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Restricted replication of herpes simplex virus in satellite glial cell cultures clonally derived from adult mice

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To determine the possible influence of satellite glial cells on restricting the spread of herpes simplex virus in the peripheral nervous system, HSV replication was studied in clonally derived cultures of satellite glial cells from adult animals. Satellite cells were purified by exploiting their close anatomical association with primary sensory neurons. Dissociated neurons from dorsal root ganglia were micro-manipulated to remove all but one of the attached satellite cells and cultured in the presence of the mitogenic stimulators bovine pituitary extract and cholera toxin. Following a lag phase of 20–30 days some of the individual satellite cells began to proliferate. Initial cultures demonstrated bipolar morphology similar to cultured Schwann cells, some of which differentiated into large astrocytic whorl-like cells on subsequent passage. Immunocytochemical and molecular studies demonstrated that these cells, designated Sat.1, express glial fibrillary acidic protein, confirming their glial origin and by electron microscopy they were shown to be phagocytic. Under single step viral growth conditions Sat.1 cells were restrictive for HSV replication, producing in the order of 1000 times less infectious virus than Vero cells, a standard permissive cell line. These results suggest that satellite cells, which tightly encase sensory neurons, play a role in restricting interneural spread of HSV within the peripheral nervous system.

Keywords: viral pathogenesis; neuropathology; ganglia; peripheral nervous system

Introduction

Herpes simplex virus (HSV) is an important human pathogen which infects not only cells at epithelial surfaces but also cells in the peripheral nervous system (PNS). During primary infection, virus spreads by axonal transport, from epithelial cells to the somas of primary sensory neurons. The histopathology of infection in skin and sensory nerve ganglia is strikingly different. In skin, as in cultured cells, HSV spreads directly from cell to cell, forming syncytia. However, syncytia are not observed in the PNS and immunohistochemical studies show that HSV infected neurons are strikingly dispersed within a single ganglion (Speck and Simmons, 1991). This is in contrast to HSV infection of cultured neurons, where all cells are susceptible to productive infection (Wilcox *et al*, 1992; Ziegler and Pozos, 1984). We reasoned that virus may be unable to spread directly between the somas of adjacent neurons because the satellite cells, which tightly ensheath each neuronal cell body, are restrictive for HSV replication. This hypothesis is supported by our observations that HSV infection is rarely if ever detected in satellite cells *in vivo* (Speck and Simmons, 1998) and electron microscopy studies reporting the presence of only unenveloped virus particles in satellite cells *in vivo* (Cook and Stevens, 1973; Dillard *et al*, 1972). Therefore the primary focus of the current research was to establish semi-continuous clonally derived satellite cell cultures from adult mice and to examine their susceptibility to HSV replication.

Satellite cells are one of three glial types found in the PNS, along with enteric glia and peripheral nerve Schwann cells (Dulac *et al*, 1992; Pannese *et al*, 1972). Studies of the phylogeny, structure and function of peripheral glial cells have generally used heterogeneous cell cultures derived from

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embryos or neonates (Brockes *et al*, 1979; Dulac *et al*, 1992; Dupin *et al*, 1990; Eccleston *et al*, 1991; Jessen *et al*, 1990; Lisak and Bealmear, 1991). Such cell cultures are relatively easy to establish but they have the disadvantages of a mixed cellular origin and the possibility that the structure and function of embryonic or neonatal cells may not be representative of adult cells. In establishing clonal satellite cell cultures, the major perceived difficulties were the differentiation of the cell of origin from a Schwann cell and the continuous replication of adult cells *in vitro*. These problems were overcome by exploiting the close anatomical association between satellite cells and neurons and culturing of the purified cells in the presence of mitogenic stimulators. Dorsal root ganglia were enzymatically dissociated and micromanipulated to a stage where only single satellite cells remained attached to neuronal cell surfaces. Single neurons, each with an attached satellite cell, were then cultured in the absence of nerve growth factor and the presence of the mitogenic stimulators cholera toxin and bovine pituitary extract. Here we describe the characterisation of one such clonal satellite cell culture derived from adult mice and examine the susceptibility of these cells to HSV replication. To our knowledge this represents the first time that cloned satellite glia from adult animals have been propagated and characterised in terms of morphology, molecular biology and virus susceptibility.

