Serological Evidence for Variation in the Incidence of Herpesvirus Infections in Different Species of Apes†

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Sera from captive lowland gorillas, chimpanzees, orangutans, and gibbons were screened by enzyme-linked immunosorbent assay (ELISA) for antibody to herpesviruses serologically related to human herpes simplex virus types 1 and 2 (HSV-1, HSV-2), a baboon virus (SA8), and a macaque herpesvirus (B virus). The incidence of herpesvirus antibodies varied considerably among the different species, gorillas having the highest incidence of seropositivity (65.4%) and orangutans the lowest. The virus specificity of positive sera was further analyzed by examining the kinetics of virus neutralization, competition of reactivity in ELISAs, and immunoblotting against HSV-1, HSV-2, SA8, and B virus antigens. Using these assays, the majority of positive gorilla sera (49 of 53, 92%) were determined to react in a manner identical to human HSV-1 immune sera. The remaining four positive gorilla sera reacted as HSV-2-positive sera. In contrast, the majority of positive chimpanzee sera (5 of 7, 71%) reacted as HSV-2 immune rather than HSV-1 immune. All positive sera from gibbon apes reacted as HSV-1 positive. No orangutan sera were identified which gave positive reactions by ELISAs to any of the four primate herpesviruses tested. Although four orangutan sera gave equivocal results against HSV-1 antigen, further analysis by immunoblotting could not confirm any specific reactivity with any of the primate herpesvirus antigens. Varied reactivity among individual animals with both SA8 and B virus proteins was observed, but none of the seropositive primates detected appeared to be infected with either of these simian viruses. Three gorilla sera had antigen recognition patterns slightly different from those of HSV-2-positive human and chimpanzee sera and another HSV-2-positive gorilla serum, raising the possibility that these animals harbor an indigenous virus related to HSV-2.

Herpesviruses have been isolated from a wide variety of mammalian and nonmammalian species (15). Many of these isolates have been classified as members of the alphaherpesvirus subgroup which includes the human herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) and varicella-zoster virus (36). While it is known that chimpanzees are susceptible to infection with HSV (31), it is not known whether any of the anthropoid ape species harbor a herpesvirus indigenous to their own species. Viruses biologically similar to HSV-1 and HSV-2 have, however, been isolated from African monkeys (herpesvirus cercopithecus, simian agent 8; SA8) and Asiatic macaques (herpesvirus simiae, B virus) (18, 23, 26, 27). Two other viruses have also been isolated from South American squirrel monkey and spider monkey populations (14, 17, 18). In their natural hosts, each of these simian viruses causes infections similar to HSV in humans typified by self-limiting oral or genital lesions or both, the establishment of latent infections in sensory ganglia following primary infection, and spontaneously recurring overt lesions or asymptomatic shedding (2, 21, 27, 32, 39). Infection of neonates or xenogeneic species by these viruses also often results in severe generalized infections involving the central nervous system (10, 13, 25, 33, 35). A particularly notable example of this is B virus which, although causing self-limiting orogenital lesions in macaques (23, 39, 40), almost invariably results in a fatal encephalitis or encephalomyelitis in humans (4, 11, 37). Similarly, fatal infections

caused by HSV have been documented in gibbons and both patas and colobus monkeys (25, 35). Thus, cross-species transmission of herpesviruses is a recognized hazard for humans working with primates.

While most herpesviruses isolated from nonhuman primates are either cytomegaloviruses or lymphotropic herpesviruses related to the human Epstein-Barr virus, several cases of varicella-zoster virus infections in gorillas and chimpanzees have been reported (12, 29, 30, 34). Isolation of viruses related to HSV from primates are, however, scarce. A number of primate species including apes have been surveyed for antibodies to many different simian and related human viruses (18-20). These studies reported that the incidence of antiherpesvirus antibody in gorillas, orangutans, and gibbons was very low, while chimpanzees had a higher incidence (still $\leq 15\%$). McClure et al. (31) also reported "natural" orogenital herpetic infections in captive chimpanzees and isolated HSV-2 from lesions. Since apes are all endangered species and captive populations represent an ever-increasing percentage of their total population, concern exists regarding the unknown consequences of the introduction of human viruses into these species. In this study, we report the screening of captive gorillas, chimpanzees, orangutans, and gibbons for the presence of serum antibodies reactive with the human HSVs and related herpesviruses of cercopithecoid monkeys to determine whether any of these species possibly harbor indigenous neurotropic herpesviruses which may represent a potential hazard to human caretakers, and to determine the extent of the problem of infection of captive apes with the human HSV-1 or HSV-2 viruses.

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MATERIALS AND METHODS

Viruses, cells, and media. Vero, CV-1, and HEp-2 cell cultures were maintained in Dulbecco modified Eagle medium supplemented as described previously (7). The herpesviruses HSV-1 (strain KOS), HSV-2 (strain 186), SA8 (strain B264), and B virus (strain E2490) were used throughout these studies. Viral isolate 5336 was obtained from gorilla 3-18, who suffered recurrent genital and orofacial lesions. HSV-1 strains F and IS (isolated from a human case of encephalitis) were also used in some experiments as noted. All viruses were propagated at 37°C (34°C for HSV-2).

Serum samples. Serum samples were obtained from lowland gorillas (*Gorilla gorilla*, $n = 81$), chimpanzees (*Pan troglodytes*, $n = 38$), orangutans (*Pongo pygmaeus*, $n = 70$), and several species of gibbon apes (*Hylobates* spp., $n = 24$) resident in zoological parks in the United States and Canada. Sera were collected by zoo personnel during routinely scheduled examinations.

