Antibody Responses to Meningococcal Polysaccharide Vaccines

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Over the past 4 years 19 lots of group C polysaccharide vaccine and five lots of group A polysaccharide vaccine have been tested for their immunogenicity in man. For each lot tested, groups of 18 to 50 men received 50 μg of vaccine subcutaneously. Sera were obtained prior to and 2 weeks after vaccination. The analytical and serological methods used in these studies were Sepharose 4B chromatography for the estimation of molecular size, the radioactive antigen binding assay, and the indirect hemagglutination (IHA) test for measuring the antibody response. Results have shown that the radioactive antigen binding assay is preferable to the IHA test as a measure of antibody response. Group C meningococcal vaccines have been highly stable when stored at 4 C in powdered form. All lots of group C vaccine tested to date have been of equal potency, with molecular weight varying from 520,000 to 2,000,000. Group A polysaccharides have been found to be unstable after 2 years of storage at 4 C. Optimal antibody response to the group A vaccines appears to be directly related to the molecular size of the preparation.

Materials and Methods

Vaccines. The group A and C vaccines were prepared by basic methodology described by Gotschlich et al. (14) and Berman et al. (7). Lots A-7, A-8, C-7, C-8, and C-9 were prepared by the Squibb Institute for Medical Research (under contract with the U.S.A. Medical Research and Development Command); lots 439-A, 440-A, 419-C, 420-C, 421-C, 422-C, 423-C, 424-C, 426-C, 438-C, 487-C, 488-C, 489-C, 490-C, 491-C, 512-C, and 513-C were prepared by Merck Sharp & Dohme Research Laboratories (under the same contract as for Squibb). Lots 1227-A and 1228-C were prepared by Merrell-National Laboratories and were kindly provided by James Sorrentino. All group A vaccines were prepared from a single strain, A-1; group C vaccines were derived from cultures of strain C-11 (14).

Subjects, vaccinations, and collection of specimens. Army recruits in the first week of basic training received either group A or group C vaccine subcutaneously in a dose of 50 μg. Vaccine lots were administered in sequence rather than by random selection. The number of subjects in any vaccine group varied from 18 to 50, depending upon the total number of recruits in the company unit that day. In accordance with Army regulations informed consent was obtained from all volunteers who received group A polysaccharide vaccines and from those who received group C vaccines prior to its designation as a mandatory procedure. Serum specimens were obtained prior to and 2 weeks after vaccination, except...
where specifically stated, and were stored at -20 C prior to and between testing. Pharyngeal cultures were obtained prior to vaccination and at the time of each postvaccination venipuncture. Isolates were characterized by techniques described previously (11). The very few individuals shown to be nasopharyngeal carriers of group C meningococci were found during these studies. Because only three or four lots of vaccine were tested at any one time, a control group which received a previously tested lot of vaccine was included in each study.

**Molecular sizing of vaccines.** Molecular size of vaccine preparations was determined by gel filtration. Chromatography was accomplished by using a 2.5 x 36 cm column of Sepharose 4B (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) equilibrated with 0.05 M tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 7.4. Flow through the column was in the ascending direction, and the flow rate was maintained at 12.5 ml/hour by using a polystaltic pump (Buchler Instruments, Fort Lee, N.J.). Samples (2 mg of group C polysaccharide or 8 mg of group A polysaccharide) were applied by using a sample injection valve loop (Chromatronic, Inc., Berkeley, Calif.). Fractions of 2.5 ml were collected in a Fractomette 200 fraction collector (Buchler Instruments, Fort Lee, N.J.). The void volume of the column was determined with blue dextran (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.), and the total volume was determined with 14C-labeled sodium acetate; dextran T-500, T-250, T-150, and T-70 were used for calibration. The average distribution coefficient ($K_v$) was calculated from the peak elution volume of the polysaccharide (Sepharose-Agarose gels in bead form, Pharmacia Fine Chemicals, Inc.). The column was monitored for carbohydrate content in the dextran fractions by the anthrone method (17), phosphorus by the method of Chen et al. (9) for the group A vaccines, or sialic acid in the group C vaccines by the method of Svennerholm (19).

**Serosal studies.** The serum specimens collected were tested for antibodies by either the indirect hemagglutination (IHA) test or by both the IHA and the radioactive antigen binding assays (RABA).

The IHA test used polysaccharide-coated human erythrocytes and was performed as previously described (4). The RABA was a modification of the method described by Brandt et al. (8); namely, a titration of 14C-labeled polysaccharide in undiluted serum. An end point was determined in the range of 40 to 60% binding of antigen, which is the portion of the antigen titration curve where the response is linear.

