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TRANSMISSION OF SIMIAN ACQUIRED IMMUNODEFICIENCY SYNDROME (SAIDS) WITH TYPE D RETROVIRUS ISOLATED FROM SALIVA OR URINE

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Saliva and urine specimens from rhesus monkeys with SAIDS were found to contain a type D retrovirus related to Mason-Pfizer monkey virus (MPMV) which has been linked etiologically to SAIDS. Virus isolates from saliva and urine were shown to have the characteristics of the SAIDS agent by their reverse transcriptase divalent cation preference for synthetic template-primers, production of characteristic cytopathology in Raji cells and antigenic relatedness to MPMV as determined by enzyme-linked immunosorbent assay (ELISA) and competition radioimmunoassay (RIA). Electron micrographs of parotid tissue from an animal with SAIDS also showed budding particles with type D retrovirus morphology. A tissue culture grown virus isolate from urine of an animal with SAIDS, produced SAIDS when inoculated into two normal juvenile rhesus monkeys. Since saliva and urine of monkeys with SAIDS contain infectious SAIDS virus, they are likely sources of virus by which the disease is naturally transmitted. Thus, care should be taken to avoid contact of normal and infected animals.

A simian acquired immunodeficiency syndrome (SAIDS), which in many respects resembles human immunodeficiency syndrome (AIDS), has been reported to occur enzootically in macque species housed at three regional primate centers in the United States (1-3). SAIDS has been transmitted to healthy rhesus monkeys (M. mulatta) with unfiltered homogenates of tissues, whole blood and serum as well as cell-free filtrates of plasma, serum or homogenates of lymphomatous tissue form rhesus monkeys with advanced disease (2,4-6). Most recently we and our collaborators at the California Primate Research Center have isolated a type D retrovirus from affected animals and have transmitted the disease with the virus grown in tissue culture (7,8).

The aim of the present study was to identify possible sources of virus which would explain the natural transmission of SAIDS among monkeys.

Materials and Methods

Urine samples were asceptically collected by transabdominal suprapubic tap from normal control animals and animals in various stages of SAIDS. Salivation was stimulated by subcutaneously inoculating animals with 0.1ml of Urecholine, 5mg/ml (Merck, Sharp and Dohme, West Point, Pennsylvania). After collection of saliva, the stimulatory effect of Urecholine on the parasympathetic nervous system was reversed by injecting animals with atropine. Prior to inoculation into tissue cultures, saliva samples were passed through a 0.45μM pore size filter to remove contaminating free-living microorganisms.

Low passages of normal rhesus monkey fibroblasts or Flow 7000 human diploid fibroblasts were used to isolate virus from saliva and urine. Reverse transcriptase activity in con-

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TABLE I. Isolation of SAIDS Retrovirus From Saliva and Urine of SAIDS Monkeys

Source and Animal No.	Animal Inoculum	Interval After Inoculation	Clinical Findings
Saliva			
E-12	Serum Pool A (Fresh)	14 Wks	Terminal SAIDS
E-16	Sentinel Exposed to SAIDS	17 Wks	Terminal SAIDS
E-17	Serum Pool A (Fresh)	6 Wks	Terminal SAIDS
B-952	Serum Pool A (Frozen/Thawed)	18 Wks	Advanced SAIDS
B-335	IDB-2 (Tissue Culture)	4,5,6 Wks	Early SAIDS
Urine			
E-12	Serum Pool A (Fresh)	14 Wks	Terminal SAIDS
B-649	Tissue Suspension #19219 (Davis)	21 Wks	Early SAIDS

* At time of virus isolation.

centrated harvests of culture medium was assayed as described previously (9). Parotid tissue from a rhesus monkey (B-335) with SAIDS was fixed in glutaraldehyde and then in osmium tetroxide, dehydrated in ethanol, embedded in Araldite 502 and cured overnight at 70°C. Thin sections were double stained with uranyl acetate and lead citrate and examined in a JEM 100 CXII electron microscope.

Results

SAIDS retrovirus was readily isolated from saliva and urine samples from animals in early, advanced and terminal stages of the disease (Table I). Virus isolations were made from animals which had been inoculated with materials from animals with SAIDS, including tissue homogenates and filtered, pooled serum. Isolations were also made from an animal inoculated with tissue culture grown SAIDS retrovirus (strain IDB-2) (7). One study animal was a normal sentinel who developed SAIDS after being caged with an animal with SAIDS.

The reverse transcriptase of these isolates had a Mg^{++} preference for the synthetic template-primers, poly (rA) · oligo$(dT)_{12-18}$ and poly (rC) · oligo $(dG)_{12-18}$, and a Mn^{++} preference for poly (rC_m) · oligo$(dG)_{12-18}$. The same divalent cation preferences were previously reported for the SAIDS type D retrovirus and prototype MPMV (7,8). Inoculation of Raji cells with several of the saliva isolates also resulted in cytopathology characteristic of that reported for SAIDS retrovirus (10). In addition, isolates were identified by ELISA and competition RIA as being similar antigenically to SAIDS retrovirus (7,8). Electron micrographs of thin sections of parotid from rhesus monkey B-335, an animal from which retrovirus was isolated from saliva, also showed typical budding type D retrovirus (Fig. 1).

