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Serologic Response in Man to Adenovirus and SV_{40} Components In Adenovirus Vaccines.* (32196)

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This report summarizes the findings in a retrospective study concerned with the serological response in man following vaccination with adenovirus vaccines prepared with adenovirus strains containing SV_{40} genetic material. The SV_{40} component was enclosed within adenovirus capsids; no detectable SV_{40} infectious virus was present in the vaccines or the seed strains. An earlier report from one of our laboratories presented findings, based on studies in hamsters concerning the occurrence of SV_{40} neoplastic and antigenic information in adenovirus type 3, strain JF, one of the virus strains employed in the manufacture of vaccine used in the current study(1).

It was reported earlier that when the vaccines employed in this investigation were given to Naval recruits, there was an approximate 50% reduction in hospitalized acute respiratory disease admission rates as compared with the rates of adenovirus disease in recruits in an unvaccinated control group (2).

Materials and methods. Vaccines used were live oral monovalent adenovirus type 4, an inactivated parenteral monovalent adenovirus type 4 and an inactivated preparation of parenteral trivalent adenovirus types 3, 4, and 7. The live type 4 vaccine was developed and described by Dr. R. M. Chanock (3). It was commercially manufactured from virus seed that had been passed only in human cell cultures. The seed material was free of SV_{40} virion and SV_{40} genetic information and devoid of oncogenicity when examined in appropriate tests(3). The vaccine was lyophilized, placed in enteric coated capsules and kept at —20 for 6 months until used.

The inactivated type 4 vaccine was prepared commercially in the conventional manner(4) in African green monkey kidney cells from a virus strain recovered in 1962 by Dr. E. L. Buescher, and subsequently passed 15 times in African green monkey kidney cells. At this passage level the virus was shown to be free of infectious SV_{40}, both in the laboratory of the manufacturer and in our laboratory. Additionally, it was demonstrated that the virus did not induce SV_{40} "T" antigen in cell cultures or in hamsters and accordingly it was assumed to be free of SV_{40} genetic material. These tests were performed with an aliquot of the virus pool which was removed for examination before the pool was inactivated for use in the manufacture of the monovalent type 4 vaccine. A portion of the same inactivated monovalent pool was used in the manufacture of the type 4 component of the trivalent types 3, 4, and 7 vaccine. The histories of the other 2 adenovirus components in the trivalent vaccine, *i.e.*, type 3, strain JF and type 7, strain LL are recorded(1,5) and are known to have been propagated early in their passage history in cultures of rhesus monkey kidney cells which were contaminated with SV_{40}. Subsequently, both strains were passed in the presence of SV_{40} antiserum and this procedure

* The field aspects of this study were part of a larger investigation of adenovirus vaccines performed by personnel of NAMRU #4, R. O. Peckinpaugh, Officer in Charge; M. Rosenbaum, E. E. Edwards and W. E. Pierce, Chiefs of Virology, Immunology and Biometric Divisions, respectively.

TABLE I. Adenovirus Vaccine Administration Schedule.

Group (No. men in group)	Vaccine: Used	Dose vol (ml)	Units/dose	Route
I (20)	Live monovalent type 4	1.0	$10^{6.5}$ $TCID_{50}$	Oral
II (22)	Killed trivalent type 3	1.0	5 $GPED_{50}$	IM
	4		125 "	
	7		125 "	
III (21)	Killed monovalent type 4	1.0	125 "	IM
IV (21)	Saline placebo	1.0	—	IM

$TCID_{50}$ = 50% tissue culture infective doses. $GPED_{50}$ = 50% guinea pig effective doses. IM = intramuscular.

appeared to have eliminated the contaminating SV_{40} virion. The procedure did not, however, rid the strains of the potential of inducing SV_{40} "T" antigen in cell cultures or tumors in hamsters(1,5). Accordingly, both strains were carriers of SV_{40} antigenic and oncogenic information.

Potency assays of vaccines. The contents of several capsules containing the lyophilized live adenovirus type 4 preparation were rehydrated and titrated for infectivity in human embryonic kidney cell cultures. The 50% tissue culture infectious dose ($TCID_{50}$) based on 21-day readings was $10^{6.5}$/1.0 ml of restored vaccine.

Aliquots of the inactivated vaccines were examined for potency in antigen extinction tests in guinea pigs in the conventional manner employed to measure the potency of inactivated adenovirus vaccines(4). The reciprocal of the 50% effective dose for the monovalent type 4 vaccine was 125 and the reciprocals of the 50% effective dose for the type 3, type 4, and type 7 components in the trivalent vaccines were 5, 125 and 125, respectively. Reciprocals of these values obtained in simultaneous assays with a standard reference trivalent type 3, 4 and 7 vaccine (4) were 125, 125 and 125. These results indicated that the potency of the type 4 and type 7 components in the trivalent vaccine were satisfactory and that of the type 3 component was below the desired level.

