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Antigenic Relationship of Human Foamy Virus to the Simian Foamy Viruses

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A foamy virus isolated by Achong et al. from a human nasopharyngeal carcinoma was studied to determine its antigenic relationship to the eight known simian foamy viruses (SFV). Using reciprocal cross-immunofluorescent and cross-neutralization techniques, we found the human isolate to be closely related to SFV type 6. In a seroepidemiological survey, neutralizing antibody to the human foamy virus was not detected in the sera of animal caretakers or laboratory personnel who routinely handled a wide variety of nonhuman primates. We conclude that the human isolate most probably represents a variant strain of SFV type 6.

In an extensive review of the simian foamy viruses (SFV), Hooks and Gibbs were able to classify the eight known serotypes into subgroups based upon serological reactions and the species of nonhuman primate from which each serotype had been isolated (3). Achong et al. (1) reported the isolation of a new virus from a cell line derived from a nasopharyngeal carcinoma of a Kenyan African. Subsequently, Epstein and his colleagues (2) characterized the agent as a new, immunologically distinct member of the foamy virus group, a finding based primarily upon biological, morphological, and one-way neutralization data. The present study was designed to explore further the antigenic relationship of the human foamy isolate to the eight known simian foamy viruses with reciprocal cross-neutralization and cross-immunofluorescent techniques. In a separate study, several biological properties of the agent also have been defined (P. Brown et al., J. Infect. Dis., in press).

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MATERIALS AND METHODS

Viruses. SFV types 1 through 5 (SFV-1 through SFV-5) were obtained from the office of Program Resources and Logistics, Viral Oncology, National Cancer Institute, National Institutes of Health, and were identified as SFV-1 (67-10), SFV-2 (68-11), SFV-3 (68-12), SFV-4 (68-13), and SFV-5 (68-14). Each serotype had undergone two additional passages in dog thymus cell cultures before use in these experiments.

SFV types 6 through 8 (SFV-6 through SFV-8) were isolated and serologically characterized in our laboratory (4, 5). SFV-6 was passaged 12 times in human embryonic kidney cells and 2 times in dog thymus cells. SFV-7 was passaged 22 times in human embryonic kidney cells and 2 times in dog thymus cells. SFV-8 underwent two passages in human embryonic kidney cells and two passages in dog thymus cells.

Human foamy virus, as it will be referred to henceforth, was kindly supplied by M. Epstein; in our hands, it had undergone three passages in human embryonic lung cells (MA-321) and seven passages in dog thymus cells.

Stock virus was prepared by inoculating drained, subconfluent dog thymus cell cultures at a multiplicity of infection of 0.01. After adsorption for 1 h at 37°C, fresh medium consisting of Eagle minimum essential medium with 10% heat-inactivated fetal bovine serum and antibiotics was added. When the cells displayed 50 to 75% cytopathic effect, the virus-containing culture fluids were collected, dispensed into vials, and frozen at −70°C until needed. The titer of the stock virus was 10^4 50% tissue culture infective doses ($TCID_{50}$)/ml.

Single-dose antisera. Concentrated virus used in the preparation of monospecific antisera was prepared by inoculating subconfluent dog thymus roller cell cultures with stock virus. When the cells displayed approximately 50% cytopathic effect, the culture fluids were harvested at 4-h intervals and stored at −70°C. Only early morning harvests were omitted. The procedure was continued until 95 to 100% of the cells were destroyed. The virus-containing culture fluids were thawed, pooled, and clarified by centrifugation at 10,000 × *g* for 15 min in a Beckman type 19 rotor. The clarified fluid was carefully layered onto a 10-ml density cushion consisting of 20% glycerol, 0.05 M tris(hydroxymethyl)aminomethane (pH 7.4), and 0.1 M KCl and centrifuged at 70,000 × *g* for 90 min in a Beckman type 35 rotor. The pellets were suspended in a small volume of phosphate-buffered saline and stored at −70°C until needed.

Concentrated virus preparations of SFV-6 or human

foamy virus were emulsified with equal volumes of Freund complete adjuvant, and 1.0-ml volumes were inoculated intramuscularly into both hind legs of adult Hartley guinea pigs. The animals were bled on day 28, and the sera were collected.

Hyperimmune antisera. Hyperimmune sera against SFV-1 through SFV-7 were obtained from the Office of Program Resources and Logistics, National Cancer Institute. The lot numbers of the SFV immune sera used in this study were: SFV-1 antiserum, 7EF-1000; SFV-2 antiserum, 3DEF-1110; SFV-3 antiserum, 68-1200; SFV-4 antiserum, 4DEF-1300; SFV-5 antiserum, 151; SFV-6 antiserum, 161; SFV-7 antiserum, 68-1600. Antisera to SFV types 1, 2, and 4 were prepared in horses, and antisera to types 3, 5, 6, and 7 were produced in rabbits. Rabbit antiserum to SFV-8 was prepared in our laboratory as previously described (4). Antiserum against human foamy virus was prepared by inoculating adult New Zealand white rabbits intravenously at weekly intervals with stock virus in 1.0-ml volumes. The animals were bled on day 56, and the sera were collected.

