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Replication of Simian Herpesvirus SA8 and Identification of Viral Polypeptides in Infected Cells

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The replication of the simian herpesvirus SA8 in Vero cells was examined. The time course of replication of the simian herpesvirus SA8 was found to be similar to that of the herpes simplex viruses. Infectious progeny virions were first detectable by 6 h postinfection and were readily released into the extracellular fluids beginning at 9 h postinfection. All cell lines tested, with the exception of Madin-Darby canine kidney cells, were permissive for SA8. Analysis of SA8-infected cells by sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed over 40 infected cell polypeptides ranging in molecular weight from 158,000 to less than 10,000. Of these proteins, 23 were present in virions. Three classes of infected cell polypeptides could be identified based on the kinetics of their synthesis. Post-translational processing of several SA8-induced proteins was also observed in pulse-chase experiments. Six distinct SA8-specific glycoproteins ranging from 118,000 to 19,500 daltons were also identified in infected cells. Of these glycoproteins, five were present in virions.

The widespread use of simians in medical research has resulted in the isolation of over 30 herpesviruses from various species of monkeys and apes (2, 16, 25). Some of the simian herpesviruses have been worked with extensively, but others have been largely ignored. Perhaps the best-known and most studied of the neurotropic simian herpesviruses is *Herpesvirus simiae* (monkey B virus). The relationship between *H. simiae* and its natural host (*Macaca* spp.) is quite similar to that of herpes simplex virus type 1 (HSV-1) and humans; the virus causes oral and labial lesions, may recur spontaneously, is shed asymptomatically in the saliva, and can be isolated from ganglia of latently infected monkeys (5, 19, 26, 31). Despite its relatively mild pathogenicity in *Macaca mulatta*, *H. simiae* has been shown repeatedly to be highly pathogenic for humans, death being the usual outcome (6, 7, 10, 34, 35). Perhaps partly because of these observations, very little research has been done on *H. simiae*, and even less has been done on the other known neurotropic herpesviruses of nonhuman primates, despite their potential medical importance to researchers using nonhuman primates.

The simian herpesvirus SA8 was originally isolated from neurological tissues of asymptomatic African vervet monkeys (21, 22) and later from asymptomatic baboons as well (28). Despite the apparently widespread infection of Old World simians with SA8 (17) and the widespread use of these animals in research, very little is known about the biological, biochemical, or immunological properties of SA8. Based on the rapid development of nuclear inclusions and later formation of syncytia in infected cells, Malherbe and co-workers concluded that SA8 was a herpesvirus, possibly representing another serotype of *H. simiae* (23, 24). SA8 has since been shown to cross-react with both *H. simiae* and HSV-1 (12, 15, 37). Other than its antigenic relatedness to these viruses, very little is known about the molecular biology of this virus. In this report, basic characteristics of the replication of SA8 and the synthesis of viral polypeptides in infected cells are described.

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MATERIALS AND METHODS

Virus, cells, and media. The 3264 strain of SA8 originally obtained from H. Malherbe was used throughout these studies. Vero and HEp-2 cells were obtained from the American Type Culture Collection, Rockville, Md. African green monkey kidney, bovine embryonic kidney, and Madin-Darby canine kidney (MDCK) cells were obtained from Flow Laboratories, Inglewood, Calif. Mouse L and RK-13 cells were obtained from R. Courtney, University of Tennessee, Knoxville; owl monkey kidney cells were obtained from B. Fleckenstein, University of Erlangen-Nürnberg, Erlangen, West Germany; and human foreskin fibroblast cells were obtained from J. Zaia, City of Hope Medical Center, Duarte, Calif. Vero, HEp-2, MDCK, L, and RK-13 cells were grown in Dulbecco modified Eagle medium supplemented with 10% calf bovine serum; African green monkey kidney, bovine embryonic kidney, owl monkey kidney, and human foreskin fibroblast cells were grown in Dulbecco modified Eagle medium containing 10% fetal calf serum. For all experiments, cells were maintained in Eagle minimal essential medium containing 2% calf serum and 0.225% $NaHCO_3$. In experiments employing ^{14}C-amino acids, minimal essential medium containing one-tenth the normal concentration of amino acids was used (8).

Chemicals and radioisotopes. All chemicals for gel electrophoresis were purchased from Bio-Rad Laboratories, Richmond, Calif. L-[1-^{3}H]fucose (2 to 10 Ci/mmol), D-[6-^{3}H]galactose (20 to 40 Ci/mmol), D-[6-^{3}H]glucosamine hydrochloride (20 to 40 Ci/mmol), [*methyl*-^{3}H]thymidine (25 Ci/mmol), D-[1-^{14}C]glucosamine hydrochloride (50 to 60 mCi/mmol), D-[2-^{7}H]mannose (10 to 20 Ci/mmol), L-[^{35}S]methionine (>800 Ci/mmol), and ^{14}C-amino acids mixture (>50 mCi/milligram-atom) were purchased from Amersham Corp., Arlington Heights, Ill.

Infection, labeling, and antigen preparation. Unless otherwise stated, an input multiplicity of infection of at least 20 was used in all experiments. After a 30-min adsorption period, the inoculum was removed and maintenance medium was added. For labeling with radioisotopes, medium was changed at the time of isotope addition as specified in the

text or the figure legends. Cells were harvested by scraping into the medium, washed once with ice-cold phosphate-buffered saline, and suspended in cold, distilled water. Samples were then sonicated for 30 s with a Heat Systems W-220F sonicator (Heat Systems-Ultrasonics, Inc., Plainview, N.Y.) equipped with a microtip probe and frozen at −20°C.

Experiments designed to determine the incorporation of radiolabeled precursors into DNA and protein were carried out as follows. Replicate uninfected and SA8-infected cell cultures were labeled at 0.5 h postinfection (p.i.) with [^{3}H]thymidine (5 μCi/ml) and [^{35}S]methionine (10 μCi/ml). Beginning at 1 h p.i., uninfected and infected cultures were harvested at hourly intervals. Cells were scraped into the medium and washed once with ice-cold phosphate-buffered saline. Cell pellets were washed twice more with ice-cold 10% trichloroacetic acid, solubilized in NCS (New England Nuclear Corp., Boston, Mass.), and assayed for radioactivity. Results are expressed as the counts per minute incorporated per 10^6 cells (= infected CPM minus uninfected CPM).

