PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 163, 498–503 (1980)

Polyvalent Pneumococcal Vaccine Given Alone and in Combination with Bivalent Influenza Virus Vaccine (40804)

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Some years ago it became apparent that the fatality rate of bacterial pneumococcal infections remained high, regardless of antibiotic treatment and that pneumococcal polysaccharide vaccines again would be of value (1). Polyvalent pneumococcal polysaccharide vaccines, recently reintroduced, are now advocated for use among high-risk populations (2–7), where case fatality rates from bacteremic pneumococcal pneumonia approach 1 in 4 and where penicillin treatment fails in the attempt to prevent such deaths. In addition, pneumococci with multiple resistance, which are beginning to appear (8, 9), further complicate the problem by posing among susceptible groups the threat of future bacterial pneumonia outbreaks that will be difficult to control.

Because morbidity from influenza virus also occurs commonly in high-risk groups, a combination vaccine comprised of pneumococcal polysaccharide and influenza A and B virus antigens would have broad application in preventing the morbidity and mortality due to these agents in such groups. Pneumococcal revaccination is recommended no more often than at 3-year intervals, and newly developed influenza virus vaccines would have to be administered on the same schedule to make combination vaccines feasible. This approach requires the capability of producing a potent combination vaccine. To investigate the immunizing potential of antigens in a combination vaccine, we have evaluated the antigenicity and reactivity of a hexavalent pneumococcal polysaccharide vaccine given either alone or in combination with a formaldehyde-inactivated bivalent influenza virus vaccine.

Materials and methods. Subjects. The study group was comprised of 68 healthy adult volunteer subjects, aged 20 to 55 years, and included medical students, student nurses, college students, and medical center employees; none of the volunteers had ever previously received a pneumococcal polysaccharide vaccine or had received an influenza virus vaccine within the previous 12 months and they had not had a serious respiratory illness in the recent past. The nature of the study was explained to the volunteers and each freely signed a consent form. Volunteers were assigned by means of random numbers to one of three vaccine groups, namely, pneumococcal polysaccharide vaccine, inactivated bivalent influenza virus vaccine, or a combination vaccine. Subjects, but not observers, were blinded to vaccine allocation. Both the pneumococcal polysaccharide vaccine and the combination vaccine groups were comprised of 23 subjects each, while the influenza virus vaccine group contained 22 subjects; mean ages of the three vaccine groups were 28, 27, and 32 years, respectively. Men and women were nearly equally divided in the pneumococcal and combination vaccine groups (12 men, 11 women in the former and 11 men, 12 women in the latter), but women comprised over three-fourths of the influenza virus vaccine group (17 women, 5 men).

Vaccines and immunization. The pneumococcal polysaccharide vaccine consisted of six capsular types—1, 3, 4, 7, 8, and 12—each at a 50-μg concentration. In-

[1] Deceased March 3, 1979.

0037-9727/80/040498-06$01.00/0

activated bivalent influenza virus vaccine (whole particle) contained 700 and 300 CCA units of strains A2/Aichi/2/68 and B/Mass/1/71, respectively. The combination vaccine contained the identical pneumococcal polysaccharide types and influenza virus strains at the same concentrations as the single vaccines. All of the vaccines, which were produced by Lederle Laboratories, were packaged in unit dose, single-use syringes and were administered intramuscularly in 0.5-ml volume doses. Twenty-milliliter samples of venous blood were collected for anitbody measurements from each vaccinee prior to immunization and at 3 weeks postimmunization.

Each subject was monitored for local and systemic reactions. Clinical reactivity was evaluated at 15 min and at 24 and 72 hr following vaccine administration by fever response and a local- and systemic-symptom profile. At each time, the vaccinees were examined by a nurse or physician for evidence of reactions and were questioned concerning symptoms which had occurred between examinations. Type-specific pneumococcal antibody for types 1, 3, 4, 7, 8, and 12 was measured by a radioimmunoassay (RIA) procedure (10). The test was performed on preimmunization and 3-week postimmunization sera. Statistical analysis of the test results for the subjects who received the influenza vaccine alone showed that with 95% confidence, 5%, at most, of future subjects receiving influenza vaccine alone could expect an increase in antibody titer (regardless of type) as large as 40%. Hence, responses to individual polysaccharide were considered significant with the RIA procedure if an increase in specific antibody nitrogen of 40% or greater was observed. Test specificity was confirmed by the closeness to 1.0 of the ratios of post- to preinoculation antibody titers (0.87 to 1.06) to the six pneumococcal types in the subjects who received the influenza vaccine alone. Influenza virus A and B antibodies were measured by a hemagglutination-inhibition assay (HI) using strains A2/Aichi/2/68 and B/Mass/1/71 as test antigens (11). Pre- and postimmunization sera from all three groups were tested together for antibody to the individual pneumococcal types or influenza A and B viruses. Antibody responses and local and systemic reactions of the three vaccines in the volunteers were compared by appropriate statistical procedures (χ^2 or t test) to determine the significance of any observed differences.

