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Presence of Antibodies to Simian Virus 40 (SV40) T Antigen in Rhesus Monkeys Infected Experimentally or Naturally with SV40* (33043)

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The tumor (T) antigen of SV40 is present in nuclei of all cells transformed by SV40 *in vivo* or *in vitro* (1,2) and it is also produced during the acute cytolytic phase of SV40 multiplication (3,4). Antibodies to T antigen, detectable by complement fixation and immunofluorescence tests, were first demonstrated in sera of hamsters bearing SV40-induced tumors and in this species were found exclusively in tumorous animals (2,5). Recently, it has been shown that T antibodies develop without the production of tumors in African green monkeys infected experimentally with SV40 (6–8).

Relatively little is known about the course of SV40 infection in rhesus, although it is the natural host of the virus. In a study now in progress, nonimmune juvenile rhesus were infected with SV40 by several routes. The present communication reports the development of SV40 T antibodies in the sera of these rhesus and its relationship to routes of infection and viremia. It also records the finding of T antibodies in a proportion of naturally infected rhesus.

Materials and Methods. Juvenile rhesus, 1–2 years old, were obtained from the free-living groups on Cayo Santiago, an islet off the east coast of Puerto Rico. This island is a part of the facilities of the Laboratory of Perinatal Physiology. These rhesus groups appear to be free of active SV40 infection (K. V. Shah and J. A. Morrison, unpublished data). A total of 16 animals were infected as follows: six each by subcutaneous (s.c.) and intranasal (i.n.) routes with 0.4 ml of a $10^{-1.0}$ dilution of virus and four by intragastric (i.g.) route by introduction of 1 ml of undiluted virus by a stomach tube. The gastric acidity was neutralized by 40 ml of 6% $NaHCO_3$ in two of four infected (serum series 103 and 107) by the i.g. route. The animals were caged individually and bled periodically for tests for viremia and antibodies. Two uninfected controls in individual cages were kept in the same room as the i.g. inoculated group.

Strain A2895 of SV40, isolated originally from a hamster tumor produced by inoculation of rhesus kidney extracts (9), was obtained from Dr. B. Eddy. Prior to use in this

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TABLE I. Frequency of Viremia and Development of Virus-Neutralizing and T Antibodies in Rhesus Infected with SV40 by Three Routes of Inoculation.

Route of inoculation	No. in group	Virus dose log TCD_{50}	No. developing: Viremia	Neutralizing antibodies	T antibodies
Intranasal	6	7.3	4	6	4
Subcutaneous	6	7.3	6	6	6
Intragastric	4	8.7	4	4	4

study, the virus had undergone at various times one additional passage in hamster tumor, one passage in HeLa cells and 10 passages in *Cercopithecus* kidney cells, either primary cultures or continuous cell line B-SC-1.

Viremia titrations and neutralization tests were performed in primary African green monkey kidney (AGMK) cultures as described earlier (10). Cultures were observed for 28 days. For demonstration of T antibodies, one or more dilutions of sera beginning at 1:5 were reacted with SV40-transformed hamster cells (Flow Laboratories) in indirect fluorescent antibody (FA) tests (1). The transformed cells were grown on 11 × 22-mm No. 2 coverslips in 60 × 15-mm Falcon plastic petri dishes, and were fixed in −20°C acetone for 10 min. They were stored at −70° for periods of up to several months. As determined by immunofluorescence tests, these cells contained T antigen in all nuclei but no viral antigen. Ascitic fluid from a hamster with SV40 tumor (Flow Laboratories) was used as a positive control.

To confirm the presence of T antibodies demonstrated by tests with transformed hamster cells, at least one postinfection serum from each animal and all sera from three infected rhesus (serum series 1,25,103) were also tested in indirect FA with 5-fluorodeoxyuridine (FUDR)-treated and -untreated AGMK cells infected with a high multiplicity of SV40 and harvested at 24–36 hours after infection. Controls were included to show that FUDR prevented the formation of viral but not of T antigen (11). The treatment with FUDR was as described by Lewis *et al.* (12).

