Anti-CD8 Impairs Clearance of Herpes Simplex Virus from the Nervous System: Implications for the Fate of Virally Infected Neurons
By Anthony Simmons and David C. Tscharke

From the Division of Medical Virology, Institute of Medical and Veterinary Science, Frome Road, Adelaide, South Australia 5000, Australia

Summary
The role of CD8⁺ T cells in resistance to herpes simplex virus (HSV) was examined. After cutaneous inoculation, HSV spreads to the peripheral nervous system (PNS) where it replicates in ganglionic neurons. In normal mice, replication of virus in the PNS was rapidly terminated and evidence of neuronal destruction, assessed by a quantitative histological assay, was sparse. Clearance of infectious virus was impaired, and a strikingly high proportion of ganglionic neurons was killed, in mice treated with an antibody that depleted them of CD8⁺ T cells. These results suggest that CD8⁺ T cells play an important role in maintaining the integrity of the sensory nervous system during primary infection with HSV. Therefore, viral epitopes recognized by CD8⁺ T cells and restricting class I major histocompatibility complex genes are, in principle, implicated as interacting genetic determinants of neurovirulence.

After inoculation of HSV into the skin, virus replicates in epidermal cells and spreads rapidly along sensory nerve fibres to infect spinal ganglia of the peripheral nervous system (PNS) (1). On reaching the PNS, HSV DNA sequences may lie dormant (latent) in primary sensory neurons for long periods creating a reservoir from which infection may periodically reactivate (2). Concurrent with establishment of latency, a proportion of sensory neurons become productively infected, judged by recovery of infectious virus from ganglia and the presence of viral antigens and nucleic acids in neurons (3, 4). Immunocompetent hosts rapidly control the productive phase of herpes simplex but infection disseminates and becomes life threatening in neonates or when cellular immunity is impaired (5). Consequently, the immunological mechanisms responsible for resolution of primary herpes simplex have received a great deal of attention.

Several lines of evidence suggest that CD8⁺ T lymphocytes may play an important role in controlling HSV infection. First, class I MHC genes, the function of which are to restrict the activity of CD8⁺ cells (6), have a substantial influence on the severity of herpes simplex (7). Second, in line with the classic paradigm that viral infections are controlled by cytotoxic T lymphocytes, class I-restricted CTL can be recovered from lymph nodes draining herpetic lesions as early as 4 d after infection (8). Finally, adoptive transfer of draining lymph node cells enriched for CD8⁺ lymphocytes can protect syngeneic recipients against potentially lethal herpes if administered before or soon after inoculation of virus (9). However, the efficacy of adoptively transferred cells is lost if their administration is delayed until 48 h after infection (9), and consequently, although CD8⁺ cells have an established immunoprophylactic role, their contribution towards recovery from infection is an issue that has not been satisfactorily resolved.

Experimental immunosuppression is commonly used for studying adaptive immunity and the use of mAbs to selectively deplete mice of lymphocyte subsets in vivo is a refinement of this approach (10). In the experiments reported here, a well-characterized anti-CD8 mAb, YTS 169.4 (10), shown previously to deplete mice of CD8⁺ lymphocytes and ablate HSV-specific cytotoxicity (11), was used to selectively immunosuppress mice before infection with HSV. Using this strategy, it is shown that CD8⁺ cells make a substantial contribution to the clearance of HSV from PNS tissues. Furthermore, it is demonstrated that CD8⁺ cells contribute to the control of cutaneous infection in H-2a but not H-2b mice. Therefore, H-2-linked genes influence the overall contribution made by CD8⁺ cells in vivo, despite the potential complexity of antigenic challenge with HSV.

