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Persistence of Neutralizing Antibody in Adult Volunteers Immunized With Adenovirus Soluble Antigens. (30747)

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A previous report has described the antigenicity and protective effects of 2 vaccines prepared from non-infectious soluble antigens of adenovirus type 1(1). Vaccine effectiveness was evaluated by homotypic virus challenge of adult volunteers early in the post-immunization period.

An important consideration in the use of such inactivated vaccines is the duration of neutralizing antibody elicited by these preparations. The following investigation was therefore undertaken to determine the persistence of neutralizing antibody in adults immunized with the type-specific and group-specific soluble antigens of adenovirus types 1 and 2.

Materials and methods. Volunteer selection and clinical procedures. The study was conducted at the Clinical Center of the National Institutes of Health with volunteers from Federal correctional institutions. Criteria of selection and clinical evaluation of the men have been previously described(1).

Antigen preparation. The type-specific antigens employed in this study correspond to the C(2) and E(3) antigens; the group-specific antigens correspond to the A(2) and L(3) antigens.

Adenovirus type 1 and type 2 strains obtained from throat specimens* of children were employed to prepare stock virus suspensions. The materials used to obtain soluble antigens were the fourth (type 1) and third (type 2) passages in primary human embryonic kidney (HEK).† Except as noted below, procedures for the preparation and safety-testing of soluble antigen vaccines were identical to those previously described(1,4). Fractions containing the type-specific and group-specific antigens of adenovirus type 1 were rechromatographed twice, as was the group-specific antigen of adenovirus type 2. The fraction containing the type-specific antigen of the latter serotype was rechromatographed a single time. Protein determinations were done by the Biuret technique(5). The type-specific antigen preparation of adenovirus 1 contained 200 μg of protein per milliliter. The other antigen preparations had less than 100 μg of protein per milliliter. The type-specific antigen vaccine of adenovirus 1 had a complement-fixing titer of 1:512 and the three other vaccine preparations had titers of 1:128, respectively, when tested by a previously described procedure(6). The absence of DNA (less than 0.5 μg per ml) was determined by Burton's modification(7) of the diphenylamine method of Dische.

Isolation of virus. Virus isolation studies of throat and rectal specimens obtained prior to and twice weekly for 5 weeks after immunization were done employing HEK cell cultures using two cultures per specimen. Each culture was examined at weekly intervals for four weeks.

* Received through the courtesy of Dr. A. Vargosko, Children's Hospital, Washington, D.C.

† Pathologic tissue was obtained through the courtesy of Dr. William Jaffurs, Columbia Hospital for Women, Washington, D.C.

TABLE I. Persistence of Neutralizing Antibody in Volunteers Following Immunization with the Type-Specific Antigen of Adenovirus 1.

Volunteer No.	Days after first injection												
	0	14	28	56	91	119	147	175	210	245	280	315	350
1	<2*	128	64	16	8	8	8	8	8	8	8	8	8
2	<2	16	16	8	†								
3	<2	64	64	32									
4	<2	16	8	32	32	32	16	16	16	16			
5	<2	16	8		4								
6	<2	64	16	16	8	16	16	8	8	8	8	8	8
7	<2	4	8	8	8	4	8	8	8	4	4		<4
8	<2	4											
9	<2	4	8		4	4	4	8	8	4	4		
10	<2	16	16	8	8	16	8	8	8	8	8	8	8
11	<2		16										

* Reciprocal of serum dilution.
† Blank spaces indicate serum not available for study.

Neutralization tests. Neutralization tests were done in Rhesus monkey kidney tissue cell cultures according to the modification of the Rowe #2 procedure(6,8). Final reading was made when the simultaneous virus titration showed a virus challenge dose of 32-100 $TCID_{50}$. Dilutions are recorded as initial dilutions of sera.

Immunization schedule. Fifteen volunteers received adenovirus 1 type-specific antigen on day 1, 1.0 ml intramuscularly and 0.1 ml intradermally; 13 of these men received an additional 1.0 ml intramuscularly on the 15th day. Eleven volunteers received adenovirus 1 group-specific antigen, as follows: 8 received 1.0 ml intramuscularly on the first and eighth days; 2 received a single injection of 2.0 ml on the first day; one man received 2 ml initially and 1 ml on the eighth day.

Ten men received adenovirus 2 type-specific antigen, as follows: 6 men received 2 ml intramuscularly on the first day and 1 ml on the eighth day; 4 received 1 ml intramuscularly on the first and eighth days. Six men received adenovirus 2 group-specific antigen, 2 ml intramuscularly on the first day and 1 ml on the eighth day.

Results. There were no local reactions to the intramuscular injections with the soluble antigens of adenovirus types 1 and 2. Six of the men who received intradermal inoculation with adenovirus 1 type-specific antigen developed a small area of erythema and induration at the site of injection, which cleared completely within a few days. Extensive laboratory evaluation including complete blood counts, urinalysis, blood urea nitrogen, glucose, cholesterol, alkaline phosphatase, cephalin flocculation, thymol turbidity, and serum proteins showed no significant abnormality. There were some transient elevations of serum transaminase values which did not correlate with the immunization schedules. Virus isolation studies of throat and rectal specimens were negative.

Eleven of 15 men given the type-specific antigen of adenovirus 1 developed neutralizing antibody. The antibody titers of these 11 men are shown in Table I. Antibody titers usually reached maximum values on day 14, with levels ranging from 1:4 to 1:128; by day 28, the levels were slightly less. Most men maintained neutralizing antibody titers near the 28-day level for as long as sera were available for study. Three of four men followed for 350 days maintained an antibody titer of 1:8; one became antibody-negative (less than 1-4). Volunteer 1, who received only one injection, developed a titer of 1:128 which has persisted at 1:8 for 350 days.

