Infection of Volunteers with Adenovirus Type 16. (28750)

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Although adenovirus type 16 has been frequently recovered from children(1), information concerning its importance in human disease is limited. Only a single case of illness with this serotype has been reported (2). Adult volunteers were inoculated with a strain of adenovirus type 16 in order to obtain additional data on the clinical and laboratory responses to infection with this serotype. This report describes the results of these experiments.

Materials and methods. Volunteer selection and clinical procedures. The participants in this study were adult male inmates (2 Negro and 1 Caucasian) between the ages of 23 and 34 from Federal correctional institutions. Selection was based on the absence of pre-existing neutralization antibody.

Urine analysis, complete blood count, chemical tests of liver and kidney function and bacterial cultures of nose, throat and rectum were done prior to inoculation and were repeated at weekly intervals for 3 weeks. X-rays of the chest and paranasal sinuses were made prior to inoculation and repeated 2 weeks after inoculation.

The 3 volunteers were isolated in a single room and except for exercise periods were kept there for 4 weeks. Oral temperature, pulse and respiratory rates were determined 4 times daily. Once daily physical examination was performed by a team of 2 physicians and this, with a record of any complaints of illness, was recorded before leaving the room. The examining physicians were unaware of the type of inoculum or the route of administration until termination of the experiment.

No aspirin or antibiotics were given the volunteers during the experiment.

Inoculum. The virus strain used to prepare the inoculum was recovered from an anal specimen of a young child residing in an institution in Washington, D. C.† The material used was the second passage in primary human embryonic kidney (HEK). The maintenance medium which consisted of Eagle's basal medium(3) also contained 250 units of penicillin and 250 μg of streptomycin per ml. Following a single cycle of freezing and thawing, fluids from cultures were pooled, clarified by centrifugation at 3000 rpm for 15 minutes, passed through a millipore filter of an 800 millimicron pore diameter and frozen at —60°C until used. Aliquots of the inoculum were safety-tested as previously described(4).

The inoculum was identified in neutralization and hemagglutination-inhibition tests with prototype immune rabbit serum.

Inoculation of volunteers was done by stroking the lower conjunctival sac of the eye 10 times with a cotton swab previously immersed in 1.5 ml of fluid containing a 10^{-3} dilution of the inoculum. Immediately following administration of the virus, a sample of the undiluted inoculum was titrated in HEK cultures. Cultures were refed once weekly and after a 28 day incubation period, the titration endpoint which was determined by the method of Reed and Muench(5) was $10^{-8.0}$ $TCID_{50}$ per ml.

Virus isolation. Specimens from both eyes were collected prior to inoculation, daily for the next 7 days and 3 times per week for 2 weeks and 2 times in the fourth week. Swabs from the throat and rectum were collected before inoculation and daily for a 3 week period and 3 times during the fourth week. Isolation attempts were made with HEK cultures after all specimens were collected as previously described(6). Two isolates from each man were identified in the hemagglutination-inhibition test using immune rabbit serum prepared from the prototype strain.

Serologic procedures. Neutralization tests

† Received through the courtesy of Dr. Leon Rosen, Pacific Research Section, Lab. of Infect. Dis., Nat. Inst. of Allergy and Infect. Dis., Honolulu, Hawaii.

TABLE I. Virus Recovery Following Conjunctival Inoculation of Volunteers with Adenovirus Type 16.

	Virus recovery (weeks after inoculation)											
	Eye				Throat				Rectum			
Volunteer	1	2	3	4	1	2	3	4	1	2	3	4
AS	2/7*	2/3	1/3	0/2	1/7	1/7	0/7	0/3	0/7	2/7	1/7	0/3
CH	2/7	2/3	0/3	0/2	2/7	2/7	1/7	1/3	0/7	3/7	1/7	0/3
HA	2/7	1/3	0/3	0/2	0/7	0/7	1/7	1/3	0/7	0/7	1/7	0/3
Totals	6/21	5/9	1/9	0/6	3/21	3/21	2/21	2/9	0/21	5/21	3/21	0/9

* No. positive/No. of samples tested.

were done with rhesus kidney cultures according to "procedure 2" as described by Rowe *et al*(7).

Hemagglutination-inhibition (HI) tests were performed by the technique described by Rosen(8).

