AN ANALYSIS OF HOSPITALIZATIONS FOR ACUTE RESPIRATORY DISEASE IN RECRUITS IMMUNIZED WITH ADENOVIRUS TYPE 4 AND TYPE 7 VACCINES¹

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(Received for publication August 19, 1971)

Dudding, B. A., F. H. Top, Jr., R. M. Scott, P. K. Russell and E. L. Buescher (Walter Reed Army Institute of Research, Washington, D. C. 20012). An analysis of hospitalizations for acute respiratory disease in recruits immunized with adenovirus type 4 and type 7 vaccines. *Am J Epidemiol* 95: 140-147, 1972.—Among 911 recruits immunized with live, oral adenovirus types 4 and 7 (ADV-4 and 7) vaccines, there were 149 hospital admissions to the acute respiratory disease (ARD) wards during the study. Eighteen admissions were not associated with ARD (15 rubella infections, 3 immunization reactions). Of the remaining 131 ARD admissions, 17 were complicated by pneumonia and 114 were judged to have uncomplicated ARD. Within the latter group, evidence for infection by a single respiratory pathogen was obtained from 68 admissions; 11 admissions were associated with multiple infectious agents; and 35 admissions yielded no infectious agent(s). Despite immunization, adenoviruses, primarily ADV-4, were still the most common cause of ARD. Among non-adenovirus associated ARD admissions, rhinoviruses were the most common (10), followed by influenza A₂ virus (7), group A streptococci (2) and herpesvirus hominis (2). Thus, in this initial study of ARD among recruits immunized with ADV-4 and ADV-7 vaccines, no other respiratory pathogens emerged to replace adenovirus types 4 and 7 as the major causes of ARD in military trainees.

adenovirus; recruits; respiratory diseases; vaccines

INTRODUCTION

The protective efficacy of live, oral adenovirus type 7 (ADV-7) vaccine when given

Abbreviations: ADV, adenovirus; ARD, acute respiratory disease; BCT, basic combat training; CF, complement-fixation; HI, hemagglutination-inhibition; N, neutralizing; TCID, tissue culture infectious dose.

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This study had the concurrence of The Surgeon General of the Army, and the Army Investigational Drug Review Board, the Vaccine Development Branch, National Institutes of Allergy and Infectious Diseases. It was made with the collabora-

tion of the Surgeon and Training Command, Fort Dix, N. J.

The authors are grateful to the following for their support and help in this study: LTC Joseph J. Smith, MC; MAJ William H. Bancroft, MC; MAJ Herbert E. Segal, MC; MAJ James Ferguson, VC; SP-6 William L. O'Brien; SSG Bennie W. Roach; and SP-4 Salvatore Romano. The authors thank Dr. Edwin Lennette of the California Dept. of Public Health for determining the rhinovirus immunotypes, and Dr. Lewis Wannamaker and Dr. Steven Chapman of the Univ. of Minnesota Medical School for identification of beta-hemolytic streptococcal strains.

(1). Earlier it had been shown that ADV-7 disease emerged following suppression of ADV-4 disease by ADV-4 vaccine in similar groups of trainees (2). Whether suppression of adenovirus-associated ARD in a population immunized with both ADV-4 and ADV-7 vaccines would foster the emergence of other respiratory pathogens is an unknown and important question. Thus, a second objective of the same study was to examine the role played by agents other than ADV-4 and ADV-7 in hospitalized trainees immunized with both vaccines.

MATERIALS AND METHODS

Study design. Five trainee cohorts (5795 men) entered the 3rd Basic Combat Training (BCT) Brigade at Fort Dix, N. J., during the study period January through April, 1970. All trainees were immunized with ADV-4 and ADV-7 vaccines in the reception center within 72 hours after arrival on post. Each cohort was formed into six training companies. One company from each cohort was studied intensively for etiology of all hospitalizations for acute respiratory disease (ARD) occurring in members of that company throughout the eight-week training period.