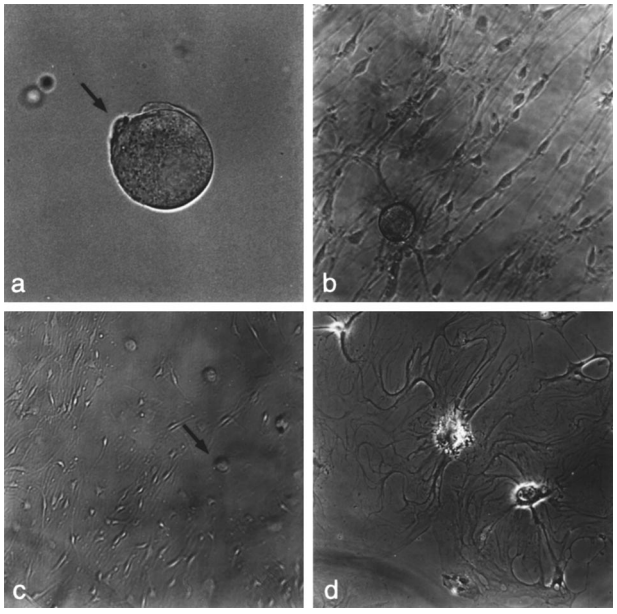

Figure 1 Development of satellite cell cultures. (**a**) A neuron with a single attached satellite cell. The nucleus of the satellite cell (arrow) is clearly visible and the cytoplasm to the right of this is starting to detach from the neuron. ×760. (**b**) After 30 days in culture the satellite cell has proliferated to form a small foci of bipolar cells. Note the tendency to parallel alignment. ×230. (**c**) Passage 4; a second larger whorl-like cell morphology (arrow) develops in the cultures. ×125. (**d**) Passage 6; these cells enlarge and show distinctive astrocytic type morphology. ×230.

Results

Establishment of satellite cell cultures

Routinely, the cellular product from five dorsal root ganglia diluted in 5 ml of culture media in a sample tube, provided a large pool of neurons which were dilute enough to enable individual neurons to be transferred to fresh culture media without contaminating cells. Occasionally the axonal debris concentration compromised this important separation step, in which case the neuron enriched fraction from the bottom of the sample tube was diluted in 1–2 mls of fresh media. Satellite cells became detached from the neurons as they were gently manipulated under microscope control using the fine end of an extruded pasteur pipette. When only one satellite cell remained attached, the neuron was transferred to fresh media to ensure that no additional cells were present and then transferred again into culture wells (Figure 1a). Neuron-satellite cell pairs were readily visualised by light microscopy. Within 3–4 days individual neurons either died and started to degenerate or remained intact and extended 2–3 neurites 50–500 microns out into the surrounding medium. These apparently viable neurons generally remained visible for several weeks of culture. Four separate experiments were done, with a total of 120 neuron-satellite cell pairs being seeded into culture wells. Of these initial cultures, 43 resulted in growth of cells to the stage where they could be passaged into larger culture wells. From these, a total of four semi-continuous cultures were established. One clone, designated Sat.1, was selected arbitrarily for further characterisation. Following seeding of neuron-satellite cell pairs into wells there was a lag phase of at least 18 days before any visible cell foci were observed and most cultures produced no cell growth until day 25–30. Early cultures demonstrated bipolar morphology similar to cultured Schwann cells with the proliferating cells displaying a tendency to align in a parallel orientation (Figure 1b). As these cells were passaged into larger cultures a second cellular morphology became apparent with the appearance of large astrocytic whorl-like cells with a cell body diameter of approximately 150–200 μm (Figure 1c and d). These morphological phenotypes remained constant over multiple passages. As this culture was established with only a single cell capable of division, these two morphologies apparently represent different stages of differentiation of the same cell.

Scanning electron microscopy studies

Phagocytosis is thought to be an important function of glial cells in the central nervous system (CNS) (Lu *et al*, 1991; Matsumoto *et al*, 1992; Montgomery,

1994) and numerous studies have demonstrated that glial cells of the CNS and PNS retain a phagocytic capability when cultured *in vitro* (Argall *et al*, 1992; Bigbee *et al*, 1987; Ford *et al*, 1993; Gold *et al*, 1995; Nikcevich *et al*, 1997). Phagocytosis studies at the light microscope level suggested that the cultured Sat.1 cells were phagocytic. However, the resolution of the light microscope was insufficient to accurately determine whether latex particles were engulfed inside the cytoplasmic membrane or attached to it (data not shown). To resolve this issue, Sat.1 cells were incubated with latex particles for 10, 30, 60 and 120 min and then washed, fixed and processed for scanning electron microscopy. Both cell types were capable of rapid phagocytosis of latex particles with uptake occurring within 10 min (Figure 2). Uptake of the latex particles was often observed more towards the end of long cellular processes with the particles eventually being transferred centripetally, suggesting active movement of the phagocytosed latex particles along the cellular processes.

Demonstration of the glial specific lineage of cultured satellite cells

The glial lineage of the Sat.1 cells was confirmed by immunocytochemistry and PCR. Cells were subjected to immunocytochemistry using an antibody against GFAP, a known glial cell specific marker. In addition, the reactivity of these cells with antibodies directed against S100, a calcium binding protein expressed at high levels in most Schwann cells (Brockes *et al*, 1979; Eccleston *et al*, 1991; Watabe *et al*, 1990) and Thy 1.1, a marker of murine fibroblasts, was also examined. Satellite cells were consistently GFAP positive and S100 and Thy 1.1 negative (Figure 3a). The Vero and 3T3 cell lines were GFAP and S100 negative whilst the 3T3 cells were Thy 1.1 positive. GFAP and S100 specific RT–PCRs were developed using cDNA from brain and dorsal root ganglia as positive controls. The GFAP specific RT–PCR amplified the correct size product (360 bp) from satellite cell RNA (Figure 3b) and the specificity of this band was confirmed by sequencing. No product could be identified from satellite cell cDNA using the S100 specific RT–PCR (Figure 3c) despite the fact that the correct size product (457 bp) was readily amplified from brain and dorsal root ganglia cDNA. These data confirm the glial origin of the Sat.1 cells and distinguish them, by the absence of S100, from Schwann cells.

