# Experimental Models to Study Varicella-Zoster Virus Infection of Neurons

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**Abstract** Varicella zoster virus (VZV) infection results in the establishment of latency in human sensory neurons. Reactivation of VZV leads to herpes zoster which can be followed by persistent neuropathic pain, termed post-herpetic neuralgia (PHN). Humans are the only natural host for VZV, and the strict species specificity of the virus has restricted the development of an animal model of infection which mimics all phases of disease. In order to elucidate the mechanisms which control the establishment of latency and reactivation as well as the effect of VZV replication on neuronal function, *in vitro* models of neuronal infection have been developed. Currently these models involve culturing and infecting dissociated

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A.M. Arvin et al. (eds.), *Varicella-zoster Virus*,
Current Topics in Microbiology and Immunology 342, DOI 10.1007/82_2010_15

human fetal neurons, with or without their supporting cells, an intact explant fetal dorsal root ganglia (DRG) model, neuroblastoma cell lines and rodent neuronal cell models. Each of these models has distinct advantages as well as disadvantages, and all have contributed towards our understanding of VZV neuronal infection. However, as yet none have been able to recapitulate the full virus lifecycle from primary infection to latency through to reactivation. The development of such a model will be a crucial step towards advancing our understanding of the mechanisms involved in VZV replication in neuronal cells, and the design of new therapies to combat VZV-related disease.

## 1 Introduction

During primary varicella-zoster virus (VZV) infection (varicella), the virus establishes a latent infection in sensory ganglia, mainly dorsal root ganglia (DRG) and trigeminal ganglia (TG), from where it can reactivate years later, resulting in re-initiation of productive replication with a consequence of the development of herpes zoster (shingles). Neurons within sensory ganglia are the primary site of latent infection, although there have also been sporadic reports of latency in ganglionic satellite cells (Hyman et al. 1983; Gilden et al. 1987; Croen et al. 1988; Schmidbauer et al. 1992; Lungu et al. 1995, 1998; Kennedy et al. 1998; LaGuardia et al. 1999; Levin et al. 2003). Sensory ganglia are part of the peripheral nervous system and sensory neurons can respond to mechanical, thermal, or chemical stimuli (Lawson 2005). Despite the critical importance of sensory ganglia in the lifecycle of VZV infection, not much is understood about the interactions between VZV, neurons, and their supporting cells. Specifically, factors controlling the establishment, maintenance, and reactivation from latency, as well as the impact of VZV replication on normal neuronal function remain poorly defined. This is largely due to the high species specificity of VZV, meaning the virus does not progress to a full cycle of lytic infection in most non-human cells (Weller and Stoddard 1952). This chapter will overview current experimental models that have been developed to study the interaction of VZV with ganglionic cells, with a particular focus on cell culture-based models of VZV infection as animal models of infection are detailed elsewhere in this volume.

## 2 Experimental VZV Infection of Primary Human Neurons from Dissociated Neural Tissue

The first *in vitro* study to examine VZV replication in human cells derived from neural tissue used cultures of human brain and ganglia cells. These cultures contained a mixed population of uncharacterised cells of mesenchymal and

neuroglial origin, although due to multiple passaging neurons were not present (Gilden et al. 1978). VZV was successfully able to infect these cells and the infection occurred in a similar fashion to human lung fibroblasts, with the exception that cells of neural origin exhibited the formation of large intracytoplasmic vacuoles (Gilden et al. 1978).

Because of the inherent difficulty in obtaining fresh adult human sensory ganglia to derive primary neurons, several studies have utilised fetal sensory ganglia-derived neurons to study VZV neuropathogenesis. Although the capacity to obtain fresh fetal sensory ganglia remains a limiting factor, this approach is advantageous as humans are the only natural host for VZV and the virus generally does not replicate efficiently in cells of non-human origin (Weller and Stoddard 1952). Aborted fetal samples between 8 and 20 weeks of gestation are generally obtained and DRG harvested. Dissociation of the neurons within DRG is achieved usually after physically removing some of the surrounding epineurium before treatment with trypsin or collagenase to disrupt cell–cell junctions. To further dissociate the cells, they have been triturated through fire-polished pipettes or a fine gauge needle to obtain a uniform single cell suspension. Mitotic inhibitors have then often been employed to eliminate dividing non-neuronal cells such as fibroblasts, resulting in a relatively pure population of neurons, although some satellite cells often remain. After plating onto an extracellular matrix such as collagen, these cells can then be cultured in the presence of nerve growth factor (NGF) to ensure their survival (Wigdahl et al. 1986; Assouline et al. 1990; Somekh et al. 1992; Somekh and Levin 1993; Hood et al. 2003, 2006).

The first studies to utilise human fetal neurons were performed by Wigdahl et al. (1986) and Assouline et al. (1990). Using cell-associated and cell-free methods of infections, respectively, both studies showed that fetal neurons could be infected *in vitro* as demonstrated by the detection of viral proteins using immunofluorescence and electron microscopy to show the presence of viral particles within infected neurons (Wigdahl et al. 1986; Assouline et al. 1990). A cytopathic effect characterised by cellular degeneration and detachment of the cells was also observed within these infected neuronal cultures, consistent with productive replication. However, both studies also noted that infection of neurons progressed more slowly than infection of fibroblasts, a cell type highly permissive to productive VZV infection. Assouline et al. (1990) went on to characterise the temporal cascade of VZV protein expression and showed that the early protein encoded by open reading frame (ORF) 36, deoxypyrimidine kinase, accumulated to detectable levels prior to the immediate early gene IE62 and remained predominant in the cytoplasm, which was in contrast to the infection of fibroblasts where ORF36 was detected predominantly in the nucleus (Assouline et al. 1990). These studies showed that there are likely to be important differences between the nature of VZV replication in neurons compared to fibroblasts and the authors suggested that neurons may be able to control some aspects of viral replication to limit virus-induced damage (Wigdahl et al. 1986). Somekh and Levin (1993) also utilised dissociated fetal neurons to demonstrate infection with the attenuated vaccine strain vOKA; however, infection with the wild-type strain resulted in a much greater percentage

of infected neurons, implying that the vaccine strain has attenuated neurotropism (Somekh and Levin 1993).

## 2.1 Modulation of Neuronal Apoptosis by VZV

Our group used primary human fetal DRG-derived neurons to examine the interaction of VZV with this cell type (Hood et al. 2003) (Fig. 1). It was shown that during productive infection with VZV, apoptosis in fibroblasts increased proportionally to the number of infected cells. In contrast, VZV infected neurons appeared to be protected from apoptosis, highlighting a functional difference between VZV replication in neurons and fibroblasts (Hood et al. 2003). In a follow-up study we demonstrated a role for the VZV protein IE63 in the protection of neurons from apoptosis during productive infection (Hood et al. 2006). IE63 is encoded by ORF63 and is duplicated in the VZV genome, with this additional copy known as ORF70. In comparison to wild-type parental virus infection, infection of primary human neurons with either an ORF63 or an ORF70 deletion virus resulted in an increase in the percentage of neurons undergoing apoptosis (Hood et al. 2006). This protection from apoptosis by IE63 was further supported by experiments showing that the induction of apoptosis by withdrawal of NGF from cultured rat neurons was suppressed when these cells were first transfected with a plasmid encoding IE63 (Hood et al. 2006). At present the mechanism by which IE63 inhibits neuronal apoptosis has not been elucidated. In addition to IE63, other VZV genes may also play a function to prevent apoptosis. For example, the ORF66 protein product, which is expressed during productive infection as well as during latent infection of neurons (Cohrs et al. 2003), has been shown to play a role in preventing apoptosis in VZV-infected T cells (Schaap et al. 2005), although additional studies will be required to determine whether this gene product functions in an anti-apoptotic manner in neurons.

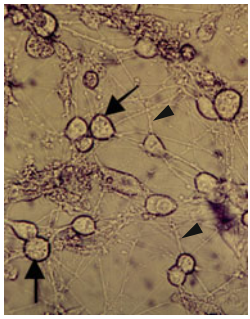

**Fig. 1** Light microscopy of dissociated human fetal dorsal root ganglia (DRG) neuronal cell culture. After culture of dissociated ganglionic cells in media containing nerve growth factor for 3 days, neurons (*arrows*) develop extensive axonal networks (*arrowheads*). Copyright © American Society for Microbiology, Hood et al. (2003), DOI: 10.1128/JVI.77.23.12852-12864.2003

Protecting neurons from apoptosis during the critical first stages of virus reactivation would likely allow for greater production of new virions for axonal transport to the skin and herpes zoster lesion formation (Hood et al. 2003). It may also be of benefit to the virus to actively resist the induction of apoptosis during the latent phase of infection. It can also be argued that resistance of neuronal apoptosis may also benefit the host, given that neurons are post-mitotic and therefore are not replaced following cell death. Interestingly, however, observations in human ganglia obtained post-mortem from patients suffering from herpes zoster and post-herpetic neuralgia (PHN) have revealed that these ganglia display regions of altered morphology, where damage to neuronal tissue and cell loss has occurred as a result of VZV reactivation (Head and Campbell 1900; Denny-Brown et al. 1944; Smith 1978; Watson et al. 1988, 1991; Steain et al. 2009). Thus, mechanisms responsible for this damage *in vivo* may be a consequence of the inflammatory process rather than VZV-induced apoptosis (Hood et al. 2003). Understanding the mechanisms that underlie VZV-induced cellular damage and PHN will ultimately require extension of *in vitro*-based findings to additional analysis of naturally infected human ganglia. In this respect, our group has gained access to adult human ganglia removed post-mortem from people suffering from herpes zoster at or near the time of death. Experiments to define the nature of the ganglionic cellular infiltrate and neuronal damage that accompanies VZV reactivation are currently underway, and it is hoped that these analyses will provide a better understanding of the factors that influence reactivation and the development of PHN.

## 3 Models of Latent Infection of Human Neurons

Another important aspect of the life cycle of VZV is the establishment of latency. In an attempt to create an *in vitro* model of latency, Somekh et al. (1992) treated neuronal cultures as well as satellite cell and mixed (neurons and satellite cells) cultures with the anti-herpes arabinosyl nucleoside analogue bromovinyl arabinosyl uracil (BVaraU) before infecting with cell-free VZV. They found that BVaraU was able to prevent a full productive infection in these cultures, and that no viral replication occurred even after BVaraU was removed 7 days post-infection. However, infectious VZV was able to be recovered from some of the mixed (neuronal and satellite cell) cultures a week after infection and BVaraU withdrawal, when the cells were trypsinized and co-cultured with human embryonic lung fibroblasts (HELFs) (Somekh et al. 1992). It was concluded from these experiments that satellite cells may play an important role in the establishment and/or maintenance of latency. However, important differences exist between this model and latency in vivo due to the requirement of BVaraU to establish latency *in vitro* and the absence of IE62, which has been detected in neurons during latency *in vivo* (Lungu et al. 1998). Since this initial report, there has been no new published work utilising cell-culture based models of latent VZV infection using cultures of dissociated human fetal DRG. Rather, the published studies of VZV latency in human neurons have

relied predominantly on examination of naturally infected adult ganglia obtained post mortem (Gilden et al. 1983, 2001; Croen et al. 1988; Vafai et al. 1988; Mahalingam et al. 1992, 1993, 1996; Schmidbauer et al. 1992; Lungu et al. 1998; LaGuardia et al. 1999; Theil et al. 2003; Cohrs et al. 2003, 2005; Hufner et al. 2006; Cohrs and Gilden 2007; Verjans et al. 2007).

## 4 Experimental VZV Infection of Intact Ganglia

Dissociation of ganglia results in separation of neurons from satellite cells, which may affect their function and slow VZV spread *in vitro*, given that *in vivo* neurons are tightly enclosed by multiple satellite cells (Hanani 2005). Hanani et al. noted that "Isolated intact sensory ganglia should be the first choice for studying SGC (satellite glial cell)–neuron interactions as there is minimal tissue disruption" (Hanani 2005). Thus, to study fetal neurons in the context of a more intact anatomical architecture, two different approaches have been developed. The Arvin group reported the development of an *in vivo* model whereby severe combined immunodeficiency (SCID) mice were used following implantation of intact human fetal DRG under the kidney capsule. This model has provided a very useful means to study different routes of infection of DRG, and has been used to provide evidence for a role of VZV-infected T cells in the transfer of virus to the DRG (Zerboni et al. 2005). VZV also establishes a persistent infection within these DRG, which can last for up to 8 weeks post-infection in the absence of detectable infectious virus production (Zerboni et al. 2005). Additional studies using this model are detailed in (Zerboni et al. 2010). Disadvantages of the SCID-hu mouse model, however, are the extended period of time required to allow for the establishment of the DRG xenograph and costs associated with housing animals.

Our laboratory has established an intact explant fetal DRG culture model (Gowrishankar et al. 2007). By extracting fetal DRG from aborted fetuses between the ages of 14–20 weeks gestation and removing the surrounding epineurium, DRG can be cultured directly on glass coverslips in the presence of NGF. After 2–3 days post-explant extensive axonal growth can be seen protruding from the entire DRG (Gowrishankar et al. 2007). Characterization of the ganglia post-explanting has shown that the architecture of the ganglia is preserved, with neurons being surrounded by supporting satellite cells. Immunohistochemical staining has also demonstrated the presence of ganglionic cell markers, with neurons staining positive for the neural cell adhesion molecule (NCAM) and satellite cells positive for S100B (Gowrishankar et al. 2007), similar to normal human adult ganglia. This model allows VZV infection of neurons and satellite cells to be studied in the context of an intact ganglion *in vitro*. Following 48 h of cell-associated infection with VZV strain Schenke, discrete VZV glycoprotein-positive neurons were detected throughout cultured ganglia, suggesting productive infection and axonal transport of the virus from the periphery (Fig. 2). The number of infected neurons

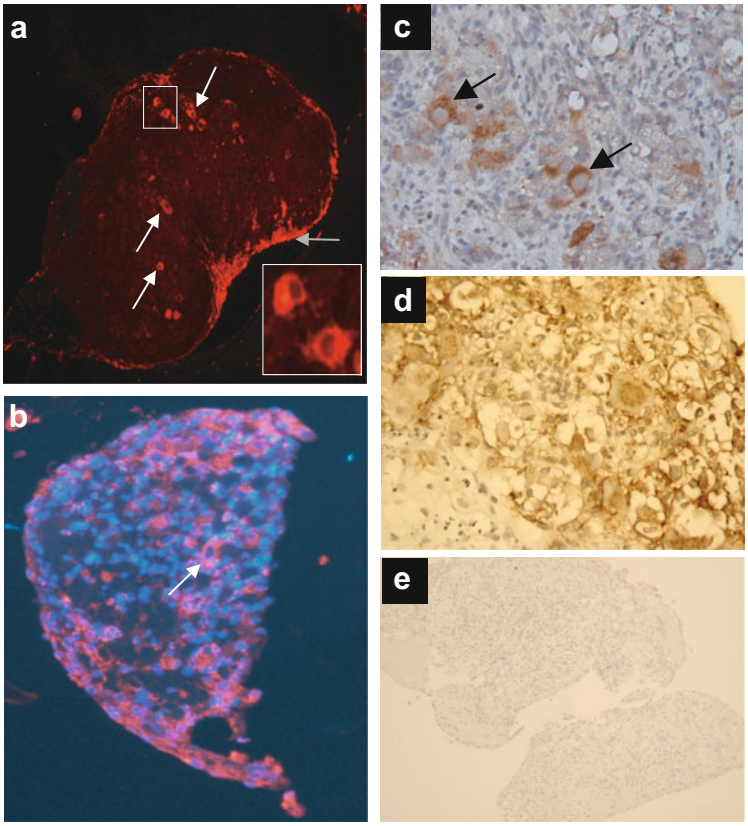


**Fig. 2** VZV antigen expression in infected intact explant human dorsal root ganglia (DRG). (**a**) Immunofluorescent staining of a DRG 48 h post infection with human VZV hyper immune serum and secondary antibody consisting of fluorescently conjugated anti-human AlexaFluor 594 (*red staining*). VZV antigen expression on the surfaces of distinct, scattered neurons (*white arrows*) and around the DRG body (*green arrows*). *Boxed inset* shows magnified image of VZV antigen positive neurons. (**b**) Immunofluorescent staining of a DRG at 48 h post infection with mouse anti-VZV gE monoclonal antibody and secondary antibody consisting of fluorescently conjugated anti-mouse AlexaFluor 594 (*red staining*). Nuclear blue DAPI staining is indicated. (**c–e**) Immunohistochemical detection of VZV infected DRG stained with mouse anti-VZV gB monoclonal antibody at 48 h post infection (**c**) and 72 h post infection (**d**) (*brown staining*) or mock infected DRG (**e**). *Black arrows* indicate infected neurons. Sections were lightly counterstained with hematoxylin (*blue staining*). 

and supporting cells increased over time, indicating that cell-to-cell spread of the virus likely occurred within the infected DRG (Gowrishankar et al. 2007). The presence of virus particles in neurons, as well as in the extracelluar space, was also demonstrated by transmission electron microscopy (TEM) (Gowrishankar et al. 2007). Importantly, cell-free virus was shown to be released from these cultures, which is a unique feature of this model, as VZV typically remains highly

cell-associated in culture (Weller 1953). The explant DRG model provides a number of opportunities to study various aspects of VZV replication within DRG, including innate immune responses to infection and effects of viral replication on neuronal function. This model could also be used to screen potential new vaccine candidates for neurotropism, and determine the basis of VZV neuropathogenesis using viral gene deletion viruses. This model may also have other uses such as studying neuronal signaling during VZV infection and anterograde and retrograde transport of VZV proteins in axons, which could be done in combination with explanted skin sections. This approach has previously been used in pioneering work by the Cunningham group to study axonal transport of herpes simplex virus (HSV) from human DRG (Penfold et al. 1994, 1996; Holland et al. 1998, 1999; Mikloska et al. 1999; Mikloska and Cunningham 2001), and a similar approach could be used to study VZV virion transport and assembly. Such studies may lead to the identification of new drug targets to limit axonal transport of the virus, which may prevent the establishment of latency or aid in the treatment of herpes zoster.

## 5 VZV Infection of Neuronal Cell Lines

An alternative approach for studying VZV infection of primary neurons has been to examine infection of neuroblastoma cell lines. Such a surrogate model of neuronal infection affords distinct advantages over working with primary neurons in terms of cost, availability, cell number, ease of manipulation, a lack of donor variation, and a reduced risk of contamination with other infectious agents. Further, by using neuroblastomas, ethical issues that arise when using fetal tissues are avoided. Cell lines in general are also easier to genetically manipulate, and thus neuroblastomas have the potential to be used to evaluate the role of cellular genes in VZV infection. Although a caveat of using neuroblastoma cell lines is whether they adequately mimic primary neurons, it remains rather surprising that there have been so few reports exploring the nature and properties of VZV infection in neuroblastomas.

VZV infection of the human derived IMR-32 and the murine neuro-2A neuroblastoma cell lines have been studied (Bourdon-Wouters et al. 1990). Infection of IMR-32 cells, in both a cell-free and a cell-associated manner using infected MRC-5 cells, resulted in a cytopathic effect, cell death, and the release of cell-free virus. In contrast, infection of neuro-2A cells using a similar approach was non-productive, with no viral antigens detected within cells, despite persistence of VZV DNA, as detected by in situ hybridization (ISH) (Bourdon-Wouters et al. 1990). These differences may be due to the non-human origin of the neuro-2A cells (Bourdon-Wouters et al. 1990).

The SK-N-SH neuroblastoma cell line, which is also of human origin, as well as one of its derivatives SH-SY5Y (Biedler et al. 1973; Ross et al. 1983), has been used for infection with VZV (Cohen and Nguyen 1998; Cohen et al. 2001; Sato et al. 2002); however, the infection of these neuroblastomas has not yet been fully characterised. SH-SY5Y cells can be readily differentiated using retinoic acid and brain-derived neurotrophic factor (BDNF) to produce growth-arrested cells which have extended

neuritic processes and express neuronal markers, rendering them similar to primary neurons (Encinas et al. 2000). SH-SY5Y cells have been shown to be more permissive to HSV-1 infection in their differentiated form due to an upregulation of Nectin-1 and -2 and HVEM (Gimenez-Cassina et al. 2006). Unpublished data from our group indicates that infection of both undifferentiated and differentiated SH-SY5Y cells with VZV results in a full productive infection, which shares many characteristics of infection with dissociated primary human fetal neurons. Mechanisms involved in the development of neuropathic pain during post-herpetic neuralgia are poorly understood. SY-SY5Y cells can be stably transfected to express transient receptor potential (TRP) vanilliod receptors (Lam et al. 2007) and the tetrodotoxin-resistant voltage-gated sodium channel alpha-subunit Nav1.8 (Dekker et al. 2005), giving properties of nociceptor (pain sensing) neurons. Therefore, these cells could be used to study the effects of VZV infection on neurotransmitter release and neuronal function, which may have implications for PHN. Thus the use of neuroblastomas may serve as a more practical alternative to using fetal neurons, at least for initial studies of various aspects of VZV infection, which could then be confirmed and extended using primary neuronal cultures or naturally infected tissue samples.

## 6 VZV Infection of Rodent Neurons

Neurons of rodent origin have also been used to study VZV infection. However, as VZV is highly species specific and humans are the only natural host for the virus, infection of cells of non-human origin may not accurately reflect the virus lifecycle *in vivo*. Ganglionic neurons of rodent origin, however, are much easier to obtain than human fetal ganglia, and this has been the predominant driving force in pursuing this approach.

The first study of VZV infection using animal neurons *in vitro* was performed by Merville-Louis et al. (1989). A mixed population of dissociated neurons and supporting cells was established, with neurons consisting of approximately 10% of the culture. Cells were infected in either a cell-free or cell-associated manner, and in both cases no cytopathic effect was observed up to 10 days post-infection. In addition, no cell-free virus was released and virus was not able to be transferred to permissive MRC-5 cells. Viral antigens were detected in a small percentage of neurons, using human serum which was known to contain anti-VZV antibodies; however, antigen was detected only up until day 5 post-infection. Despite the lack of de novo virus production, VZV nucleic acids were detected within neurons by ISH, and this increased from 20% of neurons being positive at day 1 to 50% at day 6 post-infection. No ISH signal was detected in any of the non-neuronal cells. ISH specific for RNA transcripts of immediate early, early, and late genes showed that genes from all the temporal classes were expressed in neurons, although the IE63 transcript was found to give a stronger hybridization signal. From this study the authors concluded that the infection of rat neurons resulted in a non-productive,

persistent infection, and that the IE63 gene may play a role in repressing a productive infection (Merville-Louis et al. 1989).

Subsequent studies using rat DRG neurons *in vitro* were able to achieve a productive infection using a "microculture" system (Kress and Fickenscher 2001; Schmidt et al. 2003). Neurons and infected fibroblasts were concentrated into very small volumes of media and incubated together for 4 h before additional culture media was added. Presumably this increased cell-to-cell contact between infected fibroblasts and neurons facilitated the spread of VZV. IE62, IE63, and glycoprotein E could be detected within neurons by immunofluorescence. Within 3–4 days post-infection, the majority of productively infected rat neurons died. This was characterised by a loss of viable cells within the culture, which was in contrast to control uninfected cultures, which appeared healthy. These studies went on to further examine the effects of VZV infection on rat neurons with respect to neuronal function and showed that VZV infection results in a gain-of-function conferring sensitivity to adrenergic agonists, including norepinephrine which is associated with pain (Kress and Fickenscher 2001; Schmidt et al. 2003). It was also shown that this effect was greatly reduced when rat neurons were infected with the vaccine OKA strain, despite the vaccine strain being able to infect an equal percentage of neurons as the wild-type strain (Schmidt et al. 2003). This contrasted with a prior study using human fetal neuron cultures, which showed that the vaccine strain did not infect neurons to the same degree as did the wild-type VZV strains (Somekh and Levin 1993). It was proposed that the induction of sensitivity to adrenergic stimulation following VZV infection could be responsible for heat hyperalgesia, which is commonly linked with PHN (Schmidt et al. 2003). In a separate study using rat neurons, a VZV IE63 expression construct was transfected into neurons derived from rat embryos to show that this caused an increase in calcitonin gene-related peptide (CGRP) release, which *in vivo* can increase the sensation of pain (Hamza et al. 2007). Thus expression of IE63 in neurons may play a role in the development of pain that is experienced during herpes zoster and PHN.

Isolated guinea pig and mouse enteric neurons have also been used to establish a model of lytic, latent, and reactivating VZV infection *in vitro* (Chen et al. 2003; Gershon et al. 2008). Enteric ganglia differ from dorsal root ganglia in that they not only contain afferent (sensory) neurons, but also efferent (motor) neurons and interneurons. To validate the use of enteric neurons for VZV infection, it has been shown *in vivo* that enteric neurons can harbor latent VZV (Gershon et al. 2009). Using this guinea pig-derived model it was demonstrated that infection of guinea pig enteric neuronal cultures with cell-free VZV resulted in an apparent latent infection. No cytopathic effect was observed, and transcripts for the latency-associated ORFs 4, 21, 29, 40, 62 and 63 could be detected (Chen et al. 2003). In addition, several proteins arising from these transcripts were detected in neurons by immunofluorescence where they were found in the cytoplasm of cells, which is indicative of latency (Lungu et al. 1998; Chen et al. 2003). Immunofluorescence staining also revealed that sensory neurons present in the culture, as well as other types of neurons, were able to be infected in a nonproductive manner, and these infected neurons could survive in culture for weeks, similar to uninfected controls

(Chen et al. 2003). Interestingly, when these neurons were infected in a cell-associated manner, or when other non-neuronal cells from the bowel wall were present, a productive infection resulted (Chen et al. 2003). In this scenario, glycoproteins, which have not been reported to be produced during latency, were detected and the neurons died rapidly (Chen et al. 2003). It was proposed that the absence of the non-structural ORF61 protein may enable the virus to establish a latent infection when cell-free virus was used as the inoculum. It was subsequently shown that superinfection of latently infected enteric neurons with a viral vector expressing the ORF61 protein resulted in an apparent reactivation of the virus, with concomitant expression of viral glycoprotein and neuronal death (Gershon et al. 2008). This model has also been used to demonstrate that the cellular localization of IE63 and ORF29p is cell-type dependant, with both proteins accumulating in the cytoplasm of guinea pig enteric neurons and the nucleus of epithelial cells, and that the expression of ORF61p in neurons can drive their nuclear import in a protease-dependent fashion (Stallings et al. 2006; Walters et al. 2008). Infected guinea pig enteric neurons were also used to show a link between IE63 and the human antisilencing function 1 protein, which may influence transcription of genes during VZV infection (Ambagala et al. 2009).

In addition to the use of dissociated rodent neurons to study VZV infection, a number of studies have sought to utilise whole-animal models of infection. In this respect, *in vivo* VZV infection of weanling guinea pigs via various routes of inoculation, including intranasally, subcutaneously, intramuscularly, and via corneal infection has been reported to result in viremia and nasopharyngeal shedding of virus, which can lead to transmission of VZV to other animals (Myers et al. 1980, 1985; Matsunaga et al. 1982). In addition, the presence of VZV DNA in DRG of infected guinea pigs was also demonstrated by PCR, indicating that virus can access sensory ganglia in these animals (Lowry et al. 1993). Likewise, there has also been a report of VZV infection of mice via a corneal inoculation, resulting in viremia and detection of VZV DNA in various tissues including the trigeminal ganglia, the brain stem, and the spleen, up to 33 days post-infection, with the mice not suffering from any apparent VZV-related illness (Wroblewska et al. 1993). There has also been interest in creating an *in vivo* model of VZV infection using rats, and these have been used to study VZV latency and as a behavioral model of VZV-induced pain (Sadzot-Delvaux et al. 1990, 1995, 1998; Annunziato et al. 1998; Fleetwood-Walker et al. 1999; Kennedy et al. 2001; Sato et al. 2003; Grinfeld et al. 2004; Garry et al. 2005). The development and uses of rat models of latency is discussed further in (Cohen 2010).

## 7 VZV Infection of Ganglionic Satellite Cells

Neurons within the DRG and TG are surrounded by satellite cells, and in addition to providing physical support to neurons, satellite cells appear to be capable of affecting the microenvironment of the ganglia and may even play a role in neuronal

signaling (review in Hanani 2005). Recently, satellite cells of the TG have also been shown to share some properties of antigen presenting cells, and therefore may also influence the immune response within ganglia (van Velzen et al. 2009). It is not surprising that satellite cells are required to perform immune surveillance, and antigen presentation within the ganglia, given neurons within sensory ganglia, like neurons of the central nervous system, fail to express major histocompatibility complex (MHC) molecules (Turnley et al. 2002). This may allow VZV to persist latently within neurons without recognition by the immune system. This would be beneficial for both the virus and the host as neurons are post-mitotic cells and therefore will not be replaced if killed as a result of infection (Joly et al. 1991). VZV DNA and RNA have been detected within satellite cells during latency (Croen et al. 1988; Schmidbauer et al. 1992; Kennedy et al. 1998; Lungu et al. 1998; LaGuardia et al. 1999). VZV antigens within satellite cells have also been reported in studies of explanted VZV-infected fetal ganglia (Gowrishankar et al. 2007) and in infected fetal ganglia xenographed into SCID mice (Zerboni et al. 2007; Reichelt et al. 2008). However, further studies examining the impact of VZV infection on satellite cell function are needed due to the important role these cells play in supporting neurons within the ganglia.

## 8 Conclusions and Perspectives

Productive, latent, and reactivated VZV infection of sensory neurons and their supporting satellite cells may result in alterations to normal cellular processes, which in combination with an immune-mediated response to viral replication may contribute towards the pain experienced during herpes zoster and subsequently during PHN. Thus, a greater understanding of how VZV interacts with cells of the sensory ganglia is required to develop new therapeutic strategies to prevent or manage these conditions.

The lack of an adaptive immune response is the greatest disadvantage to *in vitro* models of VZV neuronal infection, as well as the *in vivo* SCID-hu mouse model, and without an animal model that can mimic all phases of disease progression in the context of an immune response, studies of post-mortem ganglia affected by varicella, herpes zoster, and PHN will remain critical.

From the *in vitro* studies that have been conducted thus far, it is becoming clear that distinct differences exist between VZV replication in neuronal cells compared to other diploid cells. These differences could play a key role in enabling VZV to successfully replicate in neurons within sensory ganglia, for example, during the initial stages of virus reactivation, leading to herpes zoster. The precise viral and cellular mechanisms that underpin virus replication within this cell type, as well as those that facilitate latency, remain to be established.

Differences in VZV replication *in vitro* are also evident between studies and models, and which model is the most accurate representation of VZV infection *in vivo* remains unknown. Like during natural VZV replication in human skin, in

both the explant fetal DRG model and in IMR32 neuroblastoma cells, cell-free virus release has been demonstrated (Bourdon-Wouters et al. 1990; Gowrishankar et al. 2007). This is in stark contrast to infection of other cell types *in vitro* (Weller 1953), including other neuroblastoma-based culture systems, where virus remains cell-associated (Abendroth, unpublished data). This may reflect differences in the virus lifecycle in these cells and perhaps a difference in the expression of mannose-6-phosphate receptor, which has been shown to affect cell-free virus release (Chen et al. 2004). Studies comparing neuronal infection *in vitro* to naturally infected human ganglia derived post-mortem or surgically are therefore needed to establish the robustness of these models, as well as to establish the role of the adaptive immune response.

Another difference observed between models of VZV neuronal infection *in vitro* is that VZV-infected rat and guinea pig enteric neurons do not survive infection (Merville-Louis et al. 1989; Chen et al. 2003; Gershon et al. 2008), which is in contrast to studies of primary human neuronal cells, in which VZV infection has been shown not to induce apoptosis (Hood et al. 2003). The IE63 protein has been shown to protect human neurons from apoptosis; however, the precise mechanism behind this protection is unknown (Hood et al. 2006). Further studies of the different models, animal vs. human neurons, may help to elucidate how IE63 modulates the apoptotic response of neurons.

Pain as a result of herpes zoster and especially PHN can be disabling and have a major negative impact on patient's quality of life (Dworkin et al. 2001). In coming years the number of individuals suffering from herpes zoster and PHN is expected to rise, concomitant with the increasing number of patients who are elderly or immunosuppressed due to infection or therapies for cancer or transplantations. Therefore, VZV and associated diseases are likely to place a large burden on health care systems, and adequate treatment and prevention strategies are needed. To achieve this, further studies into the mechanisms involved in VZV replication in neuronal cells are necessary. In addition, given that VZV is able to readily infect neurons, like HSV, it may be a suitable vector for gene delivery into neuronal cells for the treatment of various nervous system disorders. This could be achieved only by further studies into the interaction between VZV and neuronal cells, and suitable attenuation of the virus to limit replication in other cell types.

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