Gel electrophoresis. Details of methods used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) have been described previously (8). The apparent molecular weights of SA8 infected cell polypeptides (ICPs) were estimated from samples run on gels of four different acrylamide concentrations, using commercial molecular weight standard kits (Bio-Rad) for calibration of the gels.

Preparation of antisera. Rabbit anti-SA8 serum was prepared in New Zealand white rabbits. HEp-2 cells were infected with SA8 and scraped into the medium at 24 h p.i. Cells were washed once, suspended in sterile, distilled water at 10^7 cells per ml, sonicated, and frozen. For the primary immunization, SDS was added to a final concentration of 0.5%, and the disrupted cells were mixed 1:1 with complete Freund adjuvant and injected intradermally at multiple sites along the back. Beginning 4 weeks later, booster immunizations were given intramuscularly every 2 weeks, using a 1:1 mixture of infected cells and incomplete Freund adjuvant. The first booster antigen was made 0.1% in SDS; all subsequent immunizations were with nondenatured antigen. Rabbits were bled approximately 4 months after the initial immunization. Before use, sera were adsorbed with rabbit liver powder and uninfected Vero and HEp-2 cells as described previously (8).

Viral growth curve. Vero cell monolayers grown in 35-mm plastic petri dishes were infected at a multiplicity of infection of 5 PFU per cell and placed at 37°C. After a 30-min adsorption period, the inoculum was removed and the cell monolayer was washed once for 1 min with warm trypsin-EDTA solution to inactivate virions that had adsorbed to, but not penetrated, host cells. Then cells were washed with warm phosphate-buffered saline, 1 ml of maintenance medium was added, and cells were returned to 37°C. At various times p.i., cells were scraped into the medium and pelleted. The supernatant was removed and frozen at −80°C for determination of extracellular infectious particles. The cell pellet was suspended in 1 ml of maintenance medium, sonicated for 30 s, and frozen at −80°C for assay of cell-associated infectious particles. Infectivity assays were performed in Vero cells with a 1% methylcellulose overlay and neutral red stain at 2 days p.i.

Purification of SA8 virions. SA8 virions were partially purified from the extracellular medium by methods previously described for HSV-1 (9). Briefly, SA8-infected Vero cells were labeled with [^{35}S]methionine (10 μCi/ml) from 4 to 24 h p.i. Intact cells were pelleted from the medium by low-speed centrifugation. Virions were then pelleted by centrifugation at 15,000 × *g* for 30 min. The virion pellet was resuspended and layered onto 20 to 60% continuous sucrose gradients. Gradients were centrifuged at 107,000 × *g* for 1 h, and the virion band was collected. Virions were pelleted by centrifugation at 36,000 × *g* for 15 min and suspended in disruption buffer for analysis by SDS-PAGE.

RESULTS

Replication of SA8. The time course of the intracellular production and subsequent release of infectious virus from infected cells was examined. Replicate cultures of Vero cells were infected at a multiplicity of infection of 5 PFU per cell. After a 30-min adsorption period, infected cells were washed to remove unadsorbed virus and incubated at 37°C. At various times p.i., the extracellular fluid and infected cells were assayed separately for infectious particles by using a plaque assay. The production of infectious progeny virions after infection is shown in Fig. 1A. As is typical of viral growth curves, an initial drop in PFU was observed after inoculation, presumably due to uncoating of the input virus. Intracellular progeny virus was first detectable at significant levels at 6 h p.i., with maximal titers being reached by 15 h p.i. Release of infectious virus into the extracellular fluids lagged approximately 4 h behind the production of intracellular virions, beginning at 9 to 10 h p.i.

The incorporation of [^{3}H]thymidine ([^{3}H]TdR) into DNA and of [^{35}S]methionine into protein after infection of Vero cells with SA8 was also investigated (Fig. 1B). In comparing incorporation of these isotopes into acid-precipitable material in infected cells and uninfected cells, a rapid drop in the incorporation of [^{3}H]TdR was observed in SA8-infected cells which persisted until 2 to 3 h p.i. This decrease in incorporation of DNA precursor presumably represents an inhibition of host cell DNA synthesis, as has been previously reported to occur after infection of cells with other herpesviruses (3, 33). By 4 h p.i., incorporation of [^{3}H]TdR began to increase, with peak levels of incorporation being reached at 7 to 9 h p.i. The increase in DNA synthesis both parallels the production of intracellular virions and precedes their appearance.

The incorporation of methionine into acid-precipitable material also showed an immediate drop after infection, as has been observed for HSV-1 (14, 33). This was followed by an increase in incorporation, which peaked at 8 h p.i. and subsequently decreased. The time course of incorporation of methionine into proteins followed that of thymidine incorporation and preceded the appearance of infectious virions in infected cells.

The replication of SA8 in Vero cells was also followed by immunofluorescent staining of infected cell cultures (data not shown). These experiments paralleled the results shown in Fig. 1; infected cells were easily detectable by 4 h p.i., with fluorescence localized to the nucleus and the perinuclear region. Cytopathic effect (CPE) in the form of cell retraction and rounding was evident by 6 h p.i. At 25 h p.i., the entire cell monolayer exhibited 4+ CPE, again demonstrating the rapid growth of SA8. Nuclear inclusions were also visible in infected cells, as has been previously described (23).

The ability to replicate in cell lines derived from species other than the natural host of the virus is a characteristic common to a number of neurotropic herpesviruses that are also pathogenic in xenogeneic host species. Therefore, we examined the ability of SA8 to replicate in various cell lines derived from several different species. The results of these

