THE RELATIONSHIP BETWEEN GROUP A AND GROUP C Meningococcal Polysaccharides AND SERUM OPSONINS IN MAN*. †

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Meningococcal infections continue to pose a serious medical and public health problem, due in part to the emergence of sulfa-resistant organisms (reviewed in reference 1). Since antibiotics no longer are regularly effective in eradicating meningococci from the nasopharynx of carriers (2), protection of a population from clinical infection may well necessitate acquired immunity.

Opsonization of microorganisms is considered one of the major protective functions of antibodies in the host defense system. By this mechanism, homologous opsonins specifically promote phagocytosis of bacteria by combining with antiphagocytic surface antigens (3). Such antigens have been well defined for two pathogens, the Group A streptococcus (type M protein) and the pneumococcus (type-specific capsular polysaccharide) (4, 5).

Previous studies have demonstrated that phagocytosis of meningococci by rabbit polymorphonuclear leukocytes occurs only in the presence of antisera from rabbits immunized with log-phase organisms, suggesting that these bacteria may also have surface antigens which exert an antiphagocytic effect (6). Gotschlich, Liu, and Artenstein recently purified polysaccharides from meningococci of groups A and C (7), and detected hemagglutinating and bactericidal activity in sera of human volunteers immunized with 50 μg of these polysaccharides (8).

This report describes the interaction in vitro between human granulocytes and meningococci in the presence of sera from these immunized volunteers. In addition, the relationship between groups A and C polysaccharide and meningococcal opsonins in sera from naturally infected individuals was also studied. The findings indicate that meningococcal polysaccharides produce group-specific opsonins in man and that these polysaccharides are the major antiphagocytic determinants for group A and group C meningococci.

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Materials and Methods

Prototype group A (A-1), B (B-11), and C (C-11) meningococcal strains, group A and group C polysaccharides, and sera from immunized volunteers were kindly supplied by Dr. M. S. Artstein, Department of Bacterial Diseases, Walter Reed Army Research Institute, Washington, D.C., and Dr. E. C. Gotschlich, The Rockefeller University, New York. Other meningococcal strains and human sera were generously provided by Dr. H. Feldman, Dr. D. Ivler, Dr. M. Schaeffer, and Dr. J. G. Hirsch.

Leukocyte suspensions were prepared from blood drawn from normal volunteers and mixed with an equal volume of physiologic saline containing 0.02% heparin (Connaught Medical Research Laboratories, Toronto, Canada) and 2% Dextran 250 (Pharmacia Fine Chemicals, Uppsala, Sweden). Tubes with this mixture were slanted at 45° for 15 min at room temperature to allow for red cell sedimentation. The leukocyte-rich plasma was then collected and the volume and granulocyte count determined. Leukocytes were sedimented at 200 g for 10 min, washed first in heparinized saline and then in saline alone, and resuspended to the desired concentration in sterile Gey's balanced salt solution (Microbiological Associates, Inc., Bethesda, Md.) with 0.1% gelatin (Difco Laboratories, Inc., Detroit, Mich.).

Broth cultures of meningococcal strains with less than five serial passages after primary isolation were stored in small portions at −65°C. Maintenance of daily cultures and the preparation of bacterial suspensions for phagocytosis have been described previously (6). In the present studies, 2–21 μl hr-shaking broth cultures were used for phagocytosis experiments. Human sera were stored in small portions at −20°C and thawed immediately before each experiment. Immune sera frozen for more than 4 wk were inactivated at 56°C for 30 min prior to each test and supplemented with 10% fresh normal serum.

Preparation of phagocytosis tests and the enumeration of viable bacteria have been described previously (6). Two controls were included in the present experiments: First, an immune serum control without leukocytes to rule out the bactericidal effect of serum alone, and second, a fresh normal serum control with cells to rule out phagocytosis in the presence of normal serum. A phagocytosis test was considered positive when greater than 90% of organisms were killed after 1 hr of incubation at 37°C.

For absorption studies, log-phase organisms from 3-hr-shaking broth cultures were sedimented at 12,800 g for 10 min. The supernate was removed and to the remaining 0.05 ml bacterial pellet was added 0.5 ml serum. The bacteria were resuspended gently and the bacteria-serum mixture incubated at 4°C for 2 hr. After centrifugation the serum was removed and stored at −20°C. Serum was absorbed with purified polysaccharides by two methods. Alum-absorbed polysaccharide (100 μg) prepared by Dr. Gotschlich (9) was incubated with serum (1:10 vol/vol) at 4°C for 2 hr. After centrifugation at 12,800 g for 10 min, the serum was decanted and stored as above. Most absorption experiments, however, were performed by directly adding group A (lot A-V) and group C (lot C-I) polysaccharides to other components of the phagocytosis system. Thus, in phagocytosis tubes were placed 0.6 ml leukocytes (2 × 10⁷ granulocytes/ml), 0.1 ml heat-inactivated test serum, 0.1 ml fresh normal serum, 0.1 ml bacteria (1–2 × 10⁷ organisms/ml), and 0.1 ml purified polysaccharide (0.1–100 μg/ml).

Studies of the neutralization of opsonic activity in group A and group C polysaccharide antisera were conducted with the following haptens: mannose, mannosamine, N-acetyl mannosamine, N-acetyl glucosamine, N-acetyl galactosamine (Sigma Chemical Co., St. Louis, Mo.), N-acetyl neuraminic acid (Calbiochem, Los Angeles, Calif.), N-acetyl-4-O-acetyl neuraminic acid, and N-acetyl-7-O,8-O-diacetyl neuraminic acid. The latter two free acyl-neuraminic acids were purified from horse and bovine submaxillary mucin (10) and were

1. These polysaccharides have molecular weights below 50,000 (7).
kindly supplied by Dr. Hans Faillard, Ruhr-Universität Bochum, Germany. Various concentrations of these materials (in 0.1 ml) were added to other components of the phagocytosis system as described above.

Preparations of 7S and 19S immunoglobulins were isolated from serum by gel filtration at 4°C with Sephadex G-200 in phosphate-saline buffer, pH 7.6. 2 ml fractions were collected and the protein concentration determined spectrophotometrically at 280 nm. Each fraction was tested for opsonic activity and the immunoglobulin identified by gel diffusion using goat antiserum to human 7S and 19S immunoglobulins (Hyland Laboratories Inc., Los Angeles Calif.).

Normal and immune sera were incubated with zymosan (Fleischmann Laboratories, New York) for 2 hr at 37°C (11), after which time the zymosan was sedimented and the serum removed and immediately tested.

RESULTS

Opsonins After Meningococcal Polysaccharide Immunization.—The interaction between meningococcal strain A-1, the prototype group A strain, and human leukocytes in the presence of pre- and postimmunization sera is demonstrated in Fig. 1. Strain A-1 multiplied in the presence of preimmunization serum, whereas phagocytosis readily occurred when this strain and leukocytes were incubated with group A polysaccharide antiserum. The extracellular count closely paralleled the total count (extracellular and intracellular organisms), indicating that after ingestion by leukocytes meningococci were rapidly killed. The immune serum control without leukocytes showed no bactericidal effect. In addition, if tubes were left static and not tumbled so as to preclude adequate contact between leukocyte and bacterial populations, minimal killing occurred, thus demonstrating the absence of extracellular killing in the intact system.

Similar findings were observed when strain C-11, the prototype group C strain, and leukocytes were incubated in the presence of group C polysaccharide antisera, i.e., strain C-11 multiplied in the presence of preimmunization serum and phagocytosis occurred with postimmunization serum. After ingestion by leukocytes, these organisms were also rapidly killed. Phagocytosis of strain C-11 in the presence of pre- and postimmunization sera is shown morphologically in Figs. 7a and 7b. Details of this technique have been described previously (6). In Fig. 7a, strain C-11 was incubated for 1 hr with a monolayer of leukocytes in the presence of preimmunization serum. Most organisms, stained with Wright-Giemsa remained extracellular. After incubation of this strain in the presence of leukocytes and group C polysaccharide antiserum, organisms were observed either on or in phagocytic cells (Fig. 7b). In addition, many intracellular bacteria lost their staining properties and degranulation of granulocytes occurred. Similar morphologic findings were seen when strain A-1 was incubated with human granulocytes and group A polysaccharide antiserum.

Sera from 10 immunized volunteers were tested and in all instances opsonic activity was demonstrated. Examination of serial serum samples revealed that opsonic activity appeared within 1 wk after immunization and persisted for at
least 14 months. Titers of opsonic activity ranged from 1:20 to 1:320; the highest titers were noted in 2-4 wk antisera.

**Serologic Specificity of Serum Opsonins.**—To determine the serologic specificity of opsonins in group A and group C polysaccharide antisera, five additional meningococcal strains of each serologic group isolated from both cases and carriers as well as from different geographical areas were examined. Phagocytosis of only group A and group C strains was observed in the presence of homologous antiserum.

Antisera were also absorbed with log-phase organisms and meningococcal polysaccharides; results after absorption of group A polysaccharide antiserum are shown in Fig. 2. Opsonic activity was lost after absorption with strain A-I, whereas activity remained the same after absorption with either strain B-11 or C-11. Similar findings were observed after absorption with five other strains of

![Graph showing the interaction between meningococcal strain A-1 and leukocytes in the presence of pre- and postimmunization sera.](image-url)

**Fig. 1.** The interaction between meningococcal strain A-1 and leukocytes in the presence of pre- and postimmunization sera.
each serologic group. Studies on absorption of the same antiserum with meningococcal polysaccharides revealed that absorption with group A polysaccharide removed opsonic activity, whereas no effect was observed after absorption with group C polysaccharide. Immune serum controls showed no bactericidal effect.

The findings after absorption of group C polysaccharide antiserum with log-phase organisms and meningococcal polysaccharides are recorded in Table I.

Opsonic activity was lost after absorption with either strain C-11 or group C polysaccharide, whereas no loss was observed after absorption with either strains A-1 and B-11 or with group A polysaccharide. Similar results were also obtained after absorption with other group A, B, and C meningococcal strains.

Opsonins After Group A and Group C Meningococcal Infections.—Group C meningococcal opsonins were detected in the sera of two nasopharyngeal carriers and three patients recovered from meningitis (examples shown in Fig. 3) but not in preinfection sera. Comparison of total with extracellular counts indicated that rapid intracellular killing of organisms occurred. Morphologic
TABLE I  
Phagocytosis of Strain C-11 in the Presence of Group C Polysaccharide Antisera Absorbed with Meningococci and Meningococcal Polysaccharides

<table>
<thead>
<tr>
<th>Serum absorbed with:</th>
<th>Bacteria per ml (strain C-11)</th>
<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preimmunization</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td></td>
<td>1.4 × 10^7</td>
<td>4.1 × 10^7</td>
<td>8.0 × 10^7</td>
</tr>
<tr>
<td>Postimmunization</td>
<td></td>
<td>1.3 × 10^7</td>
<td>3.0 × 10^3</td>
<td>&lt;3.0 × 10^4</td>
</tr>
<tr>
<td>Strain A-1</td>
<td></td>
<td>1.0 × 10^7</td>
<td>7.0 × 10^3</td>
<td>&lt;3.0 × 10^4</td>
</tr>
<tr>
<td>Strain B-11</td>
<td></td>
<td>1.0 × 10^7</td>
<td>7.0 × 10^3</td>
<td>&lt;3.0 × 10^4</td>
</tr>
<tr>
<td>Strain C-11</td>
<td></td>
<td>1.2 × 10^7</td>
<td>3.3 × 10^7</td>
<td>7.0 × 10^7</td>
</tr>
<tr>
<td>A polysaccharide</td>
<td></td>
<td>1.0 × 10^7</td>
<td>4.0 × 10^3</td>
<td>3.0 × 10^5</td>
</tr>
<tr>
<td>C polysaccharide</td>
<td></td>
<td>1.2 × 10^7</td>
<td>2.8 × 10^7</td>
<td>7.2 × 10^7</td>
</tr>
</tbody>
</table>

![Graph showing phagocytosis of strain C-11 in the presence of sera from a nasopharyngeal carrier and a patient recovered from meningitis.](image)

Fig. 3. Phagocytosis of strain C-11 in the presence of sera from a nasopharyngeal carrier and a patient recovered from meningitis.
findings were similar to those described for phagocytosis of strain C-11 in the presence of group C polysaccharide antisera. Opsonic activity was lost from group C infection sera after absorption with group C meningococci. Absorption with group C but not group A polysaccharide also removed opsonic activity from these sera (Fig. 4). Group A opsonic activity was also lost from the serum of naturally infected individuals after absorption with both the group-specific organism and the group-specific polysaccharide.

![Graph](image)

**Fig. 4.** The interaction between strain C-11 and leukocytes in the presence of group C infection serum absorbed with meningococcal polysaccharides.

*Neutralization of Opsonic Activity in Meningococcal Polysaccharide Antisera.*—Gotschlich et al. reported that group A meningococcal polysaccharide is a polymer of N-acetyl, O-acetyl mannosamine PO₄ (7). It was therefore of interest to study the inhibitory effect of closely related haptens on the phagocytosis of strain A-1 in the presence of group A polysaccharide antisera. Results of opsonin neutralization with increasing concentrations of low molecular weight group A polysaccharide and N-acetyl mannosamine are demonstrated in Fig. 5. Stud-
isies of this antiserum were performed at a 1:20 dilution because of the relatively high titer of opsonic activity (1:80). Phagocytosis of strain A-1 was prevented at polysaccharide concentrations of 50 and 100 μg/ml, and at N-acetyl mannosamine concentrations of 100 and 1000 μg/ml. Similar concentrations of N-acetyl mannosamine had no effect on the phagocytosis of staphylococcus albus in the presence of 10% fresh normal serum or of meningococcal strain C-11 in the presence of group C polysaccharide antiserum. Neutralization studies with other haptens are summarized in Table II. Concentrations up to 1000 μg/ml mannose, mannosamine, N-acetyl glucosamine, N-acetyl galactosamine, and N-acetyl neuraminic acid did not reverse the opsonic action of the 1:20 antiserum. At these concentrations, there was no bactericidal effect in immune serum controls without leukocytes. Group A polysaccharide antisera from four other volunteers were examined and similar findings were observed in three. However, in one group A polysaccharide antiserum N-acetyl mannosamine at concentrations up to 10 mg/ml had no neutralizing effect.
It has also been reported that group C meningococcal polysaccharide is a polymer of N-acetyl, O-acetyl neuraminic acid (7, 12). Concentrations as low as 0.1 μg/ml of this polysaccharide inhibited the phagocytosis of strain C-11 in the presence of homologous antisera. However, neutralization of opsonic activity in these antisera was not observed with 1.0–100 μg/ml of N-acetyl neuraminic acid, N-acetyl-4-O-acetyl neuraminic acid, or N-acetyl-7-O, 8-O-diacetyl neuraminic acid. Concentrations up to 1000 μg/ml mannose, mannosamine, N-acetyl mannosamine, N-acetyl glucosamine, and N-acetyl galactosamine also had no effect.

Effect of Heat-Labile Factors on Opsonic Activity.—Previous studies demonstrated that the activity of meningococcal opsonins in immune rabbit sera depended on heat-labile components present in both normal and immune sera (6); opsonic titers in these rabbit antisera were consistently low (1:10–1:20).

### Table II

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Bacteria per ml (strain A-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Mannose</td>
<td>1.5 × 10^7</td>
</tr>
<tr>
<td>Mannosamine</td>
<td>1.3 × 10^7</td>
</tr>
<tr>
<td>N-acetyl mannosamine</td>
<td>1.6 × 10^7</td>
</tr>
<tr>
<td>N-acetyl glucosamine</td>
<td>1.0 × 10^7</td>
</tr>
<tr>
<td>N-acetyl galactosamine</td>
<td>1.2 × 10^7</td>
</tr>
<tr>
<td>N-acetyl neuraminic acid</td>
<td>1.2 × 10^7</td>
</tr>
<tr>
<td>None</td>
<td>1.4 × 10^7</td>
</tr>
</tbody>
</table>

In the present studies, the activity of low-titer opsonins in human antisera after polysaccharide immunization also depended on heat-labile factors: in the presence of 10% heat-inactivated immune serum and 10% heat-inactivated normal serum, multiplication of log-phase organisms occurred.

Similar experiments were performed using polysaccharide antisera with relatively high titers of opsonins (1:160–1:320). Phagocytosis was observed in the presence of heat-inactivated antisera although the titers of opsonic activity were reduced to 1:10. Similar findings were observed after treatment of normal or immune sera with 1–5 mg zymosan. After ingestion in the presence of heat-inactivated or zymosan-treated antisera, rapid intracellular killing of meningococci was observed.

Opsonic Capacity of 7S and 19S Serum Fractions.—7S and 19S immunoglobulins were prepared by gel filtration from 3 ml of antiserum collected from volunteers 12 months after polysaccharide immunization. All fractions were tested for opsonic activity in the presence of 10% fresh normal serum. As
demonstrated in Fig. 6, opsonic activity was detected in both 19S and 7S fractions. Though in all determinations the opsonic titers were relatively low, most activity was associated with the 7S fraction. As observed in studies of heat-inactivated low-titer antisera, phagocytosis did not occur in the presence of either the 19S or 7S fraction and 10% heat-inactivated normal serum.

**DISCUSSION**

The interaction between meningococci and human leukocytes in the presence of human sera has been determined in past studies by stained smears (13-15). Although conflicting results have been reported, phagocytosis of meningococci in the presence of sera from naturally infected individuals has been described (14). Because of severe clumping and retraction of leukocytes, however, quantitation by this technique is unreliable. The methods employed in this study allow quantitative evaluation of the interaction between meningococci, leukocytes, and serum factors. Consideration of the concentration of reactants, as well as other important variables in such a phagocytosis system has been reviewed previously (6, 16).

The findings demonstrate that immunization with group A and group C meningococcal polysaccharides produces serum opsonins in man. Furthermore, these opsonins are present in both 19S and 7S immunoglobulins and persist in high titers for at least 14 months. The demonstration of opsonic activity after immunization is consistent with studies by Gotschlich, Goldschneider, and Artenstein who detected hemagglutinating and serum bactericidal activity in polysaccharide antisera (8). Concentrations of $10^8$ organisms/ml and 25% fresh serum were employed in the study of serum bactericidins by these authors. Since the presence of bactericidal activity makes it impossible to observe opsonic effects by the method which uses intracellular killing of bacteria as an index of phagocytosis, different concentrations of reactants were employed in the present studies. At concentrations of $1-2 \times 10^7$ organisms/ml and 10% fresh sera, opsonic activity could be detected, whereas bactericidal activity was not observed in serum controls.
Absorption of polysaccharide antisera with log-phase organisms and polysaccharides, as well as phagocytosis of group A and group C meningococci only in the presence of homologous antisera, suggest that meningococcal opsonins are group-specific. Group-specificity of serum opsonins has also been observed in animals immunized with groups A and C log-phase meningococci. It will be of interest to study serum opsonins after group B meningococcal polysaccharide immunization, since type-specific opsonins in sera of rabbits immunized with log-phase group B strains have been reported.

Meningococcal opsonins were also detected in the sera of nasopharyngeal carriers and of patients recovered from meningitis. Because of the small sample of patients studied, the role of these antibodies in natural immunity to meningococcal infections requires further study. Results after absorption of these sera with log-phase bacteria and meningococcal polysaccharides suggest that these polysaccharides are the major antiphagocytic determinants for groups A and C meningococci. Minor determinants should also be kept in mind since such factors have been described for other bacteria–phagocyte interactions. Previous studies demonstrated that group A meningococcal polysaccharide is a polymer of N-acetyl, O-acetyl mannosamine PO₄, and that the high molecular weight form of this polysaccharide (greater than 100,000) inhibits hemagglutinating activity at concentrations of 0.5–2.5 μg/ml. The relatively high concentrations of group A polysaccharide that were necessary to neutralize opsonic activity may be related, as suggested by these authors, to differences in the molecular weights of polysaccharide preparations. Phagocytosis of group A meningococci in the presence of group A polysaccharide antisera was also inhibited by N-acetyl mannosamine. This inhibition was specific, as similar concentrations of N-acetyl mannosamine had no effect on the phagocytosis of staphylococcus albus or group C meningococci. In addition, other closely related haptens did not neutralize group A opsonic activity. The lack of an inhibitory effect of N-acetyl mannosamine in the sera from one volunteer suggests that certain group A polysaccharide antisera recognize a determinant other than N-acetyl mannosamine. In these cases it seems likely that the O-acetylation of N-acetyl mannosamine may be necessary for neutralization of opsonic activity.

Phagocytosis of group C meningococci in the presence of group C polysaccharide antisera was inhibited by group C polysaccharide (polymer of N-acetyl, O-acetyl neuraminic acid) but not by N-acetyl neuraminic acid, N-acetyl-4-O-acetyl neuraminic acid, or N-acetyl-1-7-O, 8-O-diacyl neuraminic acid. In addition, no effect was observed when colominic acid (N-acetyl neuraminic acid extracted from Escherichia coli) was added to the phagocytosis system. These results are consistent with previous reports demonstrating the lack

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2 Colominic acid was kindly supplied by Dr. W. F. Goebel.
of biologic activity of free acyl-neuraminic acids. Studies on the phagocytosis of group C meningococci after treatment of these organisms and group C polysaccharide with bacterial neuraminidase are now in progress.

Differential centrifugation to separate leukocyte from bacterial populations and direct colony plating, as well as morphologic studies, indicate that after ingestion by human granulocytes, meningococci are rapidly killed. Similar findings have been observed in the study of phagocytosis of meningococci by rabbit polymorphonuclear leukocytes (6). The intracellular killing of meningococci does not depend on the presence of either heat-labile components or specific opsonins since similar killing rates have been observed with heat-inactivated antisera and after ingestion of stationary-phase meningococci in the presence of fresh normal serum. These findings are similar to those of Smith, Barnett, May, and Sanford who demonstrated that the effect of opsonins on the phagocytosis of Proteus mirabilis by rabbit granulocytes was due to enhanced uptake rather than increased intracellular killing of bacteria (20).

Observations on complement requirements for erythrophagocytosis by polymorphonuclear and mononuclear leukocytes have demonstrated that for efficient phagocytosis of red cells, 7S antibody and the first four components of complement are required (21, 22). Phagocytosis of pneumococci in the presence of type-specific opsonins and heat-labile factors has recently been reported by Johnston, Klemperer, Alper, and Rosen (23). These authors demonstrated that optimal phagocytosis of pneumococci requires 7S antibody, the first four components of complement, a heat-labile, dialyzable cofactor and a heat-labile, 5-6S, beta pseudoglobulin. In the presence of these heat-labile serum factors the rate of phagocytosis was similar to that observed with fresh immune serum. The present studies demonstrate that phagocytosis of meningococci occurs in the absence of heat-labile serum factors when titers of opsonic activity in fresh immune sera are relatively high. However, optimal ingestion of meningococci is observed in the presence of both antibody and heat-labile factors. Further studies to characterize the heat-labile factors required for optimal phagocytosis of meningococci are indicated.

The findings described herein support and extend recent studies suggesting that immunization with groups A and C meningococcal polysaccharides may well provide group-specific protection against meningococcal infections (8, 24).

SUMMARY

The interaction in vitro between human granulocytes and meningococci in the presence of sera from volunteers immunized by Gotschlich et al. with purified group A and group C meningococcal polysaccharides was studied. Phagocytosis of meningococci did not occur in the presence of preimmunization sera. In all volunteers tested, group-specific opsonins were detected in groups A and

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C polysaccharide antisera. Opsonic activity appeared within 1 wk after immunization and persisted for at least 14 months. Titers of opsonic activity ranged from 1:20 to 1:320; highest titers were noted in 2–4 wk antisera. Meningococcal opsonins were detected in both 19S and 7S immunoglobulins. Opsonic activity in low-titer antisera depended on heat-labile factors present in both normal and immune sera, whereas phagocytosis was observed in the presence of heat-inactivated high-titer antisera. Phagocytosis of group A meningococci in the presence of certain group A polysaccharide antisera was inhibited by N-acetyl mannosamine, but not by mannose, mannosamine, N-acetyl glucosamine, N-acetyl galactosamine, or N-acetyl neuraminic acid. