HUMAN IMMUNITY TO THE MENINGOCOCCUS

V. The Effect of Immunization with Meningococcal Group C Polysaccharide on the Carrier State

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The ideal vaccine not only protects the immunized host against clinical disease but also interrupts the chain of transmission of the pathogenetic organism, thereby protecting the nonimmunized members of the population. In the instance of a disease which is spread primarily by contact with a clinical case, a vaccine, if effective, will perform both functions. However, in order to decrease the rate of transmission of a disease which is spread primarily by the asymptomatic carrier, a vaccine must be able to interfere with the carrier state. This effect has been shown for several immunizing agents and may be correlated with the presence of secretory antibodies (1–6). A relevant example is the study of MacLeod et al. (7) showing that vaccination with purified pneumococcal polysaccharides not only prevents lobar pneumonia, but also results in a significant lowering of the carrier rate among the immunized population.

The preceding studies have shown that the group A and the group C meningococcal polysaccharides, if prepared in a high molecular weight form (8) are excellent immunogens in humans (9). It has also been shown that antibodies to the group C polysaccharide play a major role in naturally acquired immunity to meningococcal disease (10). There is reason, therefore, to hope that antibodies artificially induced with the purified polysaccharides will perform the same protective function as the ones produced as a result of the carrier state (11). However, it remains to be determined whether this immunization also will give rise to local immunity in the nasopharynx, and hence be of value in controlling the transmission of meningococcal disease. Towards this end, a small-scale field trial was carried out in military recruits to test the effect of immunization with meningococcal C polysaccharide upon the acquisition of group C meningococci.

Materials and Methods

Design of the Experiment.—The experiment to test whether immunization with the C antigen would prevent the acquisition of group C meningococci was initiated in three basic training companies at Fort Dix, N. J., at the end of April 1968. The recruits had been on

the military base for 9 or 10 days at the time the first nasopharyngeal culture and serum specimen were obtained. The purpose of the study and the nature of the vaccines were explained and the written consent of each volunteer was obtained. 50 men from Company B-6-3 and 50 from Company E-2-3 were selected at random from a greater number of volunteers and vaccinated with the C antigen. 53 additional volunteers were injected with A polysaccharide. Only 45 men in Company E-5-3 volunteered and all were injected with the C antigen. The numbers of immunized recruits and controls are shown in Table I.

All available recruits in the vaccinated and control groups were cultured 2, 4, and 6 wk after immunization; serum was obtained at 2 and 6 wk. In the course of the study, 5.9% of the population was lost to follow-up for various reasons.

Serological Methods.—Sera were stored in the frozen state. Recruits in Companies E-5-3 and E-2-3 were bled 12 days after immunization, whereas Company B-6-3 was bled at 14 days. These sera will be referred to as the 2nd wk sera. Passive hemagglutination with human erythrocytes sensitized with the C antigen was performed as described before (8). However, glutaraldehyde-fixed cells sensitized with A antigen (8) were found to be nonspecifically

TABLE I

Distribution of Recruits Injected with Group A or Group C Meningococcal Polysaccharide

Company	Number immunized with		Controls	Total
	A antigen	C antigen		
B-6-3	17	50	181	248
E-5-3	0	45	185	230
E-2-3	36	50	117	203
Total.....	53	145	483	681

agglutinated by these frozen and thawed sera. This did not occur when fresh unfixed red blood cells were sensitized with the A antigen. The donor of the fresh cells was the same person from whom the aldehyde-fixed cells were obtained.

Antibody was also measured by the bactericidal reaction as described previously (11).

Bacteriological Methods.—Nasopharyngeal cultures were performed as described by Artenstein et al. (12) except that chocolate Mueller-Hinton agar (13) was used. Meningococci were identified by colonial appearance, examination of a Gram-stained smear and carbohydrate fermentations. The serological grouping was performed both by the slide agglutination method with hyperimmune rabbit sera (14), and by the hemagglutination inhibition method described previously (8). In the occasional instance of disagreement between the two procedures, the results obtained with the hemagglutination inhibition test were accepted.

Immunization.—Recruits were injected intradermally with 0.2 ml of isotonic saline containing 50 μg of either group A polysaccharide lot A-5 or group C polysaccharide lot C-5 (8). Skin reactions were measured by a single observer at 24 and 48 hr after immunization. Each injected volunteer was questioned concerning systemic symptoms.