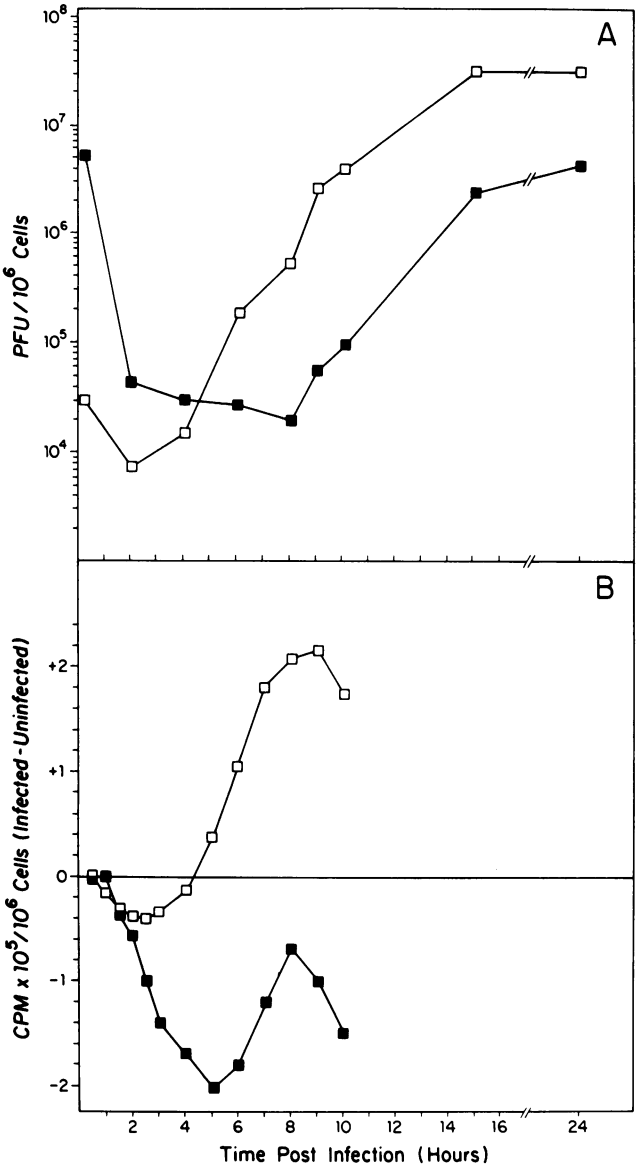

FIG. 1. Time course of SA8 replication. Vero cell monolayers (10^6 cells) were infected with 5 PFU of SA8 per cell and incubated at 37°C. (A) Production of infectious virus at different times p.i. Both intracellular and cell-associated virions (□) and virus released into the extracellular medium (■) were quantitated. (B) Incorporation of both [^{3}H]TdR (5 μCi/ml) into DNA (□) and [^{35}S]methionine (10 μCi/ml) into protein (□) in infected cells relative to their incorporation in uninfected cells.

experiments are summarized in Table 1. At 24 h p.i., all cell lines examined, with the exception of MDCK cells, exhibited extensive CPE; MDCK cell cultures maintained for up to 72 h p.i. did not exhibit any viral CPE. Quantitation of infectious particles present at 24 h p.i. revealed similar results. Excluding MDCK cells, the amount of infectious virus ranged from 8×10^5 to 8×10^7 PFU per culture. No increase in infectious particles above the inoculum residua was observed in SA8-infected MDCK cells.

The synthesis of virus-specific polypeptides in different cell lines was also examined by SDS-PAGE of infected cells labeled with [^{35}S]methionine from 5 to 24 h p.i. Again

TABLE 1. Replication of SA8 in different cell lines

Cell line[a]	CPE[b]	PFU[c]	Protein synthesis[d]	
			Host cell	Virus-specific
Vero	+	5.6×10^7	−	+ (3.9)
AGMK	+	ND[e]	−	+
OMK	+	7.2×10^6	−	+ (1.5)
HFF	+	3.3×10^7	−	+ (4.0)
HEp-2	+	8.2×10^7	−	+ (6.4)
RK-13	+	4.0×10^7	−	+ (6.3)
BEK	+	1.2×10^6	−	+ (1.7)
L	+	6.0×10^6	−	+ (2.0)
MDCK	−	3.7×10^4	+	± (6.3)

[a] Abbreviations: AGMK, African green monkey kidney; OMK, owl monkey kidney; HFF, human foreskin fibroblast; BEK, bovine embryonic kidney.

[b] Confluent cell monolayers cultures grown in 35-mm petri dishes were infected with 5 to 10 PFU per cell. Cultures were incubated for 24 h at 37°C and then examined for signs of viral CPE.

[c] At 24 h p.i., cells were harvested and infectious virus was quantitated by plaque assay. Results represent total PFU per culture.

[d] A duplicate set of cell cultures were infected and labeled with [^{35}S]methionine (3.0 μCi/ml) from 5 to 24 h p.i. At 24 h p.i., cells were harvested and analyzed by SDS-PAGE. Infected and uninfected cells were compared to determine (i) whether host cell-specific protein synthesis occurred between 5 and 24 h p.i., and (ii) whether virus-specific proteins were synthesized. Numbers in parentheses represent counts per minute $\times 10^{-6}$ of [^{35}S]methionine incorporated per infected cell culture. These values (with the exception of MDCK cultures) reflect the relative amounts of methionine-labeled viral proteins present in each of the infected cell preparations.

[e] ND, Not determined.

excluding MDCK cells, the polypeptides present in all cell lines was quite similar. There was little difference in the apparent molecular weights or relative quantities of most of the infected cell-specific proteins in the different cell lines. Some variation in the relative quantity and electrophoretic mobility of the infected cell glycoproteins was also evident, probably reflecting the differential glycosylation of these polypeptides in the different cell lines, as has been observed for HSV-1 and HSV-2 (1, 9, 30; unpublished data). Paralleling the lack of development of CPE in MDCK cells, the profile of polypeptides labeled in SA8-infected MDCK cells from 5 to 24 h p.i. was quite dissimilar to that observed in the other cell lines. SA8 failed to inhibit the synthesis of host cell-specific protein synthesis in MDCK cells. In addition, only a very limited subset of the ICPs observed in infected Vero cells was present in infected MDCK cells. These included ICPs 2, 4/5, 11, and 28/29 (see below for ICP designations). Very few of the major structural proteins of SA8 appeared to be synthesized in infected MDCK cells. Thus, MDCK cells appeared to be nonpermissive for SA8 replication, whereas all other cell lines examined were capable of supporting SA8 infection.

