Isolation of Foamy Virus Types 1 and 2 from Primary Rhesus Monkey Brain Cultures (35780)

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The existence of latent foamy viruses in monkey kidney cell cultures has been demonstrated by several investigators (1–5). Presently seven simian foamy virus serotypes are recognized (6–10). Serologic and virus isolation studies have indicated that the predominant foamy virus serotype associated with rhesus monkeys has been type 1 (7, 11–14).

Our laboratory has been involved with studies on the optimal conditions for dispersing and cultivating brain cells *in vitro* in order to study the course of measles virus encephalitis in rhesus monkeys (15) and to isolate measles virus from brain tissue (16). During the course of these investigations, nine foamy viruses were isolated from primary rhesus brain cultures or primary rhesus brain–Vero cell co-cultures. This report deals with the isolation and subsequent serological identification of these foamy virus isolates.

Materials and Methods. Source of monkeys. Rhesus monkeys (*Macaca mulatta*) were received from a commercial dealer in New Delhi, India, quarantined on arrival in one large room, and one or two animals were housed per cage. These rhesus monkeys had not been exposed to other simian species known to harbor the type 2 foamy virus. Thirty-four measles-negative monkeys, as determined by hemagglutination-inhibition tests, were assigned to 8 experimental sensitization or immunosuppression treatment groups as indicated in Table I. Serum samples were taken prior to and approximately 28 days after treatment.

Sensitization. Some monkeys were hyperimmunized by intramuscular (im) injection into each leg of 0.25 ml of a mixture of equal volumes of killed measles virus reference vaccine (DBS Lot 2) and Difco Freund's complete adjuvant (FCA). Other animals were inoculated intradermally in five sites on the shaved back with a total of 0.5 ml of a CNS antigen consisting of a mixture of equal volumes of 40% guinea pig spinal cord homogenate and FCA.

Immunosuppression. Essentially, each animal received a total of 180 mg/kg of cyclophosphamide (Cytoxan; Mead Johnson Laboratories, Evansville, Indiana) divided into 4 daily iv injections. This was supplemented with 6 injections of amethopterin (Methotrexate; Lederle Laboratories, Pearl River, New York) given 2, 4, 6, 13, 20, and 27 days after the last injection of Cytoxan.

Viruses. Known simian foamy virus types 1, 2, and 3 were obtained from Dr. P. Johnston of the University of Louisville, Louisville, Kentucky. The passage history of these viruses is as follows: type 1 (strain FV-21): *Macaca cyclopsis,* primary rabbit kidney (RK)/13, KB/1, $LLCMK_2$/7, KB spinner culture/1, primary RK/2; type 2 (strain FV-34): *Macaca cyclopsis,* primary RK/22, KB spinner culture/2, primary RK/2; type 3 [strain FV-2014; (7)]: primary RK/6, $LLCMK_2$/6, primary RK/2. The foamy viruses which were isolated from primary rhesus monkey brain cultures or rhesus brain–Vero co-cultures were passaged three additional times in primary RK cell cultures.

Stock virus pools were prepared by inoculating 2-oz bottle cultures of primary RK cells with 0.2 ml of undiluted seed virus. After adsorption at 36° for 1 hr, each bottle received 5.0 ml of maintenance medium

which consisted of Earle's balanced salt solution containing 0.5% lactalbumin, 2% unheated fetal calf serum, and antibiotics (penicillin G, 100 units/ml; streptomycin sulfate, 200 μg/ml; Mycostatin, 50 units/ml). When cytopathic effect involved 75 to 100% of the cell sheet, cells and fluid were harvested and stored in glass-sealed ampoules at −70°.

Virus infectivity. Titrations of foamy viruses were performed by inoculating primary RK tube cultures with 0.1 ml of virus preparation, four tubes for each 10-fold dilution. After adsorption at 36° for 1 hr, each tube received 1.0 ml of the maintenance medium described above. The Kärber method (17) was used to calculate the $TCID_{50}$ end points. Titers of stock virus pools of the known foamy viruses and the isolates ranged from $10^{2.3}$ to $10^{5.8}$ $TCID_{50}$/1.0 ml, when read at 3 weeks.

Cell culture. Primary rabbit kidney cell cultures were prepared in the routine manner established by the Cell Biology Section of our laboratory. Cultures were received in our laboratory 5 days after initiation, changed to the maintenance medium described above, and used after monolayers formed.

Virus isolation. Rhesus monkey brain tissue removed at autopsy was minced, washed three times with Dulbecco's phosphate-buffered saline without calcium and magnesium (18), and dissociated by trypsinization as described previously (16). A 10% brain cell suspension was prepared in Eagle's minimal essential medium with Earle's salt base (MEME) and 0.2 ml of this suspension was seeded into each of three petri dishes (35-mm diam, Falcon Plastics) containing 3.0 ml of MEME with 10% fetal calf serum and antibiotics (penicillin G, 100 units/ml; streptomycin sulfate, 100 μg/ml). Additionally, 0.6 ml of the 10% brain cell suspension was mixed with 9.0 ml of a suspension (10^5 cells/ml) of the Vero African green monkey kidney cell line (19) and seeded into three 35-mm petri dishes (co-cultivation). Some petri dishes contained coverslips for use in fluorescent antibody tests. All petri dishes were incubated in an humidified CO_2 incubator at 37°. The cultures and co-cultures were observed periodically for cytopathic changes for at least 3 weeks.

