

Behavioural changes in the rat following infection with varicella-zoster virus

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Following the establishment of a chronic varicella-zoster virus infection in the rat, behavioural allodynia and hyperalgesia were observed in the injected, but not the contralateral hind limb up to 33 days post-infection. This model may prove useful in investigating mechanisms involved in the establishment of post-herpetic neuralgia.

Following primary infection varicella-zoster virus (VZV), like other neurotropic alphaherpesviruses, establishes latency in ganglia of the peripheral nervous system. Reactivation may occur years later and present as shingles (zoster) (Gilden *et al.*, 1992; Hay & Ruyechan, 1994). However, VZV is unusual within this virus family as following reactivation it is commonly associated with the development of a long-term pain state, post-herpetic neuralgia (PHN). The pain develops and persists after the disappearance of skin lesions even though the virus becomes latent within the sensory ganglia of the affected dermatomes. This pain is often prolonged, increases in incidence with age and responds poorly to classical analgesics (Bowsher, 1997). PHN is characterized by the development of chronic hyperalgesia (an increased response to noxious stimuli) and mechanical allodynia (an abnormal pain response to what are defined as innocuous stimuli in normal, unaffected individuals), accompanied by spontaneous pain, usually of a burning or aching nature (Choi & Rowbotham, 1997; Rowbotham & Fields, 1996; Rowbotham *et al.*, 1996; Scadding, 1984).

In latent herpes simplex virus type 1 (HSV-1) infections no viral proteins are detected in the latently infected cells and the only viral transcripts detected are the latency-associated transcripts (LATs) (Stevens *et al.*, 1987). These LATs are stable intron sequences whose function is unclear and from which no protein is known to be translated. In contrast, five VZV-encoded transcripts and proteins (genes 4, 21, 29, 62 and 63)

have been shown to be present in human and rat ganglia with latent VZV infection (Lungu *et al.*, 1998; Sadzot-Delvaux *et al.*, 1990; Cohrs *et al.*, 1996). The role, if any, of these proteins in PHN is unknown.

We utilized a previously reported animal model of latent VZV infection (Sadzot-Delvaux *et al.*, 1990) to investigate if rats infected with VZV exhibit alterations in sensory responses similar to those observed in PHN. VZV (Dumas *et al.*, 1981) was propagated on CV-1 cells and harvested when cells exhibited approximately 80% cytopathic effect. Male Wistar rats (weight 220–450 g, $n = 11$) were anaesthetized with Sagatal [60 μ l/100 g, intraperitoneal (i.p.)] and injected subcutaneously in the left (ipsilateral) glabrous footpad with approximately 4×10^6 infected cells per animal in 50 μ l PBS, as previously described (Sadzot-Delvaux *et al.*, 1995). Control rats ($n = 10$) were injected with mock-infected CV-1 cells and housed separately from virus-infected animals. Behavioural tests were carried out 1–10 days prior to infection and then up to 33 days post-infection to test for the development of hyperalgesia and allodynia, and consisted of the following.

1. Paw withdrawal in response to graded innocuous and noxious mechanical stimuli. A calibrated set of von Frey nylon monofilaments, each of which exerts a known force at its bending threshold (1.5–125.9 g), was used to determine the mean mechanical force (g/unit area) to cause two or more reflex foot withdrawals over ten applications of graded filaments. The stimulus was applied at 1–2 s intervals to the ventral, glabrous surface of both the left and right hind paws (Chaplan *et al.*, 1994) and repeated three times with a 5 min interval between tests.

2. Paw withdrawal to noxious thermal stimuli (Hargreaves *et al.*, 1998). The mean latency (to the nearest 0.1 s) for hind paw withdrawal to noxious heat (range 30–55 °C) applied to the glabrous skin of the footpad was assessed using a Ugo Basile Unit. A standard cut-off latency of 15 s prevented possible tissue damage.

Statistical analysis was carried out using the Wilcoxon test for left to right hind limb comparisons, within groups, or the Mann–Whitney U-test for between group comparisons. Care was taken to ensure that there was no undue bias in the way