Fluorescein-conjugated goat antirhesus globulin (Microbiological Associates) and goat antihamster globulin (Baltimore Biological Laboratories) were used at dilutions of 1:25 and 1:40, respectively. The antihamster globulin was absorbed with African green monkey liver suspension. Coverslips were counterstained with Lissamine rhodamine conjugated bovine serum albumin (Difco) diluted 1:25. Coverslips were examined on a Zeiss optical microscope using high-intensity ultraviolet illumination by an HBO-200 mercury vapor lamp.

In addition to those from experimentally infected rhesus, 64 sera from rhesus housed at the Laboratory of Perinatal Physiology, San Juan, and 38 from recently captured rhesus in North India were also examined for virus-neutralizing and T antibodies. The ages of the laboratory rhesus were known and those of North Indian monkeys estimated from their weights. For T antibodies, the sera were screened with SV40-transformed hamster cells, and all sera positive in this test were reacted with FUDR-treated and untreated acutely infected AGMK cultures.

Results. In experimentally infected rhesus. The virus dose was $10^{7.3}$ TCD_{50} for the 12 rhesus inoculated by the i.n. or s.c. routes and $10^{8.7}$ for the 4 infected by the i.g. route. All 16 developed neutralizing antibodies by the third week after inoculation and titers of 1:256 or greater were obtained by 4–6 weeks. T antibodies developed in 4 of 6 infected i.n. and in all 12 infected by s.c. and i.g. routes. Viremia was demonstrated in all inoculated rhesus except two in the i.n. group (Table I). Except for one rhesus in the s.c. group which died of a diarrheal disease 26 weeks after infection, all others remained healthy during 8 months of observation. No visible tumors were detected.

The details of viremia and T antibody re-

TABLE II. Viremia and T Antibody Response in Rhesus Infected with SV40.

Route	Serum series	Viremia[a] Days postinfection 1	2	3	5	6	7	8	T antibody[b] Days postinfection 7	8	11	12	13	14	15	18	19	21	Weeks postinfection 6	7	9	12	13	16	29	31
i.n.	1	—		—		1.4		—		—		—		—		+		40	10	10		10		20		—
	5	—		—		1.2		1.2		—		—		—		+		20	20	10		10		20		20
	9	—		—		—		—		—		—		—		+		40	20	40		10		10		—
	13		—		1.7		2.7		—				—		—		—	—	—	—		—		—		—
	17		—		—		—		—				—		—		—	—	—	—		—		—		—
	21		—		2.2		3.2		—		—		—		—		+	40	40	10		10		10		10
s.c.	25	3.7		1.7		—		—		—		+		40		+		80	160	160		20		20		10
	29	3.7		2.2		—		—		—		+		80		+		160	320	40		80		80		40
	33	3.2		—		—		—		—		+		80		+		80	40	80		10		10		5
	37		3.7		—		—		—		+		+		40		+	40	40	20		20		20		20
	41		2.7		—		—		—		+		+		40		+	40	10	20		20		20		
	45		3.2		—		—		—		±		+		80		+	40	80	80		80		80		20
i.g.	103				1.4	2.7												20			10		—		—	
	107				2.7	1.7												40			10		+		10	
	111				2.7	3.2												80			5		±		—	
	115				1.7	2.7												40			10		10		5	

[a] Figures are positive logarithms of TCD_{50} titers/ml; — indicates $< 10^{0.7}$ TCD_{50}/ml.

[b] Figures are reciprocals of serum antibody titers in indirect FA tests with transformed hamster cells; — indicates titer of $< 1:5$; + indicates titer of 1:5 or greater.

TABLE III. Relationship between SV40 Virus-Neutralizing and T Antibodies in Rhesus Sera.