Statistical Methods.—The chi square (χ^2) method was used to evaluate the differences in carrier or acquisition rates of the different populations. The t test with the correction for small samples was used to test the differences in geometric means of reciprocal hemagglutination titers. All calculations were done on the logarithms of the titers (15).

RESULTS

The Epidemiological Setting.—A very high incidence of meningococcemia and meningitis, all attributable to group C organisms, occurred in the winter and spring of 1968 among recruits undergoing basic infantry training at Fort Dix, N. J. Consequently, the incidence of meningococcal carriers among these recruits was studied. Two companies in basic training were followed with throat cultures from February 13 to April 2. The results are presented in Table II which shows the per cent of the population which carried group C meningococci, the per cent which carried meningococci other than group C, and the total carrier rate. A sample of 50 men from each company of 200 men was followed.

TABLE II

Prevalence of Group C and Other than Group C Meningococci in Two Basic Training Companies

Company	Week of training	Per cent of cultures positive		
		Group C	Not group C	Total
B-1-3	1 st	6	25	31
	3rd	48	22	70
	8th	75	11	86
D-2-3	1st	10	14	24
	3rd	50	8	58
	8th	76	14	90

There was a rapid spread of group C organisms among the recruits, so that the final cumulative rate (the percentage of recruits carrying group C meningococci at any time during the period of observation) was 82% in Company D-2-3, and 75% in B-1-3. These levels for the meningococcal carrier rates were not unusual for a military population during the winter months. However, the preponderance of group C organisms was a situation not observed heretofore. This epidemiological setting provided the opportunity to test whether immunization with the C antigen would prevent the acquisition of group C meningococci in the nasopharynx.

The Antibody Response to Immunization with A and C Antigens.—145 recruits were injected intradermally in the forearm with group C polysaccharide and 53 with group A polysaccharide. By 24 hr every vaccinated subject had a skin reaction consisting of an area of erythema varying from 2 to 7 cm in diameter. These sites were usually slightly tender and occasionally edematous. In general the reactions elicited by the A antigen were less pronounced than the ones resulting from the C antigen. None of the recruits reported any systemic symptoms. The

reactions faded over the next 24 hr and at 2 wk only a small area of pigmentation remained in a few individuals.

Antibody production was measured by assaying the hemagglutinating and bactericidal activity of the 2nd wk sera. The sera of two of the members of the

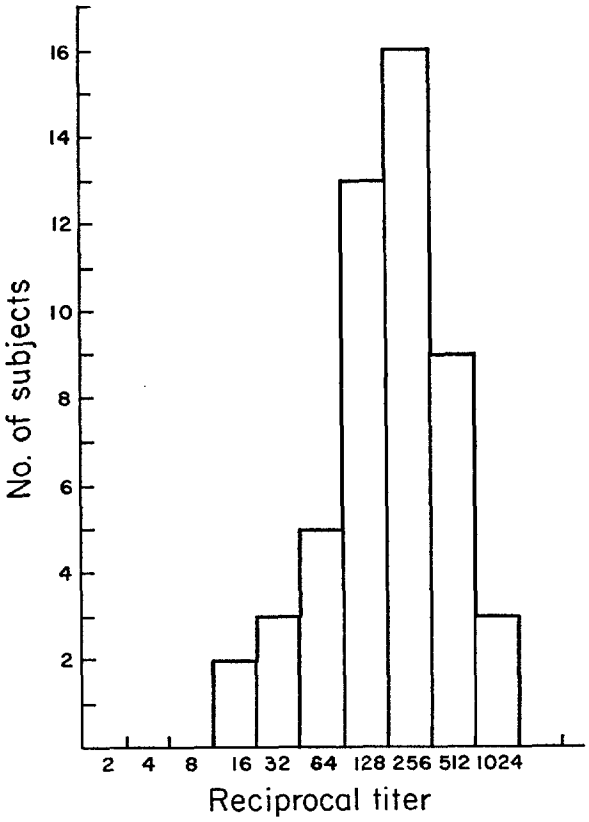

FIG. 1. The distribution of the hemagglutination titers of sera obtained from recruits immunized with group A polysaccharide.

A vaccinated group were not available. Interpretation of the hemagglutination response to the injection of the A antigen was not complicated by responses to the carrier state, since no group A organisms were found among the recruits. The histogram in Fig. 1 indicates the distribution of the reciprocal hemagglutination titers observed. The geometric mean was 182, which should be compared to the geometric mean of 7.4 of the titers observed on the preimmunization bleeding. This latter figure is included only to illustrate the magnitude of the average rise in titer and should not be construed as an indication that the first bleedings did or did not contain antibodies to A substance.

