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Antibody Responses after Immunization with Polyvalent Pneumococcal Vaccine Containing 10, 25, or 50 μg of 14 Polysaccharide Types per Dose (41690)

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Abstract. Immune responses were determined to three different doses of a pneumococcal vaccine which contained 10, 25, or 50 μg of 14 polysaccharide types (1, 3, 4, 6A, 6B, 7F, 8, 9N, 12F, 14, 18C, 19F, 20, and 23F) per dose. Pre- and 4-weeks postvaccination sera were examined by radioimmunoassay. Ratios of geometric mean titers (GMT) showed a greater than threefold increase of antibodies to each type of polysaccharide regardless of the dose of antigen. The post-GMT was highest to 12 of 14 types in recipients of the 25-μg vaccine. Subjects in the group receiving a 25-μg dose of each antigen showed more two- and fourfold titer increases than did subjects in the group receiving 10- and 50-μg doses, but, in general, seroconversion rates were similar in all the groups. All GMT values in the 25-μg vaccine group were well above what is currently considered to be the protective level of antibody. Local reactions, overall, were low in all groups. Pain, induration, and tenderness at the site of injection, which were greatest in recipients of the 50-μg dose/antigen, were progressively lower with the 25- and 10-μg dose/antigen.

Pneumococcal infection can be caused by any of more than 80 different serotypes of *Streptococcus pneumoniae*. Early vaccines contained fewer types (1–8) than the contemporary ones in which the number has been increased in order to afford protection against a greater proportion of pneumococcal disease.

Presently licensed vaccines contain 14 pneumococcal capsular polysaccharides and are designed to protect against 80% of all bacteremic pneumococcal infection. Vaccines of the future are expected to be even more complex when a greater number of polysaccharides are included (6). With this increased complexity comes the possibility of increased local or systemic reactivity. By reducing the total amount of antigen present in a vaccine, the problem might be minimized.

A twofold rationale emerged for using lower dosages of capsular polysaccharides: (i) less local or systemic reactivity might result from lower amounts of antigens, which phenomenon is known to be associated with other vaccines, and (ii) reduction of reactivity would pave the way for the inclusion of additional types of polysaccharide in a modified vaccine, thereby creating an agent with wider immunogenic coverage.

Because of the above-mentioned potential therapeutic benefits, we decided to investigate polyvalent vaccines formulated with smaller quantities of individual pneumococcal polysaccharides (SSS). The immunogenicities of three different tetradecavalent pneumococcal vaccines, each of which contained 10, 25, or 50 μg of each of 14 capsular polysaccharides (SSS) per dose, were determined in a human dose–response study.

Materials and Methods. Our study population of 105 subjects (56 women and 49 men, 22 to 84 years of age) was divided into three groups of about 30 subjects each for immunization with the tetradecavalent vaccines and one group of 10 subjects for administration of a placebo. The protocol required that the subjects be at least 18 years of age and, if females, that they not be pregnant at enrollment or become so during the study. Written consent was obtained from each participant after thorough explanation of the study.

Formulation of the polyvalent vaccine (produced by Lederle Laboratories) contained 10, 25, or 50 μg/0.5 ml of each of the following SSS types per dose: 1, 3, 4, 6A, 6B, 7F, 8, 9N, 12F, 14, 18C, 19F, 20, and 23F. A prevac-

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cination sample of blood (10 ml of venous blood) was collected from each subject for baseline evaluation of levels of type-specific pneumococcal antibodies, and then a single 0.5-cc dose of vaccine or of placebo was administered intramuscularly. Four weeks (28 to 30 days) after the injection, specimens of blood were obtained again for determination of antibody responses induced by the individual pneumococcal polysaccharides. Blood was refrigerated after collection and, within 6 hr, the serum was separated and frozen for storage at −20°c until serologic analyses could be performed. For these determinations, a radioimmunoassay, described elsewhere (7), was used. Significant seroconversions are considered generally to be those in which antibody titers increased at least twofold over prevaccination titers (1, 2). Recently, the concept of a protective level of antibody (200–300 ng of type-specific antibody/ml) has been presented (8–10). This concept may permit a more pragmatic evaluation of the type-specific antibody response and of the protective efficacy of a polyvalent pneumococcal vaccine by relating the actual levels of antibody attained by vaccinees to the suggested protective level.