Category	T antibodies		
	Present	Absent	Total
With neutralizing antibodies	32	35	67
Without neutralizing antibodies	0	35	35
		Total	102

sponses are given in Table II. After i.n. infection, viremia in one or more animals was seen on days 5, 6, 7, and 8; in two rhesus, viremia was not detected at any time tested. Following s.c. inoculation, viremia was confined to days 1, 2, and 3 postinfection and was demonstrable in all inoculated animals. After i.g. inoculation, all four rhesus were viremic on days 5 and 6, the only 2 days on which tests were made in the first 2 weeks. Neutralization of gastric acidity prior to inoculation did not affect viremia and antibody response. Viremia titers ranged from $10^{1.2}$/ml to $10^{3.7}$ ml and were higher in the s.c. group. As shown in Table II, the possible duration of viremia was between 1 and 5 days for the individual viremic animals in the i.n. and s.c. groups. Four additional serum specimens were collected from each animal in the i.n. and s.c. groups between days 8 and 21 and tested for viremia; all were negative.

For T antibodies, the results of FA tests with transformed hamster cells were in agreement with those obtained in FUDR-treated, acutely infected AGMK cultures. The T antibodies developed in 14 of 16 infected rhesus, the two exceptions being in the group infected i.n. In that group, T antibodies developed in three of four with and in one of two without demonstrated viremia.

There were differences in the time of first appearance, titers, and persistence of T antibodies which were related to the route of inoculation. After s.c. inoculation, T antibodies were demonstrable by FA tests in all rhesus by 11–13 days and persisted through the observation period of 31 weeks. In titrations of serum antibodies in FA tests, fluorescence decreased gradually with increasing serum dilutions and end points were not sharply defined. Highest FA antibody titers ranging from 1:40 to 1:360 for individual rhesus occurred 3–6 weeks after infection. After i.n. inoculation of the same dose of virus, T antibodies were first detected by 18–19 days and peak titers in individual rhesus did not exceed 1:40. The antibodies persisted in all animals through week 16 after infection but were demonstrable in only one of six at 31 weeks. After i.g. inoculation, T antibodies were present in 3-week serum specimens of all for inoculated rhesus but the time of their first appearance was not determined. Peak titers ranged from 1:20 to 1:80. The T antibodies persisted through the ninth week in all four and through the observation period of 29 weeks in two.

The relationship between the time of appearance of neutralizing and T antibodies was as follows: In all individuals infected i.n. and in two infected s.c., neutralizing antibodies appeared 4–6 days before T antibodies. In the remaining four rhesus of the s.c. inoculated group both antibodies appeared on the same day. Neutralizing antibodies persisted in all inoculated rhesus throughout the 29–31 weeks of observation.

In naturally infected rhesus. The relationship between T and virus-neutralizing antibodies to SV40 in sera of naturally infected rhesus is given in Table III. As with sera of experimentally infected rhesus, results of FA tests with SV40-transformed hamster cells agreed with those with FUDR-treated acutely infected AGMK cultures. The T antibodies were detected in a little under 50% of the sera which had neutralizing antibodies and in none of 35 without neutralizing antibodies. The distribution of T antibodies in SV40-infected rhesus by age of donor is given in Table IV. In 1–2 year old rhesus with neutralizing antibodies, 11 of 13 (85%) had T antibodies. This proportion decreased with increase in age; 14 of 30 (47%) of

TABLE IV. Frequency of T Antibodies by Age of Donor in Sera of Rhesus Naturally Infected with SV40.

	Donors with neutralizing antibodies		
	T antibodies		
Age (years)	Present	Absent	Percent
1–2	11	2	85
3–4	14	16	47
5+	7	17	29

3–4 year old and 7 of 24 (29%) of 5 year old or older had T antibodies in their sera.

Discussion. All of the 16 inoculated rhesus were readily infected with SV40 after introduction of the virus by any one of three routes: intranasal, subcutaneous, and intragastric. Viremia and T antibody response developed in all rhesus infected by the s.c. or i.g. route and in a majority of those by the i.n. route.