Data on the bactericidal activity of sera obtained two weeks after immunization with A polysaccharide are more difficult to interpret. 25 of the 51 available recipients of A substance were carriers of meningococci at the time the 2nd wk sera were obtained. All these sera had a titer of 1/64 or greater against strain A1. However, it has been shown that the majority of these individuals would be expected to show increased bactericidal activity against group A meningococci as a consequence of their carrier state (10). Of the 26 recruits who did not be-

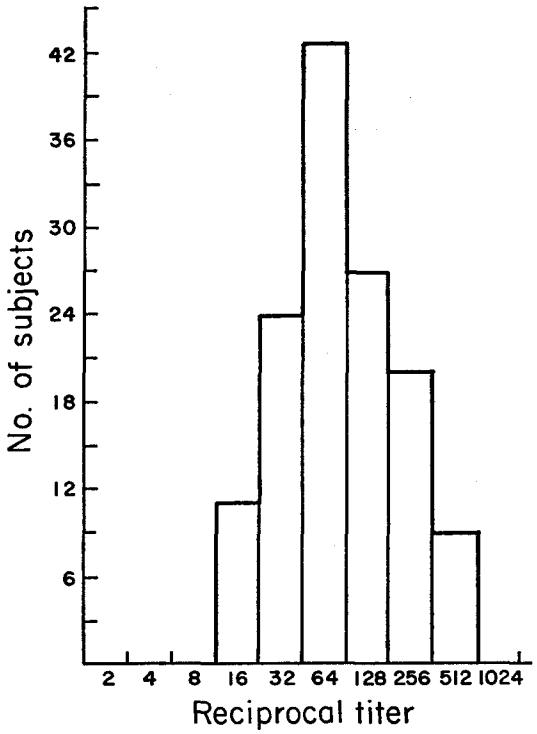

FIG. 2. The distribution of the hemagglutination titers of sera obtained from recruits immunized with group C polysaccharide.

come meningococcal carriers within the first 2 wk of the study, all but two showed significant increases in serum bactericidal activity to meningococci of strain A1. In this group, reciprocal bactericidal titers of the preimmunization sera were 16 or less, those of the 2nd wk sera 64 or more. The two men who showed no significant increase in bactericidal titer also had the lowest hemagglutination titers. They should probably be considered immunization failures.

To obviate problems in the interpretation of the antibody response to the injection with group C polysaccharide, all immunized recruits who carried group C meningococci at the beginning of the experiment, or acquired them within the

first 2 wk of the study, were excluded from this part of the analysis. This amounted to 11 men, or 7.6% of the population of 144. One vaccinated recruit was lost within the 1st wk of the study due to a preexisting medical problem. Fig. 2 shows the distribution of the reciprocal hemagglutination titers observed. The geometric mean of the titers of the preimmunization bleedings was 1.0; that of the 2nd wk sera was 82.

46 of the 133 recruits immunized with C antigen were carriers of meningococci other than group C during the first 2 wk of the study. Although all these individuals developed a reciprocal bactericidal titer of 64 or greater against a group C organism, strain C11, it cannot be stated whether this was in response to the vaccination or to the carrier state (10). The remaining 87 vaccinated recruits who were not meningococcal carriers during the first 2 wk of the study all developed bactericidal activity against strain C11, with reciprocal titers of 64 (4 men) or more (83 men). The preimmunization reciprocal titers were 16 or less.

The bactericidal and hemagglutination data indicate that all the recruits in the population of 133 who did not carry group C meningococci during the first 2 wk of the study reacted positively to immunization with C polysaccharide. There is no unequivocal method to decide whether the 11 recruits in the C-vaccinated group who were carriers of group C meningococci during the first 2 wk of the study responded to the vaccine or to the carrier state. In either event, hemagglutination and bactericidal titers were elevated in each member of this group.