Chemicals and radioisotopes. All chemical reagents for sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting were purchased from Bio-Rad Laboratories (Richmond, Calif.). L-[³⁵S]methionine (>800 Ci/nmol), [1-¹⁴C]glucosamine hydrochloride (40 to 60 mCi/mmol), [U-¹⁴C]amino acids mixture, and ¹²⁵I-labeled *Staphylococcus* protein G (SPG) were obtained from ICN Radiochemicals (Irvine, Calif.). Avidin-biotin-peroxidase ABC kits and biotinylated *Staphylococcus* protein A (SPA) were purchased from Vector Laboratories (Burlingame, Calif.). Restriction enzymes were purchased from Promega Biotec (Madison, Wis.).

Neutralization assays. The methods used for kinetic neutralization assays have been described in detail elsewhere (6). Briefly, neutralization assays were performed by incubation of 4.0×10^6 PFU of virus at 37°C under conditions of antibody excess. Samples were withdrawn at the specified times and residual PFU were quantitated by plaque assay on Vero cell monolayers. Rate constants (k) were calculated as described previously (6).

ELISA and radioimmunoassays. Antigens used in enzyme-linked immunosorbent assays (ELISAs) and radioimmunoassays were prepared in CV-1 cells. At 24 h after infection with virus or mock infection, cells were washed twice with phosphate-buffered saline, suspended in 0.1% Triton X-100, and sonicated for 30 s. Samples were assayed for protein content, adjusted with 0.1% Triton X-100 to 3.0 mg/ml (for coating of microdilution wells) or 5.0 mg/ml (for competing antigen), and stored in aliquots at -80°C.

Preliminary testing indicated that SPA bound immunoglobulin G from all species of apes; therefore, ELISAs were performed in microdilution wells as described previously (9). Plates were coated with 3 µg of antigen per well and the wells were washed once and blocked for 1 h. Serum samples were initially screened at a 1:100 dilution. Plates were washed five times and incubated sequentially with biotinylated SPA, avidin-biotinylated peroxidase complex, and *o*-phenylenediamine hydrochloride-peroxidase substrate. Sulfuric acid was added to each well to stop the enzyme reaction, and the optical density (OD) was read at 490 nm on a Molecular Biosystems automated ELISA plate reader. OD values greater than three times background levels were considered positive reactions. At the serum dilutions used, negative sera typically gave values in the range of 0.01 to 0.05 OD units. Antibody titers were determined by testing fourfold serum dilutions; titers are expressed as the highest serum dilution which gave a positive reaction.

Competition ELISAs (cELISAs) were performed by the same protocol with the following modifications. Antisera were used at a constant dilution determined for each solid-phase antigen-antibody pair as the dilution which gave approximately 80% maximal binding levels in a standard ELISA. Antisera were diluted, and 30 µl was dispensed into uncoated, preblocked wells of polyvinylchloride microdilution plates (Dynatech Laboratories, Inc., Alexandria, Va.). An equal volume of serial twofold dilutions of competing antigen was added to wells containing antiserum (final concentrations of 0.1 to 12.5 µg of competing antigen per well). After 30 min of incubation at 37°C, 50 µl of the antiserum-competing antigen mixtures was transferred in duplicate to antigen-coated, blocked microdilution plates. After incubation, plates were washed and bound antibody was detected with SPA/ABC as in standard ELISAs. Results are expressed as percent control binding = (OD in presence of competing antigen/OD in absence of competing antigen) $\times$ 100.

Immunoblot assay. The procedures for the preparation of immunoblot antigens, polyacrylamide gel electrophoresis, electrophoretic transfer of proteins to nitrocellulose membranes, and the immunological detection of viral proteins have all been described in detail previously (8, 9). Antisera prepared to purified glycoproteins of HSV-1, HSV-2, and SA8 were used to identify viral antigens (5).

Restriction enzyme analysis. DNA was prepared from virus-infected CV-1 cells by the procedure of Walboomers and ter Schagget (38). Equivalent amounts of viral DNA were digested with 8 to 12 units of restriction enzyme at 37°C for 3 h. Digests were run on 0.8% agarose gels, stained with ethidium bromide, and photographed by standard techniques (28).

RESULTS

Based on the close antigenic relationships which exist among HSV-like viruses of humans and cercopithecoid monkeys (15, 16; R. Eberle and J. K. Hilliard, submitted for publication), any herpesvirus potentially indigenous to an ape species should also be closely related to HSV, SA8, and/or B virus. Ape sera were screened for antiherpesvirus antibodies by ELISA against uninfected cell and HSV-1-, HSV-2-, and SA8-infected cell antigens. When quantities permitted, sera were also assayed for B virus-specific antibody (22). Since all positive ape sera reacted most intensely with either HSV-1 or HSV-2 antigen, titers of antiherpesvirus antibody were determined for these two antigens. The results of these serological screens of ape sera are summarized in Table 1.

Antiherpesvirus antibody was detected in 53 of 81 (65.4%) gorillas tested. All the positive sera reacted more strongly with HSV-1 and HSV-2 than with SA8 or B virus. Of these positive sera, 44 of 53 had higher titers to HSV-1 than to HSV-2, 4 of 53 had higher titers to HSV-2 than to HSV-1, and 5 of 53 had equivalent titers to HSV-1 and HSV-2. Animals with antiherpesvirus antibody were not evenly distributed among zoos, and the percentage of animals positive varied considerably among different zoos. As observed for humans, considerable variation in antibody titers were also apparent between individual animals.