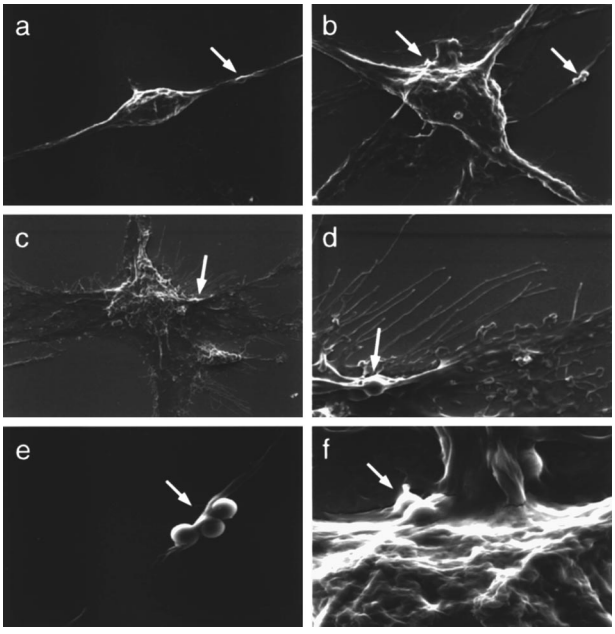

Figure 2 Phagocytic ability of Sat.1 cells. Sat.1 cell cultures were incubated for 1 h with 0.8 micron latex beads, washed, fixed and processed for scanning electron microscopy. (**a**) A typical bipolar cell with a latex particle inside one cellular extension (arrow). (**b**) A larger cell with phagocytised particles inside processes and the cell body (arrows). These are shown at a higher magnification in (**e**) and (**f**). (**c**) A larger cell with large numbers of latex particles accumulated within the cell body. The arrow in (**c**) points to a particle inside a cellular process which is shown at a greater magnification in (**d**).

HSV susceptibility of cultured satellite cells

Sat.1 and Vero cells, a well-characterised HSV permissive cell line, were compared with respect to HSV replication under single step growth curve conditions using 5 p.f.u./cell. The yield from Vero cells was approximately 1000 times the amount of input virus at 48 h post infection (Figure 4). In contrast, over this time satellite cells failed to produce a net increase in virus over input, from which it was concluded that these cells restrict HSV replication.

Discussion

To our knowledge this is the first report of a semi-continuous culture of satellite glial cells derived from sensory nerve ganglia of adult mice. The cells in these cultures were clonally derived by exploiting their close anatomical association with a large easily recognisable cell, the sensory neuron. The survival of the neuron appeared to have some effect on proliferation of the attached satellite cell as most of the initial 43 proliferating cultures were derived from wells containing an apparently viable neuron. Neuronal survival was presumably dependent on the amount of damage caused to each neuron-satellite cell pair during purification. Damage to the neuron itself may lead to rapid death whilst damage to the satellite cell could render it incapable of producing nerve growth factor and other trophic factors necessary for the neuron's survival. To date, the Sat.1 culture described in this report has been maintained for 31 cell passages. Since passage four, two morphological phenotypes have been present in all cultures. As both cell types are phagocytic,

