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Studies in Human Subjects of Polyvalent Pneumococcal Vaccines (39894)

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The pneumococci (*Streptococcus pneumoniae*) are a leading cause of pneumonia, meningitis, otitis media, and death [see Ref. (1)] in human beings throughout the world. Presently, there are at least 84 known capsular types of pneumococci that are distinguishable immunologically (2, 3), but the preponderance of pneumococcal disease is caused by only a minority of the total number (3-7). Early studies by Felton (8) showed that isolated pneumococcal capsular polysaccharides stimulated antibody in human beings. Investigations by MacLeod *et al.* (9) of a quadrivalent pneumococcal capsular vaccine provided evidence for the efficacy of pneumococcal polysaccharides for preventing pneumococcal pneumonia and nasopharyngeal carriage of pneumococci in military personnel. Studies in the contemporary period by Dr. Robert Austrian and his colleagues (2, 3, 10) have abundantly confirmed the ability of polyvalent pneumococcal vaccines to elicit homologous capsular antibody and to prevent pneumococcal disease caused by agents of the same types. The limitation in number of important pneumococcal types causing illness (2-4, 7, 16) and the fact that humoral antibody can be elicited against a number of capsular polysaccharides given together in a single dose makes pneumococcal vaccine feasible and practical. Polyvalent pneumococcal polysaccharide vaccines have been prepared in our laboratories and tested in human beings in experimental trials. The present report describes the antibody responses and clinical reactions in adults and children who received a single dose of combined 12- or 14-valent pneumococcal polysaccharide vaccine.

Materials and methods. Vaccines. Pneumococcal capsular type designations are according to the American system. The polysaccharides from which the vaccines were made were prepared in the laboratories of Drs. Thomas H. Stoudt and Dennis J. Carlo and Messrs. Bernard L. Wilker, James Lago, and William A. Sklarz of the Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey. The physical and chemical attributes of the polysaccharides were measured principally by Dr. George V. Downing. The polysaccharides were characterized for chemical and physical attributes and all were within the acceptability standards for pneumococcal vaccine now being developed by the Bureau of Biologics, U. S. Food and Drug Administration, Bethesda, Maryland (11). The vaccines were formulated into polyvalent vaccines in our laboratories at West Point, Pennsylvania. Each 0.5-ml dose of vaccine contained 50 μg of each pneumococcal polysaccharide type in physiological saline solution containing 0.25% USP grade phenol. Tests for release of final product were according to the general regulations of the Bureau of Biologics and were in compliance with the tentative specific regulations being developed by the same agency (11).

Clinical testing. All subjects were in apparent good health. Blood samples for radioimmune assays for pneumococcal antibody were taken immediately prior to, and 1 month, and, in some patients, 20 months following subcutaneous (for 12-valent vaccine) or intramuscular (for 14-valent vaccine) administration of the 0.5-ml dose of vaccine. Clinical studies of the vaccines were carried out in open populations residing in suburban Philadelphia, Pennsylvania, or in Merck & Co., Inc., employees in West Point, Pennsylvania, and Rahway, New Jersey. All tests were carried out under In-

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vestigational New Drug regulations employing informed written consent for subject participation. Temperature (0°F) readings were taken prior to and approximately 4 hr after vaccination and at daily intervals for 10 days thereafter. The injection sites were observed for local reactions by physicians and qualified nurses following vaccination at approximately 15 min, 4 hr, 24 hr, and daily until any reaction subsided.

Antibody assays. The sera were stored frozen until tested by a standard radioimmune assay (12) using radioactive type-specific pneumococcal polysaccharides obtained from Dr. Gerald Schiffman. For each type all serum samples from a particular individual were tested together in the same assay. Serum titers were measured as nanograms of antibody *N* per milliliter of serum and a twofold or greater increase in the amount of antibody between the first and second serum sample was taken as indicative of a significant antibody response.

