Simian varicella virus: characterization of virion and infected cell polypeptides and the antigenic cross-reactivity with varicella-zoster virus

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Simian varicella virus (SVV) causes a varicella-like disease in non-human primates. In this study, SVV virions were purified from SVV-infected BSC-1 cells by zonal and differential gradient centrifugation and the virion polypeptide composition was analysed by SDS-PAGE. SVV virions had a buoyant density of 1.21 g/ml, identical to the value obtained for varicella-zoster virus (VZV) virions purified by the same method. Electron microscopy of the concentrated SVV virions revealed characteristic herpesvirus morphology. SVV virions consisted of at least 30 polypeptide species ranging from 16K to >200K. The electrophoretic profiles of radiolabelled SVV and VZV virion polypeptides were very similar. Immunoprecipitations of solubilized SVV-infected cell preparations using SVV immune sera revealed at least 18 viral polypeptides with an Mr range of 12K to 142K and six glycoproteins ranging from 46K to 115K. In addition, extensive cross-reactivity between SVV and VZV proteins and glycoproteins was demonstrated by immunoprecipitation with heterologous immune sera. The high degree of antigenic relatedness between SVV and VZV provides further support for simian varicella as a model for VZV infections.

Introduction

Simian varicella virus (SVV) is the aetiological agent of an exanthematous disease of non-human primates which has pathogenic and clinical manifestations similar to those observed in human varicella-zoster virus (VZV) infections (Padovan & Cantrell, 1986). In 1967, Clarkson et al. reported a varicella-like disease in African vervet monkeys (Cercopithecus aethiops) in Liverpool, U.K. Since this initial report, several outbreaks of simian varicella have been reported in various animal facilities around the world (Ayres, 1971; Blakely et al., 1973; McCarthy et al., 1968; Riopelle et al., 1970; Takasaka et al., 1990). The disease is characterized by fever and vesicular skin rash similar to that of human varicella. Severe cases of simian varicella clinically resemble disseminated VZV infections sometimes seen in immunocompromised individuals (Padovan & Cantrell, 1986).

SVV is a highly cell-associated herpesvirus with morphological and molecular properties similar to those of VZV (Ayres, 1971). The SVV genome has an Mr of 76.5 × 10^6 (120.5 kbp) and is composed of an approximately 100 kbp long component covalently linked to an invertible 20-0 ± 0.3 kbp short (S) component (Gray et al., 1992). The S component consists of an approximately 5-3 kbp unique short sequence bracketed by approximately 7-3 kbp inverted repeat sequences. Thus, SVV DNA is similar in size and structure to VZV DNA (Gray et al., 1992). SVV and VZV are distinct viruses as indicated by comparative restriction endonuclease analysis (Gray & Oakes, 1984). However, the SVV and VZV DNAs share 70 to 75% similarity which is distributed across the viral genomes (Gray & Oakes, 1984).

A number of studies have reported antigenic relatedness between SVV and VZV. Allen et al. (1974) demonstrated fluorescent antibody staining of VZV-infected cells using SVV immune sera. Several reports have indicated that SVV and VZV share common antigenic determinants as determined by complement fixation and virus neutralization (Allen et al., 1974; Felsenfeld & Schmidt, 1975, 1977). In addition, patas monkeys immunized with VZV were protected from simian varicella following challenge with SVV (Felsenfeld & Schmidt, 1979). Recently, Soike et al. (1987) reported that immunization of monkeys with purified VZV glycoproteins conferred partial protection against simian varicella following challenge with SVV.

Although the latter studies have indicated that SVV and VZV share cross-reacting antigens, the nature of this antigenic relatedness has yet to be characterized. In fact, the protein composition of SVV has not been previously reported. The purpose of this study was to characterize the polypeptides of SVV virions and infected cells and to
define the antigenic polypeptides of the virus. In addition, the cross-reactive antigens of SVV and VZV were characterized by immunoprecipitation with heterologous immune sera.