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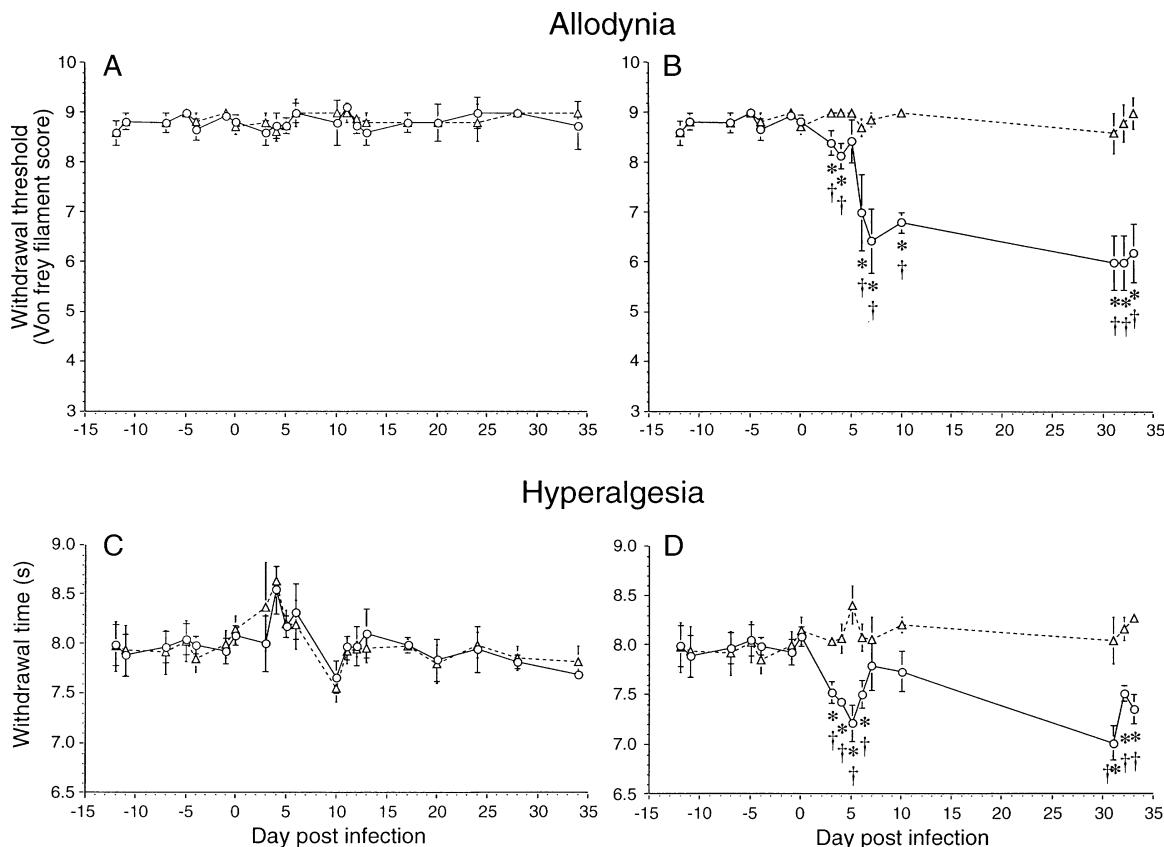


Fig. 1. Time-course of the effect of VZV injection on paw withdrawal responses to mechanical and noxious thermal stimulation. Following VZV cell injection at day 0, the time-course of changes in the two behavioural reflex responses on the injected left (s) and non-injected right (n) hind paws was followed up to day 33 post-infection. Graphs A (uninfected) and B (infected) show the threshold reflex withdrawal responses to mechanical force applied using von Frey filaments. Graphs C (uninfected) and D (infected) show the latency to paw withdrawal (seconds) in response to noxious thermal stimulation. Statistically significant differences between injected and non-injected hind paws ($*P < 0.05$, Mann-Whitney U test, $n = 7$) and from pre-injection control measurements, compared to the injected paw measurements ($\dagger P < 0.05$, Wilcoxon test; $n = 7$) are shown.

the testing procedure was performed by not allowing the animal to put its weight on the hind paw being tested (Kauppila *et al.*, 1998). At the end of 33 days, animals were killed and tissues taken for immunohistochemical processing.

Animals showed no obvious signs of discomfort and phenotypic changes were only evident on formal testing. For VZV-injected rats the thresholds for both the von Frey filament (Fig. 1B) and noxious thermal tests (Fig. 1D) showed a significant and sustained decline for the injected left hind paw compared to the uninjected right hind paw ($P < 0.05$; Wilcoxon test, $n = 5$) for up to 33 days following injection. This alteration in sensory phenotype was observed in all infected animals as indicated by the error bars in Fig. 1. In contrast, control mock-infected rats showed no significant difference between the injected and uninjected hind paw responses over this time period, or compared to preinjection control tests, (Fig. 1A, C; Wilcoxon test, $n = 5$).

These data demonstrate marked and statistically significant changes in behavioural reflex responses indicative of mechanical allodynia and hyperalgesia. The altered response

observed at 5 days post-infection may be due to a local inflammatory response at the site of injection. The sensory abnormalities are, however, undiminished 5 weeks post-infection, the time at which all evidence of local injection trauma has disappeared.