To further analyze the virus specificity of the positive gorilla sera, we performed cELISAs. Competition of sera from several animals which appeared to be HSV-1 positive is shown in Fig. 1 (top). Although the amount of competition observed for these gorilla sera did vary slightly, the overall pattern of competition (HSV-1 $\gg$ HSV-2 > SA8) was the

TABLE 1. Antibody titers to herpesviruses in ape sera

Zoo no.	No. of animals positive/total	Animal no.	Titer to:		Zoo no.	No. of animals positive/total	Animal no.	Titer to:	
			HSV-1	HSV-2				HSV-1	HSV-2
Gorillas					Chimpanzees				
1	3/4	1	1,600	6,400	1	0/8			
		2	1,600	6,400	3	0/1			
		4	1,600	6,400	4	0/5			
2	8/12	5	6,400	1,600	6	1/3	16	1,600	6,400
		6	25,600	6,400	8	3/6	19	1,600	6,400
		8	6,400	1,600			20	1,600	6,400
		11	25,600	6,400			23	6,400	6,400
		12	25,600	6,400	10	1/1	24	6,400	25,600
		13	6,400	1,600	11	0/6			
		15	25,600	25,600	12	2/6	31	1,600	400
		16	25,600	6,400			35	6,400	6,400
3	4/5	17	6,400	1,600	14	0/2			
		18	25,600	6,400					
		19	25,600	1,600	Total	7/38 (18.4%)			
		20	6,400	1,600					
4	1/5	25	25,600	6,400	Orangutans				
5	4/5	27	6,400	1,600	1	1/9	9 ^a	≤100	≤100
		28	6,400	1,600	3	1/5	13 ^a	≤100	≤100
		29	6,400	6,400	4	2/6	17 ^a	≤100	≤100
		30	6,400	1,600			19 ^a	≤100	≤100
6	0/1				6	0/2			
7	1/1	33	25,600	102,400	8	0/9			
8	3/5	36	6,400	1,600	9	0/4			
		37	25,600	6,400	10	0/4			
		38	6,400	1,600	11	0/17			
9	1/1	39	25,600	6,400	13	0/12			
10	2/11	40	25,600	6,400	18	1/2	69 ^a	≤100	≤100
		46	1,600	400					
11	6/6	51	25,600	6,400	Total	5/70 (7.1%)			
		52	25,600	25,600					
		53	102,400	6,400	Gibbons				
		54	25,600	6,400	1	4/5	2	1,600	1,600
		61	6,400	1,600			3	400	100
		62	6,400	1,600			4	400	100
12	1/2	55	25,600	6,400			5	6,400	1,600
13	2/4	59	6,400	1,600	2	1/5	9	1,600	100
		60	25,600	6,400	9	2/4	12	6,400	1,600
14	1/1	63	6,400	400			13	1,600	1,600
16	13/13	66	25,600	6,400	11	0/4			
		67	25,600	6,400	13	1/5	21	1,600	1,600
		68	25,600	6,400	18	0/1			
		69	6,400	6,400					
		70	6,400	1,600	Total	8/24 (33.3%)			
		71	25,600	25,600					
		72	6,400	1,600					
		73	6,400	1,600					
		74	6,400	1,600					
		75	6,400	1,600					
		76	6,400	1,600					
		77	6,400	1,600					
		78	6,400	1,600					
17	1/1	79	25,600	1,600					
18	2/4	80	6,400	1,600					
		81	6,400	1,600					
Total	53/81 (65.4%)								

^a These orangutan sera did not consistently give values high enough above background for titers to be determined.

same as for human HSV-1-positive serum. This pattern of competition was distinct from that obtained for HSV-2-positive human serum (not shown). Thus, HSV-1-positive gorilla sera were indistinguishable from human HSV-1-positive sera by cELISA.

Sera from three gorillas which appeared to be HSV-2 positive were also tested by cELISA with HSV-2 antigen on

the solid phase (Fig. 1, bottom). The competition patterns of the three gorilla sera were distinct from that of the human HSV-1-positive serum and similar to that of the HSV-2-positive human serum. However, the reaction of each of these gorilla sera was blocked slightly more efficiently (at lower antigen concentrations) by HSV-1 antigen and to a greater degree by SA8 antigen than was human HSV-2

FIG. 1. Analysis of seropositive gorilla sera by cELISA. cELISAs were performed as described in Materials and Methods. Sera from gorillas that reacted as HSV-1 positive by ELISA were tested against HSV-1 antigen (top), and HSV-2-positive sera were tested against HSV-2 antigen (bottom). Human HSV-1 immune serum (HS-11), HSV-2 immune serum (HS-148), and an equal mixture of HS-11 and HS-148 were included for comparison. Serial twofold dilutions of competing antigen (beginning at 12.5 μ g) were used to block the binding of sera to HSV antigen immobilized on the solid phase. Competing antigens used were from uninfected cells (+) or cells infected with HSV-1 (●), HSV-2 (○), or SA8 (■).