Identification of SA8-induced ICPs. Infection of Vero cells with SA8 resulted in a productive infection, as evidenced by the rapid development of CPE (detectable as early as 5 h p.i.), the time course of viral antigen synthesis, and the production of infectious progeny virions. In addition, infection with SA8 resulted in the rapid inhibition of host cell-specific protein synthesis (discussed below). Thus, the incubation of infected cells with [^{35}S]methionine from 5 to 24 h p.i. resulted in labeling of primarily virus-specific polypeptides. Analysis of infected Vero cell lysates on gels of

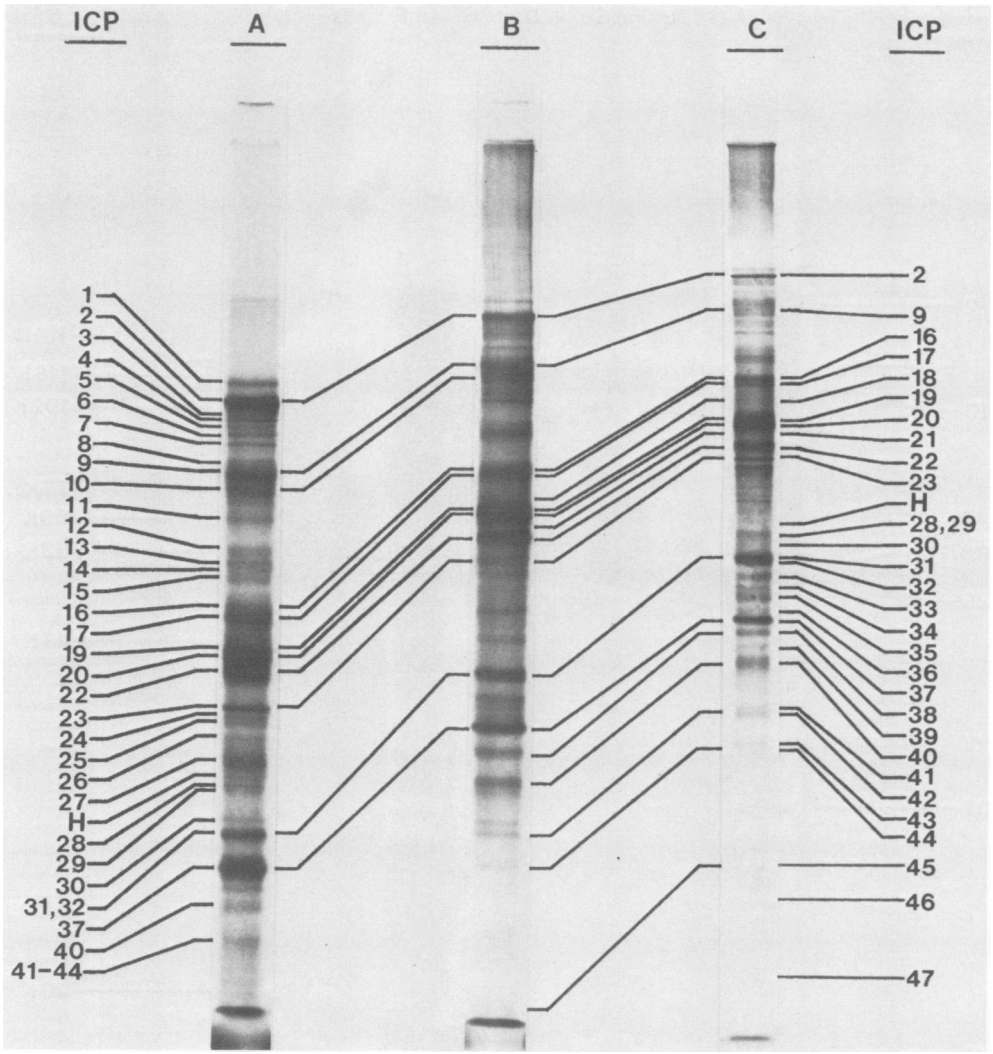

FIG. 2. Identification of infected cell-specific polypeptides in SA8-infected cells. Vero cells infected with SA8 were labeled with ^{14}C-amino acids (2.5 μCi/ml) from 5 to 24 h p.i. Infected cells were electrophoresed on (A) 8.6%, (B) 12%, and (C) 16% acrylamide gels. All bands which were either not found in uninfected cells or were more intensely labeled than comigrating bands found in uninfected cells were assigned an ICP number. Molecular weights of the ICPs identified here are given in Table 2.

different strengths permitted the identification of 47 virus-induced ICPs (Fig. 2). These polypeptides ranged in apparent molecular weight from 158,000 to <10,000, and as has been observed in other herpesvirus systems (14, 20, 27, 32), the molar ratios of the ICPs showed considerable variation. As appears typical of other herpesviruses, a methionine-labeled 145K to 160K polypeptide was presented as a major polypeptide in infected cells (ICP2; 149,000).

Identification of viral glycoproteins. To identify carbohydrate-containing SA8-specific polypeptides, infected Vero cells were labeled from 5 to 24 h p.i. with ^{3}H-labeled fucose, mannose, galactose, or glucosamine. Infected cell lysates were then analyzed by SDS-PAGE together with ^{14}C-amino acid-labeled infected cell lysates for reference (Fig. 3). Carbohydrate label was detected in seven major regions having apparent molecular weights of 119,000, 102,000, 77,000, 69,000, 63,000, 47,000, and 20,000. The two most prominent bands (119,000 and 63,000) comigrated with ICP9 and ICP22, respectively. Although they were most efficiently labeled with glucosamine, all four radiolabeled carbohydrates were incorporated into these two glycoproteins. The 77K and 69K bands also incorporated all four radioactive carbohydrates, although the 69K band appeared to have relatively more mannose label associated with it than did the 77K band, indicating that the 69K species may represent a precursor form. The 77K and 69K bands migrated in the same region of the gel as ICP16/19 and ICP19, respectively. The 20K glycoprotein was only labeled with mannose and glucosamine and comigrated with ICP45. The 102K band was labeled with galactose and glucosamine, whereas the 47K band was observed only with glucosamine label. The 47K and 102K bands did not comigrate with any readily identifiable polypeptides in cells labeled with either [^{35}S]methionine or ^{14}C-amino acids. In Fig. 3, some incorporation of glucosamine and galactose into ICP2 is also evident. The incorporation of carbohydrate precursors into ICP2 probably results from randomization of the isotope rather than bona fide glycosylation of this protein, since longer exposure of films revealed some labeling of other abundant methionine-labeled polypeptides (data not shown).