Oncogenicity of the inactivated vaccines. Tests were performed by inoculating newborn hamsters with undiluted vaccine following the procedure previously outlined(1). It has been determined by Chanock that the live oral adenovirus type 4 vaccine did not possess oncogenic potential for hamsters(3).

Recruit population and vaccine administration. Recipients of the vaccines were young healthy male recruits undergoing 9 weeks of basic training at the Great Lakes Naval Training Center. After 4 days of initial processing a company of 84 men were randomly assigned to 4 treatment groups and were vaccinated according to the vaccination schedule shown in Table I.

Serologic tests. Blood specimens were obtained from the 84 men before treatment and at weekly intervals for 7 weeks. The sera were examined for SV_{40} "T" antibody in complement-fixation tests employing the "packed-cell antigen" technique described by Huebner *et al*(6). Levels of adenovirus types 3, 4 and 7 antibody were measured in neutralization tests performed in HeLa cells according to standard procedures(4), using 10-100 cytopathic doses of virus. All titers are expressed as the reciprocal of the initial dilution of sera employed.

Results. Lack of antibody response in man to SV_{40} component in vaccine prepared with adenovirus strains containing SV_{40} genetic information. It is well established in man that when live SV_{40} virus is given by the respiratory(7) or intramuscular routes (8,9), an inapparent infection frequently results which is manifested by development of specific neutralizing antibody. The results of serologic tests presented in Table II represent measurements of the SV_{40} neutralizing antibody response in man to vaccines which contained incapsidated SV_{40} genetic information in adenovirus types 3 and 7 but no detectable live SV_{40} virus. These data show that there was no detectable serologic re-

TABLE II. SV_{40} Antibody Findings in Great Lakes Recruits.

Group (No. men in group)	Number of recruits with SV_{40} antibody					
	Before vaccination		28 days after vaccination*		Rises after vaccination	
	Neut	CF†	Neut	CF	Neut	CF
I (20)	1 (titer 1:2)	0	1 (1:2)	0	0	0
II (22)	1 (1:2)	0	1 (1:2)	0	0	0
III (21)	2 (1:2, 1:4)	0	2 (1:2, 1:4)	0	0	0
IV (21)	2 (1:4, 1:4)	0	2 (1:4, 1:4)	0	0	0

Group I recruits received live monovalent adenovirus type 4 vaccine, orally.
Group II " " killed trivalent adenovirus types 3, 4, 7 vaccine, parenterally.
Group III " " killed monovalent adenovirus type 4 vaccine, parenterally.
Group IV " " saline placebo, parenterally.

* Similar results were obtained in tests with sera collected on post-inoculation days 7, 14, 21, 35 and 42.

† In tests with SV_{40} "T" antigens of infected "packed cell" origin(6).

sponse to the encapsidated SV_{40} genome. One of 22 men had a measurable level of SV_{40} neutralizing antibody before vaccination with 1.0 ml of trivalent adenovirus types 3, 4 and 7 vaccine prepared with adenovirus types 3 and 7 strains which contained SV_{40} genetic material (Group II, Table II) and this was the only individual in Group II who possessed this antibody after vaccination. The titer of antibody in both pre- and post-vaccination specimens was identical (1:2). None of the other 21 men in Group II had SV_{40} neutralizing antibody in either their pre- or post-vaccination sera. The Table also shows the expected absence of SV_{40} neutralizing antibody rises in 62 men in control Groups I, II and IV who were injected with either live (Group I) or killed (Group III) monovalent type 4 vaccines or with placebo material (Group IV). Presence of SV_{40} neutralizing antibody in the sera of a few of the recruits in the test and control groups is thought to have resulted from previous vaccination with poliovirus vaccines, some lots of which are known to have been contaminated with live SV_{40} virus (12).

Findings which indicated that none of the men in Group II responded with virus neutralizing antibody to the SV_{40} genetic information contained in the adenovirus type 3 and type 7 strains employed in the manufacture of the trivalent vaccine were substantiated by the results obtained in complement fixation tests. Information contained in Table II shows that none of the 84 men who participated in this study had in either their pre- or post-vaccination sera antibody that fixed complement in the presence of SV_{40} "T" antigen.

