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An association between adenoviruses isolated from simian tonsils and episodes of illness in captive monkeys

By GILLIAN BULLOCK

*Virus Reference Laboratory, Central Public Health Laboratory, Colindale, London, N.W. 9**

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During recent years considerable interest has been shown in the presence of 'latent' adenoviruses in human tonsils and adenoids (Rowe, Huebner, Gilmore, Parrott & Ward, 1953; Evans, 1958; Israel, 1962). These viruses have been shown to exist in the tonsil without multiplying to any extent and to become active only under certain conditions such as culture of the infected organs. Certain types, for example 1, 2 and 5, predominated in tonsils and adenoids of young people up to puberty.

The investigation now described was done primarily to see whether the simian tonsil had a similar reservoir of 'latent' virus. Techniques selective for simian adenoviruses, the group I simian viruses (SV) of Hull, Minner & Smith (1956), were used. No illness was observed during the period of investigation of the tonsils, but 2 months after it ended acute febrile respiratory manifestations occurred in two fresh batches of monkeys. These showed nasal discharge, sometimes accompanied by conjunctivitis and bleeding gums. They refused to eat and sat listlessly in their cages. Nine out of twenty monkeys died and at autopsy were found to have pneumonia. Nose and throat swabs were taken from all the monkeys and virus isolation in tissue culture was attempted.

Some simian adenoviruses had been isolated from monkey tonsils by Pille, Yermakova, Zuyeva & Nadaichik (1961). With one exception (Tyrrell, Buckland, Lancaster & Valentine, 1959) these viruses have not been shown to cause any disease in the monkey. They have been found in various organs including the pancreas, kidney, intestines and nervous tissue and occasionally in nose or throat swabs.

MATERIALS AND METHODS

For 4 months, from October 1962, tonsils were collected from rhesus monkeys at the time of kidney excision for the preparation of cell cultures. Tonsils were placed in 10 ml. of phosphate-buffered saline (Dulbecco & Vogt, 1954) at 37° C. containing 200 units of penicillin per ml. and 200 μg. of streptomycin per ml. This concentration of antibiotics was used in all solutions unless otherwise stated. After the tonsils had been in this fluid for approximately 2 hr., they were minced with sharp scissors in fresh fluid to give small fragments, large ones being treated separately and small ones in pairs. The fragments were washed thoroughly in

* Present address: Research Division, CIBA Laboratories Ltd., Horsham, Sussex.

Hanks's balanced salt solution containing 0·15 % $NaHCO_3$ and antibiotics. Each tissue suspension was then placed in a universal container with 2 ml. of warmed medium containing 1·8 ml. of bovine amniotic fluid, 0·1 ml. of 5 % lactalbumin hydrolysate and 0·1 ml. of horse serum with antibiotics. This medium had been used successfully by Pereira (1960) for the culture of 'latent' adenoviruses in human tonsils. The containers were tightly stoppered with rubber bungs, sloped at 5° in a 37° C. incubator and the medium changed at weekly intervals.

Table 1. *Simian adenovirus antiserum pools*

Pool I	Pool II	Pool III
SV 1, 11, 15, 17, 20	SV 23, 25, 27, 30, 31	SV 32, 33, 34, 36

Fluids removed from the cultures were frozen immediately and stored at − 70° C until tested. They were then thawed rapidly in a water bath at 37° C. and 0·5 ml. was inoculated into each of three primary rhesus monkey kidney cell culture tubes. These tubes, maintained on synthetic medium 199 containing 0·15 % $NaHCO_3$ and antibiotics, were incubated at 37° C. on a roller apparatus as long as possible, the maintenance medium being changed when necessary. Any tubes with cell sheets showing an advanced stage of the characteristic cytopathic effect of simian adenoviruses were frozen and thawed rapidly (using alcohol and dry ice as a freezing mixture). The harvested fluid obtained was inoculated into two parallel series of tubes, three of monkey kidney cells and three of the continuous HeLa cell line. If satisfactory evidence of growth was found after the second passage, virus pools were made in primary vervet kidney cells for further study.

Nose and throat swabs from sick monkeys were placed in 2 ml. of phosphate-buffered saline containing antibiotics as before and also 5 μg. per ml. Amphotericin B. After contact for 2–3 hr., the fluid from each swab was used to inoculate three rhesus monkey kidney culture tubes. Tubes showing an adenovirus-like cytopathic effect were further studied in the same way as those from the tonsil cultures.

To identify the viruses isolated, complement-fixation tests were done by the method of Bradstreet & Taylor (1962) to determine whether adenovirus group complement-fixing antigen was present. Each virus isolated was tested against the serum of a patient convalescent from an adenovirus infection and against a rabbit antiserum prepared with SV 15. Neutralization tests in two stages were done in primary rhesus monkey kidney culture tubes maintained as before. Each strain was first titrated against three pools of antiserum prepared as shown in Table 1 with the serum diluted to 1/32 in each virus-serum mixture. For final identification, arising from the results of the pool tests, titrations were done with individual antisera.

In each instance 0·2 ml. of the virus at 10-fold dilutions ranging from 10^{-1} to 10^{-7} was mixed with 0·2 ml. of the antiserum pool previously diluted in medium 199 to 1/32; 0·2 ml. of the virus-serum mixture was then inoculated into each of two monkey kidney culture tubes which were incubated at 37° C. on a roller apparatus. After 6 days the tubes were examined for cytopathic effect and evi-

dence of inhibition by the antiserum pools. Control tubes of virus titrations and uninfected cells were included in all tests.

RESULTS

The necessity of washing the excised tonsils carefully is made apparent by the fact that, despite our precautions, two pairs of tonsils had to be discarded because of bacterial or fungal contamination. One pair of tonsils stored overnight at 4° C. before washing yielded no virus, but as the tonsil cells were still viable it seemed

Table 2. *Isolation of adenovirus from tonsils of healthy monkeys*

Date tonsils removed	Virus present (weeks) 1	2	3	4	5	6	Strain no.	Identification of strains. Complement-fixation test: Human convalesent serum	Rabbit SV 15 antiserum
4. x. 62 (1)	−	−	−	−	−	−	.	.	.
4. x. 62 (2)	−	−	−	−	−	−	.	.	.
10 x. 62	−	−	−	−	−	−	.	.	.
16. x. 62	−	−	−	−	−	+	1	+	+
14. xi. 62	−	−	+	−	−	−	3	+	+
17. xi. 62	−	+	+	+	−	−	2, 4, 5	+ − +	+ − +
20. xi. 62 (1)	−	−	−	−	−	−	.	.	.
20. xi. 62 (2)	−	−	−	−	−	−	.	.	.
27. xi. 62	−	−	−	−	−	−	.	.	.
29. xi. 62	Yeast contaminant								
4. xii. 62	−	−	+	−	−	−	6	+	+
3. i. 63	−	−	−	−	−	−	.	.	.
17. i. 63	−	−	−	+	−	−	7	+	+
14. ii. 63	Bacterial contaminant								

Table 3. *Isolation of adenovirus from nose and throat swabs taken from sick monkeys*

Batch no.	Virus isolated	Strain no.	Identification of strains. Complement-fixation test: Human	Rabbit SV 15 antiserum	Comments
11/63	+	8	+	+	.
12/63	+	9	+	+	.
13/63	−	.	.	.	Died of pneumonia
14/63	−	.	.	.	.
15/63	−	.	.	.	Died of pneumonia
16/63	+	10	+	+	.
17/63	+	11	+	+	Killed for kidneys
18/63	−	.	.	.	.
19/63	+	12	+	+	Ill but recovered
M 93	+	13	+	+	Diarrhoea and pneumonia; seven of this batch died

Table 4. *Results of pool tests*

Strain no.	Pool I	Pool II	Pool III
1	Neut.	–	–
2	Neut.	–	–
3	Neut.	–	–
5	Neut.	–	–
6	–	–	Neut.
7	–	–	Neut.
8	Neut.	–	–
9	–	–	Neut.
10	–	–	Neut.
11	–	–	Neut.
12	–	–	Neut.
13	–	–	Neut.

Table 5. *Specific antiserum neutralization test*

Strain no.	Type	Strain no.	Type
1	SV 15	8	SV 17
2	SV 17	9	SV 32
3	SV 17	10	SV 32
5	SV 17	11	SV 32
6	SV 32	12	SV 32
7	SV 32	13	SV 32

unlikely that release of any virus present in the cells had been affected by the treatment. The earliest release of virus appeared after 21 days incubation in one instance but usually 28 days or more elapsed. The first subculture of released virus was slow, taking 2 weeks or more before a cytopathic effect appeared, but later passages produced cell degeneration in about 5 or 6 days.

As can be seen in Table 2, seven strains obtained from the tonsil cultures all showed the group complement-fixing antigen characteristic of adenoviruses. One negative result on one occasion was preceded and followed by a virus isolation from tissue from the same tonsil. Both strains were identical and proved to be SV 17 virus. Of the other viruses isolated, one was SV 17, two were SV 32 and the remaining one was SV 15 (Tables 4 and 5).

From the nose and throat swabs six viruses were obtained (Table 3); five, from a group of monkeys received on the same date, were SV 32; one from a second group of monkeys received on another date proved to be SV 17 (Tables 4 and 5).

There was complete failure to isolate any viruses in HeLa cells.

DISCUSSION AND CONCLUSIONS

The presence of a 'latent' viral flora in the tonsils of rhesus monkeys indicates a further similarity between the simian and human adenoviruses. Both groups require a long period of incubation before release of viable particles from the tonsillar material. A further period of incubation is necessary for simian adenoviruses before cytopathic effects appear. The amount of virus liberated from the tonsils

was very small. Incubation of inoculated monkey kidney cultures for several weeks was needed before negative results could be accepted. Parallel use of HeLa cell cultures for testing the infectivity of tonsillar material from monkeys was disappointing but, unlike human adenoviruses, most simian adenoviruses failed to multiply in these cells even after subculture.

An interesting feature was the isolation of the same adenovirus serotype from the tonsils of healthy monkeys and from a monkey with acute respiratory illness. SV 17 had been previously isolated from patas monkeys with conjunctivitis and rhinorrhoea by Tyrrell *et al.* (1959) and was here implicated in one outbreak in rhesus monkeys. SV 32 was isolated from five members of one batch of monkeys suffering from a similar illness and would appear to be associated with the same type of disease. SV 15, although existing as a latent virus, was not isolated from sick monkeys.

SUMMARY

A study was made of the 'latent' viral flora of simian tonsils. This was followed by isolation of viruses from two batches of sick monkeys whose illness is described. Simian viruses 15, 17 and 32 were isolated from nose and throat swabs obtained from the sick monkeys. A connexion between human and simian adenoviruses is suggested.

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