After s.c. inoculation, viremia occurred in the first 3 days as compared to between days 5 and 8 after i.n. inoculation. Viremia titers were higher in the group inoculated s.c. In both s.c. and i.n. inoculated groups, T antibodies first appeared about 10–12 days after the period of viremia, at 11–13 days after s.c. and 18–19 days after i.n. inoculation. The T antibody response to s.c. inoculation was greater than that to i.n. inoculation as evidenced by higher peak titers in individual animals and longer persistence; 31 weeks after inoculation, T antibodies were present in all infected by the s.c. and in one of six by the i.n. route. Though fewer specimens were collected from the i.g. inoculated rhesus, the results clearly demonstrated viremia on the fifth and sixth day, T antibodies in all inoculated animals by the third week and persistence of these antibodies in two of four rhesus through the observation period of 29 weeks. This ease of infection by the i.g. route was surprising in view of the lack of antibody response in man following oral administration of SV40 (13–15). Two uninfected controls caged individually and housed with the i.g. group remained free of SV40 infection throughout the 29 weeks.

The T antibodies were also detected in a proportion of the sera of rhesus naturally infected with SV40. In juvenile rhesus 1–2 years old, as many as 85% of the SV40 infected individuals had T antibodies, a finding not unexpected since, in this age group, all SV40 infections would have to be comparatively recent. In older animals, the proportion with T antibodies decreased. The T antibodies were not encountered in sera without neutralizing antibodies.

These data clearly show that juvenile rhesus develop viremia and T antibodies as a result of a single exposure to SV40 by any one of three routes of inoculation, and in the absence of tumors, and that these antibodies may persist for 4–8 months or longer. It seems probable that unlike *Cercopithecus* (6–8) and rhesus, man does not develop T antibodies as a result of SV40 infection. Gerber (16) did not detect SV40 T antibodies in serial bleedings of 17 children who developed neutralizing antibodies to the virus after administration of SV40 contaminated inactivated poliovaccine. We have also screened 24 sera of noncancer humans naturally infected with SV40 in North India (10) with low neutralizing antibody titers (1:1 to 1:16) to SV40 and found them negative for T antibodies.

Summary. All of 16 rhesus infected with SV40 by i.n., s.c., or i.g. inoculation developed neutralizing antibodies by the third week after inoculation. All of 10 animals infected by s.c. or i.g. route and a majority of those by the i.n. route were viremic. Viremia titers were higher in the group inoculated s.c. The T antibodies developed in all except two in the i.n. inoculated group. The T antibodies appeared earlier, reached higher peaks, and persisted longer after s.c. inoculation as compared to after i.n. inoculation. The T antibodies were detected in a proportion of naturally infected rhesus. This proportion was highest (85%) in 1–2 year old rhesus and declined with increase in age of donor.

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Relative Insulinase Activity in the Liver and Kidney of the Rat* (33044)

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The term "insulinase" was proposed by Mirsky and Broh-Kahn, 1949 (1) for the enzyme or enzyme system which preferentially degrades insulin. These workers determined the relative insulinase activity in various rat tissues by measuring the blood sugar fall in rabbits injected with insulin solutions previously incubated with such tissue homogenates. They reported that in the rat the insulinase activity per gram of wet tissue was highest in the liver, second highest in the kidney, and much less in the other tissues (1). Subsequently this group has focused on rat liver insulinase activity (2,3).

Tomizawa, 1962 (4,5) investigated the degradation of insulin by utilizing insulin-^{131}I tracer in the substrate. Using beef liver as a source, Tomizawa concentrated "insulinase" activity and demonstrated that insulin degradation is initiated by the reductive cleavage of insulin into its A and B chains by glutathione insulin transhydrogenase. Using a modification of Tomizawa's procedure, preliminary investigations in our laboratory indicated that rat kidney insulinase activity per gram wet tissue was greater than that of the liver. Therefore, the relative insulinase activity of rat kidney and liver has been studied in more detail.

Materials and Methods. Insulinase activity was determined by a modification of Tomizawa's procedure (4,5). Male Sprague-Dawley rats weighing 205–270 gm that had been fed *ad libitum* on Purina chow were sacrificed by decapitation and as much blood as possible was drained from the carcass. The liver and kidneys were removed, blotted, and placed in chilled beakers until weighed. The intact liver was weighed and then a piece of approximately 3 gm was removed and weighed. This piece of liver was placed in a glass tube and homogenized in a volume of cold phosphate buffer containing 5 ×

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