**Herpesvirus tamarinus and its Relation to Herpes Simplex Virus**

By RONALD C. DESROSIERS* AND LAWRENCE A. FALK, JR

New England Regional Primate Research Center, Harvard Medical School, One Pine Hill Drive, Southborough, Massachusetts 01772, U.S.A.

(Accepted 8 May 1981)

SUMMARY

We have characterized *Herpesvirus tamarinus* (HT), a virus of New World primates related to herpes simplex virus. HT virion DNA cross-hybridized appreciably with herpes simplex virus type 1 (HSV-1) DNA. The HT–HSV-1 cross-hybridizing DNA sequences were distributed over the length of the genome; some DNA sequences, however, were apparently more homologous than others. HT virion DNA was estimated from the sum of restriction endonuclease fragments to have a mol. wt. of 98 × 10^6. Only 1 molar fragments were found with some restriction endonucleases and 0.5 and 1 molar fragments were found with other restriction endonucleases but no evidence was found for 0.25 molar fragments. Independent isolates of HT from different species of South American primates gave nearly identical restriction endonuclease cleavage patterns of virion DNA. Although cross-reacting antigenic proteins of HT and HSV-1 were not detected by neutralization or immunofluorescence tests, cross-reacting antigenic proteins were detected by SDS–polyacrylamide gel electrophoresis of immunoprecipitates of ^35^S-labelled infected cell extracts.

INTRODUCTION

The isolation of *Herpesvirus tamarinus* (HT), also known as *Herpesvirus platyrrhinae* (HVP), was first described by Holmes et al. (1964) and Melnick et al. (1964). The term *Herpesvirus tamarinus* was used because these initial isolations were made from tamarin marmosets. Subsequent investigations revealed that HT infection is relatively rare in marmosets (*Saguinus sp.*) and owl monkeys (*Aotus trivirgatus*) and frequently causes a fatal herpetic disease in these species. HT infection of squirrel monkeys (*Saimiri sciureus*) is more common but only rarely produces clinical symptoms in this species (Holmes et al., 1966; Daniel et al., 1967). A small plaque variant of HT is presently used as a live vaccine for imported owl monkeys (Daniel et al., 1978).

The biological properties of HT suggest that it is related to the human herpes simplex virus (HSV). The virus grows rapidly in a variety of cultured cells of broad host range producing multinucleated syncytial cells. Actively infected animals frequently display oral lesions. HT isolations from squirrel monkeys have been made from throat swabs, trigeminal ganglia and brain tissue. Owl monkeys and marmosets die rapidly following inoculation with HT or HSV and the histopathological lesions produced by these two viruses are indistinguishable.

HSV is the prototype of one class of the diverse herpes group. Examination of viruses related to the prototype HSV is valuable for a number of reasons. (i) The evolutionary history of the virus and of discrete genes can be examined in detail. (ii) Information on the relation of structure to function can be obtained from the differences that exist among these related viruses. (iii) It is possible that related viruses could be used as a tool in rescue, recombination or genetic mapping experiments. A number of viruses have been definitively shown to be
related to HSV on the basis of DNA-DNA cross-hybridization, antigenic cross-reactivity
and similarity in biological properties. These herpes simplex-related viruses include
pseudorabies virus, bovine mammillitis virus (BMV), and equine herpesvirus type 1 (equine
abortion virus, EHV1). This report details characterization of the New World primate virus
HT.

METHODS

Materials. Materials were obtained from the following sources: cell culture medium and
serum, Gibco; EHV1 (Kentucky D strain) and embryonic bovine tracheal cells (EBTr
NBL-4), American Type Culture Collection; nitrocellulose filters, Schleicher and Schuell;
radioactive supplies, New England Nuclear; restriction endonucleases and bacteriophage λ
dNA, New England Biolabs; fixed Staphylococcus aureus, the Enzyme Center; chemicals for
SDS-polyacrylamide gel electrophoresis, Bio-Rad; sodium salicylate, Mallinckrodt Chemical
Co.