We reasoned that classic H-2-restricted cytotoxicity cannot, on theoretical grounds, explain the activity of CD8⁺ cells in ganglia. In the experimental system used for this study, HSV infection in the PNS is largely confined to neurons (4), which unlike epidermal cells do not express MHC antigens.
been suggested that primary sensory neurons may be able to survive an abortive infection with HSV (14). Implicit in this suggestion is that the host's immune response does not destroy infected neurons, and this issue was directly addressed using a quantitative histological and histochemical approach. It is shown that the number of viral antigen-positive neurons in spinal ganglia at the peak of infection is significantly greater than subsequent neuronal loss, supporting the concept of abortive HSV replication in neurons of immunocompetent hosts. However, in mice depleted of CD8+ T cells, a strikingly high proportion of ganglionic neurons are destroyed. Thus, neuronal survival depends on adequate recognition of infection by CD8+ lymphocytes, raising the novel concept that T cell epitopes on viral proteins, together with host immune response genes, may function as interacting genetic determinants of viral neurovirulence.

Materials and Methods

Virus Strain. A well-characterized isolate of HSV type 1, strain SC16 (15), was used. This virus, which is neuroinvasive in mice (16), was grown and titrated in vero cells (17) and stored at -70°C until required.

Infection of Mice. Adult female BALB/c (H-2b), CBA (H-2k), A/J (H-2d), B10.D2 (H-2b), and B10.Br (H-2b) mice were obtained from the specific pathogen-free facility (Animal Resource Centre, Perth, Western Australia). The zosteriform model used in all experiments has been described in detail elsewhere (16, 18).

Briefly, after depilation with Nair (Carter-Wallace, NSW, 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 containing 5 x 10^4 PFU.

Titration of Virus in Tissue Samples. Skin samples and left thoracic dorsal root ganglia were removed as described previously (18) and stored at -70°C until required. Unless otherwise indicated, pooled ganglia spanning the sixth through thirteenth thoracic segments of each experimental animal were examined. Tissues were homogenized in 1-ml glass tissue grinders (Wheaton Industries, Millville, NJ) and 10-fold dilutions of the homogenates were tested for presence of infectious virus using vero cells in a standard plaque assay (17).

In Vivo Depletion of CD8+ Cells. Hybridoma YTS 169.4 (10), screening rat IgG2b anti-Lyt-2.1, was grown in pristane-primed (LOU/M x DA)F1 rats (Animal Resource Centre). Dialyzed ascites, containing 1 mg/ml active antibody, were stored at -70°C until required. The short duration of the experimental infection (8 d) enabled euthymic mice to be depleted of CD8+ cells by infusion of 1 mg of antibody 4 d before infection and again 1 and 5 d after infection. Control mice received PBS. Depletion was confirmed by immunohistochemical analysis of spleens removed from the experimental animals postmortem. Frozen sections (6 μm) were reacted sequentially with YTS 169.4, peroxidase-conjugated goat f(ab')2 anti-rat IgG (Tago Inc., Burlingame, CA) and 3,3'-diaminobenzidine (0.5 mg/ml containing 0.1% H2O2). Slides were washed (10 min) in tris buffer (pH 7.4) between steps.

Immunohistochemical Detection of Viral Antigens. Primary antibodies were: (a) rabbit antiserum to HSV-infected cells (Dakopatts, Glostrup, Denmark), and (b) mAb LPII (19) to virion polypeptide 16 (VP16). Binding of primary antibodies was detected using swine anti-rabbit or goat anti-mouse Ig as appropriate, followed by rabbit or mouse peroxidase antiperoxidase conjugate, respectively (all from Dakopatts). All reactions were allowed to proceed for 30 min at 37°C with two 5-min washes in 50 mM Tris buffer (pH 7.4) between each step. Bound antibody was detected using 3,3'-diaminobenzidine (0.5 mg/ml, containing 0.1% H2O2), and sections were lightly counterstained with hematoxylin.

Enumeration of Neuronal Profiles. The strategy for comparing the neuronal content of spinal ganglia was based on precise determination of the mean number of neuronal profiles visualized in 5-μm paraffin sections. Dorsal root ganglia (T10) were removed and fixed immediately in periodate-lysine-parafomaldehyde (PLP) for 60 min (20) and then transferred to 50% ethanol. Pooled samples from groups of 30 mice were rolled into a ball, paraffin embedded, and 5-μm sections were collected onto glutaraldehyde-activated 3-aminopropyltripethoxysilane-coated slides. Sections were stained with hematoxylin and eosin (H&E), and profiles with the characteristic appearance of primary sensory neurons were enumerated (x156) using a 1-mm graticule (519–950; E. Leitz Inc., Rockleigh, NJ). A similar strategy was applied to immunohistochemically stained sections for enumeration of antigen-positive (Ag+) neurons.

