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Adenovirus Vaccine Evaluation Study in Naval Recruits.*† (24114)

CLAYTON G. LOOSLI, VAN C. TIPTON, O. WARNER, MABEL SMITH, PAUL B. JOHNSTON AND DOROTHY HAMRE

Section on Preventive Medicine, University of Chicago School of Medicine, and Naval Preventive Medicine Unit #5, San Diego Naval Training Center, Calif.

Acute respiratory disease in military inductees continues to be a major problem (1,2,3). During the past 4 years adenoviruses, particularly types 3, 4 and 7, caused 20 to 70% of acute respiratory tract infections among military recruits(3,4,5,6,7,8). Several studies employing polyvalent adenovirus vaccines for prevention of respiratory disease have been reported(9,10,11,12,13). In their first study, Hilleman and associates evaluated a formalin-inactivated monkey kidney culture vaccine composed of types 4 and 7 adenoviruses, prepared at Army Medical Center. A subsequent study was made by them of vaccine prepared commercially(13).‡ Bell and associates used commercially prepared§ kidney cell culture vaccine containing adenoviruses types 3, 4 and 7(12). All studies have shown these vaccines highly effective in preventing infections due to types 4 and 7 adenoviruses, the only prevalent types isolated from recruit populations.

The present report gives results of an adenovirus vaccine evaluation study among inductees at Naval Training Center at San Diego, Calif., where both types 4 and 7 adenovirus infections were prevalent in the population, from March 11 to July 3, 1957.

Materials and methods. Vaccine was prepared by Eli Lilly and Co. Seed adenoviruses, types 3, 4 and 7, obtained from Dr. Robert J. Huebner at N.I.H., where they had been isolated directly from humans in monkey kidney cell cultures. Pools of each virus type were grown in 16 ounce bottles of trypsinized monkey kidney cells. After filtration each pool was inactivated with formaldehyde (1:4000) at

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‡ Lederle Laboratories.

§ Parke, Davis and Co.

37°C for 7 days. These pools were then neutralized by addition of sodium bisulfite. The trivalent vaccine was prepared by mixing equal parts of 3 monovalent types after which merthiolate 1:20.000 was added as preservative. Before mixing and inactivation each pool of virus was titered and tested with specific antiserum for purity and identification. Control procedures on the pools and trivalent vaccine were those employed for safety testing of poliomyelitis vaccine as recommended by U. S. Public Health Service. *Placebo*: Tissue culture maintenance medium to which formaldehyde (1:4000) and preservative had been added was employed. One ml of vaccine or placebo material was given intramuscularly to test and control groups. Population study comprised personnel of 39 recruit training companies activated at Training Center from Mar. 11 to May 3. Vaccine and placebo were administered within a day or two after the company was formed. Half the men in a company were given adenovirus vaccine while the other half received placebo material. Selection of vaccine and controls was determined by final digit of recruit's service number. Individuals with even numbers were given the vaccine, those with odd numbers received control material. The vaccinated group totaled 1,203 and the control group 1,249. Of these, 132 in the test and 151 in the control group were discharged some time subsequent to one week and prior to completion of ninth and final week of training. *Criteria for evaluation of vaccine*: Observations were made on study companies until the last had completed training July 6, 7 and 8. Effectiveness of vaccine was based on Dispensary admissions for adenovirus infections (based on laboratory data) from vaccinated and control groups. On each admission temperature, day and month and weeks of training were recorded along with individual's name and unit number on punch cards. *Laboratory procedures*: Throat swabs and washings with nutrient broth and acute and convalescent sera were collected on essentially all admissions up to seventh week of training. Some throat washings were collected on individuals during 8th and 9th weeks. Throat swabs with washings were frozen within an hour. Bloods were brought to Unit laboratory where serums were separated and frozen. Throat washings and paired serums were shipped at bi-weekly intervals under dry ice *via* air express to University of Chicago where they were analyzed for presence of adenoviruses and, in certain specimens, for influenza viruses, and complement fixing antibodies with adenovirus and influenza virus antigens. Hemagglutinin inhibiting (H.I.) antibodies were also determined on all paired sera employing A'/Denver/57, A/Asian/57 and B/Great Lakes/54 influenza antigens. For adenovirus isolation test tube cultures of HeLa cells were employed. Details of methods used have been published(14). Fertile egg technic was employed for isolation of influenza viruses(15). Complement fixation test for determining adenovirus and influenza antibodies was that described by Jensen(15). Antigen for complement fixation test for adenovirus was prepared by pooling cultures of adenoviruses, types 1 through 7. The hemagglutination inhibition antibody test was that recommended by Committee on Standard Serological Procedures of Armed Forces Epidemiological Board(16). For influenza antibody determinations allantoic fluid harvest, undiluted and uninactivated, of Jap/305/57, A'/Denver/57 and B/Great Lakes/57 were employed as antigens. Rapid neutralization test and colorimetric assay method for determination of adenovirus antibodies were used. These procedures have been described(17, 18, 19).