Growth of virus and preparation of virion DNA. HSV-1 strain KOS and HSV-2 strain 186
were generously provided by Dr P. Schaffer and SA8 was generously provided by Dr S. S.
Kalter. HT strain Chicago, HSV-1, HSV-2, SA8, and Herpesvirus saimiri strain 11 were
grown in owl monkey kidney (OMK) cells. The origin of HT strain Chicago is not entirely
certain but it is believed to have originated from an acutely infected marmoset. SA8 is a
baboon (Old World primate) herpesvirus (Kalter et al., 1978). BMV (also known as bovine
herpesvirus 2) and EHV 1 (Kentucky D strain) were grown in embryonic bovine tracheal cells.
The medium used for both cell types was Eagle’s minimum essential medium (MEM)
containing 10% foetal calf serum, 200 units/ml penicillin and 200 μg/ml streptomycin.
Following complete destruction of the infected cell monolayer, virus was purified from a
low-speed supernatant and DNA prepared from lysed virions by eentrifugation in CsCl
according to published procedures (Bornkamm et al., 1976). HT DNA banded in CsCl at a
density of approx. 1.725 g/ml. Labelling of HT and HSV-1 DNA in vitro by the nick-repair
method was performed with α-[3H]dTTP or α-[32P]dCTP (Mackey et al., 1977; Desrosiers
et al., 1979).

DNA–DNA hybridization. Conditions for denaturation of virion DNA, binding of DNA to
nitrocellulose filters, hybridization with homologous or heterologous 3H-labelled DNA and
rinsing of filters have been described (Tracy & Desrosiers, 1980). Hybridizations were
performed in Kimble scintillation mini-vials in 4 × SSC, 0.1% SDS (total vol. 300 μl) for 20
h at 67 °C. According to the equations of Frank-Kamenetskii (1971), this corresponds to
approx. 34 °C below the Tm of homologous hybrids. Blotting of DNA fragments separated
in agarose gels to nitrocellulose filters and hybridization with 32P-labelled DNA followed
previously published procedures (Southern, 1975; Desrosiers et al., 1979).

Agarose gel electrophoresis of restriction endonuclease-generated virion DNA fragments.
Restriction endonucleases were employed for digestion of virion DNA according to the
recommendations of the manufacturer with at least a twofold enzyme excess. Samples were
electrophoresed through agarose gels as previously described (Desrosiers et al., 1979); the
gels were stained with 1 μg/ml ethidium bromide, illuminated with a UV Products C63
transilluminator and photographed using Polaroid positive-negative film. Photographic
negatives were used for densitometer scans; for calculations of relative molarities, peaks were
cut out and weighed. Bacteriophage λDNA × HindIII fragments and various HSV-1
fragments were used as mol. wt. markers. Fragments were given letter designations according
to the nomenclature of Skare & Summers (1977).

Preparation of 35S-labelled intracellular proteins, immunoprecipitations and SDS gel
electrophoresis. OMK monolayers growing in 75 cm² flasks were inoculated with either HT
or HSV-1 at a m.o.i. of approx. 2. Virus was adsorbed for 1 h at 37 °C and 35 ml of medium
Herpesvirus tamarinus lacking methionine (with 10% foetal calf serum, penicillin and streptomycin) was added to each flask. Virus-induced c.p.e. progressed faster with HT than with HSV-1. Infected cells were labelled with 400 μCi [35S]methionine (1000 Ci/mmol) 4 to 7 h post-infection with HT and 5 to 9 h post-infection with HSV-1. The proteins of SA8 were labelled in the same way as HSV-1.

Medium was removed from flasks at the completion of labelling and the cells rinsed twice with phosphate-buffered saline (PBS). The cells were then scraped from the flasks and pelleted. Cell pellets were stored at -20 °C prior to extraction of intracellular proteins. Cell pellets were thawed in 1 ml 0.5% Nonidet P40 (NP40) buffer (0.5% NP40, 0.15 M-NaCl, 1 mM-EDTA, 20 mM-tris–HCl pH 7.5 and 100 μg/ml phenylmethylsulphonyl fluoride) and kept on ice for 20 min with vigorous intermittent vortexing. This broken cell extract was centrifuged at 20000 g for 30 min at 3 °C. The final supernatant (35S-labelled intracellular proteins) was removed and aliquots stored at -20 °C.

Human sera with HSV antibody titres of > 1:128 by indirect immunofluorescence were identified in a screen of healthy human volunteers. Similarly, squirrel monkeys with HT antibody titres > 1:128 were identified from animals housed at the New England Regional Primate Research Center (NERPRC).