Neutralization tests. Serum neutralization tests were performed by making serial twofold dilutions of serum in phosphate-buffered saline and mixing with equal volumes of 10 or 100 $TCID_{50}$ of virus. The virus-serum mixtures were incubated at ambient temperature (22 to 25°C) for 60 min and inoculated in duplicate onto subconfluent dog thymus cell cultures in 24-well plastic tissue culture plates. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Cultures were observed twice weekly and terminated at the end of 4 weeks. Neutralization titers were expressed as the reciprocal of the highest dilution of serum completely preventing the development of cytopathic effect. Preimmunization sera served as negative controls and were included in all tests.

Seroepidemiological survey. Neutralization tests were performed on sera collected from a total of 38 animal caretakers and laboratory personnel who routinely handle a wide variety of nonhuman primates. Serum was tested at a 1:4 dilution against 10 $TCID_{50}$ of virus.

Indirect immunofluorescent assay. Virus-infected cells used in the immunofluorescent study were prepared in the following manner. Subconfluent monolayers of rhesus lung cells were washed once with phosphate-buffered saline and inoculated with 0.5 ml of stock virus. After adsorption for 1 h at 37°C, the cultures were fed with minimum essential medium containing 10% heat-inactivated fetal bovine serum and antibiotics and incubated at 37°C. At the first indication of cytopathic effect, the cultures were washed, trypsinized, and planted on glass cover slips in tissue culture plates. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. When the monolayers displayed approximately 50% cytopathic effect, the cover slips were removed, washed with phosphate-buffered saline, and fixed in acetone for 2 h at −20°C. After fixation, they were air dried and stored at −70°C until needed.

For indirect immunofluorescent tests, the fixed cells were incubated with antisera at 37°C for 30 min on a rotary shaker, washed with phosphate-buffered saline, and incubated with a 1:20 dilution of fluorescein-conjugated antiglobulin for 30 min. After treatment with conjugate, the phosphate-buffered saline wash was repeated, and the cells were air dried and mounted on glass slides in buffered glycerol mounting media. A Nikon model S microscope with a fluorescence attachment was used to examine the preparations. Controls consisted of the treatment of (i) infected cells with preimmune sera, (ii) uninfected cells with immune serum, (iii) infected cells with immune serum from a different species followed by treatment with standard immune serum, and (iv) infected cells with conjugated serum only.

RESULTS

The antigenic relationship of human foamy virus to the eight SFVs as indicated by cross-immunofluorescent screening with sera diluted 1:4 is shown in Table 1.

Cover slips of rhesus lung cells infected with human foamy virus or each of the eight SFV serotypes displayed maximal fluorescent staining after treatment with homologous hyperimmune antisera. Two-way cross-reactivity was observed between human foamy virus and SFV-6, SFV-7, and, to a lesser degree, SFV-1. Two-way

TABLE 1. *Antigenic cross-reactivity by immunofluorescence: candidate human foamy virus versus SFV*

Virus type	Fluorescence intensity[a] of antisera[b] to:								
	Human foamy virus	SFV							
		1	2	3	4	5	6	7	8
Human foamy	+++	+	+	−	−	−	+++	++	−
SFV-1	+	+++	++	−	−	−	−	−	−
SFV-2	−	++	+++	−	−	−	−	−	−
SFV-3	−	++	+	+++	−	−	−	−	−
SFV-4	−	−	−	−	+++	−	−	−	−
SFV-5	−	−	−	−	−	+++	−	−	−
SFV-6	++	−	−	−	−	−	+++	++	−
SFV-7	+	−	+	−	−	−	++	+++	−
SFV-8	−	−	−	−	−	−	−	−	+++

[a] Fluorescence intensity: +++, maximal; ++, less brilliant; +, dull; −, negative.
[b] All sera were tested at a dilution of 1:4.

cross-relationships were also observed between SFV types 6 and 7 and between SFV types 1 and 2.

One-way heterologous reactions were observed between SFV-3 and SFV-1 antiserum, SFV-3 and SFV-2 antiserum, and SFV-7 and SFV-2 antiserum.

The results of cross-neutralization experiments with human foamy virus and the eight SFV types are shown in Table 2. Approximately 100 $TCID_{50}$ of human foamy virus and each of the eight SFV serotypes were completely neutralized with homologous hyperimmune antisera. A reciprocal cross-relationship was observed between human foamy virus and SFV-6, but not between human foamy virus and either SFV-7 or SFV-1, although all three viruses cross-reacted in the immunofluorescent test. A heterologous response between SFV-7 and SFV-2 antisera confirmed a previous observation made in this laboratory (4). Hull reported heterologous cross-reactivity between SFV-1 and SFV-3 antisera and in the same monograph presented data from the work of P. B. Johnston showing reciprocal cross-reactivity between SFV types 1 and 3 (6). The homologous antibody titers of SFV types 1 and 3 used in this study were lower than those used by Hull or Johnston; this probably explains our inability to corroborate their findings.

Antiserum to human formy virus or SFV 6 was prepared with only a single injection of virus to produce more highly specific antisera and to circumvent the cross-reactivity often inherent in hyperimmune or multiple-dose antisera. The results of a two-way cross-neutralization experiment with single-dose antisera are shown in Table 3. Sera were incubated with approximately 100 $TCID_{50}$ of virus. Human foamy virus was neutralized by single-dose human foamy virus antiserum at a dilution of 1:64. The same antiserum also weakly neutralized SFV-6 at a 1:2 dilution. Single-dose antiserum prepared against SFV-6 neutralized SFV-6 at a dilution of 1:16 and human foamy virus at 1:16.

A seroepidemiological survey was performed to determine whether human foamy virus or SFV type 6 or 7 infections occur in humans. Sera were collected from 14 animal caretakers and 24 laboratory personnel who routinely handle a wide variety of nonhuman primates, including chimpanzees, and were tested for neutralizing antibody to human foamy virus and SFV types 6 and 7. None was found to possess antibody to these agents, as sera diluted 1:4 failed to neutralize the infectivity of 10 $TCID_{50}$ of each virus.

DISCUSSION

The observation of Epstein and his co-workers (2) that antisera to SFV-1 through SFV-7, bovine syncytial virus, and feline syncytial virus failed to neutralize the in vitro infectivity of the human foamy virus isolate served as the serological basis for characterization of the agent as a new, immunologically distinct member of the foamy virus group. They did, however, observe slight but consistent delay in the onset of cytopathic effect (2 to 3 days) after treatment with antisera to SFV-6 and bovine syncytial virus, suggesting a slight degree of immunological relationship between the human isolate and SFV-6 and bovine syncytial virus.

We were unable to confirm the observation of Epstein regarding the immunological uniqueness of the isolate. We found the virus to be closely related to SFV-6 by reciprocal immunofluorescent and neutralization tests. Two-way neutralization tests with highly specific single-dose antisera confirmed the association. In a recently completed study, the human isolate was also indistinguishable from SFV types 6 and 7 by complement fixation testing, and sera from a

TABLE 2. *Antigenic cross-reactivity by neutralization: candidate human foamy virus versus SFV*

Virus type	Neutralization of antisera to:								
	Human foamy virus	SFV							
		1	2	3	4	5	6	7	8
Human foamy	64[a]	0[b]	0	0	0	0	32	0	0
SFV-1	0	256	0	0	0	0	0	0	0
SFV-2	0	0	32	0	0	0	0	0	0
SFV-3	0	0	0	64	0	0	0	0	0
SFV-4	0	0	0	0	32	0	0	0	0
SFV-5	0	0	0	0	0	16	0	0	0
SFV-6	4	0	0	0	0	0	64	0	0
SFV-7	0	0	8	0	0	0	0	64	0
SFV-8	0	0	0	0	0	0	0	0	32

[a] Reciprocal of the dilution that completely inhibited the cytopathic effect.
[b] 0, Failure of a 1:4 dilution of antiserum to neutralize 100 $TCID_{50}$ of virus.

TABLE 3. *Cross-neutralization antibody titers to human foamy virus and SFV type 6 with antisera prepared by single injections of virus*

Virus type	Antisera to:	
	Human foamy virus	SFV-6
Human foamy	64[a]	16
SFV-6	2	16

[a] Reciprocal of the dilution that completely inhibited CPE.

total of 256 humans, including patients with nasopharyngeal carcinoma and Burkitt's lymphoma, failed to neutralize the infectivity of the isolate (Brown et al., in press). Furthermore, we were unable to detect neutralizing antibody to human foamy virus or SFV types 6 or 7 in animal caretakers and laboratory personnel who routinely handle a wide variety of nonhuman primates.

It appears most probable, therefore, that the human isolate represents a variant strain of SFV type 6. Because of the unavailability of a serological confirmation of the agent in the individual from whom it was isolated, we can only conjecture that the isolate represents either the chance infection of a human with a simian foamy virus or the unfortunate discovery of a laboratory contaminant.

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