Results. Tests of 12-valent vaccine. Fifty adults ranging in age from 20 to 65 years (mean age 42.2 years) and 42 children, 1 to 12 years of age (mean age 5.8 years), were given 0.5 ml subcutaneously of Lot 561/C-D194 pneumococcal capsular polysaccharide vaccine. Blood samples were taken prior to, and 1 and 20 months following vaccination. The findings in the radioimmune assays for antibody against homologous pneumococcal polysaccharide are given in Fig. 1. Not all subjects' sera were tested against all serotypes, usually because of inadequate serum volume. The numbers, however, were large enough to measure representative responses. As seen in the figure, at least 90% of the adults showed a twofold or greater increase in antibody against six capsular types (Types 1, 8, 9, 12, 23, 51), at least 87% of persons against five additional capsular types (Types 3, 4, 6, 19, 56), and 79% against the remaining one capsular type (Type 14). The mean postvaccination antibody titers ranged from 1480 to 24,123. The mean fold increases in homologous antibody titer ranged from 4.3 to 13.0. Tests of sera taken 1 and 20 months after vaccination (Table I) from a random sample of 26 adult persons from the same group showed a decline in antibody amount from the peak titer in a substantial number of individuals. The decline in mean titer for the 12 capsular types ranged from 12 to 48% (average, 37%).

Greater than 90% of children showed significant increase in antibody against 10 types (Types 1, 3, 4, 6, 8, 9, 12, 14, 51, 56) following vaccination and 83 to 89% showed such rise in antibody titer against the remaining two types (Types 19, 23). The titers of antibody were generally less in children than in adults both prior to and following vaccination. The mean level of antibody following vaccination against capsular Type 23, for example, was 24,123 in adults and 10,990 in children. In one example (Type 8), the reverse was true with mean titers of 4209 in adults and 6599 in children.

Tests of 14-valent vaccine. Twenty-six adults were given 0.5 ml of 14-valent Lot 686/C-E515 pneumococcus vaccine intramuscularly and blood samples were taken prior to and 1 month after vaccination. The subjects ranged in age from 21 to 59 years and the mean age was 35 years. This particular vaccine was prepared in the most recent time period and represents vaccine prepared by the current technology. Table II shows that all of the subjects developed significant antibody responses to 11 of the capsular types (Types 1, 2, 4, 6, 8, 9, 12, 14, 19, 25, 51) in the vaccine and 92 to 96% responded to the remaining three types (Types 3, 23, 56). The mean fold antibody increase was between 6.5 and 24, and exceeded 10-fold for eight of the 14 types.

Clinical reactions. The observed clinical reactions to vaccination were generally mild and usually limited to erythema, induration, and tenderness at the injection site. No immediate local reaction was observed or reported. Most clinical reactions began 4 hr after administration of the vaccine and were comparable in onset and type to those commonly seen following killed vaccines. Table III shows that 86% of adults and nearly all children who received the 12-valent vaccine (Fig. 1) experienced reactions. The reactions were self-limited and disappeared in most persons within 4 or 5 days. Mild fever was observed to occur in a small portion of adults 4 to 72 hr after vaccination, and such mild response was observed in about 40% of the children. One child (age 9 years) who

