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Enteric Immunization with Live Adenovirus Type 21 Vaccine

I. Tests for Safety, Infectivity, Immunogenicity, and Potency in Volunteers

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Studies were undertaken in volunteers to determine whether living adenovirus type 21 (ADV-21) vaccine could be safely administered orally to susceptible young adults. In the first study, ten volunteers were fed $10^{6.4}$ tissue culture infectious $dose_{50}$ ($TCID_{50}$) of ADV-21 vaccine virus, and five received placebo tablets. Nine of ten infected volunteers shed ADV-21 in stools (mean duration, 10.1 days; range, 4 to 17 days). No pharyngeal excretion of ADV-21 was observed in any of these volunteers. Each of the nine developed type-specific neutralizing (N) antibodies to ADV-21. No evidence for person-to-person transmission of vaccine was observed. In a second study, volunteers were immunized with ADV-21 vaccines containing $10^{6.8}$, $10^{4.6}$, and $10^{2.4}$ $TCID_{50}$. ADV-21 N antibody responses were detected in nine of eleven who received the highest dose, six of twelve who received the middle dose, and two of twelve who were fed the lowest dose. None of twelve susceptible volunteers receiving the placebo capsule developed ADV-21 N antibodies postimmunization. This study established that the human infectious $dose_{50}$ for these lots of ADV-21 vaccine was approximately $10^{4.6}$ $TCID_{50}$ and that the dose response to ADV-21 vaccine was lower than those previously reported for live ADV-4 and ADV-7 enteric vaccines.

The principal causes of acute respiratory disease (ARD) requiring hospitalization in basic combat trainees in U.S. military populations are adenoviruses type 4 (ADV-4) and type 7 (ADV-7). Recent large-scale use of live enteric-coated ADV-4 and ADV-7 vaccines has been highly effective in the suppression of ARD associated with these two serotypes (7). Another adenovirus serotype that has been associated with ARD in military trainees is ADV-21. In 1967, ADV-21 caused a significant amount of disease in trainees at Ft. Dix, N.J. (5). In the past, suppression of ADV-4 disease with ADV-4 vaccine, given alone, fostered the emergence of ADV-7 disease in the immunized population. Thus, it is possible that extensive use of both ADV-4 and ADV-7 vaccines may lead to the emergence of other adenovirus serotypes as major causes of ARD. Since 85% of young adult males lack detectable neutralizing (N) antibodies to ADV-21 (F. H. Top, Jr., and E. L. Buescher, *unpublished data*), ADV-21 is a potential cause of significant disease in military training populations if it were to emerge after extensive immunization with ADV-4 and ADV-7 vaccines.

The following studies were designed to permit evaluation of the safety, infectivity, and immunogenicity, as well as the potency, of a live, oral enteric ADV-21 vaccine in man.

MATERIALS AND METHODS

Volunteers. Volunteers were chosen from enlisted military personnel. A complete and comprehensive explanation of the study and its risks was given to all volunteers by one of the principal investigators. Volunteers knew that placebo controls were included but were not told which individuals received a placebo. A consent statement was signed by each volunteer.

Vaccines. ADV-21, strain V-270, was obtained by Wyeth Laboratories from Robert Chanock. The virus was originally isolated in human embryonic kidney (HEK) cells from throat washings of a patient with pharyngitis. After isolation and identification, it was passed twice in HEK cells. At Wyeth Laboratories, the strain was passed two additional times in HEK cells, then through 11 passages in human diploid fibroblast cultures (WI-38), lyophilized, mixed with inert ingredients (principally microcrystalline cellulose and lactose), and prepared into enteric-coated capsules. The various vaccine lots used in each study and their vaccine virus titers will be described separately. Volun-

teers not receiving ADV-21 vaccine received an enteric-coated placebo capsule; this preparation was shown to contain no cytopathogenic agent when a liquid suspension was inoculated into HEK tissue culture tubes.