To monitor VZV protein expression in latently infected nervous tissue immunohistochemical analysis of the lumbar dorsal root ganglia was carried out. Animals were deeply anaesthetized with Sagatal (120 μ l/100 g, i.p.) and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4; containing 3 mM sodium nitrite and 1000 U heparin) before being perfused with 4% paraformaldehyde-PBS. Dorsal root ganglia taken from lumbar segments L3-L6 were removed and post-fixed in the same solution overnight. Tissue was then transferred to a 25% sucrose solution for a further 24 h, prior to being stored in cryoprotectant (30% ethylene glycol, 20% glycerol, in PBS, pH 5.5) at 4 °C until ready for sectioning. DRG from several infected animals were pooled, embedded in paraffin wax and 5 μ M sections cut and mounted onto Vectabond-coated slides. Uninfected animals were treated in a

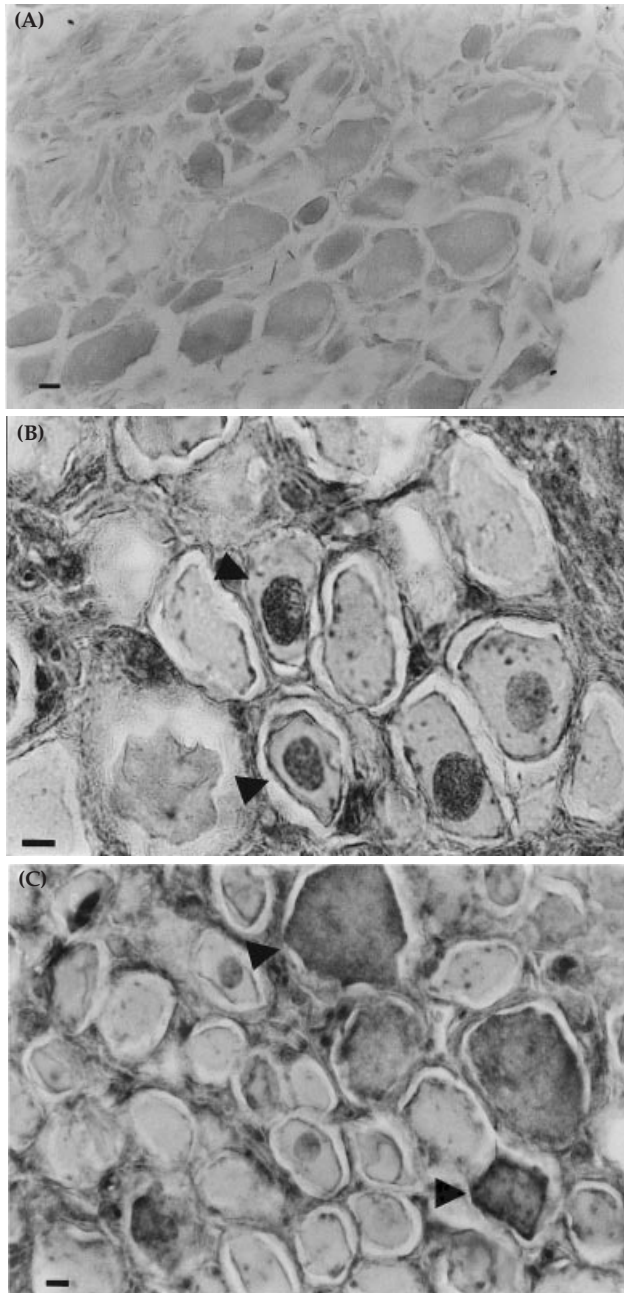


Fig. 2. VZV protein expression in infected nervous tissue. Immunohistochemical analysis of the lumbar DRG was carried out as described in Methods. (A) DRG section taken from an uninfected animal depicting a lack of specific VZV IE 63 immunoreactive labelling. (B, C) DRG sections taken from infected animals. Note the intense intranuclear labelling in (B) (arrows) compared with the specific cytoplasmic labelling in (C) (arrows). Scale bar indicates 10 μ M in each case.

similar manner. Sections were dewaxed and rehydrated, followed by a brief rinse in PBS prior to incubation with rabbit anti-IE 63 [a kind gift of C. Sadzot-Delvaux (Sadzot-Delvaux *et al.*, 1995)]. Bound antibody was detected using the Vectastain Elite ABC kit and immunoreactivity visualized by the 3,3'-

diaminobenzidine tetrachloride-immunoperoxidase method. Finally, sections were lightly counterstained with 1% eosin.

The presence of IE 63 protein was demonstrated in ipsilateral lumbar dorsal root ganglia in VZV-injected rats compared to control, uninfected rats (Fig. 2). No evidence of inflammatory infiltrates or of neuronal cell death was observed in the DRG sections. Thus, in this model, it would appear that the presence of VZV in DRG correlates with an increased sensitivity to sensory stimuli.

The mechanisms by which VZV infection causes the observed sensory changes are unknown. The duration of the responses, and their absence following injection of mock-infected cells, suggests that immune-mediated damage is not playing a major role. mRNA transcripts for three regulatory proteins, genes 62, 63 and 4, have previously been shown to be present in this model (Sadzot-Delvaux *et al.*, 1995). The expression of these regulatory proteins within the infected neurone may affect the physiological function of that neurone and its contribution to influencing central nociceptive processing.

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