Results. Following immunization with the pneumococcal polysaccharide-alone vaccine or the combination vaccine, mean pneumococcal antibody titers measured by RIA were substantially greater than preimmunization levels. In the group that received influenza vaccine, mean pneumococcal antibody levels remained essentially unchanged (Table I). Comparisons of n-fold increases of geometric mean titers (GMT) show them to be greater for each specific pneumococcal type in the pneumococcal vaccine group than in the combined vaccine group.

A comparison of mean pneumococcal antibody titers by type, 3 weeks following immunization with pneumococcal polysaccharide-alone vaccine or with the combination vaccine, demonstrated that overall antibody levels tended to be lower in volunteers who received the combination vaccine (Table I). For pneumococcal types 7 and 8, the differences were statistically significant when tested by the t test ($P < 0.02$). Antibody titers for pneumococcal types 3, 4, and 12 were lower (type 1 was slightly higher) in the group receiving the combination vaccine than in the pneumococcal polysaccharide vaccine group, but these differences were not statistically significant. Thus, these data suggest that the combination of inactivated influenza virus and pneumococcal polysaccharide exerted a slight suppressive effect on postimmunization pneumococcal antibody responses.

Frequencies of multiple pneumococcal antibody increases were similar in subjects who received pneumococcal polysaccharide vaccine alone or in combination with inactivated influenza virus. Approximately two-thirds of the vaccinees in each group developed twofold or greater antibody rises to five of the six pneumococcal types.

For each of the six pneumococcal types

TABLE I. PNEUMOCOCCAL ANTIBODY TITERS FOLLOWING IMMUNIZATION WITH PNEUMOCOCCAL VACCINE ALONE OR IN COMBINATION WITH INACTIVATED INFLUENZA VIRUS VACCINE

Vaccine group	Serum specimen	Mean antibody titer (ng of antibody nitrogen/ml) by RIA for individual pneumococcal type					
		1	3	4	7	8	12
SSS[a]	Pre	303	255	972	232	313	3079
	3 week post	1335	2308	4723	634	2265	6493
	n-Fold increase	4.4	9.1	4.9	2.7	7.2	2.1
FLU	Pre	318	923	1830	480	631	1596
	3 week post	310	805	1803	499	569	1685
	n-Fold increase	0.97	0.87	0.99	1.04	0.90	1.06
SSS–FLU	Pre	353	252	881	228	300	3248
	3 week post	1489	1691	3417	517	1550	5944
	n-Fold increase	4.2	6.7	3.9	2.3	5.2	1.8

[a] Abbreviations: SSS, pneumococcal vaccine; FLU, influenza virus vaccine; SSS–FLU, combined pneumococcal and influenza virus vaccine.

taken alone, an increase of 40% or more in serum antibody was noted in 87 to 100% of the subjects who received pneumococcal vaccine, in 57 to 91% of the recipients of combined vaccine, and in 0 to 10% of those who were given influenza virus vaccine alone (Table II). The numbers of vaccinees responding to types 1, 3, 4, 7, and 8 in the pneumococcal vaccine and combination vaccine groups were similar. Only 13 of 23 combination vaccinees responded to type 12 compared to 20 of 23 vaccinees who received pneumococcal vaccine (χ^2 (1) = 3.86, $P < 0.05$). The *n*-fold increase for type 12 antibody was lowest compared to the other types; it was only 2.1 in pneumococcal vaccinees and 1.8 in combination vaccinees. This probably reflects the high pre-vaccine mean type 12 antibody levels in both groups.

The influenza virus antibody responses to types A and B that were detected in vaccines following immunization with the bivalent inactivated influenza virus vaccine alone or in combination with pneumococcal polysaccharide vaccine were similar (Table III). A fourfold or greater rise in influenza virus HI antibody developed in more than two-thirds of the subjects in each vaccine group, and postimmunization geometric mean antibody titers to influenza virus were similar in the two vaccine groups.