Titration of vaccine viruses. Contents of vaccine capsules were emulsified with a mortar and pestle in 10 ml of Hanks' balanced salt solution. After thorough mixing, 10-fold dilutions of the virus suspension were made, and 0.1 ml of each dilution was inoculated into each of six HEK tube cultures containing Leibowitz-15 (L-15) media with 2% fetal bovine serum (FBS). Inoculated cultures were observed every other day and maintained for at least 21 days before a final reading for cytopathic effects was made. Tissue culture infectious $dose_{50}$ ($TCID_{50}$) of vaccine virus was calculated by the method of Reed and Muench (3).

Collections of specimens. Schedules for obtaining samples for virus isolation and serum antibody determinations for the two studies are shown in Table 1. Specimens for virus isolation were maintained at −70 C until tested.

Virus isolation and identification. Stool suspensions and throat washings were tested for virus by standard methods (4) in HEK cell culture tubes maintained in L-15 media with 2% FBS. All cultures were observed every other day and maintained for at least 21 days. Isolates were identified with type-specific antisera by neutralization tests.

Serological studies. Pre- and postimmunization sera from individual volunteers were tested simultaneously for (N) antibody. Serum N antibody tests were performed in HEK cells by standard techniques using the vaccine virus strain (4). Titration end points were read at a time when the test dose of virus was 3.2 to 32 $TCID_{50}$. Adenovirus complement fixation (CF) titers were determined by standard microtiter procedures with adenovirus CF antigen obtained from Microbiological Associates. Echo virus type 6 and herpesvirus N antibody tests also utilized HEK tube cultures and were read when the test dose of virus was 100 $TCID_{50}$.

Study no. 1: vaccine safety, infectivity, and antigenicity. The volunteer group consisted of 15 men found to be free of ADV-21 N antibody at a serum dilution of 1:2. Ten volunteers received ADV-21 vaccine, and five volunteers received the placebo tablet on study day 0. ADV-21 vaccine capsules (lot 16 CIX-01201, Wyeth Laboratories) contained $10^{6.4}$ $TCID_{50}$ of vaccine virus per capsule.

Volunteers were housed in individual rooms on two closed wards of the U.S. Army Research Institute for Infectious Diseases for the duration of the study; each ward contained both volunteers who received vaccine and those who received placebo enteric capsules. All volunteers shared common recreational and dining facilities. Detailed medical histories and physical examinations were performed on each volunteer upon admission to the study wards. Complete hospital records were initiated and maintained on each volunteer. Initial medical evaluation also included an electrocardiogram, chest X ray, complete blood count, urinalysis, and throat culture.

Blood specimens for white blood cell and differential counts, hematocrit, and platelet count were obtained on study days −5, −3, 0, then daily through day 14, and on day 21 of the study. Total direct and indirect bilirubin, serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase, and blood urea nitrogen (BUN) were determined on blood specimens obtained on study days −5, −3, 0, 4, 7, 14, and 21. Urinalysis was obtained on admission to the study and daily thereafter until day 14 and then on days 16, 18, and 21. The above laboratory tests were performed by standard laboratory procedures.

Study no. 2: dose response of live, oral adenovirus type 21 vaccine. Three different lots of ADV-21 vaccine were prepared for the study. Lot 16 CIX-01301 contained $10^{6.8}$ $TCID_{50}$, lot 16 CIX-01501 contained $10^{4.6}$ $TCID_{50}$, and lot 16 CIX-01701 contained $10^{2.4}$ $TCID_{50}$. Sixty-four trainees from the USAMEDTC, Fort Sam Houston, Texas, volunteered for the study. Of these, 47 were shown to have no ADV-21 N antibody at a serum dilution of 1:2. Eleven volunteers received lot 16 CIX-01301, 12 received lot 16 CIX-01501, 12 received lot 16 CIX. 01701, and 12 volunteers received a placebo capsule. These volunteers were not segregated from one another nor from trainees who did not participate in the study. The schedule for obtaining blood and stool specimens is shown in Table 1.