Results and Discussion

Treatment with Anti-CD8 Impairs Virus Clearance. In mice, as in humans, the recovery phase of primary herpes simplex typically spans the period 6-10 d after infection (1). In the experiments reported here, the effect of selective T cell subset depletion on clearance of virus from skin and PNS tissue of groups of 5-10 mice was studied 8 d after infection. Compared with controls, significantly more infectious virus (typically ~100-fold) was recovered from spinal ganglia (T6-T13) of CD8-depleted mice of all strains tested (Fig. 1), whereas, in the same animals, impaired control of cutaneous infection was mouse strain dependent (Fig. 2). Treatment with anti-CD8 increased virus yield 50-fold from the skin of BALB/c (H-2K<sup>d</sup>D<sup>d</sup>) animals (p <0.01), whereas CBA (H-2K<sup>d</sup>D<sup>d</sup>) and A/J (H-2K<sup>a</sup>D<sup>α</sup>) mice were unaffected. The behavior of the antibody-treated H-2<sup>k</sup> strains cannot be explained by inadequate depletion of CD8+ cells because virus clearance from their PNS tissues was substantially impaired. Additionally, CD8+ cells could not be detected immunohistochemically in the spleens of mAb-treated H-2<sup>k</sup> animals (Fig. 3). Therefore, it is likely that, in association with H-2<sup>k</sup> or more
specifically H-2K\(k\) gene products, CD8\(^+\) lymphocytes do not make a significant contribution to virus clearance from the skin, despite the potentially complex nature of antigenic challenge with HSV. Evidence for an association between H-2-linked genes and the observed differences in mouse strain behavior was strengthened by examining H-2 congenic mice. Anti-CD8 markedly compromised B10.D2 (H-2\(d\)) animals, but B10.Br (H-2\(k\)) mice were unaffected (Fig. 2).

**CD8\(^+\) Cells Limit the Spread of HSV in the PNS.** The contribution made by CD8\(^+\) cells in controlling HSV infection in the PNS was investigated further, by enumerating Ag\(^+\) neurons in ganglia of normal and CD8-depleted BALB/c mice 8 d after infection (Fig. 4). To measure the extent of the viral spread to spinal ganglia not directly innervating the site of cutaneous inoculation, individual spinal segments (sixth thoracic through first lumbar) were separately analyzed (Fig. 5). As expected, very few infected neurons were identified immunohistochemically in immunocompetent mice, concordant with almost complete recovery by day 8. In contrast, in mice selectively depleted of CD8\(^+\) cells, a striking number of Ag\(^+\) profiles were present at all spinal levels examined.

** Fate of HSV-infected Neurons.** The results presented in this section pertain to ganglia of the tenth thoracic (T10) neurodermatome, upon which acute infection is centered in the model system described. In H&E-stained sections, primary sensory neurons are readily identified owing to their large size and characteristic appearance (Fig. 6, a and b) and cell profile counting was facilitated by use of a 1-mm graticule. Despite the presence of a mononuclear inflammatory infiltrate surrounding many neurons in acutely infected ganglia (Fig. 6 c), neuronal architecture was well preserved and cells stained with polyclonal antiserum were normal in size, showed no obvious evidence of nuclear or nucleolar breakdown, and were not fused to surrounding support cells (Fig. 6 d). These observations suggested that neurons differ from cultured cells and epidermal cells in their response to HSV. Therefore, a quantitative histological approach was used to measure directly the extent of neuronal destruction caused by HSV infection in BALB/c ganglia. 60 animals were infected (day 0) on the left flank and 30 were killed at the peak of infection (day 5) for immunohistochemical studies. The remaining 30 mice were killed after recovery (day 8), for enumeration of T10 sensory neurons. For comparison, ganglia were removed from

![Figure 2](image2.png)

**Figure 2.** Recovery of HSV from skin of normal (●) and anti-CD8-treated (○) mice 8 d after infection. Points represent the geometric mean of values from 5-10 animals. Bars indicate range.