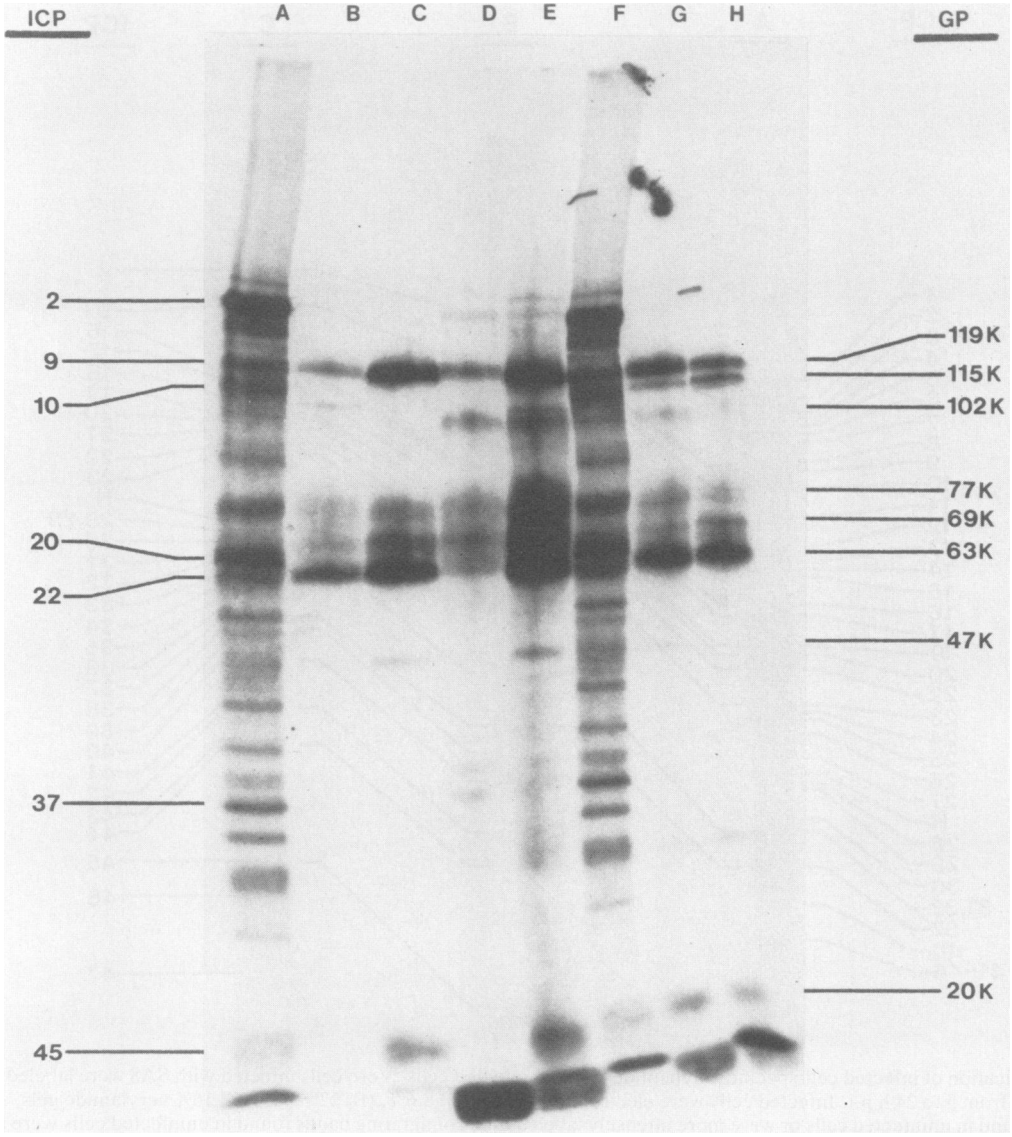

FIG. 3. Identification of SA8 glycoproteins. SA8-infected Vero cells were labeled from 5 to 24 h p.i. with ^{14}C-amino acids at 2.5 μCi/ml (lanes A and F), [^{3}H]fucose (lane B), [^{3}H]mannose (lane C), [^{3}H]galactose (lane D), or [^{3}H]glucosamine (lane E), all at 20 μCi/ml. Infected cells were also pulse-labeled at 6 h p.i. with a combination of [^{3}H]galactose (50 μCi/ml) and [^{3}H]mannose (100 μCi/ml) for 30 min and either harvested immediately (lane H) or chased for 4 h in growth medium (lane G). All samples were analyzed on 12% polyacrylamide gels. The apparent molecular weights of the major glycoprotein species present in infected Vero cells are indicated at right; some major ICPs are identified at left.

To identify any possible presursor forms of the mature glycoproteins, pulse-chase experiments were performed. Infected cells were pulse-labeled at 6 h p.i. with [^{3}H]mannose and [^{3}H]galactose and were harvested either immediately at the end of the 30-min pulse-labeling period (Fig. 3, lane H) or after a 4-h chase period (Fig. 3, lane G). Although the same number of labeled bands was present in both samples, two of the bands (115,000 and 69,000) were more prominent in the pulse sample than in the chase sample. The intensity of the 115K band decreased during the chase period, with a concomitant increase occurring in the intensity of the 119K glycoprotein. Similarly, the 69K glycoprotein was more intensely labeled in the pulse sample than in the chase samples, suggesting that it too is a precursor. Based on its increase in isotope incorporation during the chase period and its similar apparent molecular weight, the 77K glycoprotein represents the most likely product derived from the 69K precursor glycoprotein.

Kinetics of synthesis of SA8 polypeptides. For examination of the time course of synthesis of the various ICPs, cells infected with SA8 were labeled with [^{35}S]methionine for 1-h intervals beginning 1 h p.i. The time of maximal synthesis of individual virus-specific polypeptides was then determined based on the incorporation of [^{35}S]methionine into the protein bands resolved on SDS-PAGE. The results of these experiments are shown in Fig. 4. Host cell-specific polypeptides were easily identifiable both by their co-electrophoresis with proteins present in uninfected cells and by the rapid decrease in the incorporation of radiolabel into these bands with increasing time p.i. Synthesis of most host cell proteins

