Viral gene expression during acute simian varicella virus infection

Wayne L. Gray, Lisa Mullis and Kenneth F. Soike

Simian varicella virus (SVV) causes a natural varicella-like disease in nonhuman primates. Outbreaks of simian varicella occur sporadically in primate facilities. Simian varicella is used as a model for investigation of varicella-zoster virus (VZV) pathogenesis and latency. In this study, SVV gene expression and histopathology were analysed in tissues of acutely infected vervet monkeys. RT–PCR analysis demonstrated expression of specific SVV immediate early, early and late genes in the skin, lung, liver and ganglia tissues of acutely infected monkeys. Viral antigen expression and histopathology, including necrosis and inflammation, were detected in the skin, lungs, liver and spleen of infected monkeys by immunohistochemical analysis. Viral antigen expression, but little or no histopathology, was evident in the neural ganglia, the eventual site of viral latency. The study provides a foundation for further investigation on the role of viral genes in varicella pathogenesis and latency.

Simian varicella, a naturally occurring disease of Old World monkeys, is caused by a primate herpesvirus, simian varicella virus (SVV, Cercopithecine herpesvirus 7) (Oakes & d’Offay, 1988; White et al., 2001). The disease is characterized by varicella-like symptoms including fever, lethargy and vesicular rash on the face, abdomen and extremities. SVV establishes latent infection in neural ganglia during or following resolution of acute disease (Mahalingam et al., 1991). The mechanism and the time-frame for the establishment of viral latency in the ganglia are unknown. Later in life, the virus may reactivate causing secondary disease and spread of the highly contagious disease to susceptible monkeys. Simian varicella epizootics occur sporadically in captive primate populations (Soike, 1992). Some epizootics involve high morbidity and mortality, while others are associated with milder disease.

SVV is antigenically and genetically related to human varicella-zoster virus (VZV), the aetiologic agent of varicella (chickenpox) and herpes zoster (shingles). The SVV and VZV genomes are similar in size, structure and genetic organization, and share extensive DNA homology (Fletcher & Gray, 1992; Gray et al., 1992; Clarke et al., 1992; Pumphrey & Gray, 1992). The DNA sequence of the SVV genome is known and 69 distinct open reading frames (ORFs) with homology to VZV genes are defined (Gray et al., 2001b). Due to the genetic relatedness of SVV and VZV and the clinical similarities of human and simian varicella, SVV infection of nonhuman primates serves as a valuable experimental model to investigate VZV pathogenesis and latency and to evaluate antiviral strategies (White et al., 2001; Soike, 1992).

The cell-associated nature of SVV and VZV hampers investigation of viral gene expression. However, SVV and VZV gene expression is regulated into immediate early, early and late phases, based on analogy with herpes simplex virus type 1 (HSV-1) and other alphaherpesviruses (Cohen & Straus, 1996). Immediate early genes, such as VZV ORF 62 and ORF 63 (HSV-1 ICP4 and ICP22 homologues, respectively) encode regulatory proteins that are transactivators of other viral genes (Inchauspe et al., 1989; Debrus et al., 1995). Herpesvirus early gene products, such as the thymidine kinase, are generally involved in viral DNA synthesis. The viral late genes encode structural proteins such as the viral capsid proteins and envelope glycoproteins.

The molecular basis of viral pathogenesis and the role of specific viral genes during acute varicella and establishment of viral latency in the neural ganglia are not understood. In this study, SVV gene expression in tissues of acutely infected St Kitts vervet monkeys (Cercopithecus aethiops) was analysed using RT–PCR and immunohistochemistry.

Monkeys were infected by the intratracheal route of infection with $3 \times 10^4$ infected Vero cells. The details of experimental infection and the resulting clinical signs of acute disease in these monkeys have been reported previously (Gray et al., 1995b). Briefly, a transient viraemia was detected on days 3–9 post-infection (p.i.). A vesicular rash was observed on the skin of the face, thorax, abdomen and extremities beginning on day 7 p.i. Monkeys exhibited a mild fever and lethargy. A mild
Table 1. Summary of RT–PCR and histochemistry results

Detection (+) or lack of detection (−) of SVV RNA as indicated by RT–PCR products, SVV antigens as indicated by immunohistochemical staining, or pathology in tissues derived from acutely infected monkeys on day 11 or 12 p.i. Tissues from at least two different monkeys were analysed for each gene or antiserum.