Vaccines. Specifications for similar ADV-4 and ADV-7 vaccines have been reported elsewhere (3). Tablets of the ADV-4 vaccine contained between $10^{3.6}$ and $10^{4.7}$ tissue culture median infectious doses (TCID₅₀) and the ADV-7 vaccine contained between $10^{4.6}$ and $10^{4.7}$ TCID₅₀ per tablet.

Sampling. All ARD hospitalizations in five study companies were examined for respiratory disease by one of the investigators within 12 hours of admission. After a brief history and physical examination, the following specimens were obtained from each man: a throat washing for viral isolation, a throat swab for isolation of beta-hemolytic streptococci, a nasal swab for virus isolation and an acute blood sample. Convalescent blood samples were obtained

two weeks later from all individuals hospitalized before the middle of the seventh week of training.

Virology. All throat wash specimens were tested in human embryonic kidney and primary rhesus monkey kidney tissue culture monolayers. Specimens from individuals suspected of having rubella were also inoculated into African green monkey kidney tissue culture monolayers, and rubella viruses were detected by interference with an ECHO-11 virus challenge technique². Viruses isolated from these systems were identified by standard methods employing neutralization or hemagglutination-inhibition techniques. Nasal swab specimens were examined in WI-38 tissue culture monolayers, incubated on roller drums at 33 C. Viruses isolated from this system were tested for chloroform and acid sensitivity by standard methods. Those strains found to be chloroform-resistant but acid-sensitive (suspect rhinovirus strains) were identified by neutralization tests in an interlocking grid system that utilized rhinovirus immunotyping sera against rhinovirus immunotypes 1-89 and a prime strain of rhinovirus 22.

Serology. Complement-fixation (CF) tests for adenovirus, *Mycoplasma pneumoniae* and influenza A and B antibodies were performed on all acute and convalescent sera by standard techniques. Because recruits were immunized with polyvalent influenza vaccines, a soluble CF antigen of influenza A₂/HK/1/68 was used in these tests to determine influenza A infection. ADV-4 and ADV-7 neutralizing (N) antibody tests on acute and convalescent sera were performed by standard methods. Rubella hemagglutination-inhibition (HI) antibody titers were determined on acute and convalescent sera from all cases of clinical

²Standard methods refer specifically to procedures recommended in: Lennette EH, Schmidt NJ (eds): *Diagnostic Procedures for Viral and Rickettsial Infections*, 4th ed. New York, Am. Pub. Health Assoc., Inc., 1969.

cal rubella and from all other cases in which no respiratory viral or bacterial pathogens were isolated.

Bacteriology. Throat swabs were plated and streaked on 5 per cent sheep blood agar plates which were incubated for 18–24 hours at 37 C. All plates were examined for beta-hemolytic colonies with streptococcal morphology and suspicious colonies were subcultured. All suspect strains were grown in pure culture, tested for bacitracin disk sensitivity and identified by group reactivity, T-protein agglutination pattern and M-protein serotype.

RESULTS

There were 911 trainees in the five intensively studied training companies; prior to commencement of eight weeks of training, all were immunized with both ADV-4 and ADV-7 live, oral vaccines. Hospital admissions in this group, as shown in table 1, were predominately due to respiratory infections.

Non-respiratory admissions. Admission criteria for hospitalization of military trainees for acute respiratory disease vary only slightly among training posts, with temperature of 100 F or greater being the primary indication for admission. Despite this, individuals were admitted to the ARD

ward with rubella, who were only mildly febrile (98.6–99.8 F). Clinical rubella was seen in 14 of these individuals. The diagnosis was confirmed by isolation and antibody rise in nine, isolation alone in one (no convalescent serum was available) and four-fold or greater HI antibody response alone in four. One individual without overt rubella, admitted with fever, had a four-fold HI antibody response and, in the absence of other respiratory pathogens, was considered to have been admitted because of rubella.

Three individuals were admitted to the ARD ward within six hours following an initial typhoid immunization, all with fever and complaints of "sore arm." No evidence of infection by respiratory pathogens was found in these men.