For immunoprecipitation, an aliquot of 35S-labelled intracellular proteins was diluted to 50 μl with 0.05% NP40 buffer. Fifty μl of fixed S. aureus suspension (10%, w/w) were then added and this was incubated at room temperature for 20 min. (This step was performed to remove any labelled proteins that might fortuitously bind to fixed S. aureus protein.) Following centrifugation at 1400 g for 4 min, 80 μl of the supernatant were carefully transferred to another tube. Ten μl of test serum were then added to this 80 μl and incubated at 30 °C for 30 min. Ninety μl of fixed S. aureus suspension were then added (to bind all antibody with bound labelled antigen (Kessler, 1975)) and incubated for 20 min at room temperature. The supernatant was removed after centrifugation at 1400 g for 4 min and the immunoprecipitated pellet washed twice with 1 ml 0.05% NP40 buffer. The final pellet was used for SDS–polyacrylamide gel electrophoresis.

Samples containing 2.5% SDS were placed in a boiling water bath for 3 min and centrifuged at 1400 g for 4 min prior to electrophoresis. Slabs were used for electrophoresis in SDS–polyacrylamide gels with bisacrylamide cross-linking according to the procedure of Laemmli (1970). A 5% stacking gel was used. Following electrophoresis, gels were fixed for 1 h in 5% methanol and 7.5% acetic acid, rinsed for 0.5 h with several changes of water, soaked in 1 M-sodium salicylate for 1 h, dried under vacuum and placed over Kodak X-Omat film at -70 °C. Mol. wt. determinations were made using the following 14C-labelled marker proteins: myosin (200K), gamma globulin (150K), phosphorylase B (92.5K), albumin (69K), IgG heavy chain (53K), ovalbumin (46K), carbonic anhydrase (30K), lactoglobulin A (18.4K) and cytochrome c (12.3K).

Serologic studies

Indirect immunofluorescence tests. Serum samples collected from human volunteers and from non-human primates housed at NERPRC were screened in indirect immunofluorescence tests against HT- or HSV-infected cells prepared as follows: preconfluent monolayers of Vero or OMK cells were inoculated with an m.o.i. of about 0.01. Virus was adsorbed for 2 h at 37 °C and the monolayers were refed with medium and incubated at 37 °C. Cell smears were prepared after 18 to 24 h incubation: all but 2 to 3 ml of medium was removed and the cells were scraped into the small volume of medium. The cells were centrifuged, washed twice in PBS and the cell concentration was adjusted to approx. 2 x 10⁶ cells/ml. Drops of cell suspension were put into wells of laminated microscope slides, air-dried, fixed in chilled acetone and stored at -20 °C until used.
Neutralization tests. Selected human or squirrel monkey sera were assayed for virus-specific neutralizing antibodies by serum dilution, plaque reduction assays. Sera were diluted serially fourfold and an equal volume of serum was mixed with an equal volume of virus stock, diluted to contain approx. 200 p.f.u./0.2 ml. The serum–virus mixture was incubated at 4 °C overnight and then OMK monolayers growing in 35 mm dishes were inoculated with 0.2 ml virus–serum mixture; virus was allowed to adsorb for 2 h and the monolayers were overlaid with MEM containing 1% agarose. Neutral red was added after 3 days incubation and virus-induced plaques were counted with the aid of a light box. In each plaque reduction test, a negative control serum was included. The neutralization titre of a particular serum was expressed as the reciprocal of the serum dilution giving at least 50% plaque reduction compared with negative, control serum.

RESULTS

DNA–DNA cross-hybridization

$^{3}$H-labelled HSV-1 and HT DNAs were hybridized with DNAs of HSV-1, HSV-2, HT, EHV1, BMV and Herpesvirus saimiri (a T-lymphotropic virus of squirrel monkeys) immobilized on nitrocellulose filters (Fig. 1). Herpesvirus saimiri DNA did not cross-hybridize detectably (< 4%) with HT DNA under these hybridization conditions; this may be analogous to the lack of detectable cross-hybridization between the DNAs of the human viruses Epstein–Barr and herpes simplex (zur Hausen & Schulte-Holthausen, 1970). DNA of HT and HSV-1 cross-hybridized by approx. 16%; DNA of BMV cross-hybridized with HSV-1 to about the same extent (16%). The extent of BMV HSV-1 cross-hybridization found here is close to a previously published value of 14% (Sterz et al., 1973/74). DNAs of HSV-1 and HSV-2 cross-hybridized by approx. 60%; this is similar to the extent found using other procedures (Kieff et al., 1972; Ludwig et al., 1972). EHV1 DNA cross-hybridized by approx. 7 to 9% with HSV-1 and HT DNA. In separate experiments, HSV-1 DNA cross-hybridized by 31% with DNA from the baboon herpesvirus SA8 (data not shown).