Identification of viral agents. When cytopathic changes were observed, coverslip cultures were examined by the fluorescent antibody (FA) technique (15) for the presence of measles and SV_{40} viruses. Neutralization tests were used for the serological typing of the foamy virus isolates using known antisera (Foamy virus types 1, 2, and 3 immune goat sera lots Q-1 E: 881000, 882000, and 883000, respectively, were obtained from Flow Laboratories). Foamy virus type 2 immune horse serum lot 3DEF-1110 was obtained through the National Cancer Institute.

Neutralization studies. Virus neutralization studies were performed in the following manner. All reagents were kept in an ice-water bath before and during mixing. Virus diluted in Dulbecco's phosphate-buffered saline [DPBS; (18)] with 0.5% bovine plasma albumin to contain 100 $TCID_{50}$/0.1 ml was added to equal volumes of serial 2-fold dilutions of unheated serum in the same diluent. Virus-diluent controls consisted of equal volumes of the diluted virus preparation and diluent. After incubation at room temperature (23 to 25°) for 1 hr, the mixtures were returned to the ice-water bath, and 0.2 ml of each virus–serum mixture was inoculated into each of four tube cultures of primary RK cells. The virus–diluent preparation was assayed for virus by making serial 10-fold dilutions in the diluent and inoculating 0.2 ml of each dilution into each of four primary RK tube cultures. After adsorption at 36° for 1 hr, 1.0 ml of the above described maintenance medium was added to each tube. Tubes were incubated at 36° and examined on days 7, 9, and 14. The final reading was used to calculate the 50% serum-neutralizing end point by the Kärber formula (17).

Results. Table I shows the 8 treatment groups to which rhesus monkeys were assigned in the study on measles encephalitis (15). Also included in Table I are those monkeys whose brain cell cultures were positive by FA technique for measles virus and those cultures from which latent viruses (SV_{40} and foamy viruses) were isolated and

TABLE I. Summary on the Isolation of Foamy Viruses from Primary Rhesus Brain Cultures or Primary Rhesus Brain–Vero Co-cultures.[a]

Treatment group	Rhesus monkey no.	Measles virus isolation		Foamy virus-like CPE (day)		Identification of latent agents[b]	
		Primary rhesus brain cultures	Rhesus brain–Vero co-cultures	Primary rhesus brain cultures	Rhesus brain–Vero co-cultures	Foamy virus	SV_{40}
1. Untreated	672	0	0	0	0	ND	ND
	673	0	0	16	14	+	0
	674	0	0	8	8	+	0
2. Untreated; IC inoculation of measles virus	667	0	+	—[c]	—[c]	+	0
	668	0	0	0	0	ND	ND
	669	0	+	—[c]	—[c]	+	0
	670	0	0	0	0	ND	ND
3. Specifically immunosuppressed; IC inoculation of measles virus	662	0	0	21[d]	21[d]	0	ND
	664	0	0	21	21	+	0
	665	0	0	0	21	0	ND
4. Cytoxan-treated; IC inoculation of measles virus	713	+	+	0	0	0	0
	720	0	0	0	0	ND	ND
	722	0	0	0	0	ND	ND
	709	+	+	0	0	0	0
5. Measles-sensitized; IC inoculation of measles virus	774	0	0	0	0	ND	ND
	775	0	0	18[d]	0	0	ND
	777	0	0	0	18	ND	ND
6. Measles- and CNS-sensitized; IC inoculation of measles virus	766	+	+	17	17	ND	ND
	767	0	0	8	8	+	0
	772	0	0	0	0	ND	ND
	773	0	+	0	0	0	0
7. CNS-sensitized; IC inoculation of measles virus	755	0	+	0	0	0	+
	756	0	0	18	18	+	0
	757	0	0	0	0	0	0
	763	0	0	19	9	+	0
	765	0	0	0	0	ND	ND

TABLE 1 (continued)

Treatment group	Rhesus monkey no.	Measles virus isolation		Foamy virus-like CPE (day)		Identification of latent agents[b]	
		Primary rhesus brain cultures	Rhesus brain–Vero co-cultures	Primary rhesus brain cultures	Rhesus brain–Vero co-cultures	Foamy virus	SV_{40}
8. CNS-sensitized	741	0	0	0	0	ND	ND
	751	0	0	0	0	ND	ND
	752	0	0	19	9	+	0
	754	0	0	0	0	ND	ND

[a] Abbreviations: IC = intracerebral; CNS = central nervous system; ND = not done.

[b] Neutralization tests were used to identify foamy viruses. SV_{40} virus was identified by fluorescent antibody technique after passage in BS-C-1 cultures.

