Reactivation of herpes simplex virus type 1 in the mouse trigeminal ganglion: an in vivo study of virus antigen and immune cell infiltration

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The corneas of latently infected mice were UV irradiated to induce reactivation of herpes simplex virus type 1 (HSV-1) in the trigeminal ganglion (TG). On days 1 to 4 after irradiation, TG were removed, serially sectioned and double stained to identify immune cells and virus antigens. Virus antigen was detected in small numbers (most commonly one) of neurons per ganglion as early as day 1, confirming the rapidity of reactivation and the neuron as the likely site of this event. The immune response was also rapid and effective since virus antigen was identified in immune cells at day 1 and by day 4 all samples were negative. The predominant infiltrating cells on days 1 and 2, when virus antigen was present and being cleared, were T cells, both CD4+ and CD8-. Later, large numbers of B cells appeared, suggesting that local antibody production may also be involved in controlling the reactivated infection. The observations suggest that a significant proportion of reactivation events do not result in disease of the eye or shedding of virus in the tear film. However, they also suggest that as little as one reactivating neuron in the ganglion may be sufficient to lead to such disease and/or shedding.

Introduction

The ability of herpes simplex virus (HSV) to reactivate from latency is pivotal to the pathogenesis of recurrent herpetic disease. The most likely site of such reactivation is the ganglionic neuron although the biochemical and molecular events involved in this process are not fully understood and the fate of such neurons is the subject of interest and discussion (Simmons et al., 1992). In vitro studies of reactivation in explanted ganglia suggest that virus spreads from a single focus (often a solitary neuron) to form large foci containing multiple infected neurons and glial cells (Ecob-Prince & Hassan, 1994). This spread with time may explain the increasing amounts of viral DNA that are detected in such explants and the continued expression of productive cycle transcripts (Devi-Rao et al., 1994). In contrast, with in vivo systems of reactivation, only very small numbers of antigen positive neurons have been identified (Sawtell & Thompson, 1992) and only small amounts of infectious virus isolated (Shimeld et al., 1996). Moreover, viral DNA replication was undetectable and expression of productive cycle transcripts was transient (Bloom et al., 1994). The ability of the host to mount a virus specific immune response to curtail viral activity is the most likely reason for the differences observed between these two experimental systems.

In contrast to events in the ganglion during the primary infection (Simmons & Tscharke, 1992; Shimeld et al., 1995; Liu et al., 1996), the immune response during reactivation at this site has been largely unexplored. The main reason for this lack of information was the dearth of experimental models suitable for detailed immunological investigations. Such models are now available and the immune response to reactivated virus in peripheral tissue has been investigated (Shimeld et al., 1996; Nicholls et al., 1996. With respect to the immune response to HSV reactivation within sensory ganglia, very little is known, except that there is a rapid mononuclear cell infiltrate in the trigeminal ganglion (TG) of mice following reactivation of latent infection by hyperthermia (Sawtell & Thompson, 1992).

Reactivation and recurrent disease occur despite an existing virus specific immune response, although there is some evidence that defects in this response may result in more frequent disease or disease of increased severity. Moreover, it has been argued that reactivation is more likely to yield recurrent disease than asymptomatic viral shedding when subtle depressions in cytokine production are present (Simmons et al., 1992). Clearly, the immune response can operate to control infection at the site of recurrences and within the ganglion, particularly since, unlike the CNS, in the ganglion there is no equivalent of the blood–brain barrier. An
increased understanding of these mechanisms of control would be an advantage for the rational design of vaccines and other immunotherapeutic strategies.

We have investigated the early immune response to reactivated virus in the TG, using a mouse model in which reactivation and recurrent disease are induced by UV irradiation of the cornea (Shimeld et al., 1989). An immunohistochemical method was used that allows the simultaneous identification of virus antigens and a wide variety of immune cells. Our two main aims were: (i) a temporal and somatotopic investigation of the presence of virus antigen during reactivation and (ii) to make quantitative observations on the immunological events in the TG during reactivation of virus. During preparation of the tissue the somatotopic organization of the TG was carefully maintained so that the location of cells positive for viral antigens and immune cell infiltration could be precisely documented within the three parts of the TG [ophthalmic (TG1), maxillary (TG2) and mandibular (TG3)]. Examination of serial sections of entire ganglia was essential for this work: in all over 5000 sections were examined. With a less rigorous approach, reactivating neurons and small foci of infiltrating cells would have been missed.