TABLE 1. *Schedule of specimen collection for viral isolation and serology in ADV-21 vaccine studies*

Study no.	Throat wash	Stool	Serum
1	Days −4, −3, −2, 0[a] to 28	Days −4, −3, −2, 0 to 28	Days −4, 0, 7, 14, 18, 21, 28
2	Not collected	Days 11, 13, 15	Days 0, 21

[a] Specimens collected on day 0 were obtained immediately before immunization.

RESULTS

Study no. 1: vaccine safety, infectivity, and immunogenicity. Nine of the 10 volunteers who received ADV-21 vaccine excreted ADV-21 in their stools. As shown in Fig. 1, ADV-21 shedding occurred as early as study day 4 and continued through day 21 in one volunteer. The median day for virus shedding was day 12, and on day 15, all nine volunteers excreted ADV-21. Duration of fecal shedding varied between 4 and 17 days with a mean duration of 10.1 days. None of the five volunteers serving as controls excreted ADV-21 in stools during the study. All adenoviruses isolated were ADV-21. In addition, one immunized volunteer (no. 3) excreted echovirus type 6 in his stool from day −4 to day 14 of the study.

ADV-21 was not isolated from throat washings

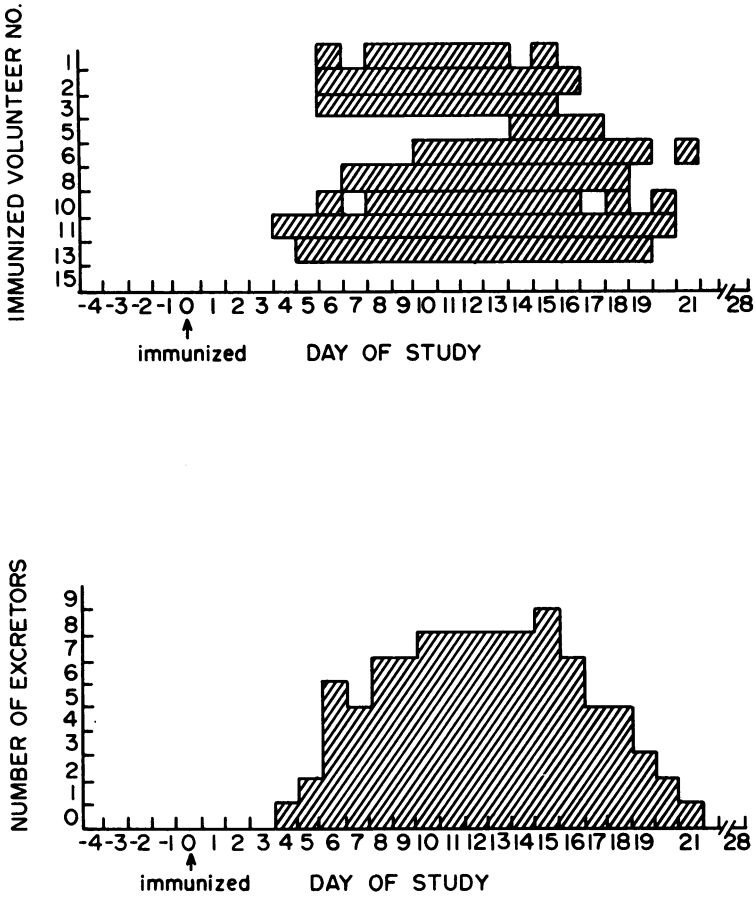

FIG. 1. *Stool excretion of ADV-21 virus in immunized volunteers. Upper graph shows excretion patterns by day of study for each volunteer. Lower graph shows the number of volunteers who excreted ADV-21 virus on each study day.*

of either immunized or control volunteers. *Herpesvirus hominis* was found in throat washings of three volunteers, all in the immunized group (volunteer no. 3, day −2 through day 8; no. 8, day −4 through day −2; and no. 10, on days 1 and 13).