Results. Detailed differential clinical study of cases admitted to Dispensary was not made. Cases were admitted generally because they had symptoms of acute respiratory disease and were febrile with temperatures of 100°F or greater. Diagnoses of adenovirus and influenza infections were made on basis of virus isolation and complement fixing or H. I. antibody responses.

Adenovirus infections in non-study population. Approximately 50 throat washings and paired bloods were collected weekly. In the beginning many were from individuals admitted to Dispensary from companies which were on board before the vaccine study began. From week ending Mar. 10 to May 5 the

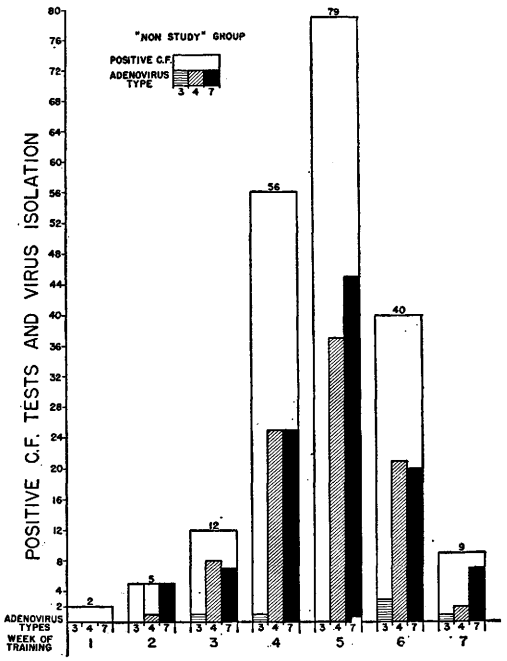


FIG. 1. Adenovirus isolation and positive complement fixation tests in relation to week of training of individuals in "non-study" companies.

weekly adenovirus rate/1,000 from this "non-study" group was 15.8 and interpolated weekly admission rate/thousand due to adenovirus was 12.6. Thus, 80% of admissions during this period were due to adenovirus infections. Fig. 1 shows adenovirus isolations from 265 throat washings and complement fixing antibody responses in 244 sets of serums from individuals from "non-study" companies recorded in relation to week of training. Two hundred and three (81%) of the paired serums showed 4-fold or greater rises with adenovirus pooled antigen. Two hundred and five (79%) of 265 throat washings yielded adenoviruses, either types 3 (6), 4 (94) or 7 (105). The great majority with adenovirus infections were admitted during fourth, fifth and sixth weeks of training. Only a few were admitted during second, third and seventh weeks. Throat washings and sera were not collected during 8th and final 9th week of training.

Antigenicity studies. Recruits of 2 companies at beginning and 3 at end of study were bled before and at 14 days after vaccination to determine antibody response to the vaccine. Paired serums selected at random from each company were tested for complement fixing and neutralizing antibody response with types 3, 4 and 7 adenovirus antigens. The results are shown in Table I. Adenovirus infections in the placebo group were low, as indicated by low complement fixing and neutralizing antibody response to the 3 antigens. Eight of 81 individuals in the vaccine group showed 4-fold or greater rise in complement fixing antibodies. There was good neutralizing antibody response to types 3 and 7 adenovirus antigens. However, only 26 or 31% of 85 individuals showed significant antibody response to type 4 antigen. This was of interest for the vaccine was not as effective against type 4 adenovirus infections as against type 7.