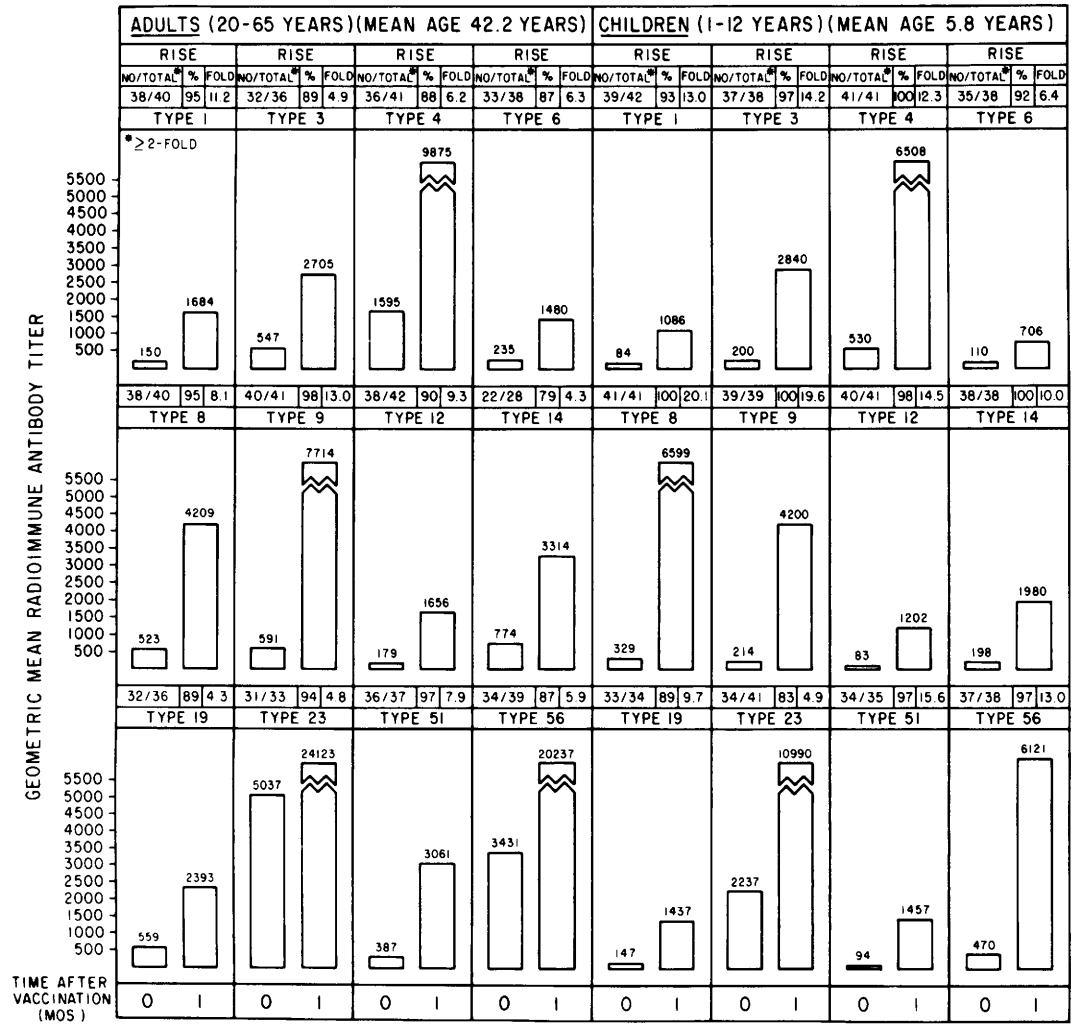

FIG. 1. Antibody responses in adults and children given a single dose of polyvalent (12-type) pneumococcal vaccine (Studies 384, 431, 454, and 482)

received the 12-valent vaccine developed headache, myalgia, and a maximum temperature of 104°F for 1 day and there were erythema, induration, and tenderness at the injection site that cleared completely within 5 days. Similar clinical reactions to those following adeministration of 12-valent vaccine were observed to follow administration of the 14-valent vaccine as well.

Discussion. Invasive pneumococcal disease is of high morbidity and mortality in spite of effective antimicrobial therapy (2, 4, 6). These effects of pneumococcal disease appear due to irreversible physiologic injury to the host caused by the bacteria (2, 4, 5, 6, 10). Older persons, individuals with chronic debilitating diseases, and persons with impaired splenic function, including those with homozygous sickle cell anemia and sickle thalassemia, may be especially susceptible to severe pneumococcal disease.

The attack rate for pneumococcal pneumonia in the United States has been estimated to be from one to five cases per 1000 persons per year (around 200,000 to 1,000,000 cases per annum) and there is about one death per 15 cases (13,200 to 66,000 deaths per year) (2, 10). According to Austrian and co-workers (2, 4, 10) about 25% of all pneumococcal pneumonia cases

TABLE I. INITIAL AND RETAINED (20 MONTHS) ANTIBODY RESPONSES IN ADULTS GIVEN A SINGLE DOSE OF POLYVALENT (12-TYPE) PNEUMOCOCCAL VACCINE (STUDY 384).