FIG. 2. Comparison of the kinetics of virus neutralization by human HSV immune sera and positive gorilla sera. Sera were tested for their ability to neutralize HSV-1 (A), HSV-2 (B), or SA8 (C). A serologically negative gorilla serum (2-9), an HSV-1 immune human serum (HS-11), an HSV-2 immune human serum (HS-148), and an SA8 immune rhesus serum (1384) were included as controls. Sera tested in panel A were 2-9 (+); HS-11 (●); HS-148 (○); 3-18 (■); 11-51 (□); 2-5 (▲); and 14-63 (△). Sera tested in panel B were 2-9 (+); HS-11 (●); HS-148 (○); 1-1 (■); 1-2 (□); 1-4 (▲); and 7-33 (△). Sera tested in panel C were 2-9 (+); HS-11 (●); HS-148 (○); 1384 (■); 7-33 (□); 1-4 (▲); 1-1 (△); 1-2 (▼); and 3-18 (▽).

immune serum. Although slight, these differences were consistently observed on repeated testing of these sera. A 1:1 mixture of human HSV-1 and HSV-2 immune sera was tested to determine how serum from an animal infected with a virus similar to but distinct from both HSV-1 and HSV-2 might react. Although not identical, the competition pattern of this artificial mixture was quite similar to that of the HSV-2-positive gorilla sera.

Kinetic neutralization tests were also used to examine the virus specificity of a number of positive gorilla sera. Neutralization of HSV-1 by HSV-1-positive gorilla and control sera is shown in Fig. 2A. Human HSV-1 immune serum clearly neutralized HSV-1 more efficiently ($k = 0.535$) than did the human HSV-2 immune serum ($k = 0.242$). Interestingly, all positive gorilla sera tested neutralized HSV-1 considerably more efficiently than human HSV-1 immune serum (approximate $k = 1.187$). Neutralization of HSV-2 by HSV-2-positive gorilla sera is shown in Fig. 2B. As expected, human HSV-2 immune serum neutralized HSV-2 more efficiently than HSV-1 immune serum ($k = 0.182$ versus 0.087 , respectively). However, unlike HSV-1-positive gorilla sera, not all HSV-2-positive gorilla sera neutralized HSV-2 with similar kinetics. Two of the sera neutralized about as efficiently as the human HSV-2 immune serum ($k = 0.137$ and 0.254), while two others neutralized considerably more effectively (approximate $k = 0.849$). Several sera were also examined for their ability to neutralize SA8 (Fig. 2C). Neutralization of SA8 by human HSV-1 and HSV-2 immune sera ($k = 0.038$ and 0.019 , respectively) was less efficient than by SA8-positive baboon serum ($k = 0.412$). All but one of the positive gorilla sera tested neutralized SA8 with kinetics similar to those of human HSV-positive sera (k values ranging from 0.038 to 0.75); the remaining gorilla serum (1-2) did not neutralize SA8 at all ($k = 0.003$).

To determine with which of the known primate viruses (if any) the seropositive animals were infected, we examined with an immunoblot assay the reactivity of sera with glycoproteins of HSV-1, HSV-2, SA8, and B virus which are known to be relatively virus-specific antigens. Control sera of known specificity for HSV-1, HSV-2, SA8, B virus and herpesvirus-negative ape sera were included in each assay. These sera served both to define the overall general pattern of reactivity of homologous immune sera to each virus antigen and to identify the virus-specific polypeptides in each of the viral antigens. Thus, of the control sera, only the human HSV-1-positive serum reacted with the HSV-1-specific gC glycoprotein of HSV-1 and only the human HSV-2-positive serum reacted with the HSV-2-specific gG glycoprotein (Fig. 3). In comparing the reactivity of the control sera with each of the four viral antigens, it was also evident that each reacted much more strongly and with a greater number of viral proteins of the homologous viral antigen than of the three heterologous viral antigens.

All HSV-1-positive gorilla sera gave a pattern of reactivity in immunoblots consistent with this conclusion (Fig. 3). These gorilla sera reacted with many more polypeptides of HSV-1 than of HSV-2, SA8, or B virus. Sera which had higher ELISA titers generally gave more intense reactions in immunoblots, although the number of viral polypeptides recognized was essentially the same as that seen with lower-titer sera. Several sera (e.g., 3-17, 10-40, and 13-60) reacted more strongly with one or more polypeptides of HSV-2 than did most other sera. Similarly, sera of several animals (e.g., 1-1, 3-19, and 5-27) recognized polypeptides of B virus which other gorilla sera did not. These B virus polypeptides were recognized by both the B virus- and SA8-positive control sera and, to a lesser extent, by human HSV-positive sera as well.

FIG. 3. Reactivity of gorilla sera in immunoblot assays. All sera were used at a 1:40 dilution. Antigens used were lysates of cells infected with HSV-1 (lanes 1), HSV-2 (lanes 2), B virus (lanes 3), or SA8 (lanes 4). Reactivity patterns obtained with human HSV-1 immune (HS-11), human HSV-2 immune (HS-148), and rhesus B virus immune (RH B) sera are also shown for comparison. All gorilla sera are identified by the zoo-animal number used in Table 1. Serum 15-65 is a gorilla serum negative for antibody reactive with any of the primate herpesviruses.

The four gorilla sera which appeared by ELISA to be more like HSV-2-positive sera (1-1, 1-2, 1-4, 7-33) gave a pattern of reactivity in immunoblot assays different from that of other gorilla sera. Although considerable variation occurred in the total number of proteins recognized by these sera, each reacted most intensely and with more HSV-2 polypeptides than other viral antigens. All the polypeptides of other viruses recognized by these four sera were cross-reactive polypeptides. However, three of these four gorilla sera (1-1,

1-2, 1-4) did not recognize the HSV-2-specific gG glycoprotein, as is typical of human HSV-2-positive sera (9). Interestingly, these same three sera recognized more B virus polypeptides than did HSV-1-positive sera or the remaining HSV-2-positive gorilla serum (7-33). This fourth HSV-2-positive gorilla serum (7-33) gave a reaction pattern typical of human HSV-2-positive sera, including recognition of the gG glycoprotein.