Uncomplicated acute respiratory disease admissions. Table 2 shows the results of the virologic, bacteriologic, and serologic evaluations of the uncomplicated ARD hospital admissions. The table summarizes three broad admission categories: first, those associated with recovery of and/or serologic response to a single respiratory pathogen; second, those admissions associated with recovery of two or more agents and/or serologic responses; and finally those admissions which could not be associated with any identifiable agent, either by isolation or serologic tests.

ARD admissions associated with single pathogens. Evidence for infection by a single agent was obtained in 68 of 131 ARD hospital admissions. Forty-seven admissions were associated with adenovirus infection: 8 with type 7, 35 with type 4, and 4 with type undetermined. Criteria for adenovirus infection included: virus isolation accompanied by a homotypic N antibody response; N antibody response alone after the third week of training; isolation alone; and finally for the four individuals with undetermined type infection, neutralizing antibody responses to both type 4 and 7 after the third week of training. Neutralizing antibody responses to either ADV-4 or

TABLE 1
*Hospital admissions, 3rd BCT brigade
study companies*

Hospital admissions	No.	Rate/100/8 weeks
Non-respiratory admissions		
Rubella	15	
Suspect typhoid		
Immunization reactions	3	
Total non-respiratory	18	2.0
Respiratory admissions		
Uncomplicated ARD	114	
ARD complicated by pneumonia	17	
Total respiratory	131	14.4
Total hospital admissions	149	16.4

TABLE 2
Uncomplicated ARD admissions

	No. of admissions								Total
	Week of training								
	1	2	3	4	5	6	7	8	
Admissions associated with single agents*									
A. ADV-7	0	0	1	1	1	4	0	1	8
B. ADV-4	0	2	1	9	8	9	6	0	35
C. ADV, type undetermined	0	0	0	1	2	1	0	0	4
D. Influenza A ₁	5	1	0	0	0	1	0	0	7
E. Rhinoviruses	1	2	4	1	2	0	0	0	10
F. Group A streptococci	0	0	0	2	0	0	0	0	2
G. Herpesvirus hominis	0	1	0	1	0	0	0	0	2
H. <i>Mycoplasma pneumoniae</i>	0	0	0	0	0	0	0	0	0
Admissions associated with multiple agents	1	2	2	3	1	0	2	0	11
Total admissions associated with infectious agents	7	8	8	18	14	15	8	1	79
Total admissions not associated with infectious agents	5	9	7	4	3	3	4	0	35

* Infection criteria: A, B—isolate plus neutralizing (N) antibody rise or N antibody rise alone after 3rd week; C—N antibody rise after 3rd week to ADV-4 and ADV-7; D—isolate and/or CF antibody; E, F, G—isolate; H—CF antibody rise.

ADV-7 during the first three weeks of training could be the expected consequence of immunization and thus could not be considered as evidence for naturally occurring adenovirus infections.

Seven individuals had evidence of influenza A₂ infections and, as expected, the majority (six) occurred in the first two weeks of training. Strains identical to influenza A₂ Hong Kong/68 virus were isolated from three of the seven cases, and six of seven showed fourfold or greater increase in influenza A₂ CF antibody titers. Recruits were immunized with a bivalent influenza vaccine during the first or second week of training.

Rhinoviruses were the only agents isolated from 10 different individuals and of those isolates, seven were typable with available rhinovirus antisera. The following types were obtained: types 2, 26, 34, 51, and 53. No single type was found in more than

two individuals. The three unidentified types were tested against 89 prototype rhinoviruses, types 1–89 and a prime rhinovirus type 22 strain. Like influenza virus, these agents were associated with illnesses that occurred early in training.

Isolation of a group A beta-hemolytic streptococcus as the sole respiratory pathogen accounted for two admissions from the same training company, during the same week. Neither strain was M-typable but both were bacitracin-sensitive and had a 12/13 T-agglutination pattern.

Two individuals proved to have herpes simplex virus as the sole respiratory pathogen. There were no influenza B virus or mycoplasma CF antibody responses.