Restriction endonuclease analysis

Digestion of HT virion DNA with restriction endonucleases HindIII, BglII, EcoRI, HpaI and BamHI produced fragment sizes that bore no resemblance to fragment sizes produced with HSV-1 virion DNA. This is to be expected from the degree of cross-hybridization of these DNAs. Densitometer scans of photographic negatives of ethidium bromide-stained gels were used to quantify the molarity of the DNA fragments (Fig. 2). The validity of this approach was borne out by the scan of HSV-1 × HindIII fragments; the mol. wt. and molarities of these fragments are known from previous work (Hayward et al., 1975; Skare & Summers, 1977). The 0.25 molar fragments C and F and the 0.5 molar fragments G, H and M were readily quantified with this procedure (Fig. 2a). The total HSV-1 × HindIII scan including the molarities of co-migrating fragments agreed with previously published results (Skare & Summers, 1977). Three of the five restriction endonucleases (BglII, EcoRI and HpaI) used with HT DNA produced only 1 molar fragments (Fig. 2, Table 1). Virion DNA averaged 98.8 × 10⁶ when the mol. wt. of the individual fragments were summed. Two of the five restriction endonucleases (BamHI and HindIII) produced 0.5 molar as well as 1 molar fragments (Fig. 2, Table 1). The mol. wt. of HT DNA using these enzymes was calculated to be 98.5 × 10⁶ with BamHI and 100.5 × 10⁶ with HindIII based on the four 0.5 molar fragments detected for each enzyme and the remainder of 1 molar fragments. No evidence was found, however, for 0.25 molar fragments. In double-digestion experiments, 0.5 molar fragments persisted when HT DNA was digested with BamHI plus EcoRI or BamHI plus HpaI.
Herpesvirus tamarinus

Fig. 1. DNA–DNA cross-hybridization of isolated virus DNAs. $^3$H-labelled HT DNA or $^3$H-labelled HSV-1 DNA was hybridized with the indicated amounts of various unlabelled virion DNAs immobilized on nitrocellulose filters. The specific activity of the $^3$H-labelled HT DNA was $7 \times 10^6$ ct/min/µg DNA and that of the $^3$H-labelled HSV-1 DNA was $1 \times 10^6$ ct/min/µg DNA. (a) $^3$H-labelled HT DNA with HT DNA (○), HSV-1 DNA (●) and Herpesvirus saimiri DNA (▲). (b) $^3$H-labelled HSV-1 DNA with HSV-1 DNA (○) and HT DNA (□). (c) $^3$H-labelled HT DNA with HT DNA (○), HSV-1 DNA (●), HSV-2 DNA (▲), BMV DNA (+) and EHV1 DNA (□). (d) $^3$H-labelled HSV-1 DNA with HSV-1 DNA (○), HSV-2 DNA (●), HT DNA (x), BMV DNA (▲) and EHV1 DNA (+).

To determine whether the HSV-1–HT cross-hybridizing DNA sequences were confined to particular regions of the genome, DNA fragments in agarose gels were transferred to nitrocellulose filters according to the procedure of Southern (1975) and hybridized with $^{32}$P-labelled DNA. HSV-1 and HT DNA digested with HindIII or BglII were blotted to a nitrocellulose filter and hybridized with $^{32}$P-labelled HSV-1 DNA. Fig. 3 (a) is a photograph of the ethidium bromide-stained gel, and the hybridization pattern following autoradiography is shown in Fig. 3 (b). Twenty times more HT DNA was required to give approximately equal intensity in the autoradiographs. Although the ethidium bromide-stained HSV-1 DNA fragments do not show in Fig. 3, all HSV-1 DNA fragments hybridized with $^{32}$P-labelled HSV-1 probe in proportion to the ethidium bromide stain intensity. All HT DNA fragments hybridized to $^{32}$P-labelled HSV DNA, although longer film exposures were required to detect hybridization of some of the HT fragments. Using the $^{32}$P-labelled HSV-1 probe, the strongly hybridizing HT fragments included HindIII fragment G and BglII.
(a) HSV-1 × HindIII