![Figure 3](image3.png)

**Figure 3.** Immunohistochemical staining of splenic sections for CD8\(^+\) cells. (a) Typical distribution of CD8\(^+\) cells (black) in an immunocompetent CBA mouse. (b) Representative example of CD8\(^+\) cell depletion in an infected CBA mouse, after a course of treatment with mAb YTS169.4.
two groups of 30 uninfected mice. Paraffin blocks, prepared from the pooled T10 ganglia of each group of animals, yielded in total ~200 slides. A large number (>30) of the slides from each block were randomly selected for analysis. The number of individual ganglionic sections on each chosen slide ranged from 10 through 21, and in total, the actual number of ganglionic sections examined for each experimental group ranged from 434 through 672. No attempt was made to determine the absolute number of neurons present in ganglia because there is dispute regarding the correction factors that must be applied to profile counts in order to generate this information (21). Instead, the mean number of neuronal profiles per ganglionic section was used as the basis for comparison between the experimental groups, on the premise that this value is related to the absolute number of neurons present in a ganglion, irrespective of cell density. Comparative data were sufficient for the purpose of this study.

At the peak of infection, a mean of 18.7 neuronal profiles/ganglionic section were antigen positive, using polyclonal antiserum to HSV-infected cells (Fig. 7a). Neuronal infection was further characterized using a mAb to the HSV late gene product VP16, expression of which signifies an advanced stage of viral replication (22). On average, 12.7 neuronal profiles/section were VP16+, most of which showed no gross abnormalities of architecture (Fig. 7b). On day 8, antigen had been cleared and comparison with uninfected tissues disclosed only a small dropout of neuronal profiles (4.7/section) (Table 1, exp. 1). It was concluded, subject to a detailed statistical analysis of these data, that the number of Ag+ neurons present in T10 ganglia at the peak of infection exceeds the number of neurons destroyed.

**Statistical Analysis.** Each histological slide, containing a minimum of 10 and a maximum of 21 individual ganglionic sections, was used to make an independent estimate of the number of neuronal profiles/section (total profiles counted divided by the number of ganglia on the slide). It was assumed that these estimates were normally distributed about the true population mean, based on the large number of samples (>30) taken from each block. In exp. 1, the mean number of neurons in ganglia of normal (uninfected) mice and animals that had recovered from infection were compared by calculating the difference between means and its SE (4.7 ± 2.0), using standard formulae. Because the difference between the means exceeds twice the SE, the null hypothesis, i.e., that there is no difference in numbers of neurons before and after HSV infection, can be rejected (95% confidence). Therefore, it was concluded that some neurons were killed by HSV infection in this experiment.

The question next addressed is whether destruction of all the Ag+ neurons present on day 5 is compatible with the observed neuronal dropout. Stringent confidence levels (99.75%; i.e., ± 3 SEM) were set for this analysis. The mean

*Figure 4.* Immunohistochemical detection of HSV antigens in BALB/c T10 ganglia 8 d after infection. (a) Typical section from a normal mouse, and (b) widespread infection characteristic of animals treated with anti-CD8. Sections were lightly counterstained with hematoxylin and photographed using a BG38 red suppression filter (E. Leitz, Inc.) to enhance contrast between antigen-positive cells (dark areas) and uninfected cells (pale areas).

*Figure 5.* Mean number of Ag+ neuronal profiles/ganglionic section (T6-L1) 8 d after infection in immunocompetent vs CD8+ cell-depleted mice. Innervation of the cutaneous inoculation site is provided predominantly by T8-T10.
dropout of 4.7 ± 2.0 profiles/section was compared with 18.7 ± 0.7 Ag⁺ profiles on day 5, and in this case, the difference between the means is 14 ± 2.2. The difference greatly exceeds 3 SE, and on this basis we reject the hypothesis (99.75% confidence level) that all Ag⁺ neurons are destroyed. On day 5, 12.7 ± 1.6 neuronal profiles/section were VP16⁺, compared with a mean loss after recovery 4.7 ± 2. The difference between the means is 8 ± 2.56. Because the difference exceeds 3 SEM, the hypothesis that all VP16⁺ neurons are destroyed was rejected.