Effect of the Injection of Group-Specific Polysaccharides upon the Acquisition of Meningococci in the Nasopharynx.—There was considerable variability in the prevalence of meningococci of various serogroups between the three basic training companies. Therefore, each company was considered separately in evaluating the cultural data. The results are set forth in Table III, showing the meningococcal carrier rates observed among the various experimental groups. Company E-2-3 had the most rapid spread of group C organisms among its members. Initially, the C carrier rates were low in all companies and evenly distributed among the C-vaccinated, A-vaccinated, and uninjected groups. But by the 2nd wk and at all times thereafter the group injected with C antigen had a lower C carrier rate than did the control group. These differences were in most instances statistically significant as indicated in the table. In addition, the prevalence for all types of meningococci tended to be lower in the group vaccinated with C antigen, whereas the prevalence of meningococci other than group C tended to be higher. This suggested that the depression of the total carrier rate due to the lower prevalence of group C organisms among the recruits vaccinated with the C antigen was partially offset by an increased prevalence of meningococci not belonging to group C. There was considerable variability in the prevalence of meningococci in the A immunized group in Company B-6-3 which was probably

due to the small size of that population. Nevertheless, from the results obtained both in Company B-6-3 and in Company E-2-3, it is clear that immunization with the A antigen did not lower the C carrier rate.

The results were also treated in terms of the cumulative acquisition rate of group C meningococci, since this allows a summary of the data over the whole 6 wk period of observation. Table IV presents the percentage of men in each population who acquired group C meningococci in the 6 wk period following

TABLE III

Prevalence of Meningococci in the Nasopharynges of Recruits Immunized with A Antigen, C Antigen, and of Controls

Company	Per cent of cultures positive											
	1st culture			2nd culture			3rd culture			4th culture		
	Group C	Not group C	Total	Group C	Not group C	Total	Group C	Not group C	Total	Group C	Not group C	Total
Co. B-6-3												
Controls	2.7	20	23	11	23	34	23	36	59	28	28	56
A vacc.	0.0	47	47	5.9	18	24	29	57	86	24	47	71
C vacc.	6.0	24	30	2.0*	22	24	8.7*	37	46	17	35	52
Co. E-5-3												
Controls	0.54	5.4	5.9	3.4	26	30	19	29	48	29	21	49
C vacc.	2.2	4.4	6.6	2.2	29	31	4.5*	45*	50	7.0‡	35	42
Co. E-2-3												
Controls	5.1	20	25	30	23	53	50	23	73	43	18	61
A vacc.	2.7	8.3	11	36	17	53	51	20	71	39	14	53
C vacc.	4.0	28	32	10‡	28	38	24‡	28	52‡	14§	27	41*

The percentages with footnote symbols are significantly different from the control group.
* $P < 0.05$.
‡ $P < 0.01$.
§ $P < 0.001$.

immunization with the polysaccharides. During the period of this study, a considerably lower percentage of recruits immunized with the C antigen acquired group C meningococci than did either the men injected with A antigen or the uninjected controls. The differences between the uninjected groups and the C-vaccinated groups were statistically highly significant.

The Relationship between Serum Antibody and the Acquisition of the Meningococcal Carrier State.—The serum antibody response to immunization with C antigen was measured by passive hemagglutination on 133 sera obtained 2 wk after injection with the C polysaccharide. As stated in a previous section, the

mean reciprocal titer rose from 1.0 to 82. None of these recruits carried group C meningococci in the 2 wk period preceding this bleeding, but 22 of them acquired group C organisms in the subsequent 4 wk of the study.

In order to determine whether there is a positive correlation between the serum antibody response and local nasopharyngeal defenses against the meningococcal carrier state, the mean of the reciprocal hemagglutination titers of the 2nd wk bleedings of the 22 vaccinated subjects who became C carriers, and of those who did not, was calculated separately and compared, using the t test. The mean of the first group was 47 and of the second group 91. The difference between these means is statistically significant ($P = 0.001$).

TABLE IV

Per Cent of Recruits in Each Group who became Carriers of Group C Meningococci

Company	Controls	A-vaccinated	C-vaccinated
	%	%	%
B-6-3	42	37	24*
E-5-3	38	—	4.6§
E-2-3	69	68	31§

The percentages with footnote symbols differ significantly from the control group.

* $P < 0.05$.

§ $P < 0.001$.