(b) HT × BglII

(c) HT × BamHI

(d) HT × HindIII

Fig. 2. Densitometer scans of photographic negatives of ethidium bromide-stained DNA fragments. The bottom-most scan of D is from a 0.3% agarose gel run 36 h at 50 V (15 mA) to obtain better resolution of the high mol. wt. products. The other scans are from 0.4% agarose gels run for 18 h at 50 V (15 mA).

Table 1. Restriction endonuclease digestion of Herpesvirus tamarinus DNA*

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Number of fragments</th>
<th>Molarity of fragments</th>
<th>Sum mol. wt. (x 10^-6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BglII</td>
<td>18</td>
<td>1</td>
<td>97.4</td>
</tr>
<tr>
<td>EcoRI</td>
<td>9</td>
<td>1</td>
<td>98.1</td>
</tr>
<tr>
<td>HpaI</td>
<td>11</td>
<td>1</td>
<td>99.1</td>
</tr>
<tr>
<td>BamHI</td>
<td>23</td>
<td>0.5 and 1</td>
<td>98.5</td>
</tr>
<tr>
<td>HindIII</td>
<td>23</td>
<td>0.5 and 1</td>
<td>100.5</td>
</tr>
</tbody>
</table>

* Data are presented only for fragments greater than 0·6 x 10^6 mol. wt.

fragment I; the weakly hybridizing HT fragments included HindIII fragments A and L and the BglII fragments, D, G and J (Fig. 3). Similar results were obtained in the reverse experiment using 32P-labelled HT probe (data not shown). Using the 32P-labelled HT probe, the strongly hybridizing HSV-1 fragments included the HindIII fragment J and the BglII fragments K, I, N and P. The weakly hybridizing HSV-1 fragments included HindIII fragments G and H and the BglII fragments A, F and H. Furthermore, HSV-1 BglII fragments N and P were considerably stronger (with 32P-labelled HT DNA probe) than BglII fragments M and O in the same region of the gel, even though these fragments are contiguous in the U_L map.

Since HT infection sometimes occurs in different species of South American primates imported to the United States, we examined various isolates to determine the degree of strain variability. HT Chicago, small and large plaque variants of HT isolated from a squirrel monkey (Daniel et al., 1967), a recent HT isolate from an imported owl monkey and HT
Fig. 3. HT and HSV-1 DNA fragments blotted from agarose gels to nitrocellulose filters and hybridized with $^{32}$P-labelled HSV-1 DNA. Restriction endonuclease-digested DNA was electrophoresed through a 0.5% agarose gel 36 h at 50 V (15 mA). (a) Photograph of ethidium bromide-stained gel; (b) autoradiogram of transferred fragments hybridized with $^{32}$P-labelled HSV-1 DNA (sp. act. about $3 \times 10^7$ ct/min/μg DNA (Cerenkov counting)). The letters indicate the positions of the various fragments. 1, 0.024 μg HSV-1 DNA × HindIII; 2, 0.500 μg HT DNA × HindIII; 3, 0.024 μg HSV-1 DNA × BglII; 4, 0.500 μg HT DNA × BglII.
isolated from the trigeminal ganglia of a marmoset experimentally infected with HT were digested with HindIII and electrophoresed through a 0.5% agarose gel (Fig. 4). The digestion products of these diverse isolates were remarkably similar. Digestion of these HT DNAs with additional restriction endonucleases confirmed the near identity in cleavage sites but did not allow differentiation of all isolates by identification of fragments whose size was unique to one or a few isolates. For example, the HindIII fragment R (1.6 x 10⁶ mol. wt.) of HT Chicago did not have any fragment co-migrating from the other isolates (Fig. 4). Also, HindIII fragment L (3.5 x 10⁶ mol. wt.) of HT Chicago had a slightly slower mobility than a similar fragment from the other isolates (Fig. 4).