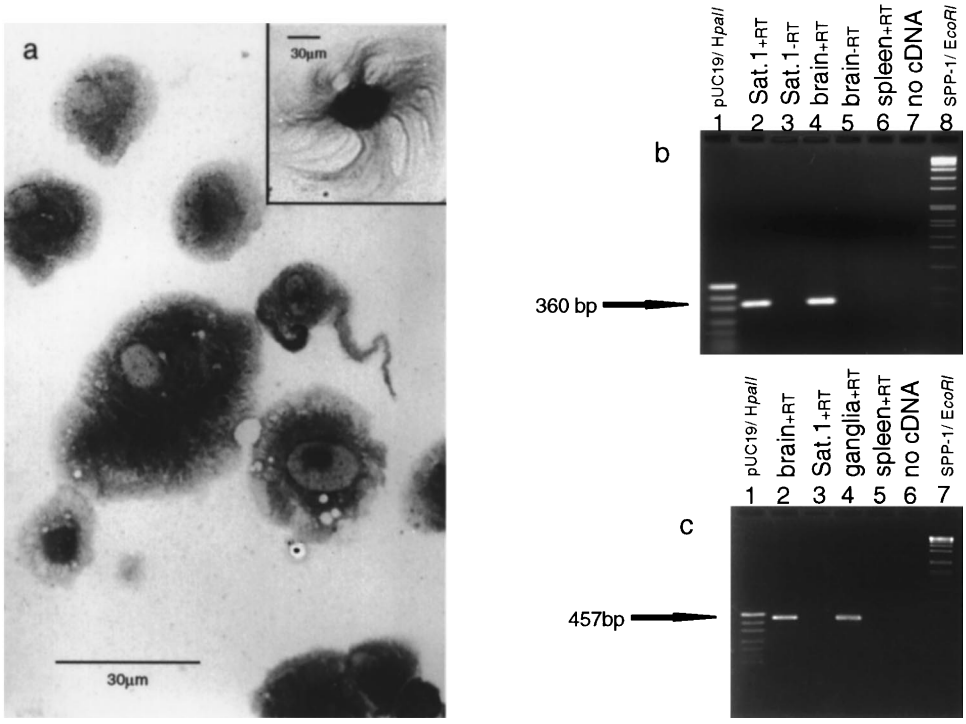

Figure 3 GFAP expression in Sat.1 cells. (**a**) Immunocytochemistry was used to demonstrate GFAP expression in cytospin preparations of Sat.1 cells. Both cell morphologies were stained with equal intensities. The inset shows a cultured Sat.1 cell stained for GFAP with typical fibril distribution. (**b**) RT–PCR detection of GFAP cDNA from Sat.1 cells. RNA was purified from cultured cells, reverse transcribed to cDNA, and following PCR amplification, products were visualised by gel electrophoresis. Controls included no RT enzyme and cDNA from brain and spleen. (**c**) S100 specific RT–PCR showing absence of S100 cDNA in Sat.1 cells.

GFAP positive and S100 and Thy 1.1 negative, they almost certainly represent two stages of differentiation of the same cell. Other reports of clonally derived cell lines have also described differing cellular morphologies which remain stable over time (MacDonald *et al*, 1996).

Sat.1 cells were restrictive for replication of HSV which may have implications for understanding how this virus spreads in the PNS. It has been established that HSV virions are able to enter satellite cells because viral antigens (Henken and Martin, 1992) and viral particles (Cook and Stevens, 1973; Dillard *et al*, 1972) have been reported from *in vivo* studies. However, the viral particles reported in these studies were unenveloped and therefore uninfectious. In the current study it is noteworthy that, following a profound lag phase in Sat.1 cells, during which virus inoculum was reduced to virtually undetectable levels, the cells were capable of limited virus replication, judged by the slope of the graph curve between 8 and 24 h after infection. However total virus yield per cell never exceeded the inoculum in stark contrast to the typical burst size (circa 1000) of Vero cells. This was unexpected as SC16 is a cytopathic virus and is highly infectious for PC12 cells, a neuronal-like cell line, which produce a mean viral titre of 100 p.f.u./cell (unpublished observations). Studies with HSV strains of low pathogenicity such as KOS or thymidine kinase deletion mutants have demonstrated productive infection in cultured neurons with 5–10-fold increases in infectious virus over input virus (Wilcox *et al*, 1992; Smith *et al*, 1992).

HSV infection within the PNS, unlike that of skin, does not result in syncytium formation. HSV enters the PNS following primary infection of the epithelial cells of broken skin or mucosal surfaces. As the virus spreads, it gains access to sensory nerve endings and travels to neuronal cell bodies within sensory ganglia. In the mouse model, infected neurons are strikingly dispersed within each infected ganglion (Blondeau *et al*, 1993; Henken and Martin, 1992; Speck and Simmons, 1991). As syncytium formation does not occur in the PNS, these infected neurons do not form the focus of a spread of infection within sensory ganglia. Further spread to other neurons within ganglia may occur via two separate pathways; (i) reactivation, where virus returns to the skin, replicates and gains access to fresh nerves (Klein, 1976), (ii) via connections within the CNS, as demonstrated in infected trigeminal ganglia of mice (Tullo *et al*, 1982). The restricted susceptibility of satellite cells to HSV replication may be a major factor in preventing syncytial formation within the PNS. Varicella-zoster virus (VZV) is another human herpes virus

