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Congenital Transmission of a Papovavirus of the Stump-Tailed Macaque

Abstract. *Stump-tailed macaque virus, a newly recognized papovavirus of the SV40 polyoma subgroup, was demonstrated in kidney cultures from each of five stump-tailed macaque fetuses in the second half of gestation and from six adult stump-tailed macaques. Such regular presence of virus in the fetus is an unusual feature for a papovavirus.*

The presence of stump-tailed macaque virus (STMV) was first recognized by thin-section electron microscopy in each of 15 kidney cultures from normal stump-tailed macaques (*Macaca speciosa*) which spontaneously developed an intense cytoplasmic vacuolated cytopathic effect (CPE) upon a few in vitro passages of the cultures (*1*). The virus was identified as a new papovavirus of the SV40-polyoma subgroup on the basis of its morphology, intracellular distribution, antigenic relationships, and DNA size and structure (*1–3*). Because the virus was found in kidney cultures of all animals, young and old, as well as in kidney cultures of a near-term stump-tailed macaque fetus (*4*), we examined the possibility of consistent congenital transmission of the virus. Kidneys and other tissues of five fetuses of the stump-tailed macaques and kidneys of six adults were cultured and monitored for virus expression. In addition to finding the virus in all the adult kidneys, we isolated STMV from every fetal kidney. These findings indicate that STMV is consistently transmitted congenitally from one generation to the next. This feature of STMV biology is an unusual one for papovaviruses which, so far, have been known to be transmitted only as horizontal infections acquired in postnatal life (*5*).

Five stump-tailed macaque fetuses of gestational ages between 84 and 133 days were obtained from the Biologic Resources Laboratory, Chicago, Illinois. The gestation period for the stump-tailed macaque is 164 days. A total of 21 tissues—kidney, lung, and skin from each of five fetuses and placenta and brain from each of three fetuses—were examined. Cultures initiated by trypsinization of minced tissues were maintained, with and without serial passage, in Eagle's minimum essential medium (Earle's salts) supplemented with fetal calf serum (10 percent) and antibiotics. In order to ensure against accidental contamination, primary cultures from 13 of these tissues, derived from three fetuses, were prepared in duplicate sets. After the first week, one set of cultures was monitored in Baltimore, Maryland, and the other set was monitored in Covington, Louisiana. Cultures from kidney, lung, skin, and brain grew vigorously and were serially passed once every week or 15 days for the first few passages; those from placenta grew very slowly and subsequent passages were less frequent. Cultures were monitored weekly for CPE (*1*) and periodically for STMV-specific nuclear immunofluorescence (IF) (*2*), for up to 130 days. At the end of the study and when indicated by the occurrence of CPE, cultures were screened for virions of papovavirus morphology by electron microscopy (EM) after negative staining. At present, no cell line is available for an infectivity assay of STMV.

The details of observations and tests on the five fetal kidney cultures are sum-

Table 1. Development of STMV cytopathic effect and immunofluorescence (IF) in kidney cultures of fetal stump-tailed macaques.

Fetus		Passage	Cytopathic effect and viral IF at week*																	
No.	Age (days)		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1238	84	Pr	−	−/*	−	−	−	−	−												
		P-1		−	−	−/*	−	−	−	−	−	−	−	−	−	−	−	⊖			
		P-2			−	−	−/*	−	−	−	+	+									
		P-3				−	−	−	−/*	−	+	⊕									
		P-4						−	−	*/−	+										
		P-5								−	−	+/*									
		P-6										−	−								
424	97	Pr	−	−/*	−	−	−	−	−	*/−	−	−	−	−	−	−	−	−	−↘	−	−
		P-1		−	−	−/*	−	−	−	−	−	−	−	*/−							
		P-2			−	−	−/*	−	−	−	−	+	+		−	−	−	−	−		
		P-3					−	−	−	−/*	+	⊕	+	+	+	⊕					
		P-4								−	−/*	+	+	+							
		P-5									−	+	+	+							
		P-6																			
1281	118	Pr	−	−/*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	*/−
		P-1		−	−	−/*	−	−	−	−	−	−	−	−	−	−	−	−	−/*	−↘	⊖
		P-2			−	−	−/*	−	−	−	−										
		P-3				−	−	−	−/*	−	−	+									
		P-4							−	−	−	−	⊕								
		P-5								−	−	+/*									
		P-6										⊕									
329	123	Pr	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
		P-1		−	−/*	−	−	−	−	−	−	−	−	−	−	*/−					
		P-2			−	−	−/*	−	−	−	−	−	−	−	+	+	+				
		P-3					−	−/*	−	−	−	−	−	−	+						
		P-4						−	−	−/*	−	−	+	⊕							
		P-5								−	−	−	⊕	+	+						
		P-6											+	+	+						
588	133	Pr	−	−	−	−	−	−	−	−											
		P-1		−/*	−	−	−	+	+	+											
		P-2			−	−/*	−	−	−	⊕											
		P-3				−	−	−/*	−	+	+										
		P-4						−	−	+/*											
		P-5							−	+	+										
		P-6																			