A tissue culture retrovirus isolate from urine of a rhesus monkey (B-649) with SAIDS produced SAIDS in two normal juvenile rhesus monkeys 20 weeks after they were intravenously

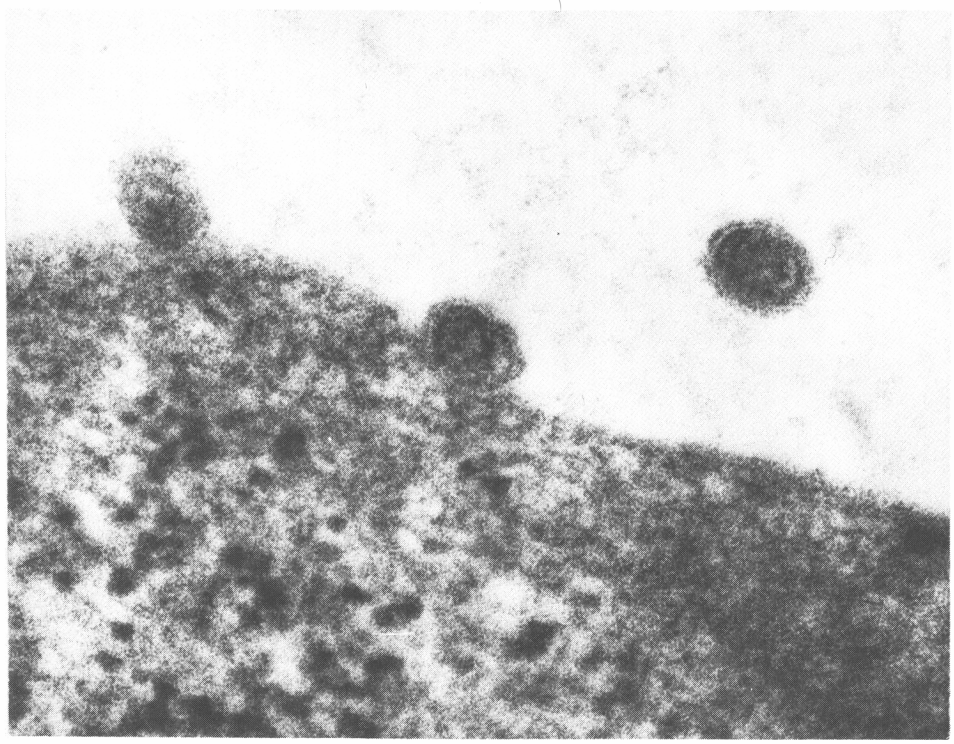

Fig. 1. Budding and free viral particles in a thin section of salivary gland tissue. X100,000.

inoculated with second passage tissue culture grown virus. SAIDS retrovirus was isolated from the serum of the one animal tested. In addition, two juvenile rhesus monkeys recently inoculated with filtered saliva, shown to contain type D SAIDS retrovirus, have developed early symptoms of SAIDS (neutropenia, lymphadenopathy, splenomegaly and intermittent diarrhea).

The urine from rhesus monkey B-649 from which retrovirus was isolated was found to contain rhesus monkey cytomegalovirus (RCMV). Both SAIDS retrovirus and RCMV were isolated in Flow 7000 cultures inoculated with urine from B-649 and passed twice in vitro. Thus, the virus inoculum contained both SAIDS retrovirus and RCMV. Our previous studies suggest that the RCMV present in the inoculum was not causally linked to the production of SAIDS. RCMV antibody negative rhesus monkeys did not develop SAIDS when inoculated with RCMV alone (5). Furthermore, animals that had antibody to RCMV were not protected against challenge with SAIDS virus (5). In contrast, RCMV antibody negative rhesus monkeys inoculated with SAIDS retrovirus developed fatal SAIDS (7,8).

Discussion

This study shows that SAIDS retrovirus replicates in the parotid glands of infected monkeys and is excreted in their saliva and urine. The excreted virus can be isolated, grown in tissue culture and when inoculated into rhesus monkeys, produces SAIDS. Saliva and urine are likely sources of virus for the natural transmission of SAIDS. Therefore, care should be taken to avoid direct contact between animals with SAIDS and normal animals. Furthermore, because of the unknown virulence of SAIDS retrovirus for humans, veterinarians, animal care technicians and laboratory personnel should avoid direct exposure to saliva and urine from monkeys with SAIDS. These studies raise the question whether saliva and urine from humans

with AIDS contain infectious virus and are sources of disease transmission.

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