Alteration in serum levels of neutralizing antibody against adenovirus types 3, 4 and 7 following vaccination. The changes in serum levels of adenovirus type 4 neutralizing antibody in the weeks following vaccination in each of the 4 vaccination groups are given in Table III. At 14 days post-vaccination 12, 11 and 12 men in Group I, II, and III, respectively, and 3 men in Group IV, who were initially without detectable levels of adenovirus type 4 neutralizing antibody, had developed significant antibody increases. At 42 days, except for 2 men in Group I, all men in each of the 4 groups who were initially antibody-free had experienced a 4-fold or greater rise in the serum level of adenovirus, type 4 antibody. The large number of men (35) in the vaccinated groups and the small number of men (3) in the placebo group with significant antibody rises 14 days post-vaccination are interpreted as due to vaccination, although some of the men in the vaccinated group might have experienced infection with a "wild" strain of

TABLE III. Adenovirus Type 4 Neutralizing Antibody Findings in Great Lakes Recruits.

Days post treatment	Group (No. men in group)	No. of recruits with: No rise in ab titer who were initially With ab	No. of recruits with: No rise in ab titer who were initially Without ab	No. of recruits with: 4-fold rise in ab titer who were initially without ab	Reciprocal of antibody titer: 10	20	40	80	160	320	Reciprocal of GMT†
14	I (20)	4	4	12	8	2	2	—	—	—	14
	II (22)	4	7	11	2	6	2	—	1	—	24
	III (21)	4	5	12	6	3	2	—	1	—	19
	IV (21)	3	15	3	—	3	—	—	—	—	20
21	I	4	2	14	6	4	3	1	—	—	19
	II	4	4	14	5	2	4	1	1	1	30
	III	4	2	15	8	2	2	1	2	—	22
	IV	3	9	9	5	2	1	1	—	—	17
28	I	4	1	15	6	3	5	1	—	—	21
	II	4	3	15	2	5	2	4	1	1	40
	III	4	1	16	3	3	4	3	3	—	40
	IV	3	3	15	4	6	3	1	1	—	24
42	I (20)	4	2	14	2	5	3	2	2	—	34
	II (20)*	2	0	18	1	3	3	5	3	3	71
	III (19)*	2	0	17	1	2	3	5	4	2	74
	IV (19)*	1	0	18	1	5	5	2	4	1	50

Group I = live monovalent adenovirus type 4, orally; Group II = killed trivalent types 3, 4, 7, parenterally; Group III = killed monovalent adenovirus type 4, parenterally; Group IV = placebo, parenterally.

ab = antibody.

* 2 men in each of these groups, not available for bleeding at 42 days.

† Geometric mean titer.

adenovirus type 4, an occurrence which is thought to have resulted in the antibody rises observed in the placebo group. It is assumed that infection with a "wild" adenovirus type 4 strain is a more likely explanation for the antibody rises observed in members of the placebo group than infection with the live adenotype 4 vaccine strain because evidence has been presented that under conditions of military recruit training the vaccine strain had a low potential for spread(3). At present, however, there is no way to differentiate conclusively the "wild" adenovirus type 4 from the vaccine strain.

Alteration in the serum levels of adenovirus types 3 and 7 neutralizing antibodies in each of the 4 treatment groups is presented in Table IV. The results show the expected increases in levels of adenovirus type 7 antibody in men in Group II who were vaccinated with the inactivated trivalent vaccine which contained the SV_{40} genetic material. Thus, the adenovirus capsid antigens elicited a response in the same men who failed to develop SV_{40} antibodies. Fifteen of the 22 men in this group experienced 4-fold or greater increases in serum level of type 7 antibody; this component of the vaccine had been shown in guinea pigs to be of satisfactory potency. The remaining 7 men had 1- and 2-fold rises. Only 11 of the 22 men experienced significant type 3 antibody rises following vaccination, but as previously noted the potency of the type 3 component in the trivalent vaccine had been shown in guinea pigs to be low compared to the NIH standard reference vaccine. The rises in serum levels of adenovirus type 3 and type 7 antibodies that occurred in men in Group II are attributable to use of vaccine. Some of the observed increases, however, could have been due to intercurrent infection with adenovirus types 3 and 7, which were active in the recruit population at the time of this study(2) as evidenced by the rises in levels of type 3 and 7 antibodies recorded in Table IV for members in Group IV. The similarity of responses to adenovirus types 3 and 7 in Groups III and IV suggests that heterotypic response from adenovirus type 4 vaccine was slight or nil.

Behavior of inactivated trivalent vaccine

TABLE IV. Findings in Neutralizing Antibody Tests with Adenovirus Types 3 and 7 and Sera from Great Lakes Recruits.

