Precipitous clearance of herpes simplex virus antigens from the peripheral nervous systems of experimentally infected C57BL/10 mice

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Clearance of herpes simplex virus (HSV) from spinal ganglia of experimentally infected mice is known to be dependent on CD8 T-cells but not on destruction of infected neurons, consistent with a non-cytolytic Tc2 response in the peripheral nervous system. Here, we demonstrate the striking rapidity of such a response in C57BL/10 mice. The number of neurons containing viral DNA and viral antigens increased until 136 h after inoculation of virulent HSV type 1 (strain SC16) into flank skin. Subsequent disappearance of HSV DNA and antigens from infected ganglia was virtually complete only 8 h later. A consistent and unexpected observation was detection of viral antigens in sensory nerve axons for at least 8 h after their disappearance from neuronal somas, raising the intriguing possibility that virus or viral proteins may be transported distally after infection has been terminated.

The pathogenesis of herpes simplex virus (HSV) infection has been studied intensively, particularly in experimentally infected animals (reviewed by Simmons et al., 1992a). During primary infection, virus is translocated intra-axonally from skin to dorsal root or cranial nerve ganglia, where it replicates in sensory neurons, creating the potential for lethal spread of virus to the brain (Whitley, 1996). However, in immunocompetent hosts, ganglionic infection is tightly controlled by timely development of an adaptive immune response. Previously, it was shown that termination of HSV infection in the peripheral nervous systems of experimentally infected mice is mediated by CD8+ T-cells but is not dependent on destruction of infected neurons (Simmons & Tscharke, 1992), implicating a non-cytolytic Tc2 response. Consistent with these findings, abortive productive infection of neurons was suggested as a likely source of the bulk of the viral DNA recovered from latently infected ganglia (Simmons et al., 1992b). During these studies, it was noticed that viral antigens appeared to be eliminated from sensory ganglia with unexpected rapidity, leading to the hypothesis that the termination of neuronal HSV infection can have an abrupt onset and short duration. This hypothesis was addressed by a detailed and quantitative temporal analysis of ganglionic infection in C57BL/10 mice, a mouse strain known to contain infection with HSV tightly (Lopez et al., 1975). It is shown that after inoculation of HSV into flank skin the number of neurons containing viral DNA or viral antigens increased until 136 h and that subsequent disappearance of detectable viral antigens was virtually complete only 8 h later. Concurrent with neuronal infection, viral antigens and DNA were detected in Schwann cells but, in contrast, infection was rarely, if ever, detected in satellite glia with the reagents used in this study. A consistent and unexpected observation was detection of viral antigens in sensory nerve axons after their disappearance from neuronal somas, which has the potential implication that virus or viral proteins are transported distally after infection has been terminated.

The experimental system was characterized initially by quantifying infectious virus in peripheral nervous system tissue at daily intervals. Nine adult female C57BL/10 mice (Specific Pathogen Free facility, Animal Resource Centre, Perth, Western Australia) were infected with $3 \times 10^4$ p.f.u. HSV-1 strain SC16 (Hill et al., 1975) by scarification of the left flank as described previously (Simmons & Nash, 1984; Speck & Simmons, 1991). Thoracic ganglia (T8–T13), together with the proximal parts of spinal nerve trunks, were removed from groups of three animals 5, 6 and 7 days after infection (day 0), homogenized and tested for the presence of infectious virus using a standard plaque assay (Russell, 1962). In this experiment, infection had reached a steady state by day 5, recovery began on day 6 and infectious virus was undetectable...
by day 7 (Fig. 1A). Therefore, subsequent experiments were focussed on the period 120–152 h after inoculation.

A series of ‘snapshots’ was generated from groups of 10 mice, killed at 8 h intervals commencing 120 h after infection. At each time, dorsal root ganglia (T8–T13) and the proximal portion of the spinal nerve trunk ipsilateral to the inoculation site were removed, pooled, fixed in periodate–lysine–paraformaldehyde for 60 min and transferred to 50% ethanol. Pooled tissues were rolled into a ball, paraffin embedded and sections (5 µm) were collected onto glutaraldehyde activated 3-aminopropyltriethoxysilane coated slides. Randomly selected sections were tested for the presence of viral nucleic acids by in situ hybridization, using a probe generated from plasmid pBAZ-1 (Simmons et al., 1992b) and consisting of an 875 bp PstI fragment from the thymidine kinase (TK) region of HSV-1 strain F, cloned into Bluescribe M13” (Stratagene). Probes were labelled to a specific activity of 5 x 10⁶ d.p.m./mg with ¹²⁵I-CTP (NEN) and hybridized overnight under denaturing conditions for DNA targets, i.e. 67 °C in the presence of 50% deionized formamide and 0·2 M Na⁺. Slides were washed 4 x 1 h in 0·1 X SSC (1 X SSC is 0·15 M NaCl and 0·015 M sodium citrate) at room temperature followed by a 20 min stringent wash at 70 °C in 0·5 X SSC containing 30% deionized formamide. All wash solutions contained 100 mM potassium iodide. Slides were exposed to nuclear emulsion (LM-1, Amersham) for 4 days at 4 °C, and developed according to the manufacturer’s instructions. Additional sections were examined immunohistochemically for HSV antigens by sequentially reacting the tissue with rabbit anti-HSV infected cells, swine anti-rabbit immunoglobulins and rabbit peroxidase–anti-peroxidase complex (all reagents from Dakopatts). Binding of antibodies was detected with 3’3’-diaminobenzidine (0·5 mg/ml), containing 0·1% H₂O₂. All reactions were allowed to proceed for 30 min at 37 °C in a humidified atmosphere, with two 5 min washes in 50 mM Tris–HCl pH 7·5 between steps, dehydrated and lightly counterstained with rapid haematoxylin. In all, serial sections covering > 80% of the tissue in each block were analysed.

In ganglia, viral DNA and antigens were detected, almost exclusively, in primary sensory neurons (e.g. Fig. 2A). Infection peaked 136 h after flank inoculation judged by the proportion of randomly selected ganglionic sections containing antigen positive profiles (Fig. 1B) or by the average number of antigen positive neuronal profiles in each ganglion (not shown). Eight hours after the peak of infection very few ganglionic sections contained detectable viral antigen and after a further 8 h infected cells could not be detected. We have considered the possibility that rapid disappearance of antigen positive cells might be an artefact caused by masking of viral antigens by the host’s developing antibody response and suggest that this hypothesis is flawed, because disappearance of viral antigens correlated closely with disappearance of HSV DNA (Fig. 1C). We conclude that infection of neurons was terminated in a precipitous fashion over 8–16 h.

In spinal nerve trunks, viral antigens were detected in long axonal tracts and Schwann cells (Fig. 2C) and Schwann cell nuclei containing HSV DNA were abundant (Fig. 2D). Like infected ganglionic neurons, infected Schwann cells were most numerous 136 h after infection. Viral proteins were distributed throughout axoplasm, rather than being confined to axonal membranes. Diffusion of viral proteins through highly viscous axoplasm cannot reasonably account for long tracts containing viral antigens and we suggest that the intense immunohistochemical staining of axons most likely represents anterograde transport of virions or virion proteins within axonal channels. An unexpected finding, reproduced in a replicate experiment, was staining of axons in tissue removed 152 h after infection, a time when antigen was not detected in neuronal somas (Fig. 2E, F).

C57BL/10 mice were selected for this study on the basis that, in comparison with other mouse strains, they best mimic the restrictive nature of HSV infection in humans (Gominak et al., 1990). A relatively simple, but thorough examination of virus clearance from spinal ganglia of C57BL/10 mice disclosed a strikingly rapid disappearance of antigen positive neurons over a period of 8–16 h and a transient infection of Schwann cells. Destruction of infected neurons, followed by rapid breakdown of debris by phagocytic cells, is one potential

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**Fig. 1.** Temporal analysis of infectious virus (A) and ganglionic profiles containing viral antigens (B) or viral DNA (C), after inoculation of mouse flanks with HSV-1 strain SC16.
Precipitous clearance of HSV from PNS of mice

Fig. 2. Photomicrographs of PLP fixed tissue sections (5 µm), stained for HSV antigens (dark or black areas) or HSV DNA (silver grains). (A) Ganglionic profile in which approximately 10% of the neuronal profiles were HSV antigen positive, typical in tissue removed 136 h post-infection. (B) Longitudinal section of spinal nerve showing viral antigens contained largely within axonal tracts. (C) Section of nerve showing numerous viral antigen positive Schwann cells. (D) Viral DNA in Schwann cell nuclei. (E, F) Immunohistochemical staining for HSV antigens 152 h post-infection, showing inflammatory infiltration in a ganglionic profile but no detectable viral antigen (E), contrasting with the presence of viral antigen in many axons at this time (F). (A, × 100; B–F, × 250; all sections were palely counterstained with haematoxylin.)

explanation for sudden disappearance of viral antigens from ganglia. However, prior data showed that virus clearance is not dependent on neuronal destruction (Simmons & Tscharke, 1992) and in the current work there was little histological evidence to support neuronal death, despite serial analysis of ganglia at 8 h intervals throughout the recovery phase. Consequently, the data presented here suggest that HSV antigens are quickly removed from neuronal somas. Rapid degradation of viral proteins might explain their disappearance but a second more intriguing possibility is intra-axonal transportation of viral proteins towards peripheral sites during the closing stages of neural infection. Strong immunohistochemical staining of axoplasm for viral antigens supports this hypothesis and further studies are required to determine
whether such a process could have biological significance, such as envelopment of virus at peripheral nerve terminals or augmentation of lesion development in the skin.

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References


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