**Methods**

**Viruses and cell culture.** The Delta herpesvirus (DHV) strain of SVV was used in this study. DHV, provided by Dr Ken Soike (Delta Regional Primate Center, Covington, La., U.S.A.), was originally isolated from the blood of an infected patas monkey during the 1973 simian varicella epizootic at the Delta Regional Primate Center (Allen et al., 1974). The virus was propagated in African green monkey kidney (Vero or BSC-1) cells and was passaged by mixing infected and uninfected cells at a ratio of 1:4, respectively. Vero and BSC-1 cells were cultured in Eagle's MEM (EMEM) supplemented with gentamicin (50 µg/ml) and 5% newborn calf serum. VZV, strain Ellen, was propagated in human foreskin or MRC-5 cells in EMEM supplemented with gentamicin and 10% foetal bovine serum (FBS). Restriction endonuclease analysis of SVV and VZV DNA isolated from the seed stocks was conducted throughout the study to ensure that no cross-contamination had occurred.

**Purification of SVV virions**

SVV or mock-infected BSC-1 cells (1.5 × 10⁷) were labelled by the addition of 10 µCi/ml [³⁵S]methionine (NEN) in methionine-deficient EMEM (Gibco) supplemented with 2% dialysed FBS, or with 10 µCi/ml [³²P]lucosamine (NEN) in EMEM supplemented with 2% FBS. Cells were incubated at 37 °C for 24 h (mock-infected) or until maximum c.p.e. (SVV-infected) was observed. Cells were harvested by scraping into the medium, washed twice with cold PBS and resuspended in 2 ml solubilization buffer (25 mM-Tris-HCl, 250 mM-NaCl, 5 mM-EDTA, 1X Triton X-100, 0.5X deoxycholate) containing protease inhibitors (1% aprotinin, 30 µg/ml PMSF). Solubilized extracts were sonicated and clarified by centrifugation at 26000 g for 30 min at 4 °C in an SW-40 rotor. Supernatants were stored at -70 °C until used.

**PAGE.** Radioactively labelled, solubilized virions and immunoprecipitates of infected cell and virion lysates were analyzed in gradient slab gels (7.5 to 15%, acrylamide) using the discontinuous SDS-PAGE system described by Laemmli (1970). Gels were soaked in fluorography film at -70 °C. Exposure times varied between 5 and 15 days. The refractive index of each were determined. Virion density was infected cell preparations, but not in the mock-infected cell preparations. A compact band was observed in the SVV-0.5 u-Tris-buffered saline (TBS pH 7.7) and centrifuged at 94000 g for 10 min at 4 °C in an SW-40 rotor. A wide band was collected from near the visible band observed in the SVV gradients. This corresponding fraction of the mock-infected BSC-1 cell virion was concentrated, and analyzed by electron microscopy. Enveloped virions with characteristic herpesvirus morphology were detected in the concentrated SVV fraction 10, but were not observed in the corresponding fraction of the mock-infected BSC-1 cell gradients. The SVV virions consisted of a viral nucleocapsid (approximately 100 nm in diameter) surrounded by an envelope giving the virion an approximate diameter of 170 to 200 nm (Fig. 1b). No virions were detected by electron microscopy in pooled fractions representing the upper and lower regions of SVV or mock-infected BSC-1 cell gradients (data not shown). The refractive index of 1.3982 determined for the virion-containing fraction corresponded to a density of approximately 1.21 g/ml.
Polypeptides of simian varicella virus

Analysis of SVV virion polypeptides

Purified \(^{35}\)S-methionine-labelled SVV virion preparations were solubilized and subjected to electrophoresis on 7.5 to 15% SDS-polyacrylamide gels for the purpose of identifying the polypeptide composition of SVV. Fig. 2 shows the electrophoretic profile of the SVV virion polypeptides which consists of at least 30 polypeptide species ranging from 16K to >200K in size. The most prominent labelled polypeptide species are indicated by an approximate \(M_r\) designation. No polypeptide bands were observed in samples of fractions 10, 11 and 12 from \(^{35}\)S-methionine-labelled mock-infected BSC-1 cell gradients (Fig. 1, data not shown). SVV and VZV virion polypeptides isolated by identical procedures were compared by SDS-PAGE (Fig. 2). The banding patterns of the SVV and VZV virion polypeptides were strikingly similar.