All 11 men who received adenovirus 1 group-specific antigen developed neutralizing antibody (Table II) that persisted for as long as sera were available for testing. The titers after 14 days were between 1:8 and 1:256; after 28 days, between 1:4 and 1:128. Thereafter, titers did not diminish appreciably.

Data from Tables I and II are presented in graphic form in Fig. 1. Since sera were

TABLE II. Persistence of Neutralizing Antibody in Volunteers Following Immunization with the Group-Specific Antigen of Adenovirus 1.

Volunteer No.	Days after first injection									
	0	14	28	49	77	112	147	182	217	252
12	<2*	32	64	32	†					
13	<2	16	16	8	4	4	4	4	4	4
14	<2	≥64	64	32	32	32				
15	<2	>16	16	8	8	8	4			
16	<2	8	8	4	4	4	4	4	4	4
17	<2	16	16	8	16	16				
18	<2	32	32	32	16	8	8	4		
19	<2	16	8	8						
20	<2	256	128	32	16	16				
21	<2	8	4	4						
22	<2	128	32							

* Reciprocal of serum dilution.
† Blank spaces indicate serum not available for study.

not available from all men for the entire observation period, the geometric mean neutralizing antibody titers for each month were calculated with reference to changes in neutralizing antibody titer from the preceding month. It can be seen that the group-specific antigen of adenovirus 1 induced slightly higher mean levels of neutralizing antibody at 14 days, but after 28 days, the mean titers obtained by either antigen vaccine were essentially identical.

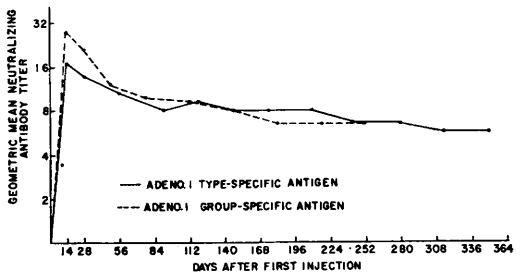

FIG. 1. Persistence of neutralizing antibody in adult volunteers immunized with type-specific and group-specific soluble antigens of adenovirus 1.

All 10 men who received the type-specific antigen of adenovirus 2 developed neutralizing antibody (Table III). The geometric mean titers at 14 and 28 days were somewhat less than that observed with adenovirus 1 soluble antigens, but 5 men followed for 77 days and 4 for 112 days retained a level of antibody similar to their 28-day values. One man whose antibody titer was 1:32 at 14 days did not retain detectable antibody at 28 days. The 6 men (volunteers 27-32) who received 2 ml of antigen initially had a higher mean antibody titer at

TABLE III. Neutralizing Antibody Titers in Volunteers Following Immunization with Adenovirus 2 Soluble Antigens.

Type-specific antigen							Group-specific antigen					
Volunteer No.	Days after first injection						Volunteer No.	Days after first injection				
	0	14	28	77	112	147		0	14	28	67	110
23	<2*	4	4	4	4	4	33	<2	128	64		
24	<2	4	4	4	4	†	34	<2	2	2		
25	<2	8	8	8	8		35	<2	64	64	32	
26	<2	4	8	4			36	<2	512	1024		512
27	<2	32	16	8	8		37	<2	2	2		
28	<2	64	32				38	<2	128	16	16	
29	<2	8	4									
30	<2	32	<2									
31	<2	16	16									
32	<2	4	4									
Geometric mean titer		10.5	6.5	4.9	4.9	—			36	26	16	—

* Reciprocal of serum dilution.
† Blank spaces indicate serum not available for study.

14 days than the 4 (23-26) who received 1 ml. This difference was less apparent at 28 days.

All 6 men who received adenovirus 2 group-specific antigen developed titers of neutralizing antibody, ranging from 1:2 to 1:512 at 14 days, and from 1:2 to 1:1024 at 28 days (Table III). Three men possessed substantial antibody titers at 67 or 110 days.

Discussion. Neutralizing antibody induced in adults by immunization with adenovirus soluble antigens was observed to persist for at least 4 to 12 months. Less adenovirus 1 group-specific antigen than type-specific antigen (protein) was needed to achieve comparable antibody titers in most instances. This indicates greater antigenicity of the group-specific antigen in man as measured by induction of neutralizing antibody. This result is consistent with the finding that the group-specific antigen of type 5 adenovirus produced a higher neutralizing antibody response in rabbits than did the type-specific antigen(9).

It has been postulated that the type-specific and group-specific antigens of adenovirus type 5 possess structurally different type-specific determinants and that neutralizing antibody developed in response to infection would consist of two species of antibody(9). Although the immunogenic significance of these antibodies has not been fully defined, available evidence indicates that neutralizing antibody produced in man in response to vaccination with the type-specific antigen and possibly the group-specific antigen of adenovirus 1 is protective(1). Nevertheless, additional experiments are required to ascertain the quality and duration of immunity induced by each of these antigens.

Summary. Eleven of 15 men given the type-specific antigen of adenovirus 1 and 10 of 10 men given type-specific antigen of adenovirus 2 developed significant levels of homotypic neutralizing antibody. All 17 men who were given the group-specific antigen of either adenovirus 1 or 2 developed antibody. Measurable levels of these antibodies persisted for extended periods of time.

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The Complement-Fixing Antigen of Rubella Virus.* (30748)

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Immunological studies on the rubella virus have been limited by the complexities and variables inherent in neutralization test procedures for this virus and also by the difficulties which have been encountered in demonstrating *in vitro* serologic reactions with the virus. Although the virus produces a cytopathic effect in the RK-13 line of rabbit kidney cells(1) the effect is variable from one passage to another and is usually seen only with low dilutions of the virus. Hence,

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