Antigenic cross-reactivity

Ten human and ten squirrel monkey sera were tested for antibodies to HSV and HT by indirect immunofluorescence and by serum neutralization tests. Generally, there was good correlation of the antibody titres obtained by the two different tests. Titres of human sera varied from 1:8 to 1:1024 against HSV and titres of squirrel monkey sera ranged from 1:8 to
Herpesvirus tamarinus

Fig. 5. SDS–polyacrylamide gel electrophoresis (10%) of $^{35}$S-labelled infected cell polypeptides. 1, 272,000 ct/min HSV-1-infected cell proteins immunoprecipitated with HT-negative squirrel monkey sera; 2, 389,000 ct/min HT-infected cell proteins immunoprecipitated with HT-negative squirrel monkey sera; 3, 342,000 ct/min SA8-infected cell proteins immunoprecipitated with HT-negative squirrel monkey sera; 4, 272,000 ct/min HSV-1-infected cell proteins immunoprecipitated with HSV-1-negative human sera; 5, 389,000 ct/min HT-infected cell proteins immunoprecipitated with HSV-1-negative human sera; 6, 342,000 ct/min SA8-infected cell proteins immunoprecipitated with HSV-1-negative human sera; 7, 190,000 ct/min uninfected OMK cell proteins; 8, 143,000 ct/min SA8-infected cell proteins; 9, 162,000 ct/min HT-infected cell proteins; 10, 113,000 ct/min HSV-1-infected cell proteins; 11, $^{14}$C-labelled protein mol. wt. markers; 12, 452,000 ct/min $H. saimiri$-infected cell proteins immunoprecipitated with marmoset immune sera; 13, 272,000 ct/min HSV-1-infected cell proteins immunoprecipitated with HSV-1-positive human sera; 14, 389,000 ct/min HT-infected cell proteins immunoprecipitated with HSV-1-positive human sera; 15, 342,000 ct/min SA8-infected cell proteins immunoprecipitated with HSV-1-positive human sera; 16, 456,000 ct/min uninfected OMK cell proteins immunoprecipitated with HSV-1-positive human sera; 17, 272,000 HSV-1-infected cell proteins immunoprecipitated with HSV-positive squirrel monkey sera; 18, 389,000 ct/min HT-infected cell proteins immunoprecipitated with HSV-1-infected cell proteins; 19, 342,000 ct/min SA8-infected cell proteins immunoprecipitated with HT-positive squirrel monkey sera; 20, 456,000 ct/min uninfected OMK cell proteins immunoprecipitated with HT-positive squirrel monkey sera.

1:256 against HT. Human sera reacted only with HSV and the squirrel monkey sera reacted only with HVP in these neutralization and indirect immunofluorescence tests.

Total and immunoprecipitated intracellular proteins of HSV-1- and HT-infected OMK cells labelled with $[^{35}$S]methionine were compared with each other and also with the proteins of SA8 (Fig. 5). The reactivity of the antisera was highly specific as negative sera did not precipitate detectable amounts of virus proteins (slots 1 to 6) and positive sera did not precipitate appreciable amounts of labelled proteins from uninfected cells (slots 16 and 20). The pattern of immune precipitable HSV-1 and HT intracellular proteins was the same as that shown in Fig. 5 with two other positive human sera and a pool of squirrel monkey sera.
The immunoprecipitated proteins appeared to be a subpopulation of the total labelled intracellular proteins (compare Fig. 5, slots 9 and 10 with 13 and 18). Also, a number of immunoprecipitated proteins that do not appear in Fig. 5 were obvious on longer film exposures; 16 HSV-1 proteins and 23 HT proteins were detected in immunoprecipitates.

The relatively simple pattern of immunoprecipitated proteins with only several major proteins evident in Fig. 5 (slots 13 and 18) probably reflects the sera used. These sera were taken from apparently healthy humans and squirrel monkeys naturally infected at some time in the past with no present signs of active infection.