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<tr>
<th>Tissue</th>
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<th>Antiserum†</th>
<th>Pathology‡</th>
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<td>ORF62 RT–PCR</td>
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<td>Ganglia</td>
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* SVV ORFs expressed, as identified by RT–PCR analysis.
† Antiserum specific for gE, gH, SVV virions or normal rabbit serum (NRS) used in histochemical analysis.
‡ Histopathology including necrosis and inflammation detected in tissues.
ND, Not determined.

hepatitis was demonstrated on day 10 p.i. as indicated by elevated serum aminotransaminase enzyme levels. Tissues were harvested on day 10 or 11 p.i., when monkeys exhibited clinical signs of acute varicella.

Expression of specific SVV genes was analysed by RT–PCR in tissues of acutely infected monkeys. Transcription of SVV immediate early genes, ORF 62 and ORF 63, in liver, lung, skin and pooled thoracic dorsal root ganglia (DRG) tissues of acutely infected monkeys was demonstrated by RT–PCR amplification of products of the expected size (Fig. 1a, b, lanes 5, 7, 9, 11, and Table 1). SVV early and late genes, as represented by the viral thymidine kinase (TK) gene and the gB and gE genes, respectively, were expressed in the same tissues of acutely infected monkeys (Fig. 1c, d, e, lanes 5, 7, 9, 11, and Table 1). In control reactions, transcription of each of the SVV genes was confirmed in SVV-infected BSC-1 cells, but not in mock-infected BSC-cells (Fig. 1a–e, lanes 1 and 3). Reactions done without RT confirmed that the products were amplified from RNA and not containing DNA (Fig. 1a–e, lanes 2, 4, 6, 8, 10, 12). Amplified SVV RT–PCR products were not detected in tissues of a mock-infected monkey (data not shown).

Immunohistochemistry was used to detect viral antigen expression in the tissues of acutely infected monkeys. The antisera employed (rabbit anti-SVV virion serum, anti-gE and anti-gH) are specific for SVV and are directed predominantly against viral structural or envelope proteins encoded by late viral genes (Gray et al., 2001a; Ashburn & Gray, 2001).

SVV antigens were readily detected in necrotic keratinocytes and multinuclear giant cells within skin vesicles of acutely infected monkeys. The viral antigens in the intra-epithelial vesicles were demonstrated using anti-SVV virion antiserum (Fig. 2a), and anti-gE and anti-gH antiserum (Table 1). Immunostaining was not detected in skin vesicles when normal rabbit serum was employed (Fig. 2b) or in normal skin adjacent to a vesicle when stained with anti-SVV virion serum, or with anti-gE or anti-gH antisera (data not shown).

Viral infection of the lung tissues was indicated by alveolar wall necrosis and thickening and the presence of intranuclear inclusion bodies in alveolar cells (Fig. 2c). SVV antigens were identified within infected alveolar cells using anti-SVV virion serum (Fig. 2c) and with anti-gE and gH sera (Table 1), but were not detected in infected lung tissues reacted with normal rabbit serum (Fig. 2d) or in mock-infected lung tissue reacted with anti-SVV virion, anti-gE or anti-gH sera (data not shown).

Histopathology, as exhibited by multifocal necrosis and intranuclear inclusion bodies within infected cells, was detected in the liver (Fig. 2e) and spleen (Fig. 2g) tissues of SVV-infected monkeys, but not in the same tissues from a mock-infected monkey (data not shown). Cytoplasmic vacuolization and pyknotic nuclei were prominent in infected liver cells. SVV antigen expression within the liver and spleen tissues of infected monkeys was detected by immunohistochemistry employing SVV-specific antisera (Fig. 2e, g and Table 1). Immunostaining was not evident in infected liver and spleen tissues reacted with normal rabbit serum (Fig. 2f, h, respectively) or in mock-infected liver and spleen tissues reacted with anti-SVV virion serum (data not shown).

In contrast to the tissues described above, histopathology was not evident in thoracic dorsal root ganglia (Fig. 3a, b, c) or in trigeminal ganglia (data not shown) of acutely infected monkeys. Ganglia did not exhibit viral necrosis, intranuclear inclusion bodies within infected neurons, or signs of tissue damage other than a mild inflammation that was detected in some ganglia of the more severely infected monkeys. However, immunostaining using anti-gE (Fig. 3a), gH (Fig. 3b) sera
and anti-SVV virion serum (Table 1) did reveal the presence of viral antigens in neurons of infected ganglia. The incidence of positive immunostaining neurons varied somewhat from ganglia to ganglia and from section to section within individual ganglia. However, approximately 10–15% of neurons in ganglia derived from acutely infected monkeys were positive for SVV antigen expression, as indicated by direct counting of positively stained cells. Viral antigens were also detected in some non-neuronal cells. Immuno-staining was not detected in infected ganglia reacted with normal rabbit serum (Fig. 3c) or in ganglia derived from a mock-infected monkey reacted with anti-SVV virion serum (Fig. 3d).

This study demonstrates SVV IE, early and late gene expression and histopathology in skin vesicles, lung, liver and spleen tissues derived from monkeys with acute simian varicella. The results confirm viral dissemination during simian varicella and indicate that active virus replication and resulting tissue damage occur in the extra-neural tissues during acute infection. Indeed, infectious virus can be isolated from SVV acutely infected skin, liver, lung and spleen tissues and cultured on susceptible cells (Allen et al., 1974).

SVV gene expression was also confirmed in trigeminal and thoracic dorsal root ganglia of acutely infected monkeys. The detection of viral IE, early and late gene transcripts and gE and gH antigens suggest active virus replication within ganglionic neurons. However, several attempts to cultivate infectious SVV from ganglia derived from acutely infected ganglia have been unsuccessful (data not shown). In addition, little or no histopathology or mononuclear cell inflammation was detected within infected ganglia. The results are in general agreement with previous findings of Dueland et al. (1992), who reported detection of SVV antigen in acutely infected ganglia with only mild inflammation and no necrosis or significant histopathology. In contrast, widespread necrosis and inflammation were reported in trigeminal and thoracic ganglia of humans who died of disseminated varicella, although this extensive tissue damage in the ganglia does not likely reflect the situation during natural varicella in children (Cheatham et al., 1956). It is
Fig. 2. SVV antigen expression and histopathology in a skin vesicle and in lung, liver and spleen tissues derived from an acutely infected monkey. Shown are immunohistochemical analyses using rabbit anti-SVV virion serum (a, c, e and g) or normal rabbit serum (b, d, f and h) reacted with an SVV-infected skin vesicle (a, b) and with lung (c, d), liver (e, f) and spleen (g, h) tissues. Immunohistochemistry was performed as previously described (Ashburn & Gray, 2001). The brown staining indicates SVV antigen detection. Representative data from one infected monkey (AI39) are shown.
likely that the lack of tissue damage in the ganglia of acutely infected monkeys is related to the ability of the virus to establish latent infection specifically in neural ganglia.

VZV gene expression in latently infected human ganglia is restricted to transcription of a limited number of viral genes (Cohrs et al., 1996; Kinchington, 1999). SVV gene expression in ganglia of latently infected monkeys has not yet been defined. However, the RT–PCR results of this study demonstrating immediate early, early and late transcription in ganglia during acute SVV infection rather than restricted viral gene expression indicate that SVV latent infection is not established in the ganglia by 10 to 11 days p.i.

The simian varicella model offers an opportunity to investigate molecular aspects involved in varicella pathogenesis. This study provides a basis for further investigation of the role of viral genes during acute varicella and the establishment and maintenance of viral latency. In addition, the immunohistochemical assays for detection of SVV antigens in tissues, along with PCR detection of SVV DNA in tissues (Gray et al., 1998a), will be useful for rapid diagnosis of simian varicella and control of epizootics in facilities housing nonhuman primates.

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References


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