Results. Ratios of geometric mean titers (GMT) of the post- to prevaccination levels of antibodies show that a greater than threefold increase to each type specific polysaccharide resulted regardless of the dose of antigen (10, 25, or 50 μg) administered (Table I). It should be noted also that the postimmunization GMT of antibody was highest for 12 of the 14 individual SSS types in recipients of 25 μg of each polysaccharide although several of the differences were small. All the responses stimulated by the 25-μg doses were well above the suggested 200- to 300-ng "protective level," a range of antibody concentration which was commented on earlier.

Examination of seroconversion rates in Table II shows that recipients of both the 10- and 50-μg doses of each polysaccharide had twofold or greater increases in titer in 93% of the subjects, whereas 98% of those immunized with the 25-μg vaccine had a twofold or greater increase in antibody titer. Fourfold increases were observed in somewhat smaller percentages of recipients of each three vaccine doses. Only scattered statistically significant differences were noted between the 10- and 25-μg, 10- and 50-μg, and 25- and 50-μg doses with respect to two- and fourfold seroconversion rates of the individual pneumococcal types.

Overall, local or systemic reactivity to the polysaccharide vaccines was low. Local reactions, which were greatest in subjects immunized with the 50-μg doses, consisted of pain, tenderness, induration, and erythema in order of descending frequency. Few systemic reactions appeared in any of the three dosage groups. No reactions occurred in subjects who were administered placebo. About half (15/32) of those inoculated with the 50-μg doses experienced pain at the site of injection within the first hour, compared to 23% (7/30) in the 25-μg, and 19% (6/31) in the 10-μg groups. Tenderness around the site of injection occurred in 25% (8/32) of the subjects in the 50-μg group, 13% (4/32) in the 25-μg group, and 6% (2/31) in the 10-μg group during the first hour. By 48 hr, local reactivity was not evident in the 10-μg group and only slightly so in the two other groups, with pain, tenderness, or induration occurring at frequencies of 10% or less.

Discussion. The accepted immunizing dose of capsular polysaccharide (50 μg) for licensed vaccines dates from the studies conducted by Heidelberger *et al.* (11) and MacLeod *et al.* (12) during the 1940s. They examined the immunogenic characteristics of amounts of polysaccharides varying between 30 and 70 μg and noted that this dosage range provided an adequate antibody stimulus; accompanying reactivity was absent to moderate.

Other studies of different dosage levels of capsular polysaccharides include one by Ekwurzal *et al.* (13), in which 2 mg total polysaccharide was administered without significant untoward effect and another (14) in which 9 μg of type 1 polysaccharide appeared to be a borderline dose in terms of its efficacy.

Presently, licensed polyvalent pneumococcal vaccine provides the base from which further investigations of more complex vaccines can proceed. These vaccines include ones with increased numbers of polysaccharides or vaccines that contain combinations of antigens from more than one bacterial species. As Austrian (6) observed, a 20-valent vaccine designed to protect against 90% of bacteremic pneumococcal infections is a future possibility. Concomitant with an increase in the number

TABLE I. GEOMETRIC MEAN TITERS (GMT) AND RATIOS OF PRE- AND POSTINOCULATION ANTIBODY CONCENTRATIONS IN RECIPIENTS OF ONE OF THREE DOSES OF A TETRADECAVALENT VACCINE OF PNEUMOCOCCAL POLYSACCHARIDES

