mouse H-$2K^b$ promoter: enhancer-like sequences and their role in the regulation of class I gene expression. *Cell.* 44:261.
35. Israel, A., A. Kimura, A. Fournier, M. Fellous, and P. Kourilsky. 1986. Interferon response sequence potentiates activity of an enhancer in the promoter region of a mouse H-2 gene. *Nature (Lond.).* 322:743.
36. Jameson, S.C., W.D. Tope, E.M. Tredgett, J.M. Windle, A.G. Diamond, and J.C. Howard. 1992. Cloning and expression of class I major histocompatibility complex genes of the rat. *J. Exp. Med.* 175:1749.
37. Kurlander, R.J., S.M. Shawar, M.L. Brown, and R.R. Rich. 1992. Specialized role for a murine class I-b MHC molecule in prokaryotic host defences. *Science (Wash. DC).* 257:678.
38. Kosz-Vnenchak, M., J. Jacobson, D.M. Coen, and D.M. Knipe. 1993. Evidence for herpes simplex virus gene expression in trigeminal ganglion neurons. *J. Virol.* 67:5383.
39. Ljungdahl, Å , T. Olsson, P.H. Van der Meide, R. Holmdahl, L. Klareskog, and B. Höjeberg. 1989. Interferon-gamma-like immunoreactivity in certain neurons of the central and peripheral nervous system. *J. Neurosci. Res.* 24:451.
40. Webb, H.E. 1992. Antiglycolipid immunity: possible viral etiology of multiple sclerosis. *In* Neuropathogenic Viruses and Immunity. S. Specter, M. Bendinelli, and H. Friedman, editors. Plenum Press, New York. 277–302.
41. Turnley, A.M., G. Morahan, H. Okano, O. Bernard, K. Mikoshiba, J. Allison, P.F. Bartlett, and J.F.A.P. Miller. 1991. Dysmyelination in transgenic mice resulting from expression of class I histocompatibility molecules in oligodendrocytes. *Nature (Lond.).* 353:566.
42. Cabrera, C.V., C. Wohlenberg, H. Openshaw, M. Rey-Mendez, A. Puga, and A.L. Notkins. 1980. Herpes simplex virus DNA sequences in the CNS of latently infected mice. *Nature (Lond.).* 288:288.
43. Breakefield, X.O., and N.A. DeLuca. 1991. Herpes simplex virus for gene delivery to neurons. *New Biol.* 3:203.