*− and + refer to negative and positive CPE, respectively. Negative IF is indicated by an asterisk, and positive IF by a circle around the CPE symbol. Two passages in week 17 of observation are indicated by arrows. The STMV-immune serum used in IF tests was reactive with STMV antigens but not to antigens of SV40, BK virus, and polyoma virus (*3*).

marized in Table 1. The STMV CPE was seen in cultures derived from every kidney, mainly after two to five passages of the cultures. The time of onset of CPE varied between days 42 and 75 after initiation of the cultures. There appeared to be no relationship between the time of onset of first STMV CPE in a culture and the gestational age of the donor fetus. The primary and first passage cultures generally remained negative for STMV CPE through the observation period, although they were monitored for 6 weeks or more beyond the period when the later passage cultures from the same kidney had already developed STMV CPE. Once a culture developed STMV CPE, it did not become completely free of vacuolated cells. The proportion of cells with cytoplasmic vacuolation varied widely from one passage to another.

The results of indirect IF tests of cells at different passage levels performed with an STMV-specific rhesus immune serum supported the CPE data. The immune serum prepared by two intravenous inoculations of a rhesus monkey that was free of antibody to SV40 was, in IF tests, reactive to STMV antigens but was not reactive to antigens of other papovaviruses (*3*). The serum of this animal before immunization was negative to both SV40 and STMV in IF tests. All 17 IF tests of CPE-negative cells harvested during the first 7 weeks from different passages of the five kidneys were negative, while seven of nine tests on cells from cultures with a history of STMV CPE, harvested between 8 and 12 weeks, were positive. The IF test detected STMV antigen in some of the CPE-negative early passage cultures maintained for long periods—for instance, in passage 2 of kidney 1281 culture in week 19 and in passage 1 of kidney 1238 culture in week 16 after initiation of the cultures. As a rule, only a small proportion of the cells of an IF-positive culture showed fluorescence. The cultures which contained cells with STMV IF were uniformly nonreactive in IF tests with SV40-immune reference serums that showed high titers of SV40 viral antibodies. The STMV IF antigen was identified in one or more cultures of each of the five kidneys.

Pellets obtained by high-speed centrifugation (100,000*g* for 2 hours) of clarified culture harvests or cell extracts (1 to 5 million cells) prepared by freezing and thawing of centrifuged cells were resuspended and examined by EM after negative staining. Large numbers of papovavirus particles were readily demonstrated in pools of CPE-positive cultures of each kidney. In addition, EM examination of early passage cultures which were held for a period of 15 to 19 weeks but were CPE-negative revealed papovavirus particles in each of four instances when these tests were made. Particles obtained from one preparation of each kidney were reacted, after treatment with Genetron-113, with antiserums to STMV and to SV40 and were examined for antibody coating by immune EM (IEM). It has been shown (*3*) that in IEM tests STMV virions react only with the homologous serums and not with antiserums to other papovaviruses. In all cases, the particles were identified unequivocally as STMV and were nonreactive with an antiserum to SV40 (Table 2).

Table 2. Identification of papovavirus particles derived from fetal stump-tailed kidney cultures by immune electron microscopy.

Source of virions	Number coated/number examined	
	STMV (rabbit)*	SV40 (rhesus)†
Kidney culture, fetus		
1238	51/52	0/59
424	26/26	0/25
1281	54/54	0/53
329	12/12	0/25
588	25/25	0/25
Virus pool, SV40	0/57	61/66

*Serum (diluted 1 : 5) reactive with homologous STMV virions but nonreactive to virions of polyoma, JC virus, BK virus, and SV40 (*3*). †Serum (diluted 1 : 5) reactive with homologous SV40 virions but nonreactive to virions of BK virus and STMV (*3*).

STMV was not identified in cultures derived from lung, skin, brain, and placenta of stump-tailed macaque fetuses. Cultures from these tissues, with the exception of the placental cultures which grew poorly, were monitored in the same way as the virus-positive kidney cultures from stump-tailed macaques described earlier. Cultures from brain and lung exhibited no CPE and were consistently negative for virus in IF and EM tests. Vacuolated cells as well as degeneration were often seen in cultures derived from skins of fetuses 1238 and 1281, but examination of such affected cultures by IF and EM tests yielded no evidence of virus.

There was good agreement between the two laboratories with respect to the cultures which they monitored simultaneously. In both places, STMV was recovered from kidney cultures of fetuses 1238, 424, and 1281, but not from cultures from other tissues of these animals.

Six adult stump-tailed macaques housed in animal facilities at Johns Hopkins Hospital, Baltimore, Maryland, were also studied; STMV was identified in the kidney cultures of each animal. The time of onset of STMV CPE in cultures of adult kidneys was variable (as it was with cultures of fetal kidneys) and ranged between 55 and 102 days. The STMV IF antigen and papovavirus particles were first detected in the cultures at about the time of the onset of CPE. The virus from each kidney was identified as STMV either by IEM test on virus particles or by IF examination of cells from vacuolated cultures.

With the data presented here, STMV has now been recovered from all of 27 stump-tailed macaques (including six fetuses) whose kidney cultures could be maintained for a few passages. The country of origin of these animals or their parents was East Asia, very likely Thailand, and they were collected from the wild over a number of years. Since STMV was identified in animals housed at Covington, Louisiana (*1*); Baltimore, Maryland; and Chicago, Illinois, it is unlikely that our findings reflect an artificial situation arising in captive laboratory animals. Virus was detected in fetal kidneys but not in any other fetal organs and there were no marked differences between fetal and adult kidneys in the time of in vitro viral expression. In the studies reported earlier (*2*), animals whose kidneys yielded STMV were without antibodies reactive to STMV antigen in IF tests. Serums from all six adults and three of five fetuses reported here were similarly tested for the presence of IF antibodies to STMV; all were negative. It therefore seems possible that the virus remains unexpressed through adult life. It is not clear whether the virus is transmitted to the fetus as an infection, as occurs with lymphocytic choriomeningitis virus (*6*), or by genetic inheritance as with mouse leukemia viruses (*7*).

KEERTI V. SHAH
Department of Pathobiology,
Johns Hopkins University
School of Hygiene and Public Health,
Baltimore, Maryland 21205
S. R. S. RANGAN
Delta Primate Research Center,
Tulane University,
Covington, Louisiana 70433
MAGDALENA REISSIG
RICHARD W. DANIEL
Department of Pathobiology,
Johns Hopkins University
School of Hygiene and Public Health,
Baltimore, Maryland 21205
FRANK Z. BELUHAN
Biologic Resources Laboratory,
University of Illinois,
Chicago, Illinois 60680

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Suppression of the Temperature-Sensitive Phenotype of a Mutant of Reovirus Type 3

Abstract. *A revertant of a reovirus group A temperature-sensitive mutant was crossed with wild type. More than 50 percent of the progeny were temperature sensitive. In all of the temperature-sensitive progeny examined by recombination tests, the temperature-sensitive lesion was in group A. The results indicate that the revertant was phenotypically suppressed.*

Conditional lethal mutations are useful tools for the study of virus replication and genetics. Growth of conditional lethal mutants under nonpermissive conditions allows an absolute selection for reverse mutation or reversion. Revertants may not be true revertants to the wild type, but may represent the acquisition of second, suppressor mutations that allow the virus to bypass the original defect (*1*). In prokaryotic systems, these suppressor mutations are often in genes other than the one with the original lesion. Such extragenic suppressors are often in genes whose products interact physically with the original gene products (*2*). We have found that a revertant of a temperature-sensitive (ts) mutant of reovirus type 3 is phenotypically suppressed by a suppressor that can be separated from the ts mutation by reassortment of genome segments.

The group A mutant ts 201 of reovirus type 3 was isolated as a result of proflavin mutagenesis (*3*); it contains $\mu 1$ and $\mu 2$ polypeptides with altered electrophoretic mobility (*4*). Reversion of ts 201 to ts^+ phenotype is often accompanied by a change in the electrophoretic mobility of the $\mu 1$ and $\mu 2$ polypeptides (*5*). In several spontaneous ts 201 revertants, reversion to ts^+ phenotype was not accompanied by a change in mobility of $\mu 1$ and $\mu 2$ polypeptides. The possibility that these revertants were not true revertants but contained the ts 201 lesion in a suppressed form was examined in one revertant clone (clone 101).

