Induction of Immunity in Man by Crystalline Adenovirus Type 5 Capsid Antigens (37438)

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Previous studies in animals and man have shown that adenoviral capsid proteins, hexon and fiber, possess immunologically unrelated antigenic determinants capable of inducing neutralizing antibody (1–4). Also, a protective effect of antibody stimulated by both antigens was suggested by resistance of vaccinated adult volunteers to experimental infection. However, the use of adenoviral capsid antigens as vaccines for prevention of adenoviral disease has not been attempted primarily because of the inability to consistently prepare vaccines with an acceptable potency and degree of purity. The recent demonstration of crystallization of adenoviral proteins now provides an additional procedure for purification and allows for further studies in man with preparations that have important attributes (5, 6). Since dosage can be specified in μg of protein, standardization and reproducibility of vaccines is assured. Such vaccines are free of adventitial proteins and thus may be less reactogenic and, as only the desired antibody response is obtained, they may be less prone to induce potentially harmful sensitization. Moreover, they are free of nucleic acid and thus presumably free of any tumorigenic potential. Finally, preparations of different serotypes may be used as polyvalent antigen preparations, an attribute desirable for immunization against adenoviral disease, particularly for pediatric age groups.

In the present study, crystalline hexon and fiber proteins of type 5 adenovirus were given to adult volunteers. Low reactogenicity was observed, a significant frequency of serum antibody responses occurred, and vaccinated volunteers exhibited resistance to challenge with live adenovirus type 5.

Materials and Methods. Vaccine preparation. A nonprototype strain of adenovirus type 5 was isolated from a throat specimen obtained from a seventeen-month-old child. The clinical specimen was kindly provided by Dr. Albert Kapikian of the National Institute of Allergy and Infectious Diseases. Cell packs collected after fourth passage of virus in monolayer cultures of primary human embryonic kidney[5] were used as a source of adenoviral capsid antigens. Maintenance medium for the first and second passage of virus was composed of 98 parts Eagle's basal medium (BME) and 2 parts of inactivated (56° for 30 min) fetal calf serum and for the third and fourth passages it consisted of 100 parts BME. All media used in tissue cultures also contained 250 units of penicillin

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[5] Cultures were obtained commercially from HEM Research, Inc., Rockville, Maryland, and Flow Laboratories, Inc., Rockville, Maryland.

and 250 μg of streptomycin/ml. Procedures for purifying and crystallizing hexon and fiber antigens were identical to those previously described (5, 6). In brief, following treatment of cell packs with fluorocarbon, soluble antigens were separated from complete and incomplete virions by banding in a discontinuous cesium chloride gradient. Hexon and fiber were then obtained by fractionation of the soluble antigen band by column chromatography with DEAE-sephadex A-50. Fractions containing the bulk of each antigen were rechromatographed separately and then further purified by rate zonal centrifugation in a sucrose gradient and crystallization. Following several washings, crystals were resolubilized, assayed for protein content by the Lowry method (7), and diluted in sterile physiologic saline. Two hexon vaccines, Lot 21947 and Lot 22429, were produced separately. Prior to use, each of these vaccines and fiber vaccine (Lot 21948) was filtered through a millipore filter with a pore diameter of 0.45 μm. Safety tests (8) with cell pack harvest fluids failed to reveal the presence of contaminating organisms. Tests in bacteriologic media and tissue culture with final container vaccine were negative for adventitious agents and for infectious adenovirus type 5. Double-gel diffusion tests and analytical polyacrylamide gel electrophoresis in a neutral SDS system (4) with each vaccine, respectively, showed a single band against antiserum to purified type 5 virus and no contaminating proteins.

Volunteer selection. The study was conducted at the Ramsey Unit of the Texas Department of Corrections. Adult male volunteers between the ages of 21 and 40 who lacked neutralizing antibody ($< 1 \log_2$) were selected for participation in the study after informed consent was obtained and after complete medical examinations revealed them to be in good health.

Study plan. An initial study in volunteers was performed using varying concentrations of a pilot lot of hexon vaccine (Lot 21947). During the vaccination period, individuals were not kept isolated from the institutional population. In the study designed to evaluate the protective effect of adenoviral vaccines, volunteers were assigned by random allocation to receive hexon (Lot 22429), fiber, or saline. As in the preliminary study, men were allowed to follow their normal duties during the immunization period. For infectious virus challenge, participants were isolated in a dormitory wing. Examinations were performed daily by physicians who were unaware of the type of vaccine given to the volunteers but were informed as to the virus used in the study.

Vaccine administration. Volunteers received two doses, 28 days apart, of either hexon or fiber vaccine intramuscularly in the concentration shown in Table I. Additional volunteers were given saline by the same route and at the same time as the adenovirus vaccines. Reactogenicity of vaccines was assessed by having each individual complete a questionnaire listing possible local and systemic reactions within 72 hr after immunization and requesting a report to sick call for any significant symptoms.