Treatment with Anti-CD8 Increases Neuronal Destruction. In view of the florid PNS infection experienced by CD8-deficient mice, ganglia removed from 30 immunocompetent and 30 CD8-depleted animals were compared with respect to neuronal dropout, 8 d after infection (Table 1, exp. 2). In this experiment, dropout was insignificant in the immunocompetent control group (0.6 ± 3.2 profiles/section). In contrast, loss of neurons was strikingly high in mice treated with anti-CD8 (50.6 profiles/section unaccounted for). As expected, selective depletion of CD8⁺ cells prevented normal clearance of viral antigen. It was concluded that depletion of CD8⁺ T lymphocytes increases the level of neuronal destruction associated with the acute phase of HSV infection.

Discussion and Implications. The mechanism of action of herpes-specific CD8⁺ cells in vivo remains an area of speculation. It has been proposed that cells with cytotoxic activity contribute to virus clearance from cutaneous lesions (23). In vitro, class I-restricted CTL preferentially recognize HSV antigens in association with H-2K (rather than H-2D) alleles (24), and in the experimental system described here, MHC restriction of CD8⁺ cells in vivo was similarly associated with H-2K. This circumstance links class I-restricted CTL activity with antiviral immunity. However, CBA (H-2k) mice ultimately clear infectious virus from cutaneous lesions, apparently without the assistance of CD8⁺ cells (11). Therefore, it is likely that other responses, such as class II-restricted cytotoxicity (28) or DTH (29), are of primary importance in the resolution of cutaneous lesions. In support of this hy-
hypothesis, mice treated with anti-CD4 cannot clear HSV from the skin (11) and cutaneous lesions fail to resolve in CD4+ cell-deficient AIDS patients (5).

Treatment with anti-CD8 had a profound influence on the severity of PNS infection. In CD8-deficient mice, a high proportion of HSV-infected ganglionic neurons were killed, and neural spread of virus was enhanced, suggesting that CD8+ T cells play an important role in maintaining the integrity of the sensory nervous system during infection with HSV. In principle, these results implicate T cell epitopes on viral proteins, together with restricting host MHC genes, as potentially interacting determinants of neurovirulence. It has been shown that viruses might escape immune surveillance by mutation of epitopes recognized by CD8+ cells (25), raising the novel possibility that neurovirulence might spontaneously be enhanced as a result of selective pressure by the immune response.

Three mechanisms can be envisaged by which CD8+ cells

Table 1. Fate of HSV-infected Neurons in Normal and Anti-CD8-treated BALB/c Mice

| Exp. | Normal mice (exp. 1 and 2) | Anti-CD8 treated (exp. 2 only) |
|------|---------------------------|--------------------------------|
| Uninfected - total | 139.4 ± 1.6 ($\bar{x}_1$) | 88.8 ± 3.3 ($\bar{x}_2$) |
| Day 8 - total : 1 | 134.7 ± 1.2 ($\bar{x}_3$) | 50.6 ± 3.7 ($\bar{x}_4$) |
| Day 8 - total : 2 | 138.8 ± 2.8 ($\bar{x}_5$) | |
| Day 8 - loss : 1 | 4.7 ± 2.0 ($\bar{x}_6$) | |
| Day 8 - Ag' : 1 | 0 | |
| Day 5 - VP16' | 18.7 ± 0.7 | 12.7 ± 4.5 |
| Day 5 - Ag' | 1 | |

* The number of sections/slide ranged from 10 through 21, and >30 slides from each histological block were examined in all cases. The total number of neuronal profiles counted from each group of animals ranged from ~60,000 through 94,000.

