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The pattern of viral persistence in monkeys intra-tracheally infected with Simian varicella virus

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Abstract In situ PCR (ISPCR) and in situ hybridisation (ISH) was performed on 32 tissues from 10 monkeys, intra-tracheally (IT) infected with simian varicella virus (SVV) and 5 tissues from 3 uninfected control animals. The results showed persistence of SVV DNA up to 2 years post-infection (pi) and the localisation of SVV to be confined to neurons except at time points 9 and 10 months pi where SVV positive satellite cells were also detected. There was no evidence for transcription of SVV ORFs 63 and 21 in the ganglia of the one IT infected and 2 naturally infected monkeys investigated using RNA ISH.

Keywords Smian varicella virus · In situ hybridisation · Persistence · Latency · Neuron

Varicella zoster virus (VZV) is a pathogenic human herpesvirus that causes varicella (chickenpox) as a primary infection following which the virus establishes latency, predominantly in neuronal cells in the spinal and trigeminal ganglia [1, 2]. Viral gene expression is restricted during human ganglionic latency, being limited to at least 5 genes (VZV genes 21,29,62,63 and 66) [3, 4]. The virus may subsequently reactivate to produce herpes zoster (shingles), which is characterised by a rash and pain in one or more sensory dermatomes. VZV is a significant cause of neurological disease including post-herpetic neuralgia which may be highly resistant to treatment [5], and small and large vessel encephalitis [6]. In order to improve understanding of the pathogenesis of VZV latency, several models of VZV latency have been studied, including rat [7–9] and monkey [10, 11]. The latter, which we used in this study, is the simian varicella virus (SVV) model of primates which has several clinical, immunological, and virological features in common with VZV. The genomes, lytic cycles, clinical appearance and pathogenesis of infection appear similar enough to allow inferences from SVV to VZV [12].

Experimental intra-tracheal (IT) inoculation of adult African green or other monkeys with SVV has been shown to lead to a persistent infection lasting up to 2 years in the blood, systemic tissues such as the liver and lung, and the peripheral ganglia [13]. A naturally transmitted infection of SVV has been shown to result in a scenario in which viral DNA is detectable only in the ganglia after 2 months [14]. We recently showed that in such naturally infected monkeys, latent virus was located exclusively in neurons in the spinal ganglia [15]. Further, a recent report documented viral reactivation in one such naturally infected monkey [16]. In the current study, we have extended this work by studying the cellular location of SVV DNA and RNA in monkeys infected by the IT and natural routes.

All inoculation and other experimental procedures were carried out in the Tulane National Primate Research Center (Covington, LA) following the appropriate guidelines. Inoculum size and sex of monkey are indicated, if known, in Table 1. Animals were killed by intravenous administration of Pentobarbital at a dose of 30–50 mg/kg, Tissues were then removed, fixed and paraffin embedded. All tissues were generously provided to us for this study by Dr R. Mahalingam.

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Table 1 Tissues from SVV IT-infected monkeys positive or negative for SVV ORF63 DNA by ISPCR

Sample no. and tissue	Sex/age	Inoculum	ORF 63 SVV	Time pi
DPRC107lung	M/3 m	10^4	+	7 d acute
DPRC107liver			+	7 d acute
DPRC50c cerv	Unk.	2×10^4	–	7 d acute
DPRC50 TG			–	7 d acute
DPRC50 Th			–	7 d acute
DPRC50 liver			+	7 d acute
DPRC50 lung			+	7 d acute
DPRC164 TG	M/2.97 y	1.5×10^3	–	2 m
DPRC164 C1			+	2 m
DPRC164 C2			+	2 m
DPRC164 lung			–	2 m
DPRC164 liver			–	2 m
DPRC164 L1			+	2 m
DPRC164 L2			–	2 m
DPRC164 Th1			+	2 m
DPRC164 Th2			+	2 m
DPRC164 Th3			+	2 m
DPRC164 sac			+	2 m
K331lumbar dorsal	Unk.	9×10^3	+	9 m
DPRC15TG	Unk.	4×10^4	–	10 m
DPRC11TG	Unk.	4×10^4	–	10 m
DPRC11cerv			+	10 m
DPRC11Th			+	10 m
DPRC11liver			–	10 m
DPRC51Th	Unk.	3×10^4	–	14 m
DPRC51TG			–	14 m
DPRC52TG	Unk.	3×10^4	–	14 m
DPRC8Th	Unk.	3×10^4	–	2 y
DPRC8TG			–	2 y
DPRC8liver			–	2 y
DPRC10 cerv	Unk.	3×10^4	+	2 y
DPRC10TG			–	2 y
CV12 cerv	M/3.16	NA	–	Uninfect
CV10 sac	Unk.	NA	–	Uninfect
HSCM3TG	Unk.	NA	–	Uninfect
HSCM3cerv				Uninfect
HSCM3liver			–	Uninfect

Key: TG: trigeminal ganglion, cerv: cervical ganglion, sac: sacral ganglion, Th: thoracic ganglion, Unk.: Unknown, pi: post-infection, d: days, m: months, y: years, NA: not applicable

In situ PCR for SVV ORF63 was performed on all tissues as previously described [1]. ISPCR for SVV ORF21 and ISH for SVV DNA was performed on those ganglia which were positive for confirmation of localisation. RNA ISH for SVV ORF 63 and ORF 21 RNA [17] was only performed on positive ganglia from DPRC 164 IT and the 2 naturally infected monkeys, which have been described in a previous study [15]. The primers and probes (digoxygenin labelled anti-sense primers) used for this study are identical to those detailed in that previous study [15].

Control tissues consisted of uninfected mouse or rat ganglia and known negative human tissue, as negative controls in addition to irrelevant (non-SVV or monkey) or HSV1 primers and tissues which had been DNased or RNased prior to ISPCR or ISH. All ISPCR runs using nonsense or HSV-1 primers were negative. They were performed on the same slide, adjacent to the sections on which SVV primers were used. In addition, human tissue was included as a positive control for the system (using VZV primers or probes) as well as acutely (7 days post-infection (pi)) SVV-infected lung or liver.

Using ISH and ISPCR for ORFs 63 and 21, we investigated 37 tissues from 13 animals including 2 acutely infected and 3 uninfected animals (Table 1). Animals DPRC107 and 164 were African Green monkeys, CV12 was C. Aethiops and the others were unknown. Ganglia from 4 of the 8 animals IT-infected by SVV were positive for SVV up to 2 year post-infection. As was the case with the ganglia from monkeys naturally infected with SVV, localisation of the SVV in latent (or potentially latent) ganglia was neuronal (Fig. 1A) with the exception of ganglia at 9 and 10 months post-infection which showed some satellite cells containing SVV DNA in addition to neuronal cells (Fig. 1B). Negative controls shown included a sample which was found negative by ISPCR for SVV ORF63 (Fig. 1C) and a sample which had been DNAsed prior to ISPCR (Fig. 1D). SVV DNA was found only in the lungs and liver from acutely infected animals. The fact that no SVV DNA was found in ganglia 7 days pi may have been due to a low level of viral replication or viremia in this monkey, or perhaps this was too early a time point for virus to have reached the ganglia. SVV DNA may have been detected at a 10, 11 or 12 day pi time point [13, 18]. The consistently negative results obtained in all the trigeminal ganglia in this study were somewhat unexpected, but may possibly relate to the anatomical location of these ganglia as well as the detection limits of the techniques used.

The presence of a neuronal localisation of the virus as well as persistence 2 years pi supports the notion that latent SVV, like VZV, persists in the neurons of ganglia for the host's lifetime. The finding that two of the samples, 9 and 10 months pi, were found to have virus in satellite cells in addition to neurons is an interesting observation which could be due to a sub-clinical reactivation, possibly in response to stress, e.g. a change in conditions such as the introduction of new monkeys or moving of cages or passing through puberty [12]. They were also given a slightly higher inoculum than the other monkeys whose tissues were studied at a time later than acute, with the exception of DPRC11, which may have had some influence on

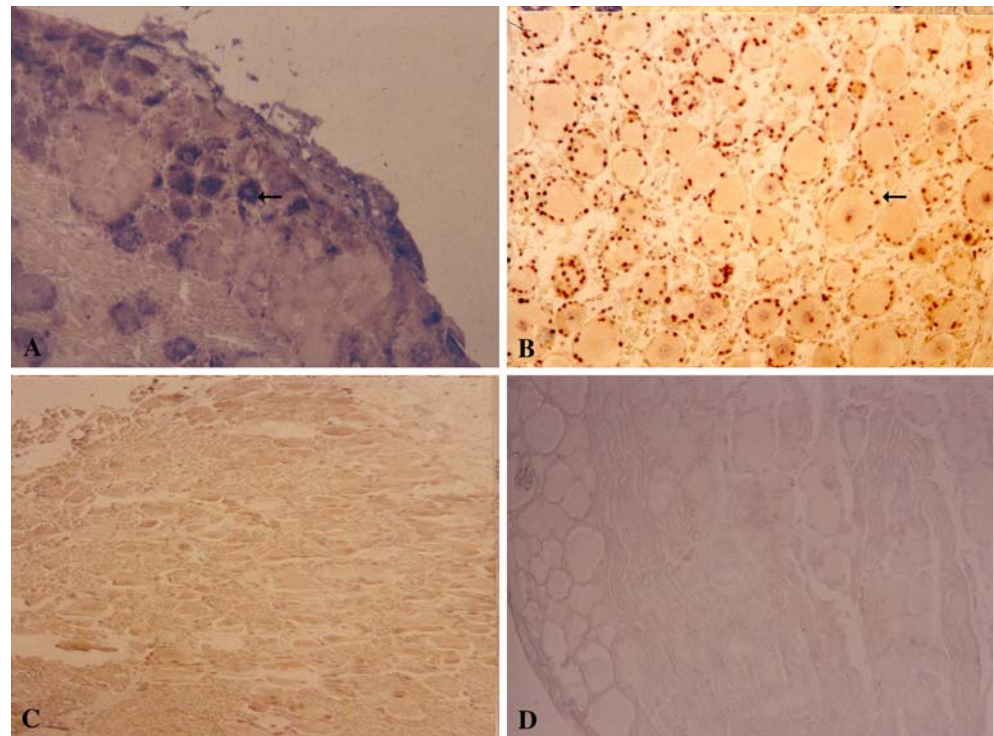

Fig. 1 SVV ORF63 ISPCR (X450) (**A**) 164Th1 ISPCR SVV 63 (2 mpi). The arrow indicates a positive neuron. (**B**) K331 lumbar dorsal SVV63 ISPCR (9 mpi). The arrow indicates a positive satellite cell. (**C**) 164 TG SVV63 ISPCR (**D**) 164c2 Dnased prior to SVV63 ISPCR

persistence or clearance of virus. In our previous study of naturally infected monkeys, we detected SVV DNA exclusively in neurons [15], however the only tissue investigated was at 2 months pi. Due to the limited amounts of tissue available to us, we were able to confirm transcription of only SVV ORF21 in one of these tissues (DPRC11 cervical ganglion).

We found no transcription of SVV ORFs 63 and 21 in ganglia of the IT-infected monkey # DPRC 164 using RNA ISH, while beta-actin probes on these tissues were strongly positive (data not shown). These results contrast with the work of White et al [13] in the IT model, where transcripts from all gene classes were found in ganglia and organs, however a more sensitive methodology was used (nested RT PCR followed by Southern blotting using a radioactive probe) than that employed in this study. In addition, it should be noted that some RNA degradation occurs during the fixation and wax embedding process. However, the absence of transcription of SVV ORF 63 is in agreement with preliminary data of Gray (W.L. Gray personal communication). VZV ORFs 63 and 21 are readily detected by ISH in both humans and rat models of VZV using RNA ISH [8, 17]. Therefore, the levels of transcription in the SVV IT model must be significantly lower than in the human or rat. We also found no evidence for transcription of these two ORFs in the naturally infected monkeys described in our previous study, [15, and unpublished data] which may be because there is no transcription of these genes in this model, or the level of transcription is below the threshold of the level of detection for RNA ISH. The criteria by which the suitability of *all* animal models in the study of VZV latency is to be judged should be very stringent. These should include the long-term persistence of the entire genome exclusively in ganglia with no infectious virus production, a predominantly neuronal localisation of latent virus, the expression of SVV genes homologous to VZV genes expressed in latency (ORFs 63, 66, 62, 29 and 21), and the reactivation of latent virus under conditions of stress and/or immunosuppression to produce infectious virus and shingles-like disease [19]. Further work needs to be carried out in the SVV models of latency to determine the precise extent to which these different criteria are met. Therefore, it is necessary to investigate the transcription of all classes of SVV genes using very sensitive techniques in the naturally infected and IT models of SVV infection at various time points pi in order to establish whether either of these models satisfy the criteria for modelling VZV latency.

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