Virus inoculation. Viral inoculum was prepared from the third passage virus described above. After two cycles of freezing and thawing, the culture suspension was clarified by centrifugation at 3000 rpm for 20 min, filtered through a millipore filter with pore diameter of 0.8 μm, vialed and frozen at −65°. Prior to use, a sample of inoculum was submitted to safety tests as previously described (8). Volunteer inoculations were performed by instilling 0.25 ml of virus suspension into each nostril (total of 0.5 ml).

Laboratory tests. Neutralization and hemagglutination inhibition (HI) tests for antibody were performed by procedures previously described (3, 9). Serum specimens for these determinations were obtained immediately prior to each administration of vaccine and 28 days after virus challenge. For men receiving hexon vaccine (Lot 21947), collections were on Days 0, 28, and 56. For those receiving saline, hexon vaccine (Lot 22429), and fiber vaccine, sera were collected on Days 0, 28, 36, and 64 of the study. Viral inoculations were performed on the latter men on Day 36. Throat swab specimens were collected before and every second day for 14 days after administration

TABLE I. Distribution of Serum Neutralizing Antibody Titers Following Vaccination with Adenovirus Type 5 Hexon and Fiber Antigens.

Antigen type	Dose No.	Dose μg	No. men[a]	No. men with indicated level of antibody (log_2) post vaccination <1	1	2	3	4	5	6	7	Geometric mean titer (log_2)[b]
Hexon	1	0.3	5	4	1	0	0	0	0	0	0	1.0
Lot 21947	2	1.0	5	4	0	0	1	0	0	0	0	3.0
Hexon	1	3.0	6	5	0	1	0	0	0	0	0	2.0
Lot 21947	2	1.0	6	5	0	1	0	0	0	0	0	2.0
Hexon	1	30	4	1	0	1	0	1	0	1	0	4.0
Lot 21947	2	1.0	4	1	0	1	0	1	0	1	0	4.0
Hexon	1	100	18	4	5	4	3	1	0	0	1	2.4
Lot 22429	2	100	16	3	3	4	3	2	0	0	1	2.9
Fiber	1	200	12	1	1	5	2	1	1	1	0	2.9
	2	100	11	0	1	1	2	4	1	2	0	3.8
Saline	1	1.0 ml	19	19	0	0	0	0	0	0	0	<1.0
	2	1.0 ml	19	19	0	0	0	0	0	0	0	<1.0

[a] All prevaccination titers were less than 1 log_2.
[b] Mean titer for those exhibiting a rise in titer.

of virus and tested for virus in primary human embryonic kidney tissue cultures. Each positive throat specimen was quantitated for virus titer and the first and last isolate from each man was typed by serum neutralization tests.

Results. Reactogenicity. Reactions within the first three days following initial vaccination were limited to three men who had received hexon vaccine; two experienced mild local and systemic reactions and one reported a more severe systemic response, although he did not consider it sufficiently severe to report to sick call. Questioning revealed that the illness consisted of headache, malaise, and weakness. No significant reactions occurred following injection of a second dose of either hexon or fiber vaccine.

Antibody response. Viral neutralizing antibody responses to both vaccines are shown in Table I. For the hexon vaccine, antibody responses to 0.3 and 3.0 μg doses occurred in only 2 of 11 men. Primary immunization with 30 or 100 μg of hexon protein resulted in similarly low seroconversion rates and levels of antibody response. In both of these groups, a second dose did not result in more seroconversions or significant increase in titer.

TABLE II. Distribution of Hemagglutination Inhibition Antibody Titers Following Vaccination with Adenovirus Type 5.

Antigen type	Dose No.	Dose μg	No. men	No. with indicated level of antibody post vaccination <10	10	20	40	80	160	320	≧640
Hexon	1	100	18	16	1	0	0	1[a]	0	0	0
Lot 22429	2	100	16	15	0	0	0	1	0	0	0
Fiber	1	200	12	6	0	2	1	0	2	0	1
	2	100	8	0	0	0	0	1	4	2	1
Saline	1	1.0 ml	19	19	0	0	0	0	0	0	0
	2	1.0 ml	19	19	0	0	0	0	0	0	0

[a] This volunteer possessed HI antibody in the prevaccination serum and no change occurred following vaccination.

TABLE III. Response of Vaccinated and Unvaccinated Volunteers to Nasopharyngeal Inoculation with Live Adenovirus Type 5.