Group	In group	No. of recruits With significant neut ab rises *vs* Type 3	Type 7
I	20	6 0/8, 4;* 0/16, 2	4 0/8, 1;* 0/16, 3
II	22	11 0/8, 3; 0/16, 2; 0/32, 2; 0/64, 2; 8/64, 1; 16/128, 1	15 0/8, 2; 0/16, 3; 0/32, 1; 0/64, 4; 0/128, 1; 0/256, 1; 8/32, 1; 8/128, 1; 16/64, 1
III	21	8 0/8, 3; 0/16, 4; 0/64, 1	8 0/8, 3; 0/16, 3; 8/32, 2
IV	21	8 0/8, 1; 0/16, 2; 0/32, 4; 8/32, 1	9 0/8, 2; 0/16, 6; 0/32, 1

* Reciprocal of prevaccination titer/Reciprocal of highest postvaccination titer, number men in category.

in newborn hamsters. In tests carried out by Rowe and Baum and by us it was shown that adenovirus type 7, strain LL(10) and type 3 strain, JF(1,13), which were employed as virus seeds for the production of the inactivated trivalent vaccine used in the current work, contained encapsidated SV_{40} material Each of these strains induced tumors which contained complement fixing antigens identical to those induced by SV_{40}. In similar tests in which newborn hamsters were inoculated intrathoracically with 0.1 ml of undiluted trivalent vaccine, none of 185 animals developed tumors during a 2-year observation period. In addition, none of an appreciable number (60-75) of hamsters inoculated with one or the other of the inactivated monovalent type 3, type 4 and type 7 components of the trivalent vaccine developed tumors during an observation period of similar duration.

Discussion. Previous workers have reported the development of subclinical infection in man inoculated nasopharyngeally or intramuscularly with relatively few 50% tissue culture doses of SV_{40} virus in the form of a viable contaminant in a pool of respiratory syncytial virus(7) and in several lots of poliovirus(8,12) and adenovirus(9) vaccines. The subclinical infections were manifested by presence of SV_{40} virus in the throat(7) and gastrointestinal(8) tract and by development of specific neutralizing antibody in the weeks following virus exposure(7,8,9). Because SV_{40} virus induces tumors in hamsters and neoplastic transformation in human cells(11), it is now required as a safety measure that vaccines destined for use in man be free of this simian virus(4). The problem of adenovirus vaccine safety was further complicated with the demonstration that SV_{40} virus genome could become incorporated into adenovirus capsids resulting in a virus particle possessing the oncogenic potential of SV_{40} virus but an exterior surface antigenically identical to adenovirus(5, 14). It thus became of interest and importance to examine retrospectively the behavior in man of these virus "hybrids."

The present work provided the opportunity to study one of the concerns associated with the use of such a vaccine, *i.e.*, the serologic response to the encapsidated SV_{40} component incorporated into vaccine strains of adenovirus which did not contain SV_{40} virion. The observations recorded in this work support the findings in studies with baby hamsters inoculated with similar formalin inactivated vaccines(1). Only the surface adenovirus antigens of the "hybrid" particle stimulated formation of antibodies in both hamsters and man. Further, it appeared that inactivated "hybrid" virus, in hamsters, was devoid of oncogenic activity. These findings are in marked contrast to the results obtained in hamsters inoculated with the living adenovirus type 3, strain JF, or type 7, strain LL, seeds prior to their inactivation for incorporation into the vaccine. The live virus seeds induced, in ham-

sters, tumors which contained SV_{40} antigens. The tumors, in turn, elicited SV_{40} antibody directed against the tumor antigens. It is emphasized, however, that there are no positive criteria at this time for assessing in man the biologic significance of the occurrence of the SV_{40} genome in its sequestered position within adenovirus particles employed in the preparation of inactivated adenovirus vaccines. Nevertheless, the data in this report indicate that by the measurements employed the SV_{40} genome is antigenically inactive in man and oncogenically inactive in hamsters. However, failure to detect antigenicity and oncogenicity does not imply safety of the product.

Summary. Volunteers were vaccinated with an inactivated trivalent adenovirus vaccine prepared with adenovirus strains containing SV_{40} genetic and oncogenic information, enclosed within adenovirus capsids, but no SV_{40} infectious virus. The volunteers showed marked increases in complement fixing and virus neutralizing antibodies directed against the adenovirus components in the vaccine, but did not respond serologically to the SV_{40} information. Suckling hamsters injected with the trivalent vaccine or with its monovalent components did not develop tumors over a prolonged observation period. Thus, the SV_{40} genome contained in the inactivated adenovirus vaccine was inactive antigenically in man and oncogenically in hamsters. The significance of the findings in relation to use in man of killed adenovirus vaccines prepared with adenovirus containing encapsidated SV_{40} information is discussed.

Addendum. Lewis *et al* (Proc. Nat. Acad. Sci., 1967, v57, 622) reported recently the detection by indirect immunofluorescent methods adenovirus "T" antibody in the sera of 16 of 18 children following infection with adenovirus types 1, 3 or 7. Dr. Lewis kindly examined for us by indirect immunofluorescent techniques for presence of SV_{40} "T" antibody post-vaccination sera obtained from 6 volunteers who received killed trivalent adenovirus types 3, 4, and 7 vaccine. All 6 sera were negative for presence of this antibody.

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