Although results of both ELISA and immunoblots suggest

FIG. 4. Identification of a gorilla herpesvirus isolate. Viral proteins and glycoproteins synthesized in CV-1 cells by a herpesvirus isolated from gorilla 3-18 (5336) and those synthesized by HSV-2, SA8, and two laboratory strains of HSV-1 (KOS and F) were compared by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (left). Infected cells were labeled from 4 to 24 h postinfection with either [14 C]glucosamine (lanes G) or [14 C]amino acids (lanes A). DNA isolated from cells infected with the same viruses as well as an additional HSV-1 strain (IS) and B virus (B) were also compared by restriction endonuclease cleavage analysis (right). Approximately equivalent amounts of DNA from each virus were digested to completion with *Kpn*I, and fragments were separated on a 0.6% agarose gel. Bacteriophage lambda DNA cut with *Hind*III was included as a size marker; fragment sizes are given in kilobase pairs at right.

that seropositive gorillas are infected with a virus very similar to HSV (type 1 or 2), isolation of the virus is necessary to confirm this. Virus was isolated from a labial lesion of a single HSV-1-positive gorilla (3-18). Using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, we compared the viral proteins and glycoproteins synthesized in cells infected with this virus with those of several human HSV-1 isolates (Fig. 4). The gorilla isolate was indistinguishable from HSV-1 strains based on the overall number and the relative sizes and quantities of infected cell polypeptides. The gorilla isolate was also compared with human HSV-1 isolates by restriction enzyme cleavage of the viral DNA. The results obtained with *Kpn*I are shown in Fig. 4. Again, the gorilla isolate showed only minor variations in its restriction pattern compared with human HSV-1 isolates. These differences were on the order of those observed among different HSV-1 isolates from humans (3). All restriction fragments of the gorilla isolate also hybridized with HSV-1 DNA under stringent conditions (data not shown).

Sera from other species of apes were also screened for antiherpesvirus antibody (Table 1). A much lower incidence of positive reaction was observed for all these species relative to the incidence seen in gorillas. In chimpanzees, only 7 of 38 (18.4%) sera tested showed specific reactivity with any of the herpesvirus antigens tested. Of these, 2 of 7 (29%) reacted as HSV-1 positive and 5 of 7 (71%) reacted as HSV-2 positive. Of the 23 gibbon serum samples tested, 8 (34.8%) were positive, all of which reacted more strongly with the HSV-1 antigen than with any of the other herpesvirus antigens. Sera from 70 orangutans were also screened. Of these, none were unquestionably positive for antiherpesvirus antibody as seen with positive gorilla, chimpanzee, and

gibbon sera. Five orangutan sera did, however, react very weakly at the lowest serum dilution tested, but only with HSV-1 antigen. On repeated testing of these sera, this borderline reactivity was consistently obtained with four samples, indicating that these animals may actually have low titers of antiherpesvirus antibody. The fifth serum sample (4-19) was positive in some tests (always just at the cutoff point) and negative in others, making the serological status of this animal questionable at best. Identical results were obtained when orangutan sera were tested with SPG (24) rather than SPA for antibody detection (data not shown). Thus, these five sera are listed as positive in Table 1.

Positive sera from species other than gorillas were also tested for their reactivity in immunoblot assays. Figure 5 shows immunoblots of chimpanzee sera. All sera from animals which were seronegative by ELISA were also negative by immunoblot, no specific reaction with any polypeptides of any of the four primate viruses being evident. Based on the relative reactivity of sera with HSV-1 versus HSV-2 antigens, five animals (6-16, 8-19, 8-20, 8-23, 10-24) appeared positive for HSV-2 and two animals (12-31, 12-35) appeared positive for HSV1. These results were consistent with ELISA results (Table 1). Sera of HSV-2-positive animals recognized the HSV-2 p40 antigen (4 of 5) and gG glycoprotein (4 of 5). Sera of both HSV-1-positive animals recognized for gC/pgC, gB, and gD glycoproteins as well as the p40 antigen of HSV-1.

Reactivity of gibbon sera by immunoblot gave results which also paralleled ELISA results (data not shown). All positive gibbon sera reacted like HSV-1 immune sera. Sera which had higher titers by ELISA reacted more strongly with HSV-1 antigen in immunoblot than did sera which had

FIG. 5. Reactivity of chimpanzee sera by immunoblot. Sera were tested at a 1:40 dilution against antigens of cells infected with HSV-1 (lanes 1), HSV-2 (lanes 2), B virus (lanes 3), and SA8 (lanes 4). The locations of the virus-specific HSV-1 gC and HSV-2 gG glycoproteins and the cross-reactive gB and gD glycoproteins were identified by using rabbit antisera specific for these viral antigens.

lower ELISA titers. Lower-titer sera recognized primarily the p40 antigen of HSV, while most higher-titer sera also recognized the pgC glycoprotein. All the orangutan sera which gave equivocal results by ELISA and a number of sera which were completely and repeatedly negative in ELISAs were tested in immunoblot assays (data not shown). Sera from two animals (4-17, 4-20) gave some reaction with the gB glycoprotein antigen of HSV-1. One of these two sera (4-20) was completely negative by both ELISA and radioimmunoassay. Orangutan serum 3-13 and to a lesser extent serum 3-11 (negative by ELISA) recognized the p40 antigens of HSV. The other two sera which gave equivocal results in ELISAs (1-9 and 4-19) did not react specifically with any polypeptides of HSV-1 or HSV-2. While most other ELISA-negative orangutan sera tested in immunoblot assays did not react with any HSV polypeptides, several of these negative sera did react nonspecifically with the VP5, gB, and/or p40 antigens as seen for sera 3-11 and 4-20.