In order to determine the immunogenic virion polypeptides, samples of \(^{35}\)S-methionine-labelled SVV virions were solubilized and precipitated with anti-SVV immune sera. The precipitates were analysed by SDS-PAGE to determine which immunogenic polypeptides comigrated with SVV-specific structural proteins. At least four SVV virion polypeptides (113K, 82K to 71K, 66K and 46K) were immunoprecipitated by the anti-SVV immune sera (Fig. 3, lane 2).
Immunoprecipitable SVV-infected cell polypeptides and glycoproteins

The immunogenic polypeptides and glycoproteins of SVV-infected cells were identified by immunoprecipitation with SVV immune sera. Mock- or SVV-infected Vero cells were radiolabelled with either [35S]methionine to label total protein or [3H]glucosamine to label glycoproteins. Normal monkey serum (NMS) or SVV immune serum was used to immunoprecipitate the mock- or SVV-infected cell lysates and the precipitates were fractionated by 7.5 to 15% SDS-PAGE (Fig. 4).

Lane 1 demonstrates the immunogenic SVV-specific polypeptides precipitated with the SVV immune sera. At least 18 [35S]methionine-labelled polypeptides ranging from 12K to 142K were detected by anti-SVV immune sera. Six [3H]glucosamine-labelled glycoproteins ranging from 46K to 115K in size were precipitated (lane 5). These glycoproteins were designated gp115, gp100, gp82, gp76, gp66 and gp46 [Fig. 4 (*)]. The predominant non-glycosylated species included polypeptides of 142K, 128K, 111K, 73K and 44K in size. A faint band at 200K could be observed after prolonged exposure times. No background was observed in the negative control immunoprecipitations except for a minor 42K band observed when uninfected Vero cell lysates were immunoprecipitated with SVV immune serum (lane 3) or NMS (lane 4).

Antigenic cross-reactivity between SVV and VZV polypeptides

Determination of cross-reactive SVV and VZV polypeptides was achieved by immunoprecipitation analysis. SVV- or VZV-infected cell extracts were labelled with [35S]methionine or [3H]glucosamine and precipitated using anti-VZV serum or SVV-specific immune sera.
The cross-reacting viral polypeptides were identified by SDS–PAGE analysis on 7.5 to 15% gradient gels.

Analyses of SVV-infected cell proteins and glycoproteins immunoprecipitated with heterologous VZV immune serum are shown in Fig. 5(a) and 5(b), respectively. Multiple [35S]methionine-labelled SVV polypeptides were precipitated by the anti-VZV serum (Fig. 5a, lane 3). The predominant cross-reactive species included polypeptides with Mr's of 115K, 111K, 66K and 46K. Each of the six [3H]glucosamine-labelled SVV glycoproteins (gp115, gp100, gp82, gp76, gp66 and gp46) was immunoprecipitated by the anti-VZV serum (Fig. 5b, lane 4). The SVV proteins (Fig. 5a, lane 2) and glycoproteins (Fig. 5b, lane 2) precipitated with the homologous SVV antisera are shown for reference.

Analyses of VZV-infected cell proteins and glycoproteins immunoprecipitated with heterologous SVV immune sera are shown in Fig. 6(a) and 6(b), respectively. SDS–PAGE of [35S]methionine-labelled VZV-specific polypeptides precipitated with anti-SVV immune sera revealed a large number of cross-reactive proteins (Fig. 6a, lane 3) with banding patterns virtually identical to those observed for VZV polypeptides immunoprecipitated with the homologous VZV immune serum (Fig. 6a, lane 4). The SVV immune sera immunoprecipitated VZV glycoproteins with apparent Mr's of 118K to 105K, 92K, 82K, 73K, 63K and 45K (Fig. 6b, lane 3).

**Discussion**

This study describes the initial purification of SVV virions and characterization of SVV virion and infected cell polypeptides. The immunogenic polypeptides of SVV virions and infected cells were elucidated and the antigenic relationship between SVV and VZV was further defined.

Analysis of SVV virions revealed properties similar to those of VZV virions. SVV virions were isolated using zonal centrifugation in glycerol followed by equilibrium flotation in glycerol/potassium tartrate gradients. Electron microscopy revealed enveloped virions consistent in size (170 to 200 nm) with that previously observed for VZV (Gelb, 1985). The virus was determined to have a calculated buoyant density of 1.21 g/ml. Shemer et al. (1980) used a similar procedure to isolate VZV virions and obtained an identical value for the density of VZV. The polypeptide composition of the purified SVV virions consisted of over 30 proteins ranging from 16K to >200K in size and the banding pattern of the proteins was similar to that observed for VZV.