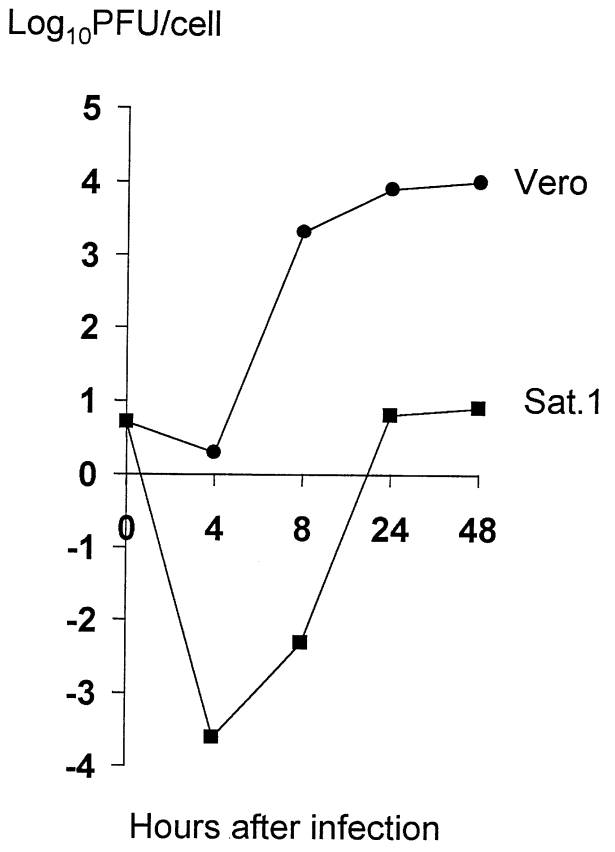

Figure 4 Replication of HSV in Sat.1 cells. A graphical representation of a typical one step viral growth curve performed with Sat.1 and Vero cells. Note that 48 h after infection Vero cells produce about 1000 times more infectious virus than Sat.1 cells.

that also infects the PNS and establishes latency in dorsal root ganglia. However, unlike HSV, reactivation of this virus results in a spread of infection within a ganglion with subsequent cutaneous eruptions generally involving an entire dermatome (Kennedy and Steiner, 1994). Although neurons are well established as the site of HSV latency (McLennon and Darby, 1980; Stevens, 1989) the cell type harbouring latent VZV is more controversial. Neurons and satellite cells have been implicated in different studies (Croen *et al*, 1988; Lungo *et al*, 1995; Mahalingham *et al*, 1996; Kennedy *et al*, 1998). It is tempting to speculate that the limited susceptibility of satellite cells to HSV infection reported in this study provides one biological reason for the observed differences in pathogenesis between HSV and VZV.

In vivo, it has been suggested that glia might play a role in recovery from injury in the nervous system through phagocytosis of debris (al-Ali and al-Hussain, 1996; Bechmann and Nitsch, 1997; Lu *et al*, 1991; Stoll *et al*, 1989). This hypothesis is supported by the intensely phagocytic nature of the Sat.1 cells *in vitro*. Further, we have observed that satellite cell proliferation is a prominent feature of HSV infection in sensory ganglia of experimentally infected mice (unpublished observation). An expanding population of glial cells within the PNS may have important immunological functions because there is substantial evidence that, *in vitro*, Schwann cells are able to take up endogenous and exogenous antigens, and following upregulation of Class I and II MHC molecules, act as antigen presenting cells (Argall *et al*, 1991, 1992; Ford *et al*, 1993; Gold *et al*, 1995; Kingston *et al*, 1989; Tsuyuki *et al*, 1998; Wekerle *et al*, 1986). Thus, it is probable that peripheral glial cells have evolved to fulfil important roles not only in the normal development and maintenance of the PNS but also in response to injury and infection (Koski, 1997; Lu and Richardson, 1991; Martin *et al*, 1988; Torvik and Skjorten, 1971). In relation to HSV, this will provide a focus of future work with Sat.1 cells.

Materials and methods

Establishment of satellite cell cultures

Dorsal root ganglia were removed from C57BL/10 mice which had been killed by CO_2 asphyxiation. A dissecting microscope was used to remove as much nerve and connective tissue as possible from five ganglia which were then partially dissociated for 30 min at 37°C in 0.5 ml HEPES Dulbecco's Modified Eagle Medium (HDMEM) containing 1% collagenase/dispase (Boehringer Mannheim, NSW, Australia). The sample was further dissociated by trituration 20 times through a sterilised pasteur pipette and diluted to 5 mls in HDMEM containing 10% foetal calf serum (FCS) and antibiotics (penicillin-100 i.u. ml^{-1} and streptomycin-1 μg ml^{-1}). Individual neurons with attached satellite cells were transferred under microscope control to a drop of fresh media, and gently manipulated until only one satellite cell remained attached to the neuron. Neurons were then seeded into 96 well tissue culture plates (Nunc, Roskilde, Denmark). Culture media consisted of HDMEM supplemented with 10% FCS, adenine (24.3 ng ml^{-1}), hydrocortisone (0.4 μg ml^{-1}), epidermal growth factor (10 ng ml^{-1}) insulin (5 μg ml^{-1} transferrin (10 μg ml^{-1}), bovine pituitary extract (33 μg ml^{-1}), cholera toxin (20 ng ml^{-1}), penicillin (100 i.u. ml^{-1}) and streptomycin (10 μg ml^{-1}). Cultures were maintained at 37°C in an atmosphere of 5% CO_2/95% air and 75% of the culture media in each well (150 μl) was carefully aspirated and replaced with fresh media at 24 h and then every 3 days. Cultures were monitored regularly and when cell foci covered approximately 50% of the well the cells were transferred to 24 well plates (Nunc). As confluency approached 90%, cultures were successively expanded into larger culture vessels until cells could be stably propagated in 150 cm^2 cell culture flasks (Corning, NY, USA).