Results. All 3 men developed conjunctivitis following conjunctival inoculation with adenovirus type 16. It was most severe in volunteer AS who developed acute conjunctivitis on the third day after inoculation accompanied by the appearance of a tender pre-auricular node on the inoculated side. At the end of a week, pronounced periorbital swelling developed. Gradual recovery occurred during the second week and all evidence of reaction was gone by the end of the third week. A moderate degree of conjunctivitis developed in the uninoculated eye during the second week. The acute phase of illness was associated with excessive lacrimation, photophobia and foreign body sensation. On day 6, two small punctate areas of the cornea stained with fluorescein. These disappeared within 2 days.

Volunteer CH developed a similar picture of illness except for the absence of corneal involvement. He also developed redness in the uninoculated eye for several days during the second week after inoculation. On day 12 herpetic lesions appeared on his upper lip which on culture yielded herpes virus.

The third volunteer, HA, had a mild conjunctivitis in the inoculated eye from which he completely recovered in two weeks.

Liver function tests, X-rays, blood counts, sedimentation rates, and urine analyses remained within normal limits. The bacterial flora of nose, throat and rectum remained essentially unchanged during illness.

In Table I is shown the virus shedding patterns of the 3 volunteers. Eye shedding began on day 4 and, with the exception of one positive culture, virus was not recovered after day 11. The incidence of throat shedding was similar but some cultures were positive during each of the 4 weeks after inoculation. Rectal isolation was limited to 8 of 42 cultures taken in the second and third week after inoculation. Cultures from uninoculated eyes were negative for virus.

Each man developed significant neutralizing antibody (Table II) after inoculation. Tests with the inoculum strain gave higher titers than those with the prototype strain. HI antibody was never demonstrated in the sera of these volunteers in repeated tests with prototype and inoculum strains.

Discussion. The present cases of conjunctivitis were similar in severity to those which have been experimentally produced with adenovirus types 1, 3, 4, 5, 26 and 27(6,9), and they were comparable in severity to many cases of naturally-occurring adenovirus

TABLE II. Serologic Response of Volunteers Following Conjunctival Inoculation with Adenovirus Type 16.

	Serologic test*			
	Neutralization *vs*		Hemagglutination-inhibition *vs*	
Volunteer	Prototype	Inoculum	Prototype	Inoculum
AS	<4†	<4	<10	<10
	8	32	<10	<10
CH	<4	<4	<10	<10
	8	16	<10	<10
HA	<4	<4	<10	<10
	16	64	<10	<10

* Reciprocal of dilution.

† First line indicates pre-inoculation titer and second line indicates post-inoculation titer (day 28).

conjunctivitis. In a case of conjunctivitis caused by adenovirus type 16 produced in the blind eye of a young adult(2), there was apparently a milder disease, but this followed an approximately 100-fold smaller dose of virus than used in the present study.

The corneal involvement in one of the present cases has not occurred previously in our experience. It was not severe, however, and disappeared without sequelae. This event probably does not indicate a special capacity of this adenovirus type for corneal invasion. The occurrence of conjunctivitis in 2 uninoculated eyes is difficult to explain. In both cases, the conjunctival involvement was limited to palpebral injection. Spread of infection would be a logical possibility but cultures were negative. In this connection it is noted that on only a single occasion following experimental inoculation of more than 40 men with adenovirus type 26 or 27 did spread to the other eye occur(6). Moreover, development of bilateral conjunctivitis among men given conjunctival inoculation in one eye with other adenovirus serotypes has occurred infrequently(10). It remains possible that there was spread of infection with adenovirus type 16 to the other eye in 2 men, but under circumstances in which virus could not be isolated.

Once isolation from the eye and rectum had been made, serial specimens tended to be positive. In contrast, isolations made from throat specimens were sporadic. Not infrequently, throat specimens were negative on days rectal specimens were positive unless serial cultures were obtained, excretion from the oropharynx might not have been detected.

The failure to demonstrate HI antibody in volunteers infected with this serotype is in agreement with a previously published report (1). In that study, extensive testing to demonstrate HI antibody with sera from children naturally infected with adenovirus type 16 was carried out with negative results.

Summary. Inoculation of 3 volunteers with adenovirus type 16 produced an afebrile, nonpurulent conjunctivitis associated in one case with slight corneal involvement. All subjects shed virus from inoculated eyes, throat and rectum. The failure to demonstrate HI antibody in these men was similar to the finding observed in children naturally infected with this same serotype.

Addendum: Review of sick call records in the resident institution showed no report of jaundice or other systemic illness during a period of at least seven months after inoculation.

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