Thus, the majority of uncomplicated ARD admissions were associated with evidence for infection by a single respiratory pathogen. Despite immunization with both ADV-4 and ADV-7 vaccines, adenovirus in-

TABLE 3
Multiple infections in hospitalized recruits

Week of training	Admissions	Pathogens				
		ADV*	Infl. A ₂ †	RV‡	Mycopl.§	Strep. A‡
1	Admission 1		pos.		pos.	
2	Admission 1		pos.¶			pos. (T-28, M-neg.)
	Admission 2			pos. (type 34)		pos. (T-11, M-neg.)
3	Admission 1	pos. (type 7)	pos.	pos. (type 53)		
	Admission 2	pos. (type 4)		pos. (type unidentified)		
4	Admission 1	pos. (type 4)	pos.			
	Admission 2	pos. (type 4)	pos.			
	Admission 3	pos. (type 4)				pos. (T-12, M-12)
5	Admission 1	pos. (type 4)		pos. (type 43)		
7	Admission 1	pos. (type 4)		pos. (type 53)		
	Admission 2	pos. (type 4)	pos.			

* Adenovirus infections established by isolation and a 4-fold rise in homotypic neutralizing antibody; italics indicated neutralizing antibody rise only.

† Influenza A₂ infections established by 4-fold rise in CF antibody titer.

‡ Rhinovirus and group A streptococcal infections established by isolation alone.

§ Mycoplasma infections established by 4-fold rise in CF antibody titer.

¶ Determined by isolation and 4-fold rise in CF antibody titer.

fections (primarily ADV-4) were associated with over twice as many hospitalizations (47) as nonadenovirus agents (21).

ARD admissions associated with multiple pathogens. Eleven of 114 uncomplicated ARD admissions (9.8 per cent) showed evidence of infection by two or more respiratory pathogens (table 3). Combinations of infectious agents followed no particular pattern and these admissions were distributed throughout the training cycle. Ten of 11 admissions associated with more than one respiratory pathogen involved two agents; one admission was associated with evidence of simultaneous infections by three pathogens (ADV-7, rhinovirus type 53 and influenza A₂). Although influenza A₂ was recovered from the throat of one individual,

evidence for infections in other individuals with influenza A₂ rested solely on a four-fold or greater rise in CF antibody.

ARD admissions not associated with infectious agents. During the course of the study, 35 admissions to ARD wards could not be associated with any respiratory disease agents, accounting for 23.5 per cent of all admissions to ARD wards and 30.7 per cent of those judged to be bonafide respiratory disease admissions. Twenty-one of the 35 admissions (60 per cent) not associated with pathogens occurred during the first three weeks of training during a time when adenovirus-associated ARD was distinctly uncommon. Likewise, 14 of 21 admissions (67 per cent) associated with nonadenovirus agents occurred in the first three weeks of

training. The timing of admissions which could not be associated with known agents of respiratory disease was similar to that of nonadenovirus admissions; both types of admissions occurred earlier in the training cycle than adenovirus-associated ARD admissions.

ARD admissions complicated by pneumonia. Seventeen individuals who were admitted to ARD wards had radiologic evidence of pneumonia. Pneumonia patients represented 11.4 per cent (17/131) of all ARD admissions and 14.9 per cent of those actually judged to have respiratory disease. Table 4 summarizes the virologic, serologic and bacteriologic data obtained from these individuals upon admission. ADV-4 was isolated from one individual and in two instances only an ADV-4 neutralizing antibody response could be documented. One individual was infected with influenza A₂ and this admission occurred during the first week of training. A rhinovirus (type unidentifiable) and a group A streptococcus were isolated from two other individuals. No agents were isolated from 10 cases. None of the 17 admissions were associated with a four-fold or greater rise in *M. pneumoniae* CF antibody.

Table 5 summarizes the total number and percentage of total (for each of the various categories already described) of all ARD hospital admissions in 3rd brigade study companies. In only 23.5 per cent of all ARD admissions was a reason for and/or possible cause for hospital admission not apparent.