Vaccine group	No. men	Prechallenge antibody status		Illness response			Virus isolation		Serum antibody response	
		No. men	Geometric mean titer (log_2)[a]	Febrile URI	Afebrile URI	Total illness	No. men	Average no. of days	No. men with ≧4-fold rise	Geometric mean titer (log_2)
Saline	19	0	0	10	0	10	14	2.8	16	3.6
Hexon	15	12	2.3	1	2	3	10	2.1	11	4.9
Fiber	8	8	4.4	0	0	0	4	3.4	1	4.4

[a] For geometric mean calculations a titer of 1 log_2 was classified as 0.

After a single injection of 200 μg of fiber antigen, fourfold or greater increases in antibody titer occurred in 10 of 12 men and a twofold rise in one. Following a booster dose, the man with a titer of 1 log_2 had an increase to 5 log_2 and the antibody negative individual exhibited a twofold response. In addition, a second dose of fiber resulted in a further increase in titer in those with measurable antibody after the first dose.

HI antibody responses to hexon and fiber vaccine are shown in Table II. One volunteer who received hexon vaccine had HI antibody in his preserum despite the absence of detectable neutralizing antibody and no change in his titer occurred following vaccination. One volunteer exhibited a twofold rise. In contrast, 6 of 12 men who received fiber vaccine developed HI antibody following the first dose and 8 of 8 following the booster dose. Moreover, titers were significantly higher following the second dose. No antibody responses to type 5 virus were noted among men who had received saline.

Response to challenge. Forty-two men in the study group who had been given either fiber, the highest dose of hexon, or placebo were available for challenge with live adenovirus type 5. Response to intranasal inoculation with 12,000 $TCID_{50}$ is shown in Table III. At the time of challenge, all men in the fiber vaccine group had detectable serum neutralizing antibody, whereas only 12 of 15 individuals in the hexon group had antibody. Among men in the saline group, 14 shed virus and an additional 2 developed a fourfold rise in serum antibody titer, indicating infection had occurred in 16 of 19 men. Four of the 8 volunteers who had previously received fiber vaccine shed virus and only one of these exhibited a subsequent rise in serum antibody following inoculation with type 5 virus. Ten of the 15 hexon vaccinated volunteers shed virus and developed a further rise in antibody titer and one developed an initial serologic response following infection.

Ten of the 19 individuals who had received saline became ill and in all instances it was accompanied by fever (≧ 100°F). The illness was characterized as febrile pharyngitis of moderate severity. In contrast, none of the

TABLE IV. Quantity of Virus in Nasopharyngeal Wash Specimens Obtained from Vaccinated and Unvaccinated Individuals Following Inoculation with Live Adenovirus Type 5.[a]

Vaccine group	No. men	Mean virus titer ($TCID_{50}$/ml) on indicated day post inoculation							
		0	2	4	6	8	10	12	14
Placebo	19	0	0	1.1	2.6	3.6	2.8	2.0	0.6
Hexon	15	0	0	1.2	1.7	2.2	1.6	1.3	0.5
Fiber	8	0	0	0.5	0.7	1.5	1.5	1.1	0.4

[a] Log_{10}.

individuals who had received fiber developed illness and the difference in the response frequency was significant ($p = 0.02$). Three illnesses occurred among men who had previously received hexon vaccine and one of these was febrile. The febrile illness and one of the two afebrile illnesses occurred among volunteers who failed to develop a neutralizing antibody response following vaccination. The difference in illness frequencies between the hexon and saline groups was not statistically significant but when the two men without antibody were excluded the difference was highly significant ($p < 0.005$).

The average number of days of virus shedding was essentially the same in all groups. However, the mean quantity of virus shed by day for each vaccine group was different (Table IV). Less virus was shed by men who had received fiber or hexon vaccine than by individuals who had received saline.

Discussion. The studies described in this report show that adenovirus type 5 crystalline subunit vaccines are essentially non-reactogenic and antigenic in man. Purity of the preparations was shown by absence of HI antibody responses among volunteers who received hexon vaccine and occurrence of HI antibody responses among those who received fiber. While different preparations were used, the frequency (60%) of neutralizing antibody response to two 100-μg doses of hexon used in this study was similar to that observed in the pilot study after vaccination with 30 μg (75%). The fiber preparation produced a higher frequency of antibody responses and appears to be a more potent preparation than hexon but quantitative data for both antigens should be obtained before such a conclusion can be accepted. Moreover, such data should be obtained in children since the epidemiological and antibody response data suggest that adults have had prior experience with adenovirus type 5 antigens (10).

Despite the lower frequency of neutralizing antibody response to the hexon preparation, it is notable that any antibody response at all was associated with a significant degree of protection against the occurrence of illness from subsequent induced infection. Moreover, the infection resulted in a boost in antibody response such that the final titers were comparable to those in the fiber vaccine group and greater than those among individuals who had not received vaccine earlier. We believe that these results are sufficiently encouraging to warrant proceeding with further studies of this type of vaccine preparation for the possibility of use in pediatric age groups.

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