Methods

Reactivation model. Details of the model have been described in detail elsewhere (Shimeld et al., 1989, 1996) and are given here in brief.

(i) Production of latently infected mice. Specific-pathogen-free, 8-week-old female NIH/OLA inbred mice were obtained from Harlan/Olac; they were maintained as a breeding colony in the Department of Pathology and Microbiology. Mice were anaesthetized as described previously (Shimeld et al., 1995) and inoculated by scarification of the left cornea with a 26 gauge needle (Tullo et al., 1983) through a 5 µl drop of medium containing 1 x 10^5 p.f.u. of HSV-1 strain McKrae (Williams et al., 1965). Control mice were inoculated in the same way with a preparation of uninjected Vero cells made in the same manner to the virus inoculum (mock inoculum). Twenty-four hours before inoculation with virus, animals were inoculated intraperitoneally with O.5 ml of rabbit serum containing antibodies to HSV-1 (Shimeld et al., 1989). The serum was diluted with PBS to give a dose of 8000 ED50. Passive immunization is used to protect the eye from the severe damaging effects of HSV-1 disease (Shimeld et al., 1999b). Only mice that survived primary infection with undamaged eyes were used for reactivation of latent infection.

(ii) Reactivation of latent infection. Forty-eight hours after corneal inoculation, mice were anaesthetized and the left cornea and lids irradiated with UV light as described previously (Shimeld et al., 1989).

(iii) Examination of eyes and isolation of virus from eyewashings. Immediately before UV irradiation and on days 3 and 4 after such treatment, mice were anaesthetized and the left cornea, iris and lids examined for signs of eye disease using a slit lamp microscope. Eyewashings were taken before UV irradiation and on days 1 to 4 after irradiation; these were put onto Vero cells for the isolation of virus (Tullo et al., 1983).

Dissection and processing of tissues. Anaesthetized mice were perfused with periodate-lysine-parafformaldehyde fixative (PLP; Whiteland et al., 1995). The left TG were carefully dissected from the skull and placed ventral surface down in cuvetteing cassettes. Spleens were removed and cut into 2 mm slices. Tissues were processed as described previously (Whiteland et al., 1995). In brief, they were fixed overnight in PLP at 4 °C, rapidly dehydrated and infiltrated under vacuum with low temperature paraffin wax. Serial transverse 6 µm sections were cut and transferred to glass microscope slides precoated with poly-l-lysine (three sections per slide).

Immunohistochemistry

(i) Double staining for HSV-1 antigens and cell surface molecules. Sections were first stained for infiltrating cells and MHC class II expression as described previously (Whiteland et al., 1995). In brief, endogenous peroxidase was blocked by 0.3% hydrogen peroxide and non-specific binding sites were blocked by 1:5% normal goat serum. Sections were incubated with primary antibody overnight at 4 °C. The rat anti-mouse monoclonal antibodies used for staining recognize the following: class II MHC (YE2/36), F4/80 antigen (C1:A3-1) on macrophages and dendritic cells, CD11b (M1/70) on granulocytes, macrophages and cells which exhibit NK activity, CD8 (KT15) on cytotoxic T cells (Serotec), CD4 (RM4-5) on helper T cells, Gr-1 antigen (RB6-8C5) on granulocytes (Pharmingen), CD45RA (RA-3-6B2), and the B220 antigen on B cells and cells which exhibit NK activity (Bdradure Biologicals, UK). The slides were then incubated with biotinylated goat anti-rat IgG, preabsorbed with normal mouse serum (diluted 1:50), followed by avidin-biotinylated horseradish peroxidase complex (ABC) and diaminobenzidine (DAB) (Sigma) with 0.01% hydrogen peroxide. The primary and secondary antibodies were diluted in PBS containing 0.1% BSA and the ABC complex in PBS. Sections were washed twice in PBS between steps. Positive control slides were sections of spleen stained in a similar manner. Negative control slides were incubated with diluent instead of primary antibody. The ABC and all sera were from Vector (Peterborough, UK) except the normal mouse serum which was from Dakopatts. Sections were then stained for HSV-1 antigens by the peroxidase–anti-peroxidase method at room temperature using in sequence 10% normal swine serum, rabbit anti-HSV-1 serum diluted 1:300 in PBS containing 0.1% BSA, swine anti-rabbit immunoglobulin diluted 1:100 in PBS, rabbit peroxidase–anti-peroxidase complex diluted 1:100 in PBS, rabbit peroxidase–anti-peroxidase complex diluted 1:100 in PBS (all sera were from Dakopatts). The chromagen was Vectastain VIP (Vector), which gives a purple end-product. Slides were incubated with the primary antibody for 2 h and with the other antibodies for 30 min. Sections were washed twice in PBS between steps. Included in each staining run were slides which were from tissue known to contain HSV infected cells; these were incubated with primary antibody or diluent to provide positive and negative staining controls. Sections were lightly counterstained with methyl green, dehydrated, cleared and mounted in Histomount (National Diagnostics).