† It was assumed that treatment with anti-CD8 did not alter the number of primary sensory neurons in ganglia of uninfected mice.
might assist in the control PNS infection: (a) class I–restricted CTL might limit the spread of virus and hence the ultimate amount of damage, by rapidly killing infected neurons; (b) cytokine release might protect uninfected neurons, thus limiting the spread of infection; and (c) CD8+ cells might promote, directly or indirectly, termination of viral gene expression in neurons without inducing cytolysis, perhaps by releasing cytokines that interfere with multiple stages of the viral replicative cycle, as described in other systems (26–28). In spinal ganglia, it is unlikely that HSV-specific CD8+ cells mediate their antiviral effect by direct cytolysis, irrespective of their mode of action in the skin. It has been proposed that lack of MHC expression protects neurons against attack by CTL (29), allowing viral infection to be terminated by nonlytic mechanisms (30). The results presented here are consistent with this hypothesis, because in normal animals we were unable to prove that all HSV antigen–positive neurons are killed. Therefore, we favor a nonlytic role for CD8+ cells in the PNS. Whatever their mechanism of action, CD8+ cells must be stimulated in the PNS by antigen presented in the context of class I MHC molecules, and capsular cells, which surround each ganglionic neuron, might fulfill this function. Capsular cells are counterparts of CNS microglia and, though not productively infected themselves (31), they strongly express MHC glycoproteins during the acute phase of herpes simplex (13).

Replication of HSV DNA is dependent on synthesis of numerous virally encoded polypeptides (22). In contrast, establishment of latency is not dependent on viral gene expression (4, 32), implying that HSV DNA replication is not a prerequisite for persistence of viral genomes in the PNS. However, a strikingly large amount of viral DNA can be recovered from latently infected tissue (33), and, in spinal ganglia of mice, two patterns of persistence of HSV DNA sequences have recently been distinguished, on the basis of location and number of viral genome equivalents per cell (34). Consequently, there may be more than one molecular pathway leading to latency. It remains to be shown whether abortive infection is responsible for most of the viral sequences that persist in the nervous system, but it is attractive to speculate that a unique interaction between neurons and HSV may have coevolved with the host’s ability to protect infected neurons from CTL. Latency conveys an enormous survival advantage to viruses (35), and preservation of virally infected neurons might be regarded as an evolutionary compromise that favors both virus and host.

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Address correspondence to Anthony Simmons, Division of Medical Virology, Institute of Medical and Veterinary Science, Box 14, Rundle Street Post Office, Adelaide, South Australia 5000, Australia.

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References
1. Wildy, P., H.J. Field, and A.A. Nash. 1982. Classical Herpes Latency Revisited. Symposium 33. Society for General Microbiology, Cambridge University Press, Cambridge, UK. 133–167.
2. Cook, M.L., V.B. Bastone, and J.G. Stevens. 1974. Evidence that neurons harbour latent herpes simplex virus. Infect. Immun. 9:946.
3. 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.
4. Speck, P.G., and A. Simmons. 1991. Divergent molecular pathways of productive and latent infection with a virulent strain of herpes simplex virus type 1. J. Virol. 65:4001.
5. Whitley, R.J. 1990. Herpes simplex viruses. In Virology, 2nd edition. B.N. Fields, D.M. Knipe, R.M. Chanock, M.S. Hirsch, J.L. Melnick, T.P. Monath and B. Roizman, editors. Raven Press Ltd., New York. 1483–1888.
6. Marrack, P., and J. Kappler. 1987. The T-cell receptor. Science (Wash. DC). 238:1073.
7. 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.
8. Nash, A.A., R. Quarter-Papafio, and P. Wildy. 1980. Cell-mediated immunity in herpes simplex virus-infected mice: functional analysis of lymph node cells during periods of acute and latent infection, with reference to cytotoxic and memory cells. J. Gen. Virol. 49:309.
9. Simmons, A., D.C. Tscharke, and P.G. Speck. 1992. Role of immune mechanisms in control of HSV infection of the peripheral nervous system. Curr. Top. Immunol. In press.
10. Cobbold, S.P., A. Jayasuriya, A. Nash, T.D. Prospero, and H. Waldmann. 1984. Therapy with monoclonal antibodies by elimination of T-cell subsets in vivo. Nature (Lond.). 312:548.
11. 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.