DISCUSSION

Although their existence is to be expected, no direct evidence exists for indigenous HSV-like viruses in any apes. In this study, we screened captive apes for antibody reactive with known primate herpesviruses. The majority of positive gorilla sera and all positive gibbon sera reacted identically to human HSV-1 immune sera. In addition, virus recovered from one seropositive gorilla was indistinguishable from human HSV-1 isolates. Sera from four gorillas from two zoos and the majority of positive sera from chimpanzees reacted like HSV-2-positive human sera. However, subtle but consistent differences were noted in both cELISA and immunoblot assays between the reactivity patterns of three

HSV-2-positive gorilla sera and human HSV-2 immune sera. The fourth HSV-2-positive gorilla serum and all HSV-2-positive chimpanzee sera reacted identically to human HSV-2 immune sera, including recognition of the virus-specific gG glycoprotein of HSV-2. None of the apes tested appeared to be infected with a virus closely related to SA8 or B virus. Thus, all antibody-positive chimpanzees, gibbons, and most gorillas appear to be infected with viruses which are antigenically indistinguishable from HSV-1 and HSV-2.

Results for orangutan sera were different from those obtained for the other ape species. Of the 70 sera screened, only 5 were positive, and these were only marginally so. Testing of these and other ELISA-negative orangutan sera by immunoblotting showed a lack of substantive reactivity with any of the four primate herpesvirus antigens tested. Since some orangutan sera which were consistently negative by ELISA reacted weakly in immunoblots, the reactivity of the five sera which gave equivocal results in ELISAs could not be considered as true positive reactions. Thus, based on combined ELISA and immunoblot analysis, none of the 70 orangutans tested were seropositive.

We observed a considerably higher rate of seropositivity in different species of apes compared with previously published serological surveys (18-20). In these earlier studies, orangutans were reported to be totally lacking in antiherpesvirus antibody when screened by complement fixation and 3.4% were positive when tested by serum neutralization. These results are comparable to those described in this study. Using complement fixation tests to screen other ape sera, very low rates of serological positives were found by other investigators; no positive gorillas or gibbons were identified and chimpanzees varied from 0.9 to 2.0% positive.

Again, a higher incidence of positives was noted (3.6 to 13.4%) with a neutralization test. While these results for chimpanzees are close to what we observed (18.4%), the results for both gorillas and gibbons are significantly different. One likely reason for this difference is the considerably greater sensitivity of ELISA relative to the complement fixation test. That this is likely to account at least in part for the observed differences in positivity rates is supported by the fact that these investigators themselves observed higher rates when sera were tested by serum neutralization (18, 19). A second potential contributory factor to the observed differences is that the studies of Kalter and co-workers (18-20) were performed 18 to 20 years ago when wild apes were still being imported into the United States from abroad, a practice which has all but ceased. Thus, the animals we screened have had a greater opportunity (temporally) to contract HSV from humans than had those tested by Kalter and co-workers. For example, hand rearing of primate infants by zoo personnel would provide ample opportunity for human-to-ape transmission of HSV-1. This suggests that human-to-ape transmission of HSV accounts in large part for the seropositive status of captive apes.

An interesting result of this study was the variation in distribution of serological positives among the different species examined. While 65.4% of gorillas had antibodies reactive with HSV, only 18.4% of chimpanzees and 0.0% (or, considering the equivocal sera as positives, 5 of 70 or 7.1% at most) of orangutans were seropositive. Given the small number of chimpanzees tested, the difference between chimpanzees and gorillas may not be biologically significant. However, the low rate (and probable total absence) of antiherpesvirus antibodies in orangutans is significant. Since HSV-like herpesviruses isolated from lower primate species (baboons and macaques) are antigenically related to HSV-1 and HSV-2, any HSV-like virus indigenous to orangutans (which are evolutionarily more closely related to humans than the cercopithecoid monkeys) should be antigenically similar to HSV-1 and HSV-2 and/or SA8 and B virus. The assay systems we employed do detect immunoglobulin G of each ape species (unpublished data), making it unlikely that the observed differences in seropositivity rates are due to a failure to detect immunoglobulin G in certain species. Given the susceptibility of chimpanzees, gibbons, and numerous species of monkeys to infection by HSV-1, HSV-2, SA8, and/or B virus (1, 10, 13, 25, 31, 32, 35), we do not consider it likely that orangutans as a species would be inherently less susceptible (or more resistant) to HSV than other ape species. Similarly, it is unlikely that orangutans as a species have less opportunity than other apes to acquire HSV via human contact. The absence of antiherpesvirus antibodies in orangutans does imply the nonexistence of an indigenous orangutan virus. However, testing of additional serum samples from additional sources may yet identify infected animals.