Pneumococcus capsular type	Blood sample (time, months)	Radioimmune antibody titer (ng of antibody N/ml)		
		Number with greater than two-fold increase/total number (percentage in parentheses)	Geometric mean	Mean fold increase
1	0		113	
	1	23/24 (96)	1743	15.4
	20	23/24 (96)	984	8.7
3	0		502	
	1	20/22 (91)	2920	5.8
	20	15/22 (68)	1606	3.2
4	0		1164	
	1	21/24 (88)	9560	8.2
	20	19/24 (79)	5385	4.6
6	0		182	
	1	19/24 (79)	1305	7.2
	20	17/24 (71)	984	5.4
8	0		498	
	1	21/23 (91)	3976	8.0
	20	18/19 (95)	2307	4.7
9	0		484	
	1	24/24 (100)	7900	16.3
	20	24/24 (100)	4138	8.5
12	0		137	
	1	21/25 (84)	1370	10.0
	20	17/25 (68)	782	5.7
14	0		881	
	1	13/18 (72)	3223	3.7
	20	11/19 (58)	2844	3.2
19	0		400	
	1	19/21 (90)	2077	5.2
	20	15/21 (71)	1386	3.5
23	0		5256	
	1	17/19 (89)	30308	5.8
	20	15/20 (75)	18764	3.6
51	0		422	
	1	23/24 (96)	4518	10.7
	20	19/24 (79)	2442	5.8
56	0		2588	
	1	22/24 (92)	15335	5.9
	20	19/25 (76)	12065	4.7

develop bacteremia with death in about 17–18% of treated patients of all ages, and in more than 25% of persons 50 years of age and older. Mufson *et al.* (6) reported a case fatality rate of 28% among 262 adult patients with bacteremic pneumococcal pneumonia or an extrapulmonary focus, or both. Pneumococcal meningitis occurs principally in young children with reported (14) rates of 3 to 11 per 100,000 per year in children less than 5 years of age. A rate of 1.4 per 100,000 persons of all ages per year was recorded (14) for Bernalillo County, New Mexico, during the period 1964–1971. It is estimated that 15 to 20% of all children develop otitis media caused by pneumococci within the first 2 years of life (10) and 50% of children develop such illness within the first 10 years of life.

The present study of 12-valent and 14-valent pneumococcal capsular polysaccharide vaccines demonstrated the high level efficacy of the vaccine for inducing specific antibody against the homologous capsular types. The imporant clinical reactions that were observed were limited to mild fever and induration and soreness at the site of injection of the kind that is ordinarily observed following administration of killed vaccines. Studies for protective efficacy of 6-valent and 12-valent pneumococcal polysaccharide vaccines for preventing pneumo-

TABLE II. ANTIBODY RESPONSES IN ADULTS GIVEN A SINGLE DOSE OF POLYVALENT (14-TYPE) PNEUMOCOCCAL VACCINE (STUDY 497).

Pneumococcus capsular type	Blood sample (time, months)	Radioimmune antibody titer (ng of antibody *N*/ml)		
		Number with greater than two-fold increase/total number, (percentage in parentheses)	Geometric mean	Mean fold increase
1	0		240	
	1	26/26 (100)	4050	16.9
2	0		479	
	1	26/26 (100)	5148	10.7
3	0		439	
	1	26/26 (96)	3556	8.1
4	0		1535	
	1	26/26 (100)	13931	9.1
6	0		181	
	1	26/26 (100)	2457	13.6
8	0		418	
	1	24/24 (100)	5761	13.8
9	0		375	
	1	25/25 (100)	4132	11.0
12	0		155	
	1	26/26 (100)	2470	15.9
14	0		300	
	1	23/23 (100)	2488	8.3
19	0		298	
	1	25/25 (100)	2540	8.5
23	0		2580	
	1	24/26 (92)	16785	6.5
25	0		137	
	1	25/25 (100)	3295	24.0
51	0		186	
	1	26/26 (100)	2396	12.9
56	0		2205	
	1	24/25 (96)	15528	7.0