[c] Possible foamy virus-like CPE would be obscured by measles-specific cytopathic changes.

[d] Before material from rhesus monkeys 662 and 775 were received for possible foamy virus isolation, the material had been frozen and thawed several times. This could have inactivated any agent which was present in the material and account for our inability to isolate any agent from these specimens.

identified.

Cross neutralization with the nine foamy virus isolates revealed two distinct foamy virus serotypes (Table II). Neutralization tests were repeated using both immune goat and immune horse serum to foamy virus type 2 against approximately 100 $TCID_{50}$ of isolates 667 and 763.

The presence or absence of foamy virus type 1 or 2 antibody in two rhesus serum samples, one obtained before treatment and one obtained approximately 1 month after treatment, showed no direct relationship to virus isolation (Table III).

Discussion. Isolation and serologic studies with rhesus monkeys have shown them to have a high incidence of foamy virus type 1 (7, 11, 13). However, previous isolation studies have employed only primary rhesus monkey kidney cell cultures (2–7, 11, 13, 14). Our present data have demonstrated the presence of foamy virus in primary rhesus monkey brain cell cultures. Although, as indicated by other investigators and supported by our data, foamy virus type 1 was the predominant latent agent in rhesus monkeys, foamy virus type 2 isolates were also recovered from the rhesus brain cultures. Previous studies on the isolation of latent viruses in chimpanzees have resulted in the isolation of foamy virus serotypes 6 (Pan 1) and 7 (Pan 2) from brain as well as other tissues (9, 20). Foamy viruses are probably as prevalent in the various tissues of rhesus monkeys, the only deterrent to their detection being the technique of virus isolation from the various primary tissues.

Although a limited number of monkeys per treatment group were used, general suppression of the immune response with Cytoxan or induction of allergic encephalitis did not appear to affect the incidence of foamy virus isolation from both the treated and control groups.

Our data are in agreement with neutralization studies by Swack *et al.* (14) who observed that a high percentage of rhesus monkey sera has a foamy virus antibody titer too low to be detected by the neutralization test. However, successful isolation from primary brain cultures might require animals with only low serum antibody titers. Further-

TABLE II. Cross Neutralization of Simian Foamy Virus by Goat or Horse Antiserum.[a]

Virus strain or isolate	Source of antibody reagent	$TCID_{50}$/0.1 ml	50% serum neutralizing titer[b] Type: 1[c]	2[c]	3[c]
SFV type 1[d]	Goat	200	150	0	0
	Horse	200	ND	0	ND
SFV type 2[d]	Goat	200	0	512	0
	Horse	300	ND	256	ND
SFV type 3[d]	Goat	200	0	0	128
664	Goat	100	102	0	0
667	Goat	60	0	256	0
667	Goat	200	0	150	0
667	Horse	100	ND	256	ND
669	Goat	300	75	0	0
673	Goat	20	211	0	0
674	Goat	300	256	0	0
752	Goat	830	96	0	0
756	Goat	200	128	0	0
763	Goat	60	0	256	0
763	Goat	200	0	150	0
763	Horse	300	ND	128	ND
767	Goat	200	256	0	0

[a] Abbreviations: SFV = simian foamy virus; ND = not done; $TCID_{50}$ = dose that gives rise to cytopathic changes in 50% of the inoculated cultures; 0 = <8.

[b] Titers are expressed as the reciprocal of the 50% serum neutralizing end point as calculated by the Kärber method (17).

[c] Known antisera obtained commercially (see Materials and Methods).

[d] Known simian foamy virus serotypes (see Materials and Methods).

TABLE III. Relationship of Antibody Titers and Simian Foamy Virus Isolations in Rhesus Monkeys.[a]

Virus strain or isolate	Serum from rhesus monkey no.	Treatment group[b]	$TCID_{50}$/0.1 ml	50% serum neutralizing titer[c] Serum 1[d]	Serum 2[e]
SFV type 1[f]	664	3	200	<8	<8
	667	2	200	<16	NA
	669	2	200	<8	NA
	673	1	200	<8	NA
	674	1	200	<8	<8
	752	8	200	<8	<8
	756	7	200	<8	<8
	763	7	200	<16	ND
	767	6	200	<8	<8
SFV type 2[f]	667	2	100	<16	NA
	763	7	100	<16	<8
667	667	2	100	16	NA
763	763	7	300	16	ND

[a] Abbreviations: NA = not available; ND = not done.

[b] See Table I.

[c] See footnote *b*, Table II.

[d] Serum samples taken prior to treatment (see Materials and Methods).

[e] Serum samples taken approximately 28 days after treatment (see Materials and Methods).

[f] See footnote *d*, Table II.

more, our observations are consistent with those of others who found a higher incidence of foamy virus isolations than of SV_{40} from rhesus monkeys (7, 13).

Summary. The isolation and serologic identification by cross-neutralization tests showed the presence of both simian foamy virus types 1 and 2 in primary rhesus monkey brain cell cultures.

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