The difference in the relative incidence of HSV-1 versus HSV-2 in gorillas, chimpanzees, and gibbons is interesting. Although the numbers of gibbons and chimpanzees tested were relatively small, 100% of gibbons and 92.5% of gorillas were HSV-1 positive compared with only 29% of chimpanzees. The distribution of HSV-1- versus HSV-2-positive animals among different zoos was similarly different for gorillas and chimpanzees. The four gorillas identified as HSV-2 positive by ELISA resided in only 2 of 16 zoos with positive animals, while HSV-2-positive chimpanzees were located in 3 of 4 zoos with positive animals. Although both HSV-1-positive gorillas and HSV-2-positive chimpanzees

were found within a single zoo (zoos 8 and 10), within a species animals were positive for only HSV-1 or HSV-2. This exclusivity of HSV serotype within species suggests that these viral infections may have derived from a common source within a species with subsequent spread from animal to animal rather than all being individual human-to-ape transmission events. However, the original introduction(s) of virus(es) may have occurred as isolated incidences of human-to-ape transmission. Consistent with this premise, HSV-2 infections have been observed to spread from chimpanzee to chimpanzee (31).

Owing to the high level of antigenic cross-reactivity expected to exist between the human HSVs likely to be contracted by animals in captivity and any indigenous ape viruses, it is difficult to draw concrete conclusions from serological studies alone unless positive ape sera react in a manner entirely distinct from human HSV immune sera. With the possible exception of three gorilla sera (1-1, 1-2, 1-4), this was not found to be the case; positive ape sera identified in this study behaved very much like either HSV-1- or HSV-2-positive human sera. The most convincing means of identifying an indigenous ape virus would be isolation of a virus from seropositive animals and molecular characterization and comparison with HSV-1 and HSV-2. However, the need for frequent and close examination of the animals would make it difficult to identify spontaneous recurrent lesions or to isolate intermittent asymptotically shed virus.

In conclusion, it can be stated that infections with HSV-like viruses are relatively common in gorillas, chimpanzees, and gibbons but apparently not in orangutans. Although the reasons for it are unclear, the incidence of such infections appears to vary considerably from species to species, with gorillas having the highest incidence. The viruses with which these animals are infected are very closely related if not identical to the human HSV-1 and HSV-2; these viruses are clearly not as closely related to either the baboon SA8 or the macaque B virus isolates. Both gibbons and gorillas were predominantly infected with an HSV-1-like virus, while an HSV-2-like virus was more prevalent in chimpanzees. Three gorillas which were infected with an HSV-2-like virus did not have the antigen recognition pattern typical of human HSV-2 immune sera (9) which was observed for all HSV-2-positive chimpanzee sera and a fourth HSV-2-positive gorilla serum, raising the possibility that these three gorillas harbor an indigenous gorilla herpesvirus related to HSV-2. Final determination whether these gorillas harbor an indigenous gorilla herpesviruses will require virus isolation or analysis of sera from feral animals without a history of human contact.