Samples were dissolved in NCS solubilizer (1 ml) (Amersham/Searle, Des Plaines, III.), washed into a counting vial with 9 ml of toluene (Matheson, Coleman and Bell, Norwood, Ohio) and Liquifluor (4.2%, vol/vol) (New England Nuclear Corp., Boston, Mass.), and then were counted at 4 C in a Packard TriCarb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). After the background counts/min were subtracted from both the test serum counts/min and the antigen control counts/min, the quantity of antigen bound was calculated by the following equation: test serum counts per minute per antigen control counts per minute = fraction of antigen bound x specific activity of antigen (counts per minute per nanogram)/antigen control counts per minute = nanograms of antigen bound per 50 uliters of serum. The specific activity of the group C antigen was expressed as counts per minute per nanogram of sialic acid, whereas the specific activity of the group A antigen was counts per minute per nanogram of A polysaccharide. The A polysaccharide concentration was calculated on the basis of its phosphorus content of 8% (14).

**Statistical analysis.** The results of the RABA analysis of each experimental group were distributed in a skewed pattern; therefore, these data were transformed into log_{10} values which were normally distributed. The mean, variance, and correlation coefficients were computed on a Hewlett-Packard 9810A calculator (Hewlett-Packard Calculator Products Div., Loveland, Colo.). The unpaired Student's $t$ test and the analysis of variance (18) were used to compare two geometric mean titer increases or groups of geometric mean titer increases.

**RESULTS**

**Variation in individual response.** After vaccination with purified meningococcal polysaccharide antigens, there was a wide variation in individual response. Figure 1 shows pre- and postvaccination responses of 24 volunteers to meningococcal polysaccharide antigen. All men received the same dose of the same lot of vaccine on the same day.

![Fig. 1. Individual variability in antibody responses of 24 volunteers to meningococcal polysaccharide antigen. All men received the same dose of the same lot of vaccine on the same day.](http://iai.asm.org/)
two-week postimmunization antibody titers in a group of 24 young adult volunteers who received a single subcutaneous injection of 50 μg of lot C-7 group C polysaccharide. Both the IHA and RABA assays showed the phenomenon. Similar patterns have been observed with all group C and A vaccines tested.

**Comparison of IHA and RABA.** A positive relationship between the RABA and IHA has been noted. By using approximately 70 pairs of sera (pre- and postvaccination) for each serogroup, a positive correlation coefficient of 0.928 ($P < 0.001$) has been calculated for the group C tests and 0.766 ($P < 0.001$) for the group A tests (Fig. 2).

**Antibody response to group A and C vaccines as measured by IHA.** Over the past 4 years, 19 lots of group C polysaccharide vaccine and five lots of group A polysaccharide vaccine were tested for their immunogenicity in man. On

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**Fig. 2.** Correlation of hemagglutination and radioactive antigen binding assays for antibodies to groups A and C polysaccharides. For each figure two vaccine groups are represented.
the basis of the IHA tests, no significant differences in the immune response were observed between any of the test lots of vaccine with one exception; lot A-439 induced a fourfold rise in IHA titer in only 50% of recipients. All other group A vaccines provided fourfold increase in ≥ 86% of the men.

**Stability of polysaccharide vaccines upon storage.** Stability of polysaccharide vaccines has been tested over a period of 2 to 3 years after storage of the lyophilized, final, packaged product at 4°C. Results of RABA tests after immunization of groups of men with lot C-9 at intervals over a 2-year period are shown in Table 1. Two measures of response were examined: (i) geometric mean titer (GMT) increase and (ii) proportion of subjects showing various-fold increases. From inspection of the data it is apparent the potency did not decline under these conditions of storage. Overall analysis of variance confirmed that there was no significant difference among the six GMTs.

Group A vaccines, lots A-7 and A-8, had been stored in the lyophilized state at 4°C for 3 and 2 years, respectively. Antibody responses of volunteers (groups of 18 to 36 men) immunized in successive years are shown in Fig. 3. RABA GMTs at varying intervals after a 50-μg dose of lot A-7 showed no significant changes during the first 2 years of storage at 4°C. Peak response occurred at about two weeks and fell slightly after 7 weeks. The GMT increase of men vaccinated in 1972 was significantly lower than the response observed in 1969 (P < 0.005). Lot A-8 did not appear to deteriorate in the 1st year of storage (although strict comparisons cannot be made because sera were obtained at 11 days postvaccination in 1971 rather than at 14 days). However, the 14-day response was significantly lower after 2 years of storage (P < 0.005). When analyzed on the basis of fold increases in titer (Fig. 4), the data for lot A-7 showed less striking changes with time of storage, although many fewer men had fivefold or greater antibody rises after 3 years. The poor response to lot A-8 after 2 years of storage is also very apparent. Differences in various lots of vaccine can also be categorized on the basis of percentage of men who failed to show a twofold increase in titer at 14 days postvaccination. For example, lot 439-A gave a 38% failure rate; lot 1227-A gave 3%.

