APPLIED MICROBIOLOGY, Jan. 1971, p. 50-52

Vol. 21, No. 1
Printed in U.S.A.

Occurrence of a Carrier State for *Herpesvirus tamarinus* in Marmosets

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Received for publication 29 July 1970

The ecology of herpesviruses in marmosets and other nonhuman primates is important today, for colonies of these animals are being established for biomedical research. This paper presents the first reported isolations of *Herpesvirus tamarinus* from throat swabs of a healthy white-lipped marmoset carrier (*Saguinus nigricollis*) during a 2-month period. Infectivity studies with this virus in both white-moustached (*S. mystax*) and white-lipped marmosets demonstrated that the virus is not lethal to white-moustached marmosets (perhaps a more resistant species) at 1,000 $TCID_{50}$. Which environmental conditions trigger the unmasking of herpesviruses in marmosets is not known. However, intermittent *H. tamarinus* shedding may help explain spontaneous infections in established colonies as well as suggest an additional mechanism for transmission of virus between marmosets under natural conditions.

A fatal disease in white-lipped marmosets (*Saguinus nigricollis*) and cotton-topped marmosets (*S. oedipus*) attributed to *Herpesvirus tamarinus* was described in 1964 by Holmes et al. (2) and Melnick et al. (5). Holmes and coworkers (3) also conducted a serological survey of small South American nonhuman primates to determine the natural hosts of this virus. Serum antibodies were detected in 53% of squirrel and spider monkeys, 37% of ringtail monkeys, and 15% of marmosets. The large proportion of squirrel, spider, and ringtail monkeys with *H. tamarinus* antibodies suggested to Holmes that these animals were the reservoir hosts. In contrast, the small proportion of marmosets with antibodies seemed to represent survivors of a much larger population experiencing high mortality from *H. tamarinus* infection under natural conditions.

This paper reports the isolation of *H. tamarinus* (Phoenix) from throat swabs of a healthy white-lipped marmoset during a 2-month period and describes infectivity and immune response in both white-lipped and white-moustached (*S. mystax*) marmosets.

MATERIALS AND METHODS

Virologic examination. Marmosets (*S. nigricollis* and *S. mystax*) were acquired from two Florida importers. Routine throat swabs and blood specimens were obtained from these animals upon admission and at monthly intervals.

Throat swabs were collected in 3 ml of Tryptose phosphate broth containing 0.5% gelatin, 1% chicken serum, and antibiotics. Specimens were passed into primary monkey kidney, HEp-2, and Wistar-38 cell cultures for a minimum of three serial transfers.

H. tamarinus identifications were made by serum neutralization tests by using *H. tamarinus* antisera supplied by W. Holmes, Rush-Presbyterian-St. Luke's Medical Center, Chicago. In addition to the Phoenix viral isolate, an *H. tamarinus* isolate (MV8-RK1) was obtained from W. Holmes for serum cross-neutralization tests and marmoset inoculation.

Neutralization tests. Marmoset serum antibody titers against *H. tamarinus* were determined by serum neutralization tests. All sera were inactivated at 56 C for 30 min and tested (screening level 1:5) against 100 $TCID_{50}$ of the Phoenix strain of *H. tamarinus* and *H. hominis*. Tests were done in tube cultures of HEp-2 cells. End points were determined when virus control tubes showed 100% cytopathic effect, and titers were expressed as the reciprocal of the highest dilution which protected 50% of the cells from infection.

Rabbit antiserum. Antiserum to Phoenix isolate of *H. tamarinus* was prepared in rabbits as previously described (1). The final concentration of antigen used was 10^7 $TCID_{50}$.

Herpesvirus-T inoculations in marmosets. Six *S. nigricollis* and four *S. mystax* without detectable serum antibody to *H. tamarinus* or *H. hominis* received intratracheal *H. tamarinus* inoculations. Four of six *S. nigricollis* were inoculated with 0.25 ml of 1,000 $TCID_{50}$ *H. tamarinus* Phoenix, whereas the remaining two received 0.25 ml of 1,000 $TCID_{50}$ *H. tamarinus* MV8-RK1.

Two *S. mystax* received inoculations of 0.25 ml of 100 $TCID_{50}$ *H. tamarinus* Phoenix and two received 0.25 ml of 1,000 $TCID_{50}$ of this material. The two marmosets which received 100 $TCID_{50}$ *H. tamarinus*

Phoenix were challenged 90 days later with 0.25 ml of 1,000 $TCID_{50}$ *H. tamarinus* MV8-RK1.

RESULTS

Sera from 66 individual marmosets (*S. nigricollis*) were examined for *H. tamarinus* antibody. Sixteen of these (24%) had *H. tamarinus* serum antibody at screening dilution. In one shipment of 15 marmosets, which included the *H. tamarinus* Phoenix carrier, 11 of 13 examined (85%) had *H. tamarinus* antibody. In two of these animals, decreasing serum neutralizing antibody titers over the period of observation suggested recent infection.