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LITERATURE CITED

- Barahona, H., L. V. Melendez, and J. L. Melnick. 1974. A compendium of herpesviruses isolated from non-human primates. *Intervirology* 3:175-192.
- Boulter, E. A. 1975. The isolation of monkey B virus (*Herpesvirus simiae*) from the trigeminal ganglia of healthy seropositive rhesus monkeys. *J. Biol. Stand.* 3:279-280.
- Chaney, S. M. J., K. G. Warren, and J. H. Subak-Sharpe. 1983. Variable restriction endonuclease sites of herpes simplex virus type 1 isolates from encephalitic, facial and genital lesions and ganglia. *J. Gen. Virol.* 64:2717-2733.
- Davidson, W. L., and K. Hummeler. 1960. B virus infection in man. *Ann. N.Y. Acad. Sci.* 85:970-979.
- Eberle, R., and R. J. Courtney. 1980. Preparation and characterization of specific antisera to individual glycoprotein antigens comprising the major glycoprotein region of herpes simplex virus type 1. *J. Virol.* 35:902-917.
- Eberle, R., and R. J. Courtney. 1980. gA and gB glycoproteins of herpes simplex virus type 1: two forms of a single polypeptide. *J. Virol.* 36:665-675.
- Eberle, R., and J. K. Hilliard. 1984. Replication of simian herpesvirus SA8 and identification of viral polypeptides in infected cells. *J. Virol.* 50:316-324.
- Eberle, R., S.-W. Mou, and J. A. Zaia. 1984. Polypeptide specificity of the early antibody response following primary and recurrent genital herpes simplex virus type 2 infections. *J. Gen. Virol.* 65:1839-1843.
- Eberle, R., S.-W. Mou, and J. A. Zaia. 1985. The immune response to herpes simplex virus: comparison of the specificity and relative titers of serum antibodies directed against viral polypeptides following primary herpes simplex virus type 1 infections. *J. Med. Virol.* 16:147-162.
- Emmons, R. W., D. H. Gribble, and E. H. Lennette. 1968. Natural infection of an owl monkey (*Aotus trivirgatus*) with herpes-T virus. *J. Infect. Dis.* 118:153-159.
- Gay, F. P., and M. Holden. 1933. The herpes encephalitis problem. *J. Infect. Dis.* 53:287-303.
- Heuschele, W. P. 1960. Varicella (chicken pox) in three young anthropoid apes. *J. Am. Vet. Med. Assoc.* 136:256-257.
- Holmes, A. W., R. G. Caldwell, R. E. Dedmon, and F. Deinhardt. 1964. Isolation and characterization of a new herpes virus. *J. Immunol.* 92:602-610.
- Holmes, A. W., J. A. Devine, E. Nowakowski, and F. Deinhardt. 1966. The epidemiology of a herpesvirus infection of New World monkeys. *J. Immunol.* 90:668-677.
- Honess, R. W., and D. H. Watson. 1977. Unity and diversity in the herpesviruses. *J. Gen. Virol.* 37:15-37.
- Hull, R. N. 1973. The simian herpesviruses, p. 389-426. In A. S. Kaplan (ed.), *The herpesviruses*. Academic Press, Inc., Orlando, Fla.
- Hull, R. N., A. C. Dwyer, A. W. Holmes, E. Nowakowski, F. Deinhardt, E. H. Lennette, and R. W. Emmons. 1972. Recovery and characterization of a new simian herpesvirus from a fatally infected spider monkey. *J. Natl. Cancer Inst.* 49:225-231.
- Kalter, S. S., and R. L. Heberling. 1971. Comparative virology of primates. *Bacteriol. Rev.* 35:310-364.
- Kalter, S. S., J. Ratner, G. V. Kalter, A. R. Rodriguez, and C. S. Kim. 1967. A survey of primate sera for antibodies to viruses of human and simian origin. *Am. J. Epidemiol.* 86:552-568.
- Kalter, S. S., J. J. Ratner, A. R. Rodriguez, R. L. Heberling, and N. B. Guilloud. 1969. Antibodies to human and simian viruses in the gorilla (*Gorilla gorilla*). *Lab. Anim. Care* 19:63-66.
- Kalter, S. S., S. A. Weiss, R. L. Heberling, J. E. Guajardo, and G. C. Smith. 1978. The isolation of herpesvirus from trigeminal ganglia of normal baboons (*Papio cynocephalus*). *Lab. Anim. Sci.* 28:705-709.
- Katz, D., J. K. Hilliard, R. Eberle, and S. L. Lipper. 1986. ELISA for detection of group-common and virus-specific antibodies in human and simian sera induced by herpes simplex and related viruses. *J. Virol. Methods* 14:99-109.
- Keeble, S. A. 1960. B virus infection in monkeys. *Ann. N.Y. Acad. Sci.* 85:960-969.
- Langone, J. J. 1982. Protein A of *Staphylococcus aureus* and related immunoglobulin receptors produced by streptococci and pneumococci. *Adv. Immunol.* 32:157-252.
- Loomis, M. R., T. O'Neill, M. Bush, and R. J. Montali. 1981. Fatal herpesvirus infection in patas monkeys and white colobus monkey. *J. Am. Vet. Med. Assoc.* 179:1236-1239.
- Malherbe, H., and R. Harwin. 1958. Neurotropic virus in African monkeys. *Lancet* ii:530.
- Malherbe, H., and M. Strickland-Cholmley. 1969. Simian herpesvirus SA8 from a baboon. *Lancet* ii:1427.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Marennikova, S. S., N. N. Maltseva, E. M. Shelukhina, L. S. Shenkman, and V. I. Korneeva. 1973/74. A generalized herpetic infection simulating smallpox in a gorilla. *Intervirology* 2:280-286.
- McClure, H. M., and M. E. Keeling. 1971. Viral diseases noted in the Yerkes Primate Center colony. *Lab. Anim. Sci.* 21:1002-1010.
- McClure, H. M., R. B. Swenson, S. S. Kalter, and T. L. Lester. 1980. Natural genital *Herpesvirus hominis* infection in chimpanzees (*Pan troglodytes* and *Pan paniscus*). *Lab. Anim. Sci.* 30:895-901.
- Melnick, J. L., and D. D. Banker. 1954. Isolation of B virus (herpes group) from the central nervous system of a rhesus monkey. *J. Exp. Med.* 100:181-194.
- Melnick, J. L., M. Midulla, I. Wimberly, J. G. Barrera-Oro, and B. M. Levy. 1964. A new member of the herpesvirus group isolated from South American marmosets. *J. Immunol.* 9:596-602.
- Myers, M. G., L. W. Kramer, and L. R. Stanberry. 1987. Varicella in a gorilla. *J. Med. Virol.* 23:317-322.
- Ramsay, E., E. L. Stair, A. E. Castro, and M. I. Marks. 1982. Fatal *Herpesvirus hominis* encephalitis in a white-handed gibbon. *J. Am. Vet. Med. Assoc.* 181:1429-1430.
- Roizman, B., L. E. Carmichael, F. Deinhardt, G. de-The, A. J. Nahmias, N. Plowright, F. Rapp, P. Sheldrick, M. Takahashi, and K. Wolf. 1981. Herpesviridae. Definition, provisional nomenclature, and taxonomy. *Intervirology* 16:201-217.
- Sabin, A. B., and A. W. Wright. 1934. Acute ascending myelitis following a monkey bite, with the isolation of a virus capable of producing the disease. *J. Exp. Med.* 59:115-135.
- Walboomers, J. M., and J. ter Schagget. 1976. A new method for the isolation of herpes simplex type 2 DNA. *Virology* 74:256-258.
- Zwartouw, H. T., and E. A. Boulter. 1984. Excretion of B virus in monkeys and evidence of genital infection. *Lab. Anim.* 18:65-70.
- Zwartouw, H. T., J. A. MacArthur, E. A. Boulter, J. H. Seamer, J. H. Marston, and A. S. Chamove. 1984. Transmission of B virus infection between monkeys especially in relation to breeding colonies. *Lab. Anim.* 18:125-130.