Pneumococcal type	GMT and ratio of pre- and postinoculation antibody concentrations											
	Pneumococcal vaccine											
	10 μg (N = 31)			25 μg (N = 32)			50 μg (N = 32)			Placebo (N = 10)		
	Pre	Post	Ratio	Pre	Post	Ratio	Pre	Post	Ratio	Pre	Post	Ratio
1	93	295	3.2	118	514	4.3	107	432	4.0	88	88	1.0
3	27	374	13.7	89	733	8.2	73	577	7.9	28	28	1.0
4	231	1222	5.3	526	2791	5.3	385	1742	4.5	591	129	0.2
6A	66	422	6.4	106	858	8.1	85	506	5.9	64	67	1.1
6B	92	501	5.5	96	772	8.0	158	823	5.2	100	77	0.8[a]
7F	85	603	7.1	130	785	6.1	146	696	4.8	97	89	0.9
8	49	501	10.3	72	935	12.9	84	923	11.0	40	38	1.0
9N	109	798	7.3	152	1681	11.0	126	1111	8.8	62	67	1.1
12F	43	278	6.5	55	565	10.3	50	369	7.3	33	40	1.2
14	222	1176	5.3	394	1259	3.2	185	901	4.9	260	181	0.7
18C	766	3353	4.4	922	4978	5.4	676	3274	4.8	563	622	1.1
19F	68	404	5.9	92	536	5.8	56	353	6.3	90	52	0.6
20	88	598	6.8	75	579	7.7	88	773	8.8	72	69	1.0[a]
23F	402	1859	4.6	692	3223	4.7	368	1708	4.6	500	218	0.4

[a] GMTs and ratios based on eight subjects.

of polysaccharides, however, there will be a rise in the aggregate quantity of immunizing material present per dose, so that a theoretical vaccine of 20 types each in a dose of 50 μg would contain 1 mg of combined antigens compared to 700 μg in the contemporary 14-

TABLE II. PERCENTAGES OF VACCINEES WITH TWO- AND FOURFOLD INCREASES IN ANTIBODY CONCENTRATIONS IN A STUDY OF THREE DOSES OF A TETRADECAVALENT VACCINE OF PNEUMOCOCCAL POLYSACCHARIDES[a]

Pneumococcal type	Percentage of vaccinees							
	Pneumococcal vaccine							
	10 μg (N = 31)		25 μg (N = 32)		50 μg (N = 32)		Placebo (N = 10)	
	Twofold	Fourfold	Twofold	Fourfold	Twofold	Fourfold	Twofold	Fourfold
1	90	32	100	53	97	47	0	0
3	94	87	97	78	91	72	10	0
4	84	42	100	56	91	47	0	0
6A	90	68	100	72	94	75	0	0
6B	81	55	94	72	94	59	0	0[a]
7F	97	84	100	66	91	59	0	0
8	97	81	100	91	94	84	10	10
9N	97	55	100	69	97	78	0	0
12F	97	52	100	84	97	72	10	0
14	94	45	100	19	97	53	0	0
18C	94	29	97	53	94	50	20	10
19F	97	48	94	66	88	69	0	0
20	94	65	97	84	97	75	0	0[a]
23F	97	39	94	59	84	56	0	0
Overall[b]	93	56	98	66	93	64	4	1

[a] Percentage based on eight subjects.
[b] Overall percentages based on pooled titer values across vaccinees and pneumococcal types.

valent vaccine. Local or systemic reactivity might be expected to increase as the complexity of the vaccine increases.

To overcome these problems, vaccines containing lesser amounts of each individual polysaccharide are being investigated. For example, the recent study of patients with nephrotic syndrome by Fikrig *et al.* (4) showed that antibody responses after immunization of normal control subjects with pneumococcal vaccines in dosages of 25 or 50 μg of each antigen were essentially the same in recipients of either dose; twofold or greater increases in GMT of antibodies to 11 of 12 pneumococcal types were observed. In a more recent study by Pankey and Schiffman (15), it was found that the immunogenicity of a 22-valent vaccine administered in a dose of 25 μg of each polysaccharide was comparable to a 50-μg dose of each antigen in the same vaccine. One year follow-up of this study indicates that antibody persistence is adequate and similar for both groups (unpublished observations). In our present study, we compared the immunizing potentials of 10- and 25-μg doses/antigen in a tetradecavalent vaccine to that of a 50-μg dose/antigen of the same vaccine and found that, overall, adequate responses of at least threefold or greater increases in antibody titers to each of the 14 pneumococcal types were obtained with all three doses of vaccine. Although differences among the three groups were not significant, the 25-μg formulation provided a slightly better antibody response than did the two other dosages and also induced increases in GMT of antibody that were well above what is now considered to be the protective level (8–10).

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