N antibody responses to ADV-21 are shown in Table 2 along with adenovirus CF antibody titers. N antibody was not detected in the 28-day sera of the five placebo volunteers or in the one immunized volunteer who failed to excrete ADV-21. The magnitude of N antibody responses among the nine immunized volunteers varied between titers of 1:2 (two individuals) and 1:64 (three individuals). The reciprocal of the geometric mean N antibody titer at 28 days was 14.8.

No significant change in CF antibody titer occurred in either the immunized or control volunteers. None of the individuals who developed ADV-21 N antibodies showed fourfold or greater rises in CF antibody titer. More detailed studies of immunologic responses in these same individuals after administration of ADV-21 vaccine are presented in the following paper by Scott et al. (6).

Volunteer no. 3, who, in addition to excreting ADV-21, also excreted echovirus type 6, had an increase in echovirus type 6 N antibody titer from 1:4 on day −4 to 1:128 on day 14. None of the individuals from whom *Herpesvirus hominis* was isolated had fourfold or greater rises in homotypic N antibody during the study.

Mild, afebrile, upper respiratory disease or diarrhea, was noted in three immunized and two control volunteers. The relationship of these illnesses to ADV-21 stool excretion is summarized in Table 3. Volunteer no. 15, who failed to excrete ADV-21, had intermittent diarrhea from day 9 to day 16 of the study. Diarrhea in three other volunteers was observed between day 16 and day 20. No viral pathogens or ADV-21 were

TABLE 2. *Experimental ADV-21 infection in man: neutralizing (N) and complement-fixing (CF) antibody responses after virus ingestion*

Volunteer status	Volunteer no.	Days of ADV-21 excretion	ADV-21 N titer (reciprocal)		ADV CF titer (reciprocal)	
			Day 0	Day 28	Day 0	Day 28
Immunized excretors ADV-21	1	8	<2	2	5	5
	2	11	<2	32	10	20
	3	10	<2	64	10	10
	5	4	<2	4	<5	<5
	6	11	<2	8	10	10
	8	10	<2	2	10	10
	10	13	<2	32	10	20
	11	17	<2	64	10	10
	13	15	<2	64	10	20
Immunized nonexcretor	15	0	<2	<2	20	10
Placebo	4	0	<2	<2	10	10
	7	0	<2	<2	10	20
	9	0	<2	<2	10	20
	12	0	<2	<2	10	10
	14	0	<2	<2	20	40

TABLE 3. *Experimental ADV-21 infection in man: illness in five volunteers*

Volunteer no.	Stool excretion of ADV-21 (study days)	Respiratory symptoms (study days)	Diarrhea (study days)
Immunized			
15	None	+7	+9–+16
10	+6, +8–+16, +18, +20	+13–+14	
1	+6, +8–+18, +15		+16–+18
Control			
4	None	−4–0	+16
12	None		+20

isolated from stools collected during these periods. The volunteer who excreted echovirus type 6 (day −4 to day 12) and ADV-21 (day 6 to day 15) in his stool and *Herpesvirus hominis* (day −2 to day 8) in throat washings was asymptomatic throughout the study period.

No abnormalities in hematocrit, complete blood count, platelet count, total direct and indirect bilirubin, SGOT, SGPT, alkaline phosphatase, BUN, or urinalysis were found in any volunteers during the course of the study.

Study no. 2: dose response of live, oral ADV-21 vaccine. The results of ADV-21 stool isolation and ADV-21 serum N antibody response for the four groups of ADV-21-susceptible volunteers are shown in Table 4. Stool excretion of ADV-21 on at least one of the three study days was detected

TABLE 4. *ADV-21 stool excretion and serum N antibody response in susceptible volunteers*

Vaccine lot	Virus titer of vaccine (Log_{10} of $TCID_{50}$)	No. of volunteers immunized	Stool excretion	N antibody response		
				No.	Per cent	Range of convalescent titers (Reciprocal)
16CIX-01301	6.8	11	6	9	82	2–64
16CIX-01501	4.6	12	0	6	50	2–4
16CIX-01701	2.4	12	0	2	17	2
Placebo		12	0	0	0	

in 6 of 11 susceptible volunteers given the highest-dose vaccine, but in none of the susceptible volunteers given the intermediate- or low-dose vaccine. No control volunteers excreted ADV-21.