(ii) Counting of cells stained with monoclonal antibodies. Cells were counted in the area of maximum staining of immune cells in the TG using a × 40 objective in two grid areas of 0.04 mm² on each of two sections.

(iii) Statistics. Normal probability plots showed that the data did not conform to a normal distribution, and the variances of different groups differed widely. Square root transformation resolved both of these features and allowed comparisons by analysis of variance. Multiple unplanned comparisons were made by the method of Tukey (Snedecor & Cochran, 1972); the level of significance was set at 5%.

Results

Experimental protocol

On each of days 1 to 4 after UV irradiation, ten latency
infected mice and three mice given mock inoculum were killed and the TG removed and processed for immunohistochemistry. Serial sections of entire ganglia were cut and double stained for immune cells and HSV-1 antigens. Similar serial sections of TG from five latently infected mice that had not been UV irradiated were stained as a control for resident immune cells.

**Eye disease and isolation of virus from eyewashings**

Virus was isolated from 4/30 eyewashings on day 2, from 5/20 on day 3 and 4/10 on day 4. No virus was isolated from eyewashings taken before, or 1 day after, UV irradiation. Of the 40 latently infected mice irradiated with UV, eight shed virus on at least one occasion, two from the group killed on day 3, and six from the group killed on day 4. Amounts of virus isolated from individual animals varied from 1 p.f.u. on 1 day to > 100 p.f.u. on each of three consecutive days.

Of the ten mice killed on day 3, six had signs of recurrent corneal disease, viz. epithelial ulceration underlain by haze or slight opacification. All six animals had irid hyperaemia and two also had recurrent lid lesions. Six of ten mice killed on day 4 had similar signs of recurrent corneal disease and one had recurrent lid disease alone. Of the 13 animals with disease, eight shed virus. None of the mice which appeared normal on clinical examination shed virus in tears.

**Immunohistochemistry**

**Virus antigen**

One day after treatment, 6/10 ganglia had virus antigen in the TG. The incidence of such antigen then declined with 3/10 positive on day 2 and 2/10 on day 3. No antigen was detected on day 4 after UV irradiation or in ganglia from mock inoculated animals or in samples taken from latently infected mice not stimulated by UV irradiation. Antigen was detected in neurons and in immune cells. Rarely, a neuron and its associated satellite cell were positive. With respect to antigen in neurons, five ganglia each showed a single positive cell, one ganglion had three positive cells and one ganglion had four positive cells. The majority of antigen positive cells were detected within foci of infiltrating cells; however, three positive neurons, two on day 1 and one on day 3 were in areas devoid of such cells (Fig. 1a). In ten of the 11 ganglia that showed antigen such antigen was in the dorsal part of the TG. In seven of the 11, antigen was in TG1 and located in the rows of neuronal cell bodies that lie at the medial edge of this ganglion. The remaining four areas of antigen were deeper in TG1 or in the area bordering TG1/TG2. No antigen was detected in TG3.

**Immune cells**

(i) **Ganglia from control mice.** Very low numbers of CD4+ cells and B cells (equivalent to < 1 to 2 cells/grid area) were found in TG of non-UV irradiated latently infected mice and these cells were scattered throughout the ganglion. The numbers and distribution of other cell types were similar to those found in ganglia from uninfected animals (Shimeld et al., 1995) (Fig. 2a–f). The number and distribution of stained cells in ganglia from mock inoculated mice (Fig. 2a–f) was similar to that in uninfected animals at all time points tested.

(ii) **Ganglia from infected mice.** Immune cell infiltration was very rapid. On day 1 after UV irradiation, 4/10 ganglia had small foci of infiltrating cells in TG1, in close association with antigen positive cells. Two other ganglia, in which antigen was not detected, showed a similar pattern of infiltration (Fig. 1b). During days 2 to 4, these foci became larger but remained localized. The majority of cells infiltrating on day 1 were T cells, both CD4+ and CD8+ (Fig. 1c, d; Fig. 2e, f) (transformed cell count 2-5, equivalent to 6-25 cells/grid area). There was considerable variation between TG samples in the numbers of such cells/grid area; CD4+ cell numbers varied from 0 to 28 and CD8+ cells from 0 to 48). Within these foci of infiltrates, increased numbers of CD11b+ cells (Fig. 1e) and F4/80+ cells, compared to controls, were also seen. During the following days, there was an increase in the numbers of infiltrating T cells (CD4+ cells: transformed cell count 4-2, equivalent to 20 cells/grid area; CD8+ cells: transformed cell count 5-0, equivalent to 31 cells/grid area). The numbers of CD4+ cells rose significantly (P < 0.05) between days 1 and 4.

On days 1 and 2, there did not appear to be a difference in the number of cells expressing class II compared to controls (Fig. 2c). However, the number of cells with such expression then rose significantly [between day 1 and day 4 (P < 0.05) and between day 2 and day 4 (P < 0.05)]. At the earlier time-points, MHC class II expression was seen mainly on cells with dendritic morphology; later, the majority of MHC class II+ cells appeared to be lymphocytic. The infiltration of B cells was less rapid than that of T cells with only small numbers present on day 1 (transformed cell count 0-65, equivalent to < 1 cell/grid area). However, the numbers of these cells rose significantly between days 1 and 3 (P < 0.05) (Fig. 1f; Fig. 2d) and by day 4 large numbers were present (transformed cell count 7-73, equivalent to 60 cells/grid area).

There was no significant difference in the numbers of CD11b+ cells or F4/80+ cells between mock inoculated controls and experimental ganglia. However, the numbers for both cell types were consistently higher in the experimental tissue (Fig. 2a, b). In controls and experimental tissue, only an occasional Gr-1+ cell (granulocyte) was detected (data not shown).

Most experimental ganglia had an easily recognized infiltrate of immune cells. However, at all times tested, there was a small number of experimental ganglia in which antigen was not detected and the number and distribution of immune cells appeared to be similar to that seen in the control ganglia. There were two on each of days 1 and 4 and one on each of days 2 and 3 with these characteristics. It is likely that virus had
Fig. 1. Immunohistochemical detection of HSV-1 antigens (purple) and immune cells (brown) in the ophthalmic parts of trigeminal ganglia from latently infected mice, 1 day (a–e), and 3 days (f) after UV irradiation of the cornea. Scale bars represent 16 μm (a, d, e), 25 μm (c, f) and 50 μm (b). (a) Neuron at the medial edge of the ganglion expressing virus antigen. There is no evidence of infiltrating cells. (b) Focus of F4/80+ cells (arrow) surrounded by tissue with a normal even distribution of such cells. No evidence of virus antigen was found in this ganglion. (c) Neuron expressing virus antigen in focus of infiltrating cells, some of which are CD4+ T cells. (d) Virus antigen, probably in immune cells, at the centre of a focus of infiltrating cells, some of which are CD8+ T cells. (e) Neuron expressing virus antigen surrounded by CD11b+ cells with dendritic morphology. (f) A focus of infiltrating B cells in an area containing neurons. No evidence of virus antigen was found in this ganglion.
not reactivated in these ganglia. Using the presence of HSV antigen and/or an infiltrate of immune cells as evidence for virus reactivation then the overall incidence of reactivation for all the ganglia was 34/40 (85 %). All animals which shed virus in eyewashings and/or had signs of recurrent disease had TG with an easily recognized infiltrate of immune cells.

Discussion

A variety of patterns of virus antigen expression and immune cell infiltration was seen in the TG following UV irradiation of the cornea of latently infected mice. Although there are dangers in using static observations to understand a dynamic process, the results suggest the following sequence of ganglionic reactivation: stage 1, antigen positive neuron with no detectable immune cell infiltrate; stage 2, antigen positive neuron surrounded by an immune cell infiltrate; stage 3, immune cell infiltrate with some immune cells containing virus antigen; stage 4, immune cell infiltrate with no virus antigen. Reactivation did not appear to be synchronous, since ganglia from the same time-points yielded results representing different examples of the above stages. Moreover, in a few cases different stages were seen within a single ganglion. However, there was a general trend over the 4 days examined with stage 1 seen only on days 1 and 2, and by day 4 all ganglia showing reactivation were at stage 4.

In previous studies using this model, small amounts of infectious virus were isolated from TG as early as day 1 after UV irradiation, with the peak incidence of isolation on day 2. In the mouse hyperthermia model of reactivation, others have reported virus isolation as early as 14 h and antigen positive neurons at 24 h after stimulation (Sawtell & Thompson, 1992), and our results show a similar timing of detection after UV irradiation. Our observations thus provide additional evidence that reactivation can occur very rapidly and that the neuron is the most likely site of this event. Unlike reactivation following hyperthermia we also detected an occasional antigen positive satellite cell. Such cells were never seen in isolation but were closely associated with neurons expressing virus antigen, suggesting that they become infected via spread from reactivated neurons. In our model, the time difference between reactivation and first appearance of virus in the tears (day 2) is most easily explained by the time needed for virus transport in axons and a round of virus replication in peripheral tissue.

UV irradiation of the eye is likely to affect the nerve endings of neurons that supply the cornea, iris and eyelids. Since the cell bodies of these neurons lie within the mediodorsal parts of TG1 and TG2 it was not surprising that the great majority of reactivating neurons were found in these areas. Reactivation, as assessed by the presence of antigen, occurred in only a small proportion (most commonly the number of such cells per ganglion was only one) of those neurons that had the potential to reactivate. This suggests that the conditions required to induce reactivation are precisely
prescribed. Even using a systemic stimulus, hyperthermia, which theoretically should affect all latently infected neurons, a similar low number of reactivating neurons was recorded (Sawtell & Thompson, 1992). The small amounts of infectious virus isolated from ganglia during reactivation in the mouse (Shimeld et al., 1996) and the rabbit (Shimomura et al., 1985) can also be accounted for by such small numbers of antigen positive cells. It is possible that not all reactivation events lead to the presence of detectable antigen but measurement of viral DNA by PCR during reactivation in vivo also suggests that only a small proportion of neurons produce infectious virus (Bloom et al., 1994). Despite the small numbers of neurons reactivating per ganglion, these cells are clearly capable of producing sufficient virus to initiate infection and clinical disease in peripheral tissue.

Clearance of virus antigen from the ganglion was very rapid. Such antigen was identified in immune cells as early as day 1 after UV irradiation and by day 4 all TG were negative. Immune cells positive for antigen were usually within a focus of infiltrate and were sometimes identified as either CD11b+ or F4/80+ cells. The immune response to virus reactivating in ganglia may depend, at least in part, on the number, distribution and type of immune cells already present in the ganglion at that time. Without the cover of immune serum, large numbers of immune cells infiltrate the TG (Shimeld et al., 1995) following corneal inoculation of virus and these cells, particularly T and B cells and cells expressing MHC class II persist, well into the period of latency. Moreover, sustained production of INF-γ at this time has also been reported (Cantin et al., 1995). Whether a similar infiltration and persistence occurs in TG of mice passively immunized before corneal inoculation with virus is not known. However, ganglia taken during the period of latency, from mice infected under cover of immune serum and just prior to UV irradiation, showed very little evidence of immune cell persistence. The number, type and distribution of the majority of immune cells in TG from such mice were similar to those found in ganglia from control animals. However, in ganglia from passively immunized mice there were low numbers of CD4+ cells and B cells scattered evenly throughout the ganglion. Such cells were rare or absent in TG taken from mock inoculated or uninfected animals (Shimeld et al., 1995).

It has been suggested, from studies in non-immunized mice, that the persistence of lymphocytes in the TG, and in particular, those which surround neurons, may be associated with maintenance of latency (Shimeld et al., 1995; Liu et al., 1996) or a response to limited HSV antigen expression during latency (Cantin et al., 1995). The lack of such cells in latently infected ganglia from passively immunized mice poses a problem for these hypotheses. Alternatively, the reduced persistent infiltrate in passively immunized animals may reflect the decreased viral replication during primary infection in these animals (Shimeld et al., 1990) and/or the lower number of latently infected neurons in their ganglia (about half the number of LAT positive neurons in TG of non-immunized mice (E. Grinfeld, unpublished results).

Informative comparisons can be made between the nature and timing of the immune infiltrate which occurred following reactivation in the ganglion and that which has been described following primary infection in this tissue and in recurrent disease in the eye. For example, very few granulocytes were detected in TG during reactivation suggesting they do not play a role in virus clearance. Moreover, these cells made only a transient appearance in the ganglion during primary infection (Shimeld et al., 1995; Liu et al., 1996). In contrast, in the cornea, during both primary infection in the mouse (Tumpey et al., 1996) and recurrent infection in the mouse (Shimeld et al., 1996) and the rat (Nicholls et al., 1996) granulocytes play a significant role in virus clearance. These findings suggest differences between ganglia and peripheral tissue in the regulation of immune cell extravasation in response to HSV via, for example, expression of chemokines and adhesion molecules. The relative inability of granulocytes to infiltrate ganglia may reduce the chances of unwanted non-specific damage to terminally differentiated neurons.

Early during primary infection in the ganglion, there was an increase in the numbers of cells expressing MHC class II and de novo expression on satellite cells in areas that stained for virus antigens (Shimeld et al., 1995); such expression was not observed during reactivation. However, the amount of virus antigen and cell damage in the ganglion during reactivation is far less than during primary infection so it is possible that there is also less release of the mediators, such as INF-γ, necessary to up-regulate MHC class II expression. Therefore, during reactivation, the initial presentation of viral antigen is likely to be mediated by resident MHC class II expressing immune cells. A likely candidate for this function would be F4/80+ dendritic cells, which are the largest group of immune cells normally resident in the ganglion; increased numbers were observed in experimental ganglia compared to controls. Very low numbers of B cells were present from day 1; however, these were not seen in close association with viral antigen and were therefore unlikely to be involved in early presentation of antigen. A rise in the number of cells with MHC class II expression was observed on days 3 and 4 after reactivation when virus antigen was almost gone. This increase was predominantly on lymphocytic cells, probably B cells, since increasing numbers of infiltrating CD45R+ cells were identified at these times. This suggests that local production of antibodies to the virus is also involved in control of the infection.

During primary infection, T cells do not infiltrate the ganglion in large numbers until days 7 to 10 after infection (Shimeld et al., 1995; Liu et al., 1996). In contrast, during reactivation, the T cell response was extremely rapid, possibly within 12 h of appearance of virus antigen, and they were the predominant infiltrating cells on days 1 and 2 when virus antigen was present and being cleared. There were equal numbers of CD8+ and CD4+ cells and they were invariably
tightly clustered. Usually, the clusters were around either neurons or immune cells expressing virus antigen. The presence of T cells (which are probably memory cells and likely to be specific for the virus) together with MHC class II cells and F4/80+ cells (mostly dendritic) would provide the potential for a secondary immune response. It has been suggested that CD11b+ (M1/70+) NK cells may play a role in clearing virus from infected ganglia during primary infection (Liu et al., 1996). Our study suggests that such NK cells were unlikely to play an initial role during reactivation since the CD11b+ cells seen, including those in close association with reactivating neurons, had a dendritic morphology and were therefore more likely to be macrophages than infiltrating NK cells. In addition, at this time CD45R+ cells (which may include cells with NK activity) were present in very low numbers and were not observed in association with antigen positive neurons. However, later during reactivation, a population of CD45R+, MHC class II- cells appear to infiltrate the ganglion; these are likely to be NK cells.

Studies based on cell transfer or depletion experiments suggest that, during primary infection, CD8+ T cells are important in the clearance of virus from the nervous system. It has been argued that neurons are protected from the direct cytotoxicity of these cells by their inability to express MHC class I antigens and by the protective covering of their satellite cells (Shimeld et al., 1995). It has also been suggested that CD8+ T cells may be involved in the maintenance of latency via the secretion of cytokines in response to HSV antigenic peptides in conjunction with MHC class I on satellite cells (Liu et al., 1996). Further experiments to investigate cytokine production during reactivation are in progress and these may elucidate the role of CD8+ T cells in clearance of virus from neurons. The fate of neurons after reactivation is unclear but in the few cases where the detailed nuclear structure of the reactivating neurons was clear enough to observe, it was obvious that the cells would not survive. Hence, evidence from such studies which rely on incidence of virus shedding in the eye, following a stimulus, in judging differences in the reactivation phenotypes of different viral mutants.

In conclusion, these studies show that the immune system responds very rapidly to reactivation of virus in ganglion. In circumstances where this response is compromised, it seems likely that the less effective immune control of reactivation in the ganglion, as well as of infection in the peripheral tissues, will be responsible for the increased severity, and sometimes incidence of recurrent herpetic disease, that may follow immunosuppression. In normal circumstances, development of methods to enhance the already efficient immune response in the ganglion may provide further means of intervention to prevent recurrent disease.

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References


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