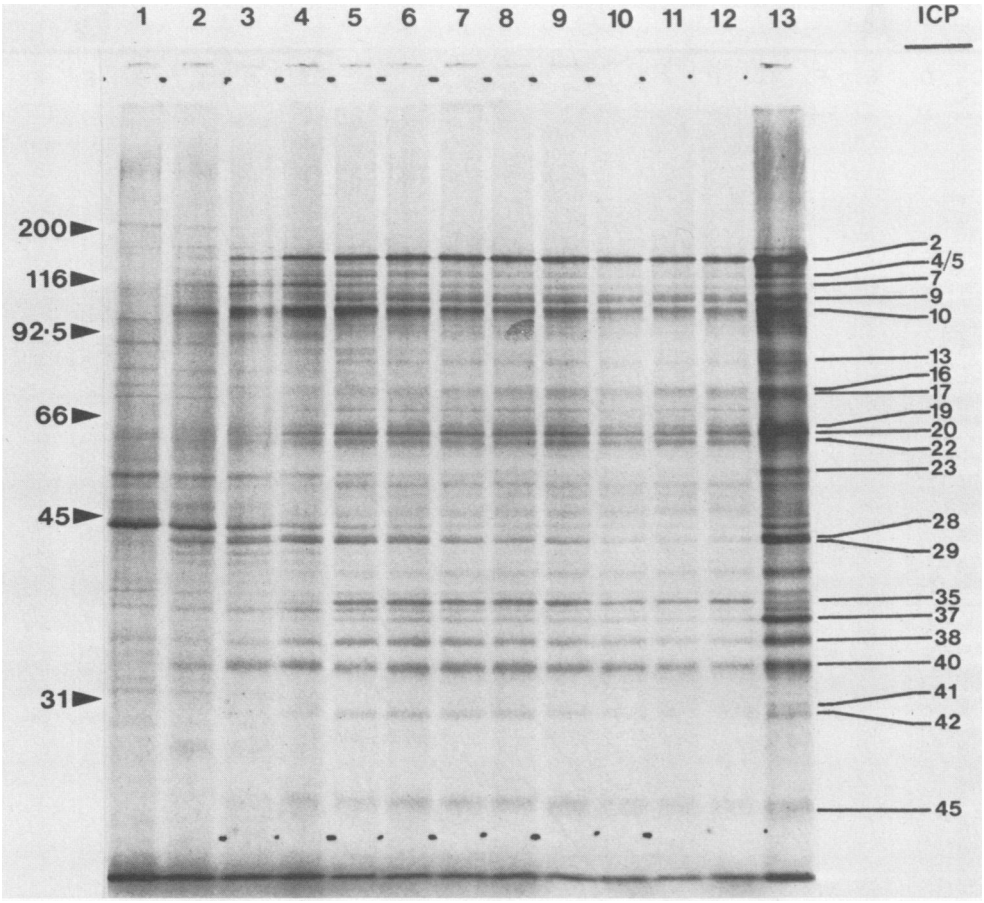

FIG. 4. Kinetics of synthesis of SA8-induced polypeptides. Vero cells infected with SA8 were labeled with [^{35}S]methionine (20 μCi/ml) at hourly intervals and harvested at the end of the labeling period. Samples were analyzed on a 12% acrylamide gel. The positions of molecular weight standards are indicated at left; the positions of certain ICPs in the infected cell lysates are identified at right. Uninfected cells labeled for 1 h are shown in lane 1. Lanes 2 to 13 are of SA8-infected cells labeled during the following time intervals: 2, 1 to 2 h p.i.; 3, 2 to 3 h p.i.; 4, 3 to 4 h p.i.; 5, 4 to 5 h p.i.; 6, 5 to 6 h p.i.; 7, 6 to 7 h p.i.; 8, 7 to 8 h p.i.; 9, 8 to 9 h p.i.; 10, 9 to 10 h p.i.; 11, 10 to 11 h p.i.; 12, 11 to 12 h p.i.; 13, 4 to 26 h p.i. (5 μCi of [^{35}S]methionine per ml).

was undetectable by 4 h p.i., although synthesis of a few proteins such as actin (43,000) could be detected up to 6 h p.i. Based on the time-related pattern of their synthesis, three major groups or synthetic classes of viral polypeptides were observed. The synthesis of one class of proteins typified by ICPs 7, 11, and 28/29 reached maximal levels by 2 to 4 h p.i. and decreased steadily thereafter. Synthesis of a second class of viral proteins including ICPs 4/5, 24, 37, and 41/42 was first detected 3 to 4 h p.i. and slowly dropped off thereafter. The third synthetic class of proteins was represented by ICPs 2, 9, 10, 20, 35, and 45. This group of polypeptides was synthesized continuously throughout the infectious cycle, some beginning as early as 2 to 3 h p.i. These proteins are primarily the structural proteins of the virions (see below).

Post-translational modification of SA8 polypeptides. To determine whether post-translational modifications other than glycosylation of SA8 polypeptides occurred in infected cells, we performed pulse-chase experiments. Vero cells infected with SA8 were pulse-labeled for 15 min with [^{35}S]methionine at 6 h p.i. and chased for various time intervals with excess unlabeled methionine as described previously (9). An autoradiograph of SDS-PAGE analysis of one such experiment is shown in Fig. 5. In five areas of the gel, time-related differences in the distribution of isotope between closely spaced bands was noted. In the region of ICP10, two distinct bands were present in the pulse sample which became a single, more diffuse band (ICP10) with time (Fig. 5A). In the region of ICP9, a single band was present in the pulse sample with two bands of different molecular weights appearing after approximately 1 h of chase. These two bands comigrated with the 119K and 115K glycoproteins described above. ICPs 29 and 28, 34 and 33, and 35 and 37 also appeared to represent precursor-product pairs as there was a gradual decrease in label associated with the former polypeptide and a corresponding increase in intensity of the latter polypeptide band during the chase period (Fig. 5B). In addition, there were differences in isotope distribution in the region of ICPs 3 to 7; whether this represented the occurrence of some post-translational modification of these polypeptides was not clear.