Most HSV-1 and HT antigenic proteins detected with homologous sera could also be detected with heterologous sera, although longer film exposures were usually required. The major exceptions to the antigenic cross-reacting proteins were HT proteins of 31 x 10^3 and 49 x 10^3 mol. wt. (31K and 49K) which were not detected with human sera, even at long film exposures (compare Fig. 5, slots 14 and 18) and HSV-1 proteins of 84K, 67K and 40K, which were not detected with squirrel monkey sera (Fig. 6, slots 13 and 17—these proteins were prominent with human sera on longer film exposures). HSV-1-, SA8- and HT-infected cells all contained a major antigenic, co-migrating polypeptide of 155K; this is the top band evident in slots 13 to 15 (Fig. 5) and their co-migration was especially evident in 6% SDS–polyacrylamide gels (data not shown). We have as yet made no attempts to correlate the labelled HSV-1 proteins with HSV-1 virion proteins or with the detailed numbering system of HSV-1-infected cell polypeptides. The important point here is that cross-reacting antigenic proteins of HT and HSV-1 were detected by SDS gel electrophoresis of immunoprecipitates. The recent results of Yeo et al. (1981) suggest that the predominant cross-reactive protein shown in slots 17 and 19 may, at least in the case of HSV-1 (slot 17), be the major DNA-binding protein.

**DISCUSSION**

The results of DNA cross-hybridizations, restriction endonuclease digestions and antigenic protein analyses show that HT is distinct from but related to HSV. Although several species of New World primates can become infected with HT, infection of New World primate species other than squirrel monkeys is much more likely to result in overt disease (Holmes et al., 1966; Daniel et al., 1978) Comparison of isolates from various sources by the very sensitive technique of restriction endonuclease digestion of virion DNA (Fig. 4) shows that these various isolates, even from different species, are different strains of the same virus, HT. The degree of HT strain variability appears to be similar to the degree of strain variability found with HSV (Buchman et al., 1978). The degree of HT strain variability, however, is much less than that observed for Herpesvirus saimiri, also indigenous to squirrel monkeys (R. Desrosiers & L. Falk, unpublished results).

The extent of cross-hybridization determined by heterologous DNA–DNA reassociation cannot be used to estimate actual sequence homology; this can be achieved only through direct DNA sequencing. The amount of hybrid that can be formed between distantly related sequences depends on the stringency of sequence matching imposed by the reassociation and assay conditions (Yang & Wu, 1979a). Commonly employed stringent techniques for DNA–DNA reassociation showed 11 to 20% cross-hybridization between the DNAs of the papovaviruses BK (human) and SV40 (simian) but direct sequencing has shown 70% sequence homology (Yang & Wu, 1979a, b). The actual sequence homology among herpes simplex-related viruses is probably considerably higher than the numbers obtained here and elsewhere from cross-hybridizations. Any given hybridization procedure, however, is valid for qualitative or comparative data, as used here for ranking relatedness of herpesviruses (Yang & Wu, 1979a). It is curious that, except for BMV, the extent of DNA cross-hybridization of these herpes simplex-related viruses with HSV-1 appears to correlate with evolutionary
distance. Others have noted an unusually high antigenic cross-reactivity of BMV with HSV (Killington et al., 1977). The HT–HSV cross-hybridizing sequences appear to be distributed over the length of the genome (Fig. 3).

The antigenic cross-reactivity of HT and HSV-1 is apparently not strong enough to be detected by our standard neutralization or indirect immunofluorescence tests. Cross-reacting antigenic proteins were specifically detected, however, by immunoprecipitation followed by SDS–polyacrylamide gel electrophoresis (Fig. 5). Using high-titred rabbit sera, Blue & Plummer (1973) have reported a weak cross-reactivity between HSV-1 and HT using neutralization and indirect immunofluorescence tests similar to ours.

The molarity of DNA fragments produced in single- and double-digests with restriction endonucleases suggests that HT DNA may exist in two isomeric arrangements with inversion in a short segment only. Preliminary results in which HT DNA was denatured and renatured to allow fold-back of inverted repeat segments are consistent with this; electron microscopic examination revealed inverted repeats bracketing a short stretch of unique DNA at one end only (R. Desrosiers, N. Berthelot, J. M. Mouchel & P. Sheldrick, unpublished observation). Detailed restriction endonuclease mapping, however, is necessary to determine sequence arrangement in HT DNA.

This work was supported by Grants RR-00168-17, Animal Resources Branch, and RO1 CA 27225-02 from the National Institute of Health and by a Fellowship from the Boston Medical Foundation, Inc. We thank Sharon Tracy, Daniel Silva, Roy Byington and Tricia Hellman for assistance and Dr M. D. Daniel for generously providing virus stocks used in Fig. 4. The art work and photography of June Armstrong is greatly appreciated.

REFERENCES


(Received 26 January 1981)