The development of ADV-21 serum N antibody 3 weeks postimmunization was detected in 9 of 11 susceptible volunteers given the highest-dose vaccine, 6 of 12 susceptible volunteers given the intermediate-dose vaccine, and 2 of 12 susceptible volunteers given the low-dose vaccine. Reciprocals of geometric mean ADV-21 N antibody titers were 7.5, 2.8 and 2.0, respectively, for the three immunized groups. None of the 12 ADV-21-susceptible volunteers given the placebo capsule developed ADV-21 serum N antibodies. Significant transmission of wild ADV-21 did not occur in the trainee population studied during the 3 weeks of the study, and thus serologic rises detected in the immunized volunteers were due to immunization and not due to naturally acquired ADV-21 infections. In this study, the human infectious $dose_{50}$ for ADV-21 vaccine was $10^{4.6}$ $TCID_{50}$ per capsule.

DISCUSSION

These studies show that ADV-21 vaccine virus can infect the gastrointestinal tract of susceptible male adults and that infection of the respiratory tract by vaccine virus did not occur. Vaccine virus was not transmitted to susceptible volunteers who received placebo capsules and who were quartered with infected vaccinees in study no. 1, in which transmission was closely monitored. Finally, no untoward reactions were observed in the immunized volunteers. In these respects ADV-21 vaccine is similar to ADV-4 and ADV-7 vaccines studied previously in similar experiments (1, 8).

The pattern of ADV-21 stool excretion in immunized volunteers in study no. 1 was similar to

that of ADV-4 and ADV-7 stool excretion after live, enteric immunization as reported by Chanock et al. (1) and Top et al. (8) for ADV-4 and ADV-7 vaccines, respectively. The failure to detect vaccine virus excretion in stools of those volunteers receiving the two lower-dose vaccines in study no. 2 and the smaller number of excretors among those who received the high dose (6/11) was unexpected. Failure to recover virus in those volunteers who developed type-specific N antibodies after immunization may have been the result of mistimed stool collections. Days 11, 13, and 15 postimmunization were chosen because in study no. 1 the median day for stool excretion of vaccine virus was day 12, and only on day 15 did all nine excretors have virus in their stool specimens. It is entirely possible that excretion of vaccine virus occurred earlier than day 11 or later than day 15 in some or all of those men who had antibody responses.

Titers of ADV-21 N antibody present in 28-day sera of the nine immunized volunteers who excreted vaccine virus in study no. 1 are comparable to N antibody titers in men immunized with live, enteric, ADV-4 and ADV-7 vaccines, respectively (1, 8). However, the lots of ADV-21 vaccine used in study no. 2 were clearly less immunogenic than comparable doses of ADV-4 and ADV-7 tested in previous studies (2, 8). In addition, the high-dose lot (16 CIX-01301) was less immunogenic than a similar dose but different lot of vaccine (16 CIX-01201) used in study no. 1.

A similar problem was encountered during the development of ADV-7 vaccine. In the initial study reported by Top et al., 11 of 16 susceptible volunteers (69%) were infected when a vaccine dose of $10^{4.9}$ $TCID_{50}$ per tablet was used (8). Yet, in subsequent studies, a similar vaccine dose, but different manufactured lot, infected 95% of susceptibles (8). This suggests that availability of vaccine virus, determined by release from the enteric-coated capsule or tablet into the intestinal tract, may vary among different manufactured lots, for unknown reasons, and result in differences in infectivity for comparable vaccine doses. This documents the importance of determining efficacy in man of different vaccine lots irrespective of their $TCID_{50}$.

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