Structural proteins of SA8 virions. SA8 virions were partially purified from the extracellular medium of infected cells at 24 h p.i. before extensive cell lysis occurred, thereby avoiding much of the problem of contamination by cellular debris and incomplete, intracellular virions. Virions were coelectrophoresed on polyacrylamide gels with infected cell lysates to identify ICPs which corresponded to structural

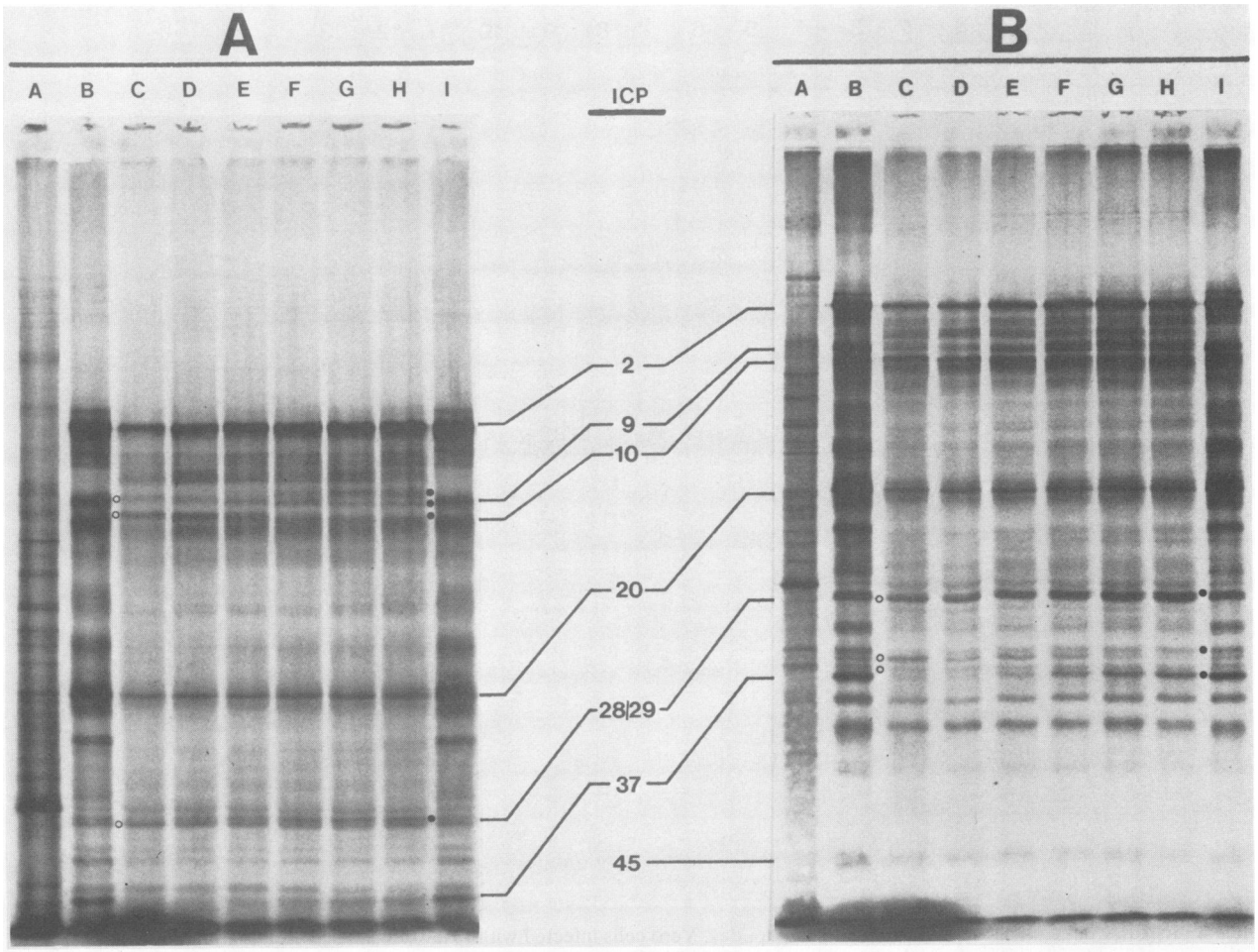

FIG. 5. Post-translational processing of SA8-infected cell proteins. Infected Vero cells were labeled with [^{35}S]methionine (30 μCi/ml) from 6.00 to 6.25 h p.i. One sample was harvested immediately (pulse sample, lanes C), and others were chased in cold medium containing 10 times the normal concentration of methionine. Samples were chased for 15 min (lanes D), 30 min (lanes E), 1 h (lanes F), 2 h (lanes G), and 4 h (lanes H). Uninfected (lanes A) and SA8-infected Vero cells labeled from 4 to 25 h p.i. with [^{35}S]methionine (5 μCi/ml) (lanes B and I) were co-electrophoresed for reference. The samples were analyzed on 8.6% (A) and 12% (B) acrylamide gels to resolve polypeptides of different molecular weights. Apparent precursor (○) and product (●) polypeptides are indicated in the pulse and 4-h chase samples. Certain infected cell polypeptides have also been identified for reference.

components of SA8 virions. One such comparative analysis is shown in Fig. 6. One of the polypeptides present in the greatest abundance in SA8 virions was ICP2. Not only its relative abundance but also its similar apparent molecular weight and immunological cross-reactivity with VP5 of HSV-1 and HSV-2 (unpublished data) suggest that this polypeptide represents the major structural component of the virion nucleocapsid, being analogous in function to VP5 of HSV-1 and other neurotropic herpesviruses (11, 20, 27, 36). Several other of the major polypeptide components of SA8 virions are glycoproteins, specifically the 119K, (ICP9), 63K (ICP22), and 20K (ICP45) species. Other polypeptides found in infected cells which are also present in virions in lesser amounts are identified in Table 2. Of the 47 viral proteins identified in infected cells, approximately 22 to 24 were also present in virions. No structural polypeptides were identified which were not also present in infected cells.

DISCUSSION

After the isolation of SA8 by Malherbe and Harwin (21, 22), studies of SA8 have been limited primarily to the investigatin of its cross-reactivity with HSV (12, 13, 15, 37). Despite its obvious antigenic relatedness to HSV, this virus has received relatively little attention. As part of our ongoing studies on the antigenic interrelationships among the primate herpesviruses, we have examined some of the basic characteristics of the replication of SA8 and the identification of viral polypeptides and glycopolypeptides.

