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Neuronal changes induced by Varicella Zoster Virus in a rat model of postherpetic neuralgia

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ABSTRACT

A significant fraction of patients with herpes zoster, caused by Varicella Zoster Virus (VZV), experience chronic pain termed postherpetic neuralgia (PHN). VZV-inoculated rats develop prolonged nocifensive behaviors and serve as a model of PHN. We demonstrate that primary rat cultures show a post-entry block for VZV replication, suggesting the rat is not fully permissive. However, footpads of VZV infected animals show reduced peripheral innervation and innervating dorsal root ganglia (DRG) contained VZV DNA and transcripts of candidate immediate early and early genes. The VZV-infected DRG showed changes in host gene expression patterns, with 84 up-regulated and 116 down-regulated genes seen in gene array studies. qRT-PCR validated the modulation of nociception-associated genes Ntrk2, Trpv1, and Calca (CGRP). The data suggests that VZV inoculation of the rat results in a single round, incomplete infection that is sufficient to induce pain behaviors, and this involves infection of and changes induced in neuronal populations.

Introduction

Herpes zoster (HZ), a painful and debilitating disease seen most often in the elderly and immune compromised, is the clinical manifestation of reactivation of the human alphaherpesvirus Varicella Zoster Virus (VZV) from a neuronal latent state that was established during the primary infection, chickenpox (varicella). HZ affects about a quarter of the population in their lifetimes, with incidence rising with age and/or cellular immune decline (Harpaz et al., 2008; Johnson and Rice, 2014). Of numerous neurological complications associated with HZ, the most common by far is pain, which is the major contributor to HZ morbidity. Most HZ patients suffer from acute pain near the time of appearance of skin lesions, with >90% of unvaccinated individuals being prescribed pain-alleviating medication (Haanpaa et al., 1999; Johnson and Rice, 2014). However, more problematic and difficult-to-treat chronic pain states develop in a third of HZ cases, termed postherpetic neuralgia (PHN). These may become so severe that they lead to disparate secondary consequences that affect quality of life, causing depression, withdrawal from society and even suicide (Drolet et al., 2010; Dworkin et al., 2008; Johnson et al., 2010; Schmader, 1998; Schmader et al., 2010). While there is a vaccine for zoster, it is only partially effective, reducing HZ incidence by 51% and the "burden of illness" (which includes pain and PHN) by 68% (Oxman et al., 2008). A significant fraction of PHN patients gain little to no relief from any current treatment strategy, often at the cost of unwanted side effects (Kim et al., 2014). Most adults worldwide harbor wild-type VZV within their ganglia and are at risk of developing HZ and PHN. As such, there remains an urgent public health need to find the cause of VZV-induced pain and to develop more effective treatments (Kawai et al., 2014; Kelly et al., 2014).

Why HZ is so frequently complicated by pain and what leads to the more protracted pain state of PHN remains unclear. It is clear that reactivation of VZV is followed by extensive intraganglionic spread that involves many neurons and ganglionic support cells. The VZV infected neurons can deliver VZV to the periphery at many points to generate large, widespread lesions (Gilden et al., 2003; Kinchington et al., 2012; Steiner et al., 2007). Inflammation, which occurs at both the periphery and at the ganglia, is thought to contribute to the pain states, but it has also been suggested that

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HZ causes changes in neuronal anatomy and circuitry (Opstelten et al., 2010). However, VZV pain states have been difficult to investigate, because the virus exhibits a high-degree of human species specificity that precludes the use of small animal modeling of latency, reactivation and zoster-like disease.

One exception to animal modeling of VZV-induced disease has been the rat. Despite the lack of appearance of any HZ-like disease in the VZV inoculated rat, animals develop prolonged behavioral responses suggesting the establishment of a chronic pain state after inoculation of live high-titer cell-associated VZV into the glabrous region of the footpad. These states are measurable and quantifiable, and recapitulate some aspects of mechanical and thermal hypersensitivity that develop in PHN patients (Baron et al., 2009; Haanpaa, 2008; Haanpaa et al., 1999). In particular, the mechanical hypersensitivity in the rat model mirrors the most common and distressing pain associated with PHN, mechanical allodynia (MA). MA is defined as pain resulting from what is considered to be normally innocuous stimuli. The rat model has permitted the evaluation of novel and established pharmacological agents for treatment of VZV induced pain, and we have used the rat model to establish gene therapy strategies for the treatment of VZV-induced pain (Garry et al., 2005; Guedon et al., 2014; Hasnie et al., 2007; Medhurst et al., 2008; Zhang et al., 2011). However, virus infection in the rat model has not been extensively evaluated. We reported that VZV infectivity and gene expression are required for the onset of pain behaviors (Guedon et al., 2014), and several studies have reported detection of viral proteins in the ipsilateral DRG of the inoculated footpad (Dalziel et al., 2004; Garry et al., 2005; Hasnie et al., 2007; Kinchington and Goins, 2011; Sadzot-Delvaux et al., 1995, 1990). However, the level of expression and number of neurons expressing VZV proteins have varied extensively, from a majority of neurons (Garry et al., 2005; Hasnie et al., 2007) to only a small fraction (Kinchington and Goins, 2011). Intriguingly, two studies report the development of prolonged VZV-induced pain in animals undergoing treatment with high doses of antivirals to block viral DNA replication. This suggests VZV-induced nocifensive responses do not require ongoing viral genome replication (Garry et al., 2005; Zhang et al., 2011).

Here we further address several important aspects of the rat model, including permissivity of the model host to VZV infection, ganglionic access of virus and neuronal changes that develop and correlate with nocifensive indicators of pain. Rat primary cell cultures derived from multiple tissues of the Sprague Dawley rat using in the pain model were found to be restricted for full VZV replication, with the block occurring at a post-entry stage after the initiation of viral gene expression. The innervating DRG of VZV inoculated rat footpads with pain contained low levels of VZV genomes and transcripts suggesting limited viral gene expression. We also report that VZV footpad inoculation induced peripheral neurite retraction at the paw skin, and triggered changes in host transcription programs at DRG that innervate the ipsilateral rat footpad. These data suggest that behavioral indices of pain in this model are not the consequence of continued viral replication, but rather the result of incomplete single round of infection in cells and neurons of the rat host.

Results

Primary cells of rat origin exhibit a post-entry block to VZV replication

We initially verified that the stock of VZV used throughout these studies was proficient at inducing behavioral response indicators of pain. Rats receiving 2×10^5 PFU of VZV cell-associated inocula developed mechanical hypersensitivity in the ipsilateral but not contralateral paw, and continued to show sensitivity for the duration of the study out to at least 43 days (Fig. 1). Mechanical hypersensitivity did not develop in animals receiving equivalent numbers of live uninfected cells, and the 50% gram weight threshold obtained from animals receiving uninfected cells was similar to that seen for the untreated contralateral paws in both sets of animals (Fig. 1A and B). These results are similar to that seen in our previous studies (Guedon et al., 2014) and establish that VZV used here induces prolonged hypersensitivity only within the infected paw.

No other clinical sign of VZV infection or skin disease other than pain was seen here, and none have been reported in studies using rat models of VZV infection by others (Cohen, 2010; Cohen et al., 2004; Cohen and Nguyen, 1998; Dalziel et al., 2004; Fleetwood-Walker et al., 1999; Garry et al., 2005; Hasnie et al., 2007). Given that VZV is generally considered to have high specificity for the human host (Cohen, 2010), we considered the permissivity of this host to VZV infection an important aspect to address. Attempts to detect infectious VZV in the inoculated footpad after day 2 postinfection (pi), or from the corresponding DRG, from peripheral blood mononuclear cells (PBMC) or from organs such as spleen and kidney at various times postinfection, all met with no success (data not shown). To further address if cells derived from the Sprague-Dawley rat strain used in the pain model can support VZV productive infection, low pass primary cultures were established from multiple tissues obtained from euthanized uninfected Sprague-Dawley rats, including the cornea, glabrous paw skin, lung, kidney, and liver. Sub-confluent cultures infected with the same VZV used to induce the pain response did not develop plaques or any visible signs of a cytopathic effect over 10 days. To more closely monitor the VZV infection process dynamically, we used a recombinant VZV in which the gene

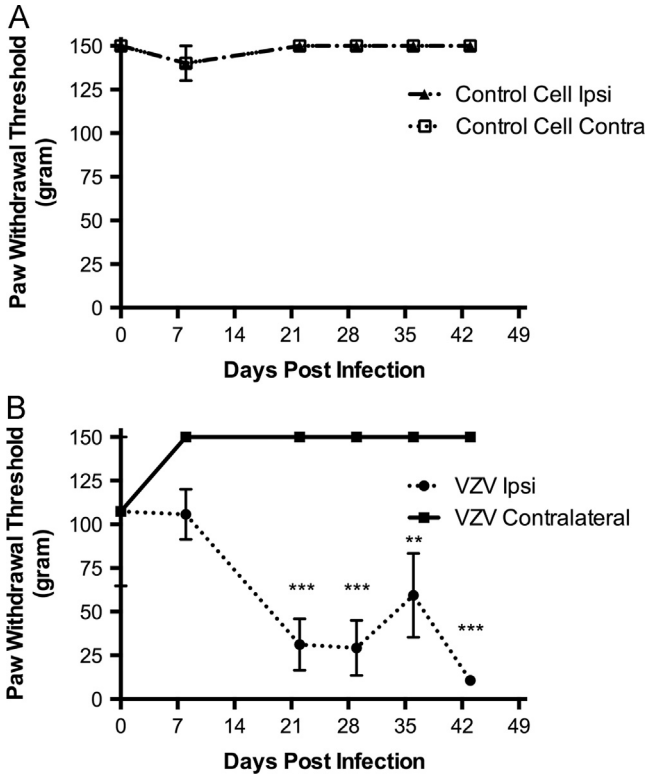

Fig. 1. Sprague-Dawley rats inoculated with VZV become hypersensitive to mechanical stimuli. Animals that were pre-tested prior to inoculation for baseline paw withdrawal responses (grams) were then inoculated ($n=3$ per group) at day 0 with uninfected cell equivalents (control cells) (A) or 2×10^5 PFU of VZV (B). Mean ± SEM plotted. This study is representative of multiple similar studies. Statistics used One-Way ANOVA with Tukey's Post-Test. $^{***}p<0.001$, $^{**}p<0.01$.