The frequencies of local and systemic reactions were greater in vaccinees immunized with the combination pneumococcal polysaccharide and inactivated in-

TABLE II. VACCINEES RESPONDING WITH A 40% OR GREATER INCREASE IN SERUM ANTIBODY TO EACH PNEUMOCOCCAL POLYSACCHARIDE AS DETERMINED BY RADIOIMMUNOASSAY

Pneumococcal type	Vaccinees responding					
	SSS		SSS–FLU		FLU	
	No.	Percentage	No.	Percentage	No.[a]	Percentage
1	21/23	91	19/23	83	0/10	0
3	22/23	96	20/23	87	1/10	10
4	21/23	91	20/23	87	1/10	10
7	23/23	100	20/23	87	1/10	10
8	22/23	96	21/23	91	1/10	10
12	20/23	87	13/23	57[b]	0/10	0

[a] Ten of twenty-two in the group were assayed for antibody to each pneumococcal polysaccharide.

[b] Significantly fewer SSS–FLU vaccinees responded to type 12 compared to SSS vaccinees; χ^2 (1 df) = 3.86, $P < 0.05$.

TABLE III. INFLUENZA VIRUS ANTIBODY TITERS FOLLOWING IMMUNIZATION WITH INACTIVATED BIVALENT INFLUENZA VIRUS VACCINE ALONE OR IN COMBINATION WITH PNEUMOCOCCAL POLYSACCHARIDE VACCINE

Vaccine group	Serum specimen	Antibody titer by HI assay for indicated influenza virus strain expressed as reciprocal geometric mean titer	
		A2/AICHI/2/68	B/MASS/1/71
FLU	Pre	97	35
	3 week post	320	150
SSS–FLU	Pre	150	38
	3 week post	420	160

fluenza virus vaccine than they were in those who received only the pneumococcal polysaccharide vaccine or only the influenza virus vaccine. This increased reactivity was mainly attributable to systemic reactions which occurred in 7 of 23 volunteers who received the combination vaccine (Table IV). Two of these seven vaccinees had fevers of 102°F, three had fever of 99°F, and two had malaise without fever. Three of the vaccinees who received combination vaccine were sufficiently ill to remain away from work or school for 1 or 2 days. The systemic reactions included fever, malaise, chills, myalgias, and headache, while the local reactions were pain at the injection site, erythema, induration, and tenderness. Local reactions were especially severe in the subjects who received the combination vaccine. At the inoculation site, they mainly experienced intense pain, erythema, induration, and tenderness which frequently persisted 72 hr postinoculation. Local reactions had abated by that time in the pneumococcal polysaccharide-alone vaccine and the influenza virus-alone vaccine groups. None of the vaccinees who received either the pneumococcal polysaccharide or the influenza virus vaccine alone experienced local reactions to such a degree of severity.

TABLE IV. FREQUENCY OF LOCAL AND SYSTEMIC REACTIONS IN VACCINEES FOLLOWING IMMUNIZATION WITH PNEUMOCOCCAL POLYSACCHARIDE VACCINE ALONE OR IN COMBINATION WITH INACTIVATED INFLUENZA VIRUS VACCINE

Vaccine group	No. in group	Number of vaccinees with indicated reaction		
		Local	Systemic	Neither
SSS	23	10	2	11
FLU	22	7	4	11
SSS–FLU	23[a]	13	7	3

[a] Significantly more local and systemic reactions in the SSS–FLU group; χ^2 (4 df) = 10.75, $P < 0.05$.

Discussion. A pneumococcal polysaccharide vaccine comprising types 1, 3, 4, 7, 8, and 12, administered alone or in combination with bivalent inactivated influenza virus vaccine, induced twofold or greater rises in type-specific antibody (determined by RIA (10)) in most vaccinees. Our results are in accord with those of Smit and associates (12), who tested hexavalent (also dodecavalent) pneumococcal vaccine and observed that 74 to 95% of those immunized with the hexavalent vaccine responded (twofold or greater rise in antibody titer) to the individual polysaccharide types.