Immunocytochemistry
Cell monolayers were trypsinized, washed once in HDMEM containing 1% FCS, adjusted to 5×10^4 cells ml^{-1} and 0.5 ml aliquots used to prepare cytospin cell preparations on a Shandon cytofuge. Vero (Green monkey kidney) and 3T3 (Murine fibroblasts) cell lines were included as controls in all staining experiments. Slides were air dried and fixed in acid alcohol (5% acetic acid in ethanol) for 10 min at -20°C. Staining for S100 antigen included a fixation step in 4% formaldehyde for 20 min at room temperature. When staining cultured cells were grown on coverslips, they were washed twice in phosphate buffered saline (PBS) and fixed as above. Cells were blocked in 10% normal goat serum for 30 min prior to staining with the following primary antibodies; anti-GFAP (1 : 500, DAKO), anti-S100 (1 : 400, DAKO) and anti-Thy 1.1 (1 : 20, Serotec). Controls included omission of primary antibody, and the replacement of primary antibody with normal rabbit and mouse serum at 1 : 100 dilution. Following overnight incubation at 4°C, cells were washed twice in PBS for 15 min and antibody reactivity revealed using the Vector Universal ABC kit (Vector Laboratories, Burlingame, CA, USA) and DAB substrate before being lightly counterstained with haematoxylin.

Scanning electron microscopy
Satellite cells were seeded onto collagen coated glass coverslips in 24 well plates and cultured for 3–5 days. Latex particles (0.8 micron, Bangs Laboratories, Fishers, IN, USA) were added to cultures at various time points before fixation. The coverslips were washed three times in PBS before overnight fixation in PBS containing 4% paraformaldehyde, 1.25% glutaraldehyde and 4% sucrose, pH 7.2. Following fixation, the cells were washed in two changes of PBS/4% sucrose for 30 min each prior to dehydration through graded ethanols. Coverslips were then stub mounted and critical point dried before being sputter coated with gold and examined with a field emission scanning electron microscope (Phillips XL 30) at an acceleration voltage of 2 or 5 kV.

Reverse transcription polymerase chain reaction
RNA was extracted from 10^7 satellite cells and a range of control tissues including brain, dorsal root ganglia, and spleen using the one step method of Chomczynski and Sacchi (1987). Contaminating DNA was degraded with DNase (Boehringer Mannheim, NSW, Australia) and the RNA recovered using phenol/chloroform extraction following standard protocols (Maniatis *et al*, 1982). Reverse transcription (RT) to cDNA was performed according to the enzyme manufacturer (Gibco BRL Gaithersburg, MD, USA) instructions. GFAP and S100 specific oligonucleotide primers were designed from sequence data obtained from Genbank (accession nos X02801 and L22144 respectively) with both sets of primers spanning two introns to ensure the detection of cDNA only. The following primers and polymerase chain reaction (PCR) conditions were used for each gene: GFAP, (5′-GGCCACCAGTAACATGGCAAG-3′ and 5′-GTGGCGATCTCGATGTCCAG-3′) 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, extension at 70°C for 1 min and a final 7 min extension at 70°C; S100, (5′-ACTCGGACACTGAAGCCAGA-3′ and 5′-TGTCTAGAGAGCTCAGCTCC-3′) 35 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 1 min and a final 7 min extension at 72°C. The products of amplification resulted in a 360 bp and 457 bp fragment for GFAP and S100 respectively. The oligonucleotide primers were prepared by the Molecular Pathology Section of the Institute of Medical and Veterinary Science (Adelaide, Australia) and purified according to the method of Sawadogo and Van Dyke (1991). The amplification products and standards (pUC19 DNA/*Hpa*II and SPP-I Phage DNA/*Eco*RI, Bresatec, Adelaide, Australia) were analysed by electrophoresis in a 1.5% agarose gel and visualised with ethidium bromide staining. Dye terminator sequencing was performed using an automated sequencer (PE Applied Biosystems, Foster City, CA, USA).

Susceptibility of cultured cells to HSV infection
Satellite cells and control Vero cells were seeded at 10^4 cells per well in Nunc tissue culture plates in culture medium containing 1% FCS. Following adherence of the cell monolayer, 5 plaque forming units (p.f.u.)/cell of a virulent cytopathic HSV type 1 strain (SC16) were added and incubated with gentle rocking for 1 h at 37°C. The culture medium was then changed twice to remove non-adherent virus and the infective virus was quantified from total well lysates at time 0, 4, 24 and 48 h post infection using a standard plaque assay (Russell, 1962).

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References

al-Ali SY, al-Hussain SM (1996). An ultrastructural study of the phagocytic activity of astrocytes in adult rat brain. *J Anat* **188:** 257–262.

Argall KG, Armati PJ, King NJC, Douglas MW (1991). The effect of West Nile virus on the major histocompatability complex class I and II molecule expression by Lewis rat Schwann cells in vitro. *J Neuroimmunol* **35:** 273–284.

Argall KG, Armati PJ, Pollard JD, Watson E, Bonner J (1992). Interactions between CD4+ T cells and rat Schwann cells in vitro: 1 antigen presentation by Lewis rat Schwann cells to P_2-specific CD4+ T cell lines. *J Neuroimmunol* **40:** 1–18.