The immunogenic polypeptides of SVV virions and infected cells were determined by immunoprecipitation with anti-SVV immune sera. At least 18 viral polypeptides with an Mr range of 12K to 142K and six glycoproteins ranging from 46K to 115K were detected by SDS–PAGE analysis. Of particular interest was the determination of the viral glycoproteins of SVV-infected cells, for the glycoproteins of the family herpesviridae...
have been shown to be dominant immunogenic viral antigens (Eberle & Mou, 1983; Spear, 1984; Stinski, 1990). Six SVV-specific glycoproteins were identified and were designated gp115, gp100, gp82, gp76, gp66 and gp46 (Fig. 4, lane 5). The number and size range of the SVV glycoproteins immunoprecipitated with SVV immune sera is consistent with that observed for the glycoproteins of VZV-infected cells immunoprecipitated with VZV immune serum (Fig. 6b, lane 4; Davison et al., 1986; Forghani et al., 1990).

A number of studies employing immunofluorescence (Allen et al., 1974), virus neutralization (Felsenfeld & Schmidt, 1975, 1977), complement fixation (Blakely et al., 1973; Felsenfeld & Schmidt, 1975, 1977) and in vivo protection assays (Felsenfeld & Schmidt, 1979; Soike et al., 1987) have demonstrated antigenic relatedness between SVV and VZV. The present study defined the specific cross-reactive polypeptide species of SVV- and VZV-infected cells by immunoprecipitation with heterologous immune sera. Extensive cross-reactivity was observed between a large number of SVV and VZV polypeptides. VZV immune serum precipitated each of the six SVV glycoproteins (Fig. 5b, lane 4). In the reciprocal study, SVV immune sera reacted strongly with a VZV 63K glycoprotein, which corresponds in size with VZV gpII (Fig. 6b, lane 3). VZV gpII and gB of herpes simplex virus type 1 (HSV-1) have been shown to be antigenically related (Kuhn et al., 1990) and to have DNA sequence similarity (Davison & Scott, 1986). In turn, the HSV-1 gB has been previously shown to be antigenically conserved among a number of other herpesviruses (Balachandran et al., 1987; Eberle et al., 1989). The SVV immune sera also exhibited partial reactivity to the remaining VZV glycoproteins with apparent Mr’s of 118K to 105K, 92K, 82K, 73K and 45K (Fig. 6b, lane 3). The 118K to 105K and 92K VZV glycoproteins correspond in size to VZV gpIII and gpI, respectively (Davison et al., 1986; Forghani et al., 1990; Grose, 1990). The 82K and 73K VZV glycoproteins correspond in size to VZV gpI precursor forms, and the 45K VZV glycoprotein corresponds in size to VZV gpI and gpIV precursor products (Davison et al., 1986; Grose, 1990). These results suggest that SVV and VZV possess analogous glycoproteins that may perform similar functions. Studies using monospecific and monoclonal antisera will further define the cross-reactive SVV and VZV glycoproteins. Recent studies involving DNA hybridizations of specific VZV glycoprotein gene probes (gpI, gpII, gpIII and gpIV) to SVV DNA have provided further evidence that SVV and VZV share homologous glycoprotein genes (C. Y. Pumphrey & W. L. Gray, unpublished results).

The molecular characterization of SVV proteins and glycoproteins will be important in future studies to determine which viral polypeptides are effective in eliciting both humoral and cell-mediated immune responses in SVV-infected monkeys. In addition, the elucidation of the immunogenic SVV polypeptides could lead to the eventual development of a subunit vaccine which might be useful in eliminating future epizootics of simian varicella in primate centres. Studies of the immune response to the viral polypeptides in SVV-infected monkeys may, by analogy, lead to a greater understanding of the proteins important for the induction and maintenance of immunity in VZV-infected individuals.

SVV infection of non-human primates has been used as a model for the study of VZV antiviral agents and vaccines (Arvin et al., 1983; Lake-Bakaar et al., 1988; Soike et al., 1987, 1990). A recent study demonstrating that SVV DNA can be detected in the dorsal root ganglia of latently infected monkeys indicates that the SVV model may be useful for investigating VZV latency (Mahalingam et al., 1991). The extensive cross-reactivity between SVV and VZV polypeptides reported in this study provides further support for simian varicella as an animal model for VZV infections.

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References


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