One of the primary aims of these studies was to identify and characterize the proteins synthesized in SA8-infected cells. In this communication, we have identified over 45 virus-specific proteins in infected Vero cells. Since it is useful to have some means of referring to specific viral proteins, particularly for complex viruses such as the herpesviruses, we have assigned each ICP a number designation. This nomenclature system avoids the use of molecular weights for polypeptide identification since these values may vary for a given protein depending not only on the electrophoresis conditions employed but also among different strains of virus and the host cell in which the virus is grown (1, 29, 32). Realizing that the relative quantities of some ICPs may also show variation in different cell lines and virus strains and that future additions of more virus-specific polypeptides would complicate matters considerably, we

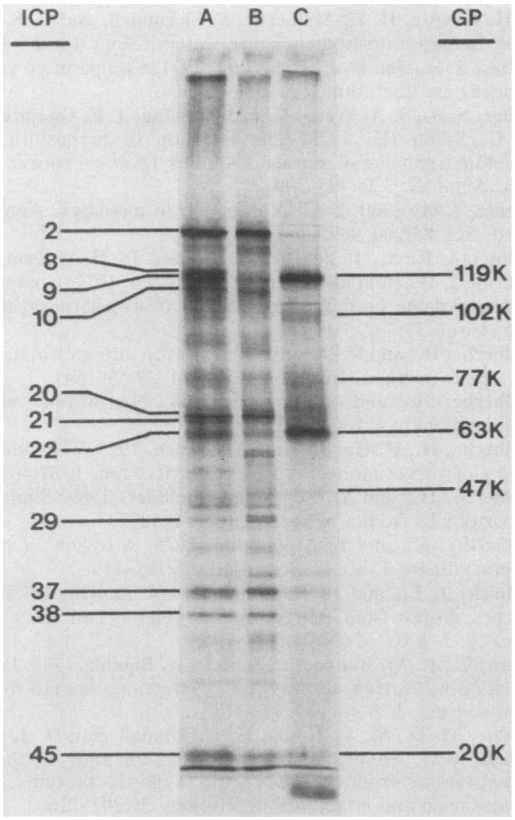

FIG. 6. Identification of structural proteins in SA8 virions. Virions purified from extracellular fluids of infected Vero cells labeled with [^{35}S]methionine (10 μCi/ml) and electrophoresed on 10% gels are shown in lane A. Lysates of SA8-infected Vero cells labeled with [^{35}S]methionine (5 μCi/ml) (lane B) or [^{14}C]glucosamine (5 μCi/ml) (lane C) are also shown. Some structural polypeptides of virions are identified by their ICP numbers at left; infected cell glycoproteins are identified at right. All structural proteins are identified in Table 2.

TABLE 2. Summary of properties of SA8-induced ICPs

ICP no.	MW[a]	Structural protein	Glycoprotein	Synthetic class[b]	ICP no.	MW	Structural protein	Glycoprotein	Synthetic class
1	158			1(host?)	24	55			3
2	149	+		3	25	54			
3	143				26	52	+		3
4	140	+		2	27	47.5		47K (?)	
5	138				28	43			1–2
6	135				29	42.5	+		
7	130			1	30	41.5	+		1
8	124	+			31	40.5			
9	118	+	119K	3	32	40	+		
10	113	+		3	33	38.5	+		3
11	111	+		1	34	37.5			2
?[c]	102	+	102K		35	37			3
12	94				36	36			1
13	90	+		2	37	35.5	+		2
14	87.5	+			38	34	+		3
15	85				39	33			
16	78	+	77K	3	40	32	+		3
17	75				41	29			2
18	72		69K	3	42	28.5			2
19	68.5	+		3	43	26			1
20	66	+		3	44	26.5			
21	65	+			45	19.5	+	20K	3
22	63	+	63K	3	46	14			
23	57			1 (host?)	47	<10			

[a] MW, Apparent molecular weight (× 10^{-3}) as determined on gels of four different acrylamide concentrations.

[b] The synthetic class of proteins is described in the text and shown in Fig. 4.

[c] This glycoprotein was not detectable in infected cells labeled with [^{35}S]methionine or ^{14}C-amino acids.

have chosen to enumerate all possible ICPs. Thus, although ICP1 and ICP23 may actually be host cell proteins (see Fig. 4), they are more intensely labeled in infected cells than in uninfected cells, leaving open the possibility that they are viral polypeptides which comigrate with host cell proteins. Additionally, although some proteins are admittedly very minor constituents of infected cells (e.g., ICP27 and ICP30), these have also been included. Although the precursor nature of some of the identified proteins such as ICP29, ICP34, and ICP35 is suggested by pulse-chase experiments, we have not designated these as such but rather as independent proteins since they are all detectable in infected cells and apparently are not totally processed to the product form. Thus, although the polypeptides listed in Table 2 undoubtedly represent an overestimate of the primary gene products of the virus to be found in infected cells, this should serve as a fairly complete listing of SA8-induced polypeptides for future reference.

The replication of SA8 appears to be quite similar to that described for HSV-1. Infectious virus and CPE were detectable by 8 h p.i. SA8 also efficiently inhibits host cell-specific DNA and protein synthesis while inducing synthesis of viral components with kinetics similar to those of HSV-1 (14, 33). The complexity of SA8 was also comparable to that of HSV, as evidenced by the large number of virus-specific proteins detected in infected cells (14, 27, 32, 33). We have not examined this particular aspect of SA8 protein synthesis in great detail, but we were able to identify three classes of viral proteins which differed in the kinetics of their synthesis. These kinetic classes are similar to several of those described by investigators examining protein synthesis in HSV-1- and HSV-2-infected cells (4, 14, 30). A number of SA8-induced proteins were also found to undergo post-translational modification. By comparing the polypeptide compositions of infected cells and virions, 23 infected cell-specific proteins were identified as structural components of SA8 virions—a number of proteins comparable to that described for other neurotropic herpesviruses (11, 27, 28, 36). Also, as appears typical of the neurotropic herpesviruses, a protein of approximately 150,000 daltons (ICP2; 149,000) is present in both virions and infected cells (11, 14, 20, 28, 32). This protein thus probably represents the major structural protein of the virion capsid, being analogous to VP5 of HSV-1 (11, 14). Even the molecular weight distribution of the viral glycoproteins was strikingly similar to that of HSV (38), with prominent glycoproteins in the molecular weight ranges of 115,000 to 120,000 and 60,000 to 70,000. Despite the marked overall similarity of SA8 and HSV, the two viruses are unquestionably distinct from one another. This is evidenced by the differences in their proteins and glycoproteins as well as their antigenic nonidentity.

Serological evidence indicates that infection by SA8 is widespread among African simians (17). The virus has also

been shown to exist in a latent state in the trigeminal ganglia of asymptomatic animals (18). In addition, certain surface and internal structural proteins of HSV-1 have been shown to cross-react antigenically with similar proteins of SA8 (12, 13, 37; unpublished data). These data, taken together with the evidence presented in this communication on the biochemical and biological properties of SA8, indicate that SA8 probably represents a simian analog of the human herpesvirus HSV-1.

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