Bechmann I, Nitsch R (1997). Astrocytes and microglial cells incorporate degenerating fibres following entorhinal lesion: a light, confocal and electron microscopical study using a phagocytosis-dependent labeling technique. *Glia* **20:** 145–154.

Bigbee JW, Yoshino JE, Deries GH (1987). Morphological and proliferative responses of cultured Schwann cells following rapid phagocytosis of a myelin-enriched fraction. *J Neurocytol* **16:** 15–24.

Blondeau JM, Aoki FY, Nagy JI (1993). Immunohistological characterisation of acute herpes simplex virus infection of lumbar dorsal root ganglia in the rat. *J Med Microbiol* **38:** 265–269.

Brockes JP, Fields KL, Raff MC (1979). Studies on cultured rat Schwann cells. 1 Establishment of purified populations from cultures of peripheral nerve. *Brain Res* **165:** 105–118.

Chomczynski P, Sacchi N (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162:** 156–159.

Cook ML, Stevens JG (1973). Pathogenesis of herpetic neuritis and ganglionitis in mice: Evidence for intra-axonal transport of infection. *Infect Immun* **7:** 272–288.

Croen KD, Ostrove JM, Dragovic LJ, Straus E (1988). Patterns of gene expression and sites of latency in human nerve ganglia are different for varicella-zoster and herpes simplex viruses. *Proc Natl Acad Sci* **85:** 9773–9777.

Dillard SH, Cheatham WJ, Moses HL (1972). Electron microscopy of zosteriform herpes simplex infection in the mouse. *Lab Invest* **26:** 391–402.

Dulac C, Tropak MB, Cameron-Curry P, Rossier J, Marshak DR, Roder J, Le Douarin NM (1992). Molecular characterisation of the Schwann cell myelin protein, SMP: structural similarities within the immunoglobulin superfamily. *Neuron* **8:** 232–234.

Dupin E, Baroffio A, Dulac C, Cameron-Curry P, LeDouarin NM (1990). Schwann cell differentiation in clonal cultures of the neural crest, as evidenced by the anti-Schwann cell myelin protein monoclonal antibody. *Proc Natl Acad Sci* **87:** 1119–1123.

Eccleston PA, Mirsky R, Jessen KR (1991). Spontaneous immortalisation of Schwann cells in culture: short-term cultures of Schwann cells secrete growth inhibitory activity. *Development* **112:** 33–42.

Ford AL, Britton WN, Armati PJ (1993). Schwann cells are able to present exogenous mycobacterial hsp70 to antigen specific T lymphocytes. *J Neuroimmunol* **42:** 151–160.

Gold R, Toyka KV, Hartung HP (1995). Synergistic effect of INF-γ and TNFγ on expression of immune molecules and antigen presentation by Schwann cells. *Cell Immunol* **165:** 65–70.

Henken DB, Martin JR (1992). Herpes simplex virus infection induces a selectable increase in the proportion of galanin-positive neurons in mouse sensory ganglia. *Exp Neurobiol* **118:** 195–203.

Jessen KR, Morgan L, Stewart HJS, Mirsky R (1990). Three markers of adult non-myelin forming Schwann cells, 217c(Ran-1), A5E3 and GFAP: development and regulation by neuron-Schwann cell interactions. *Development* **109:** 91–109.

Kennedy PG, Steiner I (1994). A molecular and cellular model to explain the differences in reactivation from latency by herpes simplex and varicella-zoster viruses. *Neuropathol Appl Neurobiol* **20:** 368–374.

Kennedy PGE, Grinfeld E, Gow JW (1998). Latent varicella-zoster virus is located predominantly in neurons in human trigeminal ganglia. *Proc Natl Acad Sci* **95:** 4658–4662.

Kingston AE, Bergsteindottir K, Jessen KR, Van der Meide PH, Colston MJ, Mirsky R (1989). Schwann cells co-cultured with stimulated T cells and antigen express major histocompatability (MHC) class II determinants without interferon-gamma pretreatment: synergistic effects of interferon-gamma and tumour necrosis factor on MHC class II induction. *Eur J Immunol* **19:** 177–183.

Klein RJ (1976). Pathogenic mechanisms of recurrent herpes simplex virus infections. *Arch Virol* **51:** 1–13.

Koski CL (1997). Mechanisms of Schwann cell damage in inflammatory neuropathy. *J Infect Dis* **176** (suppl 2): 169–172.

Lisak RP, Bealmear B (1991). Antibodies to interleukin-1 inhibit cytokine-induced proliferation of neonatal rat Schwann cells in vitro. *J Neuroimmunol* **31:** 123–132.

Lu X, Richardson PM (1991). Inflammation near the nerve cell body enhances axonal regeneration. *J Neurosci* **11:** 972–978.

Lu B, Yokoyama M, Dreyfus CF, Black IB (1991). NGF gene expression in actively growing brain glia. *J Neurosci* **11:** 318–326.