DISCUSSION

The studies reported in the present series have indicated that the susceptibility of the human being who develops meningococcal disease is due, at least in part, to the lack of circulating antibodies to the particular strain of meningococcus with which he becomes infected (11). The natural immunity which the majority of the adult population enjoys probably depends on repeated nasopharyngeal contact with the meningococcus. The carrier state has been shown clearly to be an immunizing process which gives rise to antibodies to several meningococcal antigens, among them the group-specific polysaccharides (10). The interest of the latter antigens is that they have been partially defined immunochemically and that a method has been developed for producing them in large molecular weight form and high purity (8). They have proved to be good immunogens in humans giving rise to antibodies belonging to the three major classes of immunoglobulins. The sera of the injected volunteers became highly bactericidal to all meningococci belonging to the group represented in the vaccine. This response has remained essentially undiminished for as long as 8 months (9). This is in keeping with the studies showing that, in man, the precipitating antibody response to pneumococcal polysaccharides persists for at least 3 yr and the demonstration that the bactericidal activity against meningococci is mediated by antibodies belonging to the immunoglobulin G class (16, 9).

It should be noted that the mean hemagglutination response of the recruits to these antigens was less than that of six laboratory volunteers (9). The explanation for this is unknown. One factor which should be considered in future studies is that the recruit population may have experienced the phenomenon of antigenic competition, as they had all their routine immunizations immediately preceding the injection with polysaccharide (17, 18).

The present report has delineated another desirable property of the group C polysaccharide when used as an immunogen in a population with a high meningococcal transmission rate. It has shown that systemic immunization with this material gives rise to increased local defenses in the nasopharynx against meningococci, as evidenced by the fact that a significantly lower percentage of vaccinated recruits became carriers of group C meningococci when compared to the control group. This difference applies only to organisms belonging to group C. The specificity of this local immunity suggests that a recognition mechanism is involved, and it seems plausible that secretory immunoglobulin A antibody accomplishes this function. Studies are in progress to determine whether immunization with the meningococcal polysaccharide gives rise to the production of secretory antibody. Whether other mechanisms are involved in making the nasopharyngeal environment unsuitable for the group C meningococci is unknown. One possibility is that there is a selective pressure causing the group C meningococcus rapidly to lose the property of making the C antigen. These organisms would then be untypable. This hypothesis was tested by seeing whether the proportion of untypable meningococci among the organisms isolated was significantly higher in the group vaccinated with the C antigen. No significant differences were noted, indicating that this hypothesis is not tenable.

An intriguing sidelight of these studies was the observation that there is a correlation between the degree of serum immunity as measured by passive hemagglutination and the degree of local nasopharyngeal resistance to the acquisition of group C meningococci. Such a correlation has been noted previously for poliovirus (2), rhinovirus (19), and streptococci; in the latter instance, it has been shown that preexisting antibody to the M protein markedly lowered the rate of throat infection with streptococci of the homologous type (20).

Four recruits from the companies studied developed meningitis. These individuals had not been vaccinated with either polysaccharide. Because of the relatively small size of this study, no significance can be attached to this occurrence. Much larger field trials will be required to determine whether administration of these antigens prevents meningococcal disease. Hospitalizations for other illnesses (mainly acute respiratory disease) were also monitored and these were equally common in the immunized and uninjected recruits.

Group A meningococci at the present time are very rarely isolated in the United States, and it was impossible to determine whether immunization with this polysaccharide would interfere with the acquisition of group A meningococci. This important information should be obtained as soon as possible by a

field trial in a location where there is a high prevalence of group A strains. This knowledge would be helpful in the planning of a field trial to test the effectiveness of these polysaccharides as vaccines for the prevention of meningococcal disease. The success of such studies might be jeopardized by immunizing too large a proportion of the population, as it seems likely that this measure would effectively diminish the transmission of meningococci in the control populations. There is, in fact, evidence that just such a phenomenon occurred in the study by MacLeod et al. (7) where half the population was vaccinated with pneumococcal polysaccharides. It should also be borne in mind that the group A meningococcus has been responsible for the majority of epidemic meningococcal disease, and now that sulfonamide-resistant strains have been isolated overseas (21), this organism again represents a major public health hazard.

SUMMARY

Purified meningococcal polysaccharides were administered to army recruits. No adverse reactions were observed in 145 men who received group C polysaccharide, and in 53 men who were injected with group A polysaccharide. Hemagglutinating and bactericidal activity developed in the sera of all individuals with the exception of two recruits injected with A polysaccharide.

During the 6 wk period of observation, the proportion of unvaccinated recruits who acquired group C meningococci in the three companies studied was 38, 42, and 69 per cent. A significantly lower proportion of the individuals vaccinated with group C polysaccharide acquired group C meningococci; 4.6, 24, and 31 per cent respectively.

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