Phoenix and MV8-RK1 *H. tamarinus* viruses appeared to be antigenically similar. Serum cross-neutralization tests produced identical end points in both the homologous and heterologous crosses.

The results of *H. tamarinus* inoculations are shown in Table 1. The four *S. mystax* inoculated with 100 or 1,000 $TCID_{50}$ *H. tamarinus* Phoenix survived; however, discrete signs of illness were observed. Onset of illness occurred 7 to 10 days postinoculation and was characterized by fever (>105 F, ca. 41 C), lethargy, loss of appetite, dermatitis, and cutaneous scab formations. Dermatitis appeared early in the disease sometimes followed by eruptions and scab formations as early as 14 days postinoculation. The remission of signs was complete in 30 days after onset. In general, the dosage of 1,000 $TCID_{50}$ brought about signs of illness earlier than with the use of 100 $TCID_{50}$, but no difference in severity of illness was observed. *H. tamarinus* was isolated from throat swabs of all except one *S. mystax* 3 days postinoculation and commonly persisted as long as 21 days postinoculation. The *S. mystax* showed serum neutralizing antibody titers of 1:20 to 1:40 at 10 to 14 days post-*H. tamarinus* inoculation. Serum neutralizing antibody titer increased to 1:320 to 1:640 at 30 days postinoculation and remained at this level in the 90-day postinoculation blood samples. The MV8-RK1 challenge of *S. mystax* vaccinated with *H. tamarinus* Phoenix did not produce specific signs of illness, and no increases in serum antibody titer were observed.

The four *S. nigricollis* inoculated with *H. tamarinus* Phoenix died, as did one of the two *S. nigricollis* inoculated with *H. tamarinus* MV8-RK1. Acute signs of illness were observed as early as 2 days after inoculation and death occurred 9 to 15 days postinoculation. The surviving *S. nigricollis* was recovered by the 20th day after illness onset. *H. tamarinus* was isolated from all inoculated *S. nigricollis*, including necropsy specimens of lymph nodes, lung, and spleen from the animals which died.

No antibody response was observed in sera obtained 9 to 15 days before death in the *S. nigricollis* dying after intratracheal inoculation. The one *S. nigricollis* which did survive developed a serum neutralizing antibody titer of 1:1,280 14 days after inoculation.

DISCUSSION

The carrier state for herpesviruses in man has been well demonstrated. Recently, herpesvirus carriage in marmoset kidney was demonstrated by Meléndez and co-workers when spontaneous degeneration of marmoset kidney tissue culture yielded isolations of a new herpesvirus serotype, *H. saguinus* (4). We have demonstrated the

TABLE 1. *Result of marmoset inoculations with Herpesvirus tamarinus isolates*

Marmoset	Species	Herpes virus	$TCID_{50}$[a]	Day of onset of symptoms	Day of death	Titer of antibody produced	Virus isolated[b]
1	*Mystax*	Phnx	100	7		1:640	+
2	*Mystax*	Phnx	100	7		1:640	+
1[c]	*Mystax*	MV8RK1	1,000			1:640	+
2[c]	*Mystax*	MV8RK1	1,000			1:640	−
3	*Mystax*	Phnx	1,000	10		1:640	+
4	*Mystax*	Phnx	1,000	7		1:640	+
5	*Nigricollis*	Phnx	1,000	3	15	<1:5	+
6	*Nigricollis*	Phnx	1,000	3	9	<1:5	+
7	*Nigricollis*	Phnx	1,000	2	9	<1:5	+
8	*Nigricollis*	Phnx	1,000	2	15	<1:5	+
9	*Nigricollis*	MV8RK1	1,000	3	9	<1:5	+
10	*Nigricollis*	MV8RK1	1,000	3		1:1,280	+

[a] Given 0.25 ml intratracheally.
[b] Symbols: +, positive; −, negative.
[c] Previously vaccinated with 100 $TCID_{50}$ Phnx.

presence of an *H. tamarinus* carrier in a cohort of white-lipped marmosets under conditions that would allow virus transmission to other marmosets. Also, our data show no antigenic difference between *H. tamarinus* Phoenix and *H. tamarinus* MV8-RK1. Conclusions by Holmes et al. (3) in 1966 indicated that the appearance of *H. tamarinus* antibodies in 15% of the marmosets probably represented survivorship from larger cohorts of animals experiencing generally high mortality by the introduction of the virus from other natural hosts, such as owl, squirrel, and ringtail monkeys. Our data suggest that spontaneous outbreaks of *H. tamarinus* infections which occur in stabilized marmoset colonies as well as sylvatic virus transmission between marmosets may be, in part, explained by intermittent shedding of virus from surviving marmoset carriers. The high prevalence of serum antibody and serological evidence of recent infection in the cohort of animals from which the carrier derived suggests this mechanism for viral transmission within the cohort. Under these conditions, ndigenous viral reservoiring could occur in this species despite a naturally high mortality from herpesvirus infection.

This study did not demonstrate a dose relationship in disease severity, but it did suggest that there may be a species difference in susceptibility between *S. mystax* and *S. nigricollis*.

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