The number of our vaccinees in whom antibody increases developed varied with the pneumococcal type, and the order of types by decreasing frequency was 3, 8, 7, 1, 4, and 12. We determined that mean postimmunization levels of pneumococcal antibodies to types 7 and 8 in the group that received the combination vaccine were significantly less than the corresponding antibody levels in the group immunized with pneumococcal polysaccharide vaccine alone. Geometric mean antibody levels for types 3, 4, and 12 also were observed to be lower in the combination vaccine group, but the differences were not statistically significant. Influenza virus antibody responses were similar in the subjects who received either bivalent inactivated influenza virus vaccine alone or the combination vaccine. Although we tested only a hexavalent pneumococcal vaccine alone or

in combination with influenza virus vaccine, which was the available vaccine when we initiated these studies, it is unlikely that including more capsular polysaccharides in the combination vaccine would have made it less reactive or more antigenic.

Several clinical trials have been completed elsewhere in "at risk" and normal subjects (4–6, 12, 13) using polyvalent pneumococcal polysaccharide vaccines comprised of from 6 to 14 pneumococcal types. The results were encouraging in most instances and show that adequate antibody responses can be induced by pneumococcal polysaccharides administered parenterally.

None of the recent studies, however, has addressed the concept of a combination vaccine. Adults who are at high risk from pneumococcal pneumonia are also at high risk from influenza virus pneumonia and its complications; therefore, the use of a combination vaccine would have wide application in the immunization of these subjects and could effectively reduce mortality from both diseases. Although influenza virus vaccines are winter-use vaccines, with high-risk groups requiring reimmunization each year, pneumococcal vaccine is not. Combination vaccines have as a drawback the need for the influenza virus component to be administered annually. For maximum utilization and acceptability of a combination vaccine, any influenza-virus component used should provide long-lasting immunity like the pneumococcal polysaccharides and incorporate the prevalent H and N antigen types. As new influenza virus strains emerge, they may have to be given separately. Alternatively, two injections, one of pneumococcal vaccine and one of influenza virus vaccine, given at the same time but in different sites might cause less reactivity than a combination vaccine and the antibody responses to the pneumococcal antigens might be as good as those induced in individuals given only penumococcal vaccine. Under these circumstances, a combination vaccine would not be needed, thereby simplifying the manufacture and distribution of each vaccine. Carlson and colleagues recently administered to adults 14-valent pneumococcal polysaccharide vaccine and whole virus inactivated polyvalent influenza virus vaccine simultaneously in separate sites (14). Antibody responses of these vaccinees to the pneumococcal antigens and to influenza virus were little different from those observed in vaccinees who received only a single vaccine; local or systemic side reactions were no more frequent in the dual vaccine group. Post vaccine GMT for nine pneumococcal types were lower in the group of vaccinees who received the two vaccines, but only the type 8 level was significantly lower.

In a combination vaccine, the presence of two differing species of organisms might result in potentiation of the antigenicities and reactivities of both immunogens. Unfortunately, in this antigenicity trial the combination vaccine did produce disturbingly intense side reactions. The lower mean antibody responses of pneumococcal antibody following immunization with the combination vaccine suggests that certain combination vaccine preparations may also diminish the effect of the individual components. Perhaps these effects develop by interaction of widely different components or by the presence of impurities in the influenza virus vaccine. The effect of impurities in the influenza virus vaccine could be assessed by additional testing using column chromatography-purified vaccine, which is now available. However, combination vaccines of pneumococcal polysaccharides and influenza viruses do not appear to have practical applicability at this time. None the less, this investigation provides the basis for further study of the usefulness of such combined vaccines.

Summary. Sixty-eight normal subjects were immunized by the intramuscular route with either hexavalent pneumococcal polysaccharide vaccine, bivalent influenza virus vaccine, or a combined pneumococcal polysaccharide and influenza virus vaccine. Serum specimens collected before and 3 weeks after inoculation were analyzed by radioimmunoassay and hemagglutination-inhibition assay to determine antibody response. Although both the pneumococcal vaccine and the combined vaccine induced greater mean pneumococcal antibody titers

following immunization, the titers of five of the six pneumococcal types were lower in the combined vaccine group. Postimmunization influenza virus antibody titers were similar in subjects who received the influenza virus-alone vaccine or the combination vaccine. Local and systemic side effects occurred in more subjects of the combined vaccine group than in those of the single vaccine groups; however, fewer effects appeared in the individual pneumococcal vaccine group than in the influenza virus vaccine group.

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Received January 16, 1980. P.S.E.B.M. 1980, Vol. 163.