Lungo O, Annunziato P, Gershon A, Staugaitias SM, Josefson D, LaRussa P, Silverstein SJ (1995). Reactivation and latent varicella-zoster virus in human dorsal root ganglia. *Proc Natl Acad Sci* **92:** 10980–10984.

MacDonald KPA, Mackay-Sim A, Bushell GR, Bartlett PF (1996). Olfactory neuronal cell lines generated by retroviral insertion of the *n-myc* oncogene display different developmental phenotypes. *J Neurosci Res* **45:** 237–247.

McLennan JL, Darby G (1980). Herpes simplex virus latency: the cellular location of virus in dorsal root ganglia and the fate of the infected cell following virus activation. *J Gen Virol* **51:** 233–243.

Mahalingam R, Wellish M, Cohrs R, Piette J, Rentier B, Gilden DH (1996). Expression of protein encoded by varicella-zoster virus open reading frame 63 in latently infected human ganglionic neurons. *Proc Natl Acad Sci* **93:** 2122–2124.

Maniatis T, Fritsch EF, Sambrook J (1982). Molecular Cloning: A Laboratory Manual. Cold Springs Harbour: CPH Laboratory Press.

Martin DP, Schmidt RE, Distefano PS, Lowry OH, Carter JG, Johnson EM (1988). Inhibitors of protein synthesis and RNA synthesis prevent neuronal death caused by nerve growth factor deprivation. *J Cell Biol* **106:** 829–844.

Matsumoto Y, Ohmori K, Fujiwara M (1992). Microglia and astroglial reactions to inflammatory lesions of experimental autoimmune encephalomyelitis in the rat central nervous system. *J Neuroimmunol* **37:** 23–33.

Montgomery DL (1994). Astrocytes: form, function and role in disease. *Vet Pathol* **31:** 145–167.

Nikcevich KM, Hurst SD, Barrett TA, Gordon KB, Miller SD, Tan L, Kroepfl JF, Gardinier M (1997). Interferon-γ activated promary murine astrocytes express B7 costimulatory molecules and prime naive antigen-specific T cells. *J Immunol* **158:** 614–621.

Pannese E, Bianchi R, Calligaris B, Ventura R, Weibel ER (1972). Quantitative relationships between nerves and satellite cells in spinal ganglia. An electron microscope study. I. Mammals. *Brain Res* **46:** 215–234.

Russel WC (1962). A sensitive and precise plaque assay for herpes virus. *Nature* **195:** 1028–1029.

Sawadogo M, Van Dyke MW (1991). A rapid method for the purification of deprotected oligodeoxynucleotides. *Nuc Acid Res* **19:** 74.

Smith RL, Pizer LI, Johnson EM, Wilcox CL (1992). Activation of second-messenger pathways reactivates latent herpes simplex virus in neuronal cultures. *Virology* **188:** 311–318.

Speck PG, Simmons A (1991). Divergent molecular pathways of productive and latent infection with a virulent strain of herpes simplex virus type 1. *J Virol* **65:** 4001–4005.

Speck P, Simmons A (1998). Precipitous clearance of herpes simplex virus antigens from the peripheral nervous systems of experimentally infected C57BL/10 mice. *J Gen Virol* **79:** 561–564.

Stevens JG (1989). Human herpesviruses: a consideration of the latent state. *Microbiol Rev* **53:** 318–332.

Stoll G, Griffin JW, Li CY, Trapp BD (1989). Wallerian degeneration in the peripheral nervous system: participation of both Schwann cells and macrophages in myellin degradation. *J Neurocytol* **18:** 671–683.

Torvik A, Skjorten F (1971). Electron microscopic observations on nerve cell regeneration and degeneration after axon lesions. II. Changes in the glial cells. *Acta Neuropathol* **17:** 265–281.

Tsuyuki Y, Fujimaki H, Hikawa N, Nagata T, Minami M (1998). IFN-γ induces coordinate expression of MHC class I mediated antigen presentation machinery in adult mouse schwann cells. *Neuro report* **9:** 2071–2075.

Tullo AB, Shimeld C, Blyth WA, Hill TJ, Easty DL (1982). Spread of virus and distribution of latent infection following ocular herpes simplex in the non-immune and immune mouse. *J Gen Virol* **63:** 95–101.

Watabe K, Yamada M, Kawamura T, Kim SU (1990). Transfection and stable transformation of adult mouse Schwann cells with SV-40 large T antigen gene. *J Neuropath Exp Neuro* **49:** 455–467.

Wekerle H, Schab M, Linnington T, Meyermann R (1986). Antigen presentation in the peripheral nervous system: Schwann cells present endogenous myelin autoantigens to lymphocytes. *J Immunol* **16:** 1551–1557.

Wilcox CL, Crnic LS, Pizer LI (1992). Replication, latent infection and reactivation in neuronal culture with a herpes simplex virus thymidine kinase-negative mutant. *Virology* **187:** 348–352.

Ziegler RJ, Pozos RS (1984). Inability of various immune mechanisms to establish in vitro latent infection in sensory neurons infected by HSV-1. *Arch Virol* **79:** 123–130.