DISCUSSION

Earlier epidemiologic studies showed that adenoviruses (types 4, 7 and to a lesser extent type 21) are the major causes of acute febrile respiratory disease requiring hospitalization of military trainees in both the United States and in The Netherlands (2, 4). Many other viral and bacterial pathogens (influenza A and B viruses, parainfluenza viruses, rhinoviruses, coxsackie A-21 virus, group A streptococci and *M. pneumoniae*) have caused sporadic ARD and some-

TABLE 4
*ARD admissions complicated by pneumonia,
by week of training*

Infectious agents	No. of admissions*							
	Week of training							
	1	2	3	4	5	6	7	8
ADV-4			1	(1)		(1)		
Influenza A ₂	1				1†	(1)		
Rhinovirus					1†			
Group A streptococcus					1†			
No infectious agents	1	4	2	1	0	2	0	0
Total admissions	2	4	3	2	2	2	0	0

* Numbers in parentheses indicate serologic evidence of infection alone.

† Type unidentified, tested against rhinovirus immunotypes 1-89.

‡ T agglutination pattern, 3/31/41, M nontypable.

TABLE 5
Percentage distribution of ARD admissions

Admission category	No. of admissions	% of total
Non-ARD admissions (rubella, shot reactions)	18	12.1
ARD admissions		
ARD complicated by pneumonia	17	11.4
Uncomplicated ARD		
ADV associated ARD	47	31.5
Non-ADV associated ARD (Influenza, rhinovirus, group strep, etc.)	21	14.1
ARD associated with multiple agents	11	7.4
ARD not associated with infectious agents	35	23.5
Total admissions	149	100

times epidemics in military populations. However, unlike the adenoviruses, none of these agents has exhibited predictable yearly patterns of epidemic disease in military populations. Further, the basis for the rather restrictive relationship between adenoviruses and ARD in recruit populations is not fully understood. Thus, when the first large group of trainees was immunized with ADV-4 and ADV-7 vaccines, it was essential to monitor closely the effects of immunization, not only to determine vaccine efficacy, but also to see if, under these circumstances, other respiratory pathogens

would emerge and exhibit a capacity to produce a major new disease problem.

The results clearly indicate that under the conditions of this particular study no other respiratory pathogens emerged to rival the prevalence of adenoviruses as the major cause of ARD hospitalizations. Adenovirus-associated ARD accounted for nearly one-third of the admissions and these were primarily due to ADV-4. Adenovirus types other than ADV-4 or ADV-7 were not isolated. The ADV-4 vaccine used was of marginal potency, and clearly less effective than the ADV-7 vaccine (1).

Although rubella virus is transmitted by the respiratory route, it seemed appropriate to exclude men with rubella from the analysis of ARD admissions. Fever and/or respiratory symptoms were minimal in all cases. It is probable that the exanthem prompted admission in most instances because of concern that any exanthem might be an early indication of meningococcal disease. That exanthem was the primary criteria for admission is further suggested by the number of clinical cases (14) versus the number of subclinical cases (one) among these 149 hospital admissions. A previous study in military recruits showed that subclinical infection with rubella virus occurred 6 to 7 times more frequently than clinical infection (5). Thus, many more subclinical cases of rubella should have been found among those hospitalized had some criteria other than exanthem been an important factor leading to hospitalization.

As expected, small numbers of influenza A₂ virus and rhinovirus-associated ARD admissions occurred early in the training cycle, whereas adenovirus-associated ARD was found during the latter part of training. Other potential viral respiratory pathogens such as influenza B, parainfluenza and coxsackie A-21 viruses were not found despite the use of appropriate isolation techniques. Herpesvirus hominis and group A streptococci were found only occasionally. Serologic evidence of *M. pneumoniae* infection was demonstrated in only one individual in

the entire study. However, 14-day convalescent sera are not optimal for detection of antibodies following *M. pneumoniae* infections.