To show that a revertant clone contains a suppressed ts lesion, it was necessary to show (i) that the reversion event occurred outside the gene with the ts lesion, and (ii) that the clone still contained the original ts lesion.

Since the genome of reovirus is segmented (*6*) and recombinants have been shown to arise by reassortment of genome segments in two-factor (*3, 7, 8*) and three-factor crosses (*5*), we reasoned that the suppressed ts lesion could be separated from its suppressor by reassortment if the ts lesion and the suppressor lay on different genome segments. Once separated from the suppressor mutation, the ts phenotype of the ts lesion would once against be expressed. Accordingly, clone 101 was backcrossed to wild type, and progeny plaques were picked from plates grown at permissive temperature. After two passages at permissive temperature, we determined the titer of the progeny clones. As controls, single infections were performed with clone 101, wild type, and ts 201. Progeny plaques were picked and passaged, and their titers were determined (Fig. 1). The distribution of temperature phenotypes shows two populations among the progeny of the cross; one population has a wild-type efficiency of plating (EOP) and the other has an EOP that is temperature sensitive. [The ts 201 control clones (data not shown) had an EOP ranging from 5×10^{-3} to 5×10^{-5}.] This result represents unequivocal evidence that clone 101, although phenotypically ts^+, contains a phenotypically suppressed ts lesion.

Since clone 101 was selected as a revertant of the group A mutant ts 201, one would expect the ts lesion appearing among the progeny to be group A. However, it has been shown that extragenic suppressors often have intrinsic temperature phenotypes (*2*). Thus ts lesions among the progeny would be expected to be either the ts lesion from which the revertant was selected or a new ts phenotype associated with the suppressor mutation. To test whether the ts progeny clones contained group A ts lesions or lesions in other groups, selected clones were crossed (*3*) against the group A mutant ts 201 and the group B mutant ts 352 at permissive temperature. All progeny clones tested failed to recombine with the group A test virus, whereas they did recombine with the group B test virus, indicating that they contained the group A lesion (data not shown).

Thus clone 101 does contain a phenotypically suppressed ts lesion, and this le-

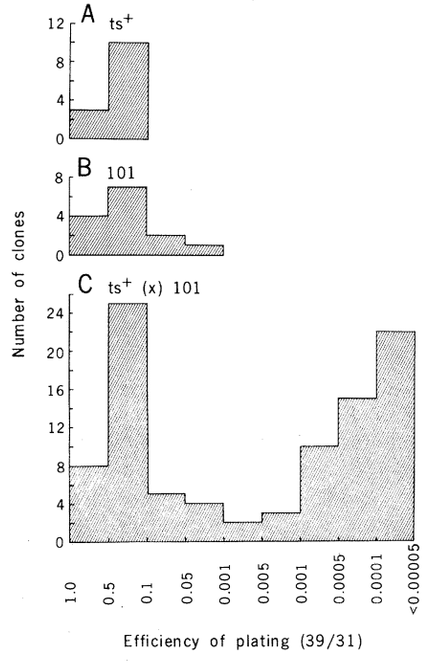

Fig. 1. Distribution of EOP of progeny and control clones. A suspension culture of 10^7 mouse L cells were mixedly infected with a multiplicity of infection of 10, each with freshly cloned 101 and wild type. Two hours after infection, unadsorbed virus was removed by centrifuging the infected cells and resuspending them in fresh medium. Forty-eight hours after infection, the cells were sonicated to release cell-associated virus and to disrupt viral aggregates. Appropriate dilutions were plated on L cell monolayers and incubated for 13 days at 31°C. The culture plates were overlaid with neutral red agar and, after overnight incubation, plaques were picked. The plaques were passaged twice on L cell monolayers at 31°C. The titer and efficiency of plating of second passage virus was determined by plating on L cell monolayers at 39°C and 31°C. Plates at 39°C were overlaid and counted on day 5; plates at 31°C were overlaid and counted on day 13 after infection. Wild-type and clone 101 controls were the same, except that for single infection a multiplicity of infection of 20 was used. The EOP is the ratio of the titer at 39°C to that at 31°C. (A) EOP of wild-type control clones. (B) EOP of clone 101 control clones. (C) EOP of clone 101 (×) wild-type progeny clones.