Adenovirus isolation. As seen in Table II, during the first 2 weeks post vaccination, throat washings were taken on 95% of admissions from the vaccine group and 82% from control group. Only 2 adenoviruses, one type 4 and one type 7, were obtained from vaccinated individuals. None was obtained from

TABLE I. Adenovirus Antibody Response following Vaccination with a Polyvalent Adenovirus Vaccine Containing Types 3, 4 and 7.*

	Vaccine group				Control group			
		Neutralization test with adenovirus types				Neutralization test with adenovirus types		
Co.	C.F. test	3	4	7	C.F. test	3	4	7
125	0/16	13/16	4/15	11/16	1/20	1/20	1/20	1/20
130	3/22	20/27	10/27	22/27	0/21	0/24	1/24	0/24
156	1/12	8/11	5/12	7/12	1/ 7	1/ 5	1/ 7	0/ 7
157	2/14	7/14	3/14	6/12	0/11	2/11	2/11	1/ 9
158	2/17	12/17	4/17	10/17	0/ 9	0/ 9	0/ 8	0/ 8
Total	8/81	60/85	26/85	56/84	2/68	4/69	5/70	2/68
	10%	71%	31%	67%	3%	6%	7%	3%

* No. showing 4-fold or greater antibody rise/No. tested.

TABLE II. Adenovirus Isolation and Complement Fixation Antibody Response in Vaccine and Control Groups.

	2 wk post-vaccination		3 thru 9 wk post-vaccination	
	Vaccine	Control	Vaccine	Control
Avg weekly population	1197	1242	1168	1210
Admissions, total	38	27	184	221
Throat washings	36	22	122	168
% admissions	95	82	66	76
Adenovirus isolation	2	0	16	69
% isolation	5.2	0	13.1	41.0
Adenovirus types				
3	0	0	1	5
4	1	0	13	36
7	1	0	2	28
Paired sera	36	22	103	139
% admissions	95	82	56	63
C. F. positive	10	6	15	69
% positive	27	27	14.6	47.5

unvaccinated individuals. During 3 through 9 week post-vaccination period throat washings were obtained on 66% of admissions from the vaccine group and 76% from control group. Only 13.1% of throat washings from the vaccinated group yielded adenovirus viruses, while adenoviruses were obtained from 41% of throat washings from unvaccinated individuals. These data and the adenovirus types isolated are also shown in Table II. Of 16 adenoviruses isolated from vaccinated individuals, one was type 3, 13 were type 4, and 2 were type 7. The 69 adenoviruses from the control group comprised 5 type 3, 36 type 4 and 28 type 7.

Adenovirus antibody response to infection. Complement fixing adenovirus antibody rises in admitted cases from vaccinated and control groups are shown in Table II. During the first 2 weeks of training, 27% of paired serums from both groups showed 4-fold or greater complement fixing antibody rises to the adenovirus antigen. Fourteen % of paired serums from vaccinated individuals admitted during 3 to 9 week period showed significant rises compared to 47% from unvaccinated individuals. Distribution of virus isolation and complement fixation antibody rises in individuals according to week of post vaccination, is shown in Fig. 2. In the vaccine group the greatest number of infections occurred during 5th, 6th and 7th week post vaccination, all due to type 4 adenovirus except for one type 3 and one type 7. In the control group the greatest number of infections also occurred during these weeks while a moderate number also occurred during third and eighth week of training.

Dispensary admissions from vaccine and control groups due to adenoviruses. During the first 2 weeks post-vaccination the weekly admission rate/1,000 was 15.8 from the vaccine group and 10.8 from control group (Table III). During the 3 through 9 post-vaccination period, weekly admission rate was 22.3 from the vaccine group and 26.2 from control group. This represents a difference of 15% in favor of the vaccine group. When admissions due to adenoviruses were determined

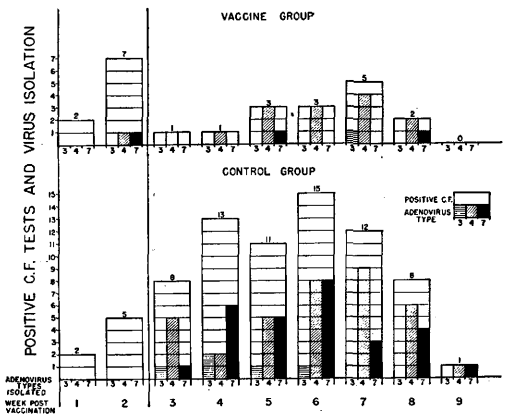
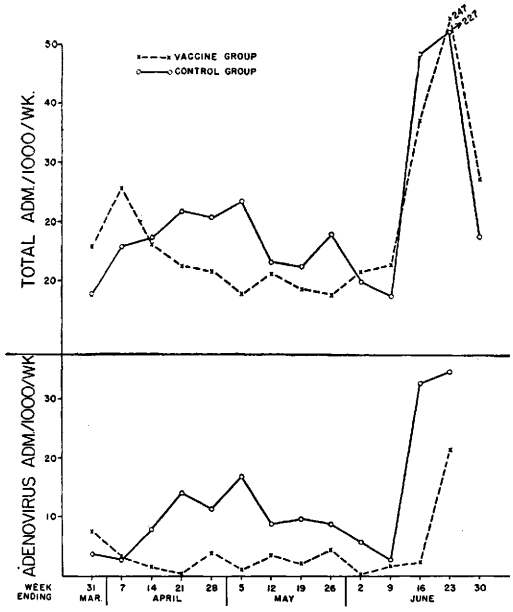