Almost one of every 10 uncomplicated ARD admissions was associated with evidence for the simultaneous occurrence of two or more respiratory infections. This points out a significant difficulty in establishing the etiologic basis of ARD hospitalizations in military training populations and raises the question of whether synergism may occur. In two previous studies of respiratory disease in military populations, the incidences of multiple infections were 8.3 and 21.0 per cent, indicating that in these populations multiple infections are not infrequent occurrences (6, 7).

Sixty per cent of ARD admissions not associated with a recovered agent or serologic evidence of infection occurred during the first three weeks of training, a time when rhinovirus infections are most common. It is entirely possible that the addition of organ culture isolation techniques as well as rhinovirus and coronavirus serology to the battery of tests employed would reduce the number of undiagnosed cases even further. Difficulties in isolating rhinoviruses are well known (8) and optimal isolation and recovery may be achieved only when two or three different tissue culture cell lines and organ culture systems are employed simultaneously.

Few, if any, conclusions are warranted from the isolation and serologic data obtained from the 17 ARD admissions complicated by pneumonia. The mere presence of organisms in the upper respiratory tract does not necessarily reflect the cause of lower respiratory tract disease. The etiologic spectrum of pneumonia in hospitalized recruits has not been firmly established except in those few instances where the evidence for *M. pneumoniae* infections (in epidemic proportions) has been obtained (9).

ADV-4 and ADV-7 vaccines are intended for control but not eradication of the two most common causes of ARD requiring hos-

pitalization in recruit populations. Given ADV-4 and ADV-7 vaccines of adequate and equal potencies, coupled with proper timing of administration based upon epidemiologic data, control of disease caused by these two viruses can be achieved. This study suggests that immunoprophylaxis for non-adenovirus associated ARD (with the exception of influenza) may be more difficult because of: 1) the multiplicity of agents; 2) the unknown number of agents that are yet unidentified; and 3) the tendency for these infections to occur early in training before immunoprophylactic measures would be effective. In the absence of a simple, safe "universal" chemoprophylactic, effective against multiple respiratory pathogens, it must be questioned whether the control of non-adenovirus associated ARD (influenza excepted) in military recruit populations is a realistic and practical goal.

REFERENCES

1. Top FH Jr, Dudding BA, Russell PK, et al: Control of respiratory disease in recruits with types 4 and 7 adenovirus vaccines. *Am J Epidemiol* 94:142-146, 1971
2. Rose HM, Lamson TH, Buescher EL: Adenoviral infection in military recruits. *Arch Environ Health* 21:356-361, 1970
3. Top FH Jr, Buescher EL, Bancroft WH, et al: Immunization with live type 7 and 4 adenovirus vaccines: Evaluation of antibody response and protective effects against naturally occurring adenovirus type 7 acute respiratory disease. *J Infect Dis* 124:155-160, 1971
4. Van Der Veen J, Oei KG, Abarbanel MFW: Patterns of infections with adenovirus types 4, 7 and 21 in military recruits during a 9-year survey. *J Hyg (Camb)* 67:255-268, 1969
5. Buescher EL: Behavior of rubella virus in adult populations. *Arch Gesamte Virusforsch* 16:470-476, 1965
6. Rosenbaum MJ, Edwards EA, Frank PF, et al: Epidemiology and prevention of acute respiratory disease in naval recruits. I. Ten years' experience with microbial agents isolated from naval recruits with acute respiratory disease. *Am J Public Health* 55:38-46, 1965
7. McDonald JC, Miller DL, Zuckerman AJ, et al: Coe (Coxsackie A21) virus, para-influenza virus and other respiratory virus infections in the R.A.F., 1958-1960. *J Hyg (Camb)* 60:235-248, 1962
8. Tyrrell DAJ, Bynoe ML, Hoorn B: Cultivation of difficult viruses from patients with common colds. *Br Med J* 1:606-610, 1968
9. Chanock RM, Fox HH, James WD, et al: Epidemiology of *M. pneumoniae* infections in military recruits. *Ann NY Acad Sci* 143:484-496, 1967