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

13. Weinstein, D.L., D.G. Walker, H. Akiyama, and P.L. McGeer. 1990. Herpes simplex virus type I infection of the CNS induces major histocompatibility complex antigen expression on rat microglia. *J. Neurosci. Res.* 26:55.

14. Steiner, I., and P.G.E. Kennedy. 1991. Herpes simplex virus latency in the nervous system: a new model. *Neuropathol. Appl. Neurobiol.* 17:433.

15. Hill, T.J., J.H. Field, and W.A. Blyth. 1975. Acute and recurrent infection with herpes simplex virus in the mouse: a model for studying latency and recurrent disease. *J. Gen. Virol.* 28:341.

16. 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.

17. Russell, W.C. 1962. A sensitive and precise assay for herpesvirus. *Nature (Lond.)* 195:1028.

18. 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. Gen. Virol.* 52:816.

19. McLean, C., A. Buckmaster, D. Hancock, A. Buchan, A. Fuller, and A. Minton. 1982. Monoclonal antibodies to three non-glycosylated antigens of herpes simplex virus type 2. *J. Gen. Virol.* 63:297.

20. McLean, I.W., and P.K. Nakane. 1974. Periodate-lysine-paraformaldehyde fixative. A new fixative for immunoelectron-microscopy. *J. Histochem. Cytochem.* 22:1077.

21. Smolen, A.J., L.L. Wright, and T.J. Cunningham. 1983. Neuron numbers in the superior cervical sympathetic ganglion of the rat: a critical comparison of methods for cell counting. *J. Neurocytol.* 12:739.

22. Knipe, D.M. 1989. The role of viral and cellular nuclear proteins in herpes simplex virus replication. *Adv. Virus Res.* 37:85.

23. Nash, A.A., J. Phelan, and P. Wildy. 1981. Cell-mediated immunity in herpes simplex virus-infected mice: H-2 mapping of the delayed-type hypersensitivity response and the antiviral T cell response. *J. Immunol.* 126:1260.

24. Jennings, S.R., P.L. Rice, S. Pan, B.B. Knowles, and S.S. Tevethia. 1983. Recognition of herpes simplex virus antigens on the surface of mouse cells of the H-2d haplotype by virus-specific cytotoxic T-lymphocytes. *J. Immunol.* 132:475.

25. Pircher, H., D. Moskophidis, U. Rohrer, K. Burki, H. Hengartner, R.M. Zinkernagel. 1990. Viral escape by selection of cytotoxic T cell-resistant virus variants in vivo. *Nature (Lond.)* 346:629.

26. Stewart, W.E. 1979. The Interferon System. Springer Publishing Company, New York. 493 pp.

27. Maheshwari, R.K., and R. Friedman. 1981. Assay of effect of interferon on viruses that bud from plasma membrane. *Methods Enzymol.* 79:451.

28. Lengyel, P. 1982. Biochemistry of interferons and their actions. *Rev. Biochem.* 51:251.

29. 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.

30. 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.

31. 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.

32. Leib, D.A., D.M. Coen, C.L. Bogard, K.A. Hicks, D.R. Yager, D.M. Knipe, K.L. Tyler, and P.A. Schaffer. 1989. Immediately regulatory gene mutants define different stages in the establishment and reactivation of herpes simplex virus latency. *J. Virol.* 63:759.

33. Efstathiou, S., A.C. Minson, H.J. Field, J.R. Anderson, and P. Wildy. 1986. Detection of herpes simplex virus-specific DNA sequences in latently infected mice and in humans. *J. Virol.* 57:446.

34. Simmons, A., B. Slobedman, P. Speck, J. Arthur, and S. Efstathiou. 1992. Two patterns of persistence of herpes simplex virus DNA sequences in the nervous systems of latently infected mice. *J. Gen. Virol.* In press.

35. Hope-Simpson, R.E. 1965. The nature of herpes zoster: a long term study and a new hypothesis. *Proc. R. Soc. Med.* 58:9.