TABLE III. LOCAL AND FEBRILE REACTIONS IN ADULTS AND CHILDREN WHO WERE GIVEN A SINGLE DOSE OF POLYVALENT (12-TYPE) PNEUMOCOCCAL VACCINE (STUDIES 384, 431, 454, AND 482. SEE FIG. 1).

Group	Reaction	No. with reaction on day:					
		0	1	2	3	4	5
Adults (50 persons)	Local (arm)	32	43	32	24	7	2
	Fever (maximum, °F; 0)						
	<99	43	43	49	46		
	99-99.9°F	6	6	1	3		
	100-100.9		1				
Children (42 persons)	Local (arm)	21	41	16	14	10	8
	Fever (maximum, °F; 0)						
	<99	21	29	33	36		
	99-99.9	17	9	6	5		
	100-100.9	1	3	2	1		
	101.4			1			
	104		1				

coccal pneumonia were carried out in collaborative studies with Dr. Pieter Smit and his colleagues in gold miners in South Africa. Preliminary analyses of the studies (to be reported) showed protective efficacy against pneumococcal pneumonia caused by the homologous capsular types to be 76 and 92%, respectively. In similar studies carried out by Austrian *et al.* (3) using tridecavalent vaccine of different sources, the reduction in

pneumonias caused by the homologous vaccine capsular types was 78.5%, and the reduction in homologous pneumococcal bacteremias afforded by hexavalent and tridecavalent vaccines was 82.3%. Incomplete data (15) suggest efficacy of the vaccine in preventing severe pneumococcal disease and bacteremia in persons with sickle cell anemia and in individuals without spleens or who have impaired splenic function.

A vaccine containing 14 appropriately selected pneumococcal capsular polysaccharides may be expected to be protective against those types causing 80% or more of pneumococcal pneumonia in the United States and Europe (3, 11, 16). The findings in these and additional, as yet unpublished, studies from our laboratories are consistent with those of Austrian and co-workers (2, 3, 10), Ammann *et al.*, (15), and Bentley *et al.* (17) and indicate immunogenicity of the vaccine for most persons from about 2 years of age through old age. The broad coverage of the vaccine against pneumococcal capsular types and the broad age responsiveness in vaccinated persons suggest considerable practical usefulness for the vaccine under wide discretionary application.

The duration of protective effect of the vaccine is presently unknown, but it has been shown in previous studies (2, 18) with different pneumococcal vaccines that antibody induced by the vaccine may persist at one-fifth to one-half or more of its peak values 2 to 3 years after a single injection. In some individuals, abundant residual antibody persisted 8 years after vaccination. The present study demonstrated 37% average decline in specific antibody 20 months after vaccination compared with the titer achieved after 1 month, but the titers were substantially above the prevaccination levels in almost all recipients who showed an initial response. It is not possible at this time to define precisely the need or proper time for revaccination. We have found, however, that the severity of local reactions at the site of injection correlates roughly with the height of circulating antibody and that such local reactions may occur more frequently and with greater severity on revaccination at 1 year. These unpublished data from our laboratories suggest that revaccination should not be carried out at less than 3-year intervals, especially in persons who have retained high antibody levels.

Summary. Clinical studies of 12- and 14-valent pneumococcal capsular polysaccharide vaccines were carried out among 76 adults and 42 children. All but a small proportion of persons developed significant increases in homologous antibody against all capsular types in the vaccine. Clinical reactions consisted mostly of mild fever and self-limiting local reactions at the injection site, such as commonly seen following administration of killed vaccines. Antibody persisted remarkably well with only slight decline 20 months after the vaccine was given. The vaccine shows great promise for preventing disease and death caused by pneumococci and merits wide discretionary application.

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