**Molecular-size determination.** Molecular weight (mol wt) estimations were determined by a comparison of the elution volume profile of vaccines with the elution volume profile of

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**Table 1.** Antibody responses (RABA) to group C meningococcal vaccine (lot C-9, Squibb) over 2 years

<table>
<thead>
<tr>
<th>Date tested</th>
<th>No. of men</th>
<th>Geometric mean titer increase*</th>
<th>Fold rise(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug. 1970</td>
<td>30</td>
<td>335</td>
<td>≥2</td>
</tr>
<tr>
<td>Nov. 1971</td>
<td>25</td>
<td>389</td>
<td>≥10</td>
</tr>
<tr>
<td>Feb. 1972</td>
<td>43</td>
<td>331</td>
<td>≥100</td>
</tr>
<tr>
<td>July 1972</td>
<td>20</td>
<td>258</td>
<td>≥500</td>
</tr>
<tr>
<td>Sept. 1972</td>
<td>33</td>
<td>315</td>
<td></td>
</tr>
<tr>
<td>Nov. 1972</td>
<td>38</td>
<td>318</td>
<td></td>
</tr>
</tbody>
</table>

*Increase of serum-binding capacity (post minus prevaccination) expressed in nanograms of antigen bound.

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![Fig. 3. Mean radioactive antigen binding assay responses to group A polysaccharide vaccines after storage. Each lot of vaccine was initially tested within a few months of its preparation.](http://iai.asm.org/Downloaded from http://iai.asm.org on September 13, 2017 by guest)

![Fig. 4. Percentage of men showing radioactive antigen binding assay antibody increases following inoculation of group A vaccines. Each lot of vaccine was initially tested within a few months of its preparation.](http://iai.asm.org/Downloaded from http://iai.asm.org on September 13, 2017 by guest)
known dextran standards (Fig. 5). The range in mol wt for the group C vaccines was \(2.2 \times 10^6\) to \(5.2 \times 10^4\). Fig. 6A presents the mol wt of 11 lots of group C vaccine and the antibody response as measured by RABA. By overall analysis of variance, no significant difference between GMT increases was found. These data suggest that group C polysaccharide vaccines of \(5.2 \times 10^5\) to \(2.2 \times 10^4\) mol wt will be of equal immunogenicity.

The mol wt of the group A vaccines was determined in 1972. At this time A-7 vaccine had been stored for 3 years at 4°C and A-8 vaccine for 2 years. The range in mol wt for the five lots of A vaccine was \(4.2 \times 10^5\) to \(1.4 \times 10^5\) (Fig. 6B). The number of individuals in each test group was as follows: A-7, 35; A-8, 27; 439-A, 34; 440-A, 30; 1227-A, 33. When antibody response (GMT increase) of these five lots of A vaccine was analyzed by the overall analysis of variance, a significant \(F\) value was calculated (\(F = 3.75, df 4, 158\)). Thus, the GMT increases among these five lots were found to be significantly different. Because of unequal sample sizes, no further statistical analysis was made.

![Fig. 5. Sepharose 4B elution profile of lot C-7 polysaccharide and dextran standards.](image)

![Fig. 6. Antibody responses to group A and C polysaccharide vaccines as a function of molecular size.](image)
It should be noted, however, that lot 439-A, which had the smallest mol wt, was also least immunogenic (38% failed to develop a twofold antibody increase; Fig. 4). Those lots of largest mol wt (A-7 and 1227) had the least number of failures (9 and 3%, respectively).

**DISCUSSION**

The IHA test, because of its simplicity, has been used as the major antibody assay for comparison of various lots of vaccines, dosages, and routes of administration (4). RABA was subsequently developed to attain greater sensitivity and more precise quantitation (8). Comparison of the two assays has shown a significant positive correlation. However, the analysis of IHA test results failed to detect loss of potency in group A vaccines after prolonged storage, a finding which was distinctly shown when the RABA test was used. Thus, the IHA test is no longer recommended for this type of investigation.

At the time of the present writing all lots of group C polysaccharide vaccine tested were of equal immunogenicity in man; molecular sizing by the Sepharose 4B method described has shown mol wt values of 5.2 × 10⁶ to 2.2 × 10⁶, based upon dextran standards; one lot tested on many occasions over a 2-year period of storage has shown no loss of potency. Group A polysaccharide vaccines have been less stable; significant losses in potency, as measured by antibody responses in groups of young adults, were observed after 2 and 3 years of storage. Storage of lyophilized powder at -20 C or lower is recommended for this vaccine, and further studies of this problem are needed.

Molecular sizing of meningococcal polysaccharides is still an imprecise science. Chromatography though Sepharose 4B has provided one means of recording relative sizes of various preparations based upon comparisons with dextran standards. Further improvements in methodology are needed to more accurately determine true mol wt of these compounds. Nevertheless, the current data suggest that group C polysaccharides with estimated mol wt of 5.2 × 10⁶ or greater are of similar potency in man. Group A vaccines with an estimated mol wt of 1.4 and 1.6 × 10⁶ were less immunogenic than lots of vaccine of 2.5 and 4.2 × 10⁶. However, lot A-7 which had a mol wt of 4.2 × 10⁶ in 1972 had been even more immunogenic 2 years earlier (when mol wt data was not available), suggesting that it had even greater molecular size at that time. Therefore, more definitive data on mol wt-potency comparisons are necessary to establish vaccine specifications.

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**LITERATURE CITED**

15. Gotschlich, E. C., M. Rey, R. Triau, and K. J. Sparks. 1972. Quantitative determination of the human immune response to immunization with meningococcal...