FIG. 2. Adenovirus isolation and complement fixing antibody response in vaccinated and unvaccinated individuals in relation to week post-vaccination.

TABLE III. Total Admissions and Admissions Due to Adenovirus Infections from Vaccine and Control Groups.

	2 wk post-vaccination		3 thru 9 wk post-vaccination		%
	Vaccine	Control	Vaccine	Control	difference
Avg weekly population	1197	1242	1168	1210	
Admissions, total	38	27	184	221	
Admission rate/1000/wk					
Total	15.8	10.8	22.3	26.2	15
By adenovirus isolation	1.0	0.0	3.4	10	66
By C. F. antibody rises	4.8	2.8	3.4	12	72

from laboratory data, including virus isolation and complement fixation antibody response, admission rates were significantly lower in the vaccine group. On the basis of adenovirus isolations, during 3 to 9 week post-vaccination period, weekly admission rate was 3.4 from the vaccinated group compared to 10 from control group. This represents a difference of 66%. When 4-fold or greater complement fixation antibody responses to adenovirus antigen were employed as evidence of infection the weekly admission rate was 3.4 from the vaccinated group, and 12 from the control group, a difference of 72% in favor of the vaccine. Weekly admission rates from vaccine and control groups are also shown in Fig. 3. The weekly adenovirus admission rate after April 7, 4 weeks after vaccination began, was consistently lower from the vaccinated group. The sharp rise in total admissions from both vaccinated and control groups during June represents Asian influenza infections as shown below. There was also a corresponding rise in adenovirus admission rates in both vaccine and control groups during this time. Weekly admission rates according to week post-vaccination are shown in Fig. 4. After the second week, admissions for adenovirus infections from the vaccinated group were low and remained so except for 7th and



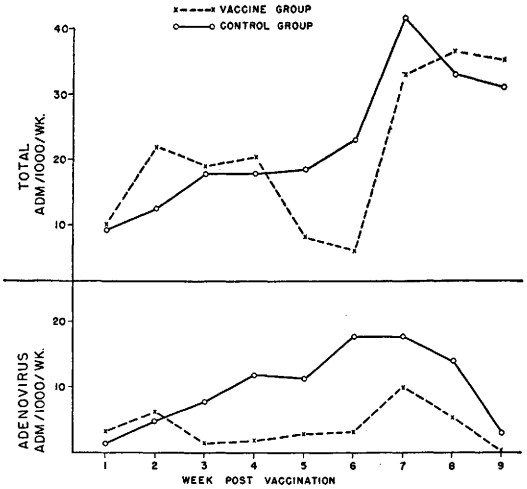


FIG. 3 (left). Total and adenovirus infection admission rates from vaccine and control groups.

FIG. 4 (right). Total and adenovirus infection weekly admission rates in relation to week post-vaccination.

8th week of training. Increase in total admissions during 7th. thru 9th week represent admissions due to Asian influenza.

Influenza infections. During the latter part of the investigation an outbreak of influenza occurred at the Training Center. To identfy the increased admission rate as influenza, complement fixing antibody determinations were carried out on paired sera from vaccine and control groups using A/Jap/305/57 and B/GL/54 as antigens. Paired serums having 4-fold or greater rises were then tested for H. I. antibodies with A'/Denver/57, A/Jap/305/57 and B/GL/57 antigens. Thirty-two % of sera from the vaccinated group and 27% from control group showed significant complement fixing antibody rises with A and B antigens. With the H.I. procedure the majority of influenza infections were found to be due to Asian influenza viruses.

Discussion. This study confirms those of others that adenovirus infections are a major cause of acute respiratory disease in military inductees. Eighty % of admissions from "non-study" companies admitted to the Dispensary from Mar. 5 to May 5 were due to either type 3, 4 or 7 adenoviruses. Forty % of control population of study companies March 31 to July 3 were also admitted because of adenovirus infection due to one of the above 3 types. During this same period only 15% of admissions from vaccinated group were due to adenovirus infections. As periods of observation were not the same, it cannot be assumed that the 40% admission rate in the 50% vaccinated companies compared to an 80% admission rate from the "non-study" companies was due to "herd immunity" effect.

The vaccine was effective against all 3 types as indicated by differences in numbers of 3, 4 and 7 adenoviruses isolated from vaccine and control groups. Of 16 adenoviruses isolated after second week from vaccinated individuals 13 were type 4. This was of interest for antibody response studies showed that the vaccine elicited significant rises to type 4 antigen in only one-third of the individuals. Thus, the isolation of type 4 viruses from vaccinated individuals may reflect a failure on the part of the vaccine to produce a significant protective antibody response.

The overall reduction in admission rates for adenovirus infection was 66% when virus isolations were used as a criterion and 72% when complement fixing antibody response was employed. These results compare favorably with previous studies reported by Hilleman and associates(13) and Bell and colleagues(12). In a controlled field study at Fort Dix, N. J., Hilleman obtained 98% reduction in acute respiratory disease due to adenoviruses(9,11) by use of type 4 or 7 adenovirus vaccine. In other studies(8,13) at Fort Leonard Wood, Mo., during 1957 employing bivalent types 4 and 7 adenovirus vaccine, adenovirus infection was reduced by 90%. At Great Lakes Bell, Hantover, and colleagues found polyvalent types 3, 4 and 7 adenovirus vaccine reduced total febrile respiratory disease incidence by 55%, and 65% of such illnesses requiring hospitalization during third to ninth week of training(12).

In addition to adenovirus infections, influenza infections occurred in study population with equal frequency in test and control groups.

Conclusions. 1) An adenovirus vaccine evaluation study was carried out at San Diego Naval Training Center. Recruit population was studied. Kidney cell tissue culture vaccine containing adenovirus types 3, 4 and 7 was used, inactivated with formalin. Half of the men in 39 companies were given vaccine while the other half were given placebo. Observations were made from March 11 to July 3, 1957. Adenovirus isolations and complement fixing antibody response in acute and convalescent serums were employed as indices of infection. Types 4 and 7 adenovirus infections were prevalent in the control population. 2) During 3 through 9 week post-vaccination period, weekly admission rate/1,000 was 22.3 from the vaccine group, and 26.2 from the control group, a 15% difference. However, on the basis of adenovirus isolation the weekly admission rate was 3.4 from the vaccinated compared to 10 from the control, a difference of 66%. When 4-fold or greater complement fixing antibody rises to adenovirus antigen were employed as evidence of infection, the weekly admission rate was 3.4

from the vaccinated group and 12 from the control group, a difference of 72% in favor of the vaccine. On the basis of above findings, adenovirus vaccine significantly reduced number of adenovirus infections in the vaccinated group compared to the control group. 3) In non-study companies from Mar. 5 to May 5 adenovirus infections accounted for 80% of Dispensary admissions. Forty % of admissions from the control group of the study companies were due to either types 3, 4 or 7 adenovirus while only 15% from the vaccine group were due to these agents.

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Epidemic Asian A Influenza in Naval Recruits.*† (24115)

CLAYTON G. LOOSLI, DOROTHY HAMRE AND O. WARNER
Section of Preventive Medicine, University of Chicago School of Medicine, and Naval Preventive Medicine Unit #5, San Diego Naval Training Center, Calif.

Asian influenza was first recognized in the United States in mid-June, 1957(1). One of the first outbreaks occurred among recruits at Naval Training Center at San Diego, Calif. (2). At time of outbreak a study of evaluation of an adenovirus vaccine was being made (3). During the week ending June 15 a sharp rise in admission rates for acute respiratory disease occurred in both non-study and study companies. From the study companies the admissions came from both adenovirus vaccinated and placebo vaccinated groups. The illnesses resembled epidemic influenza. As it had been pointed out by Hilleman(4,5) that this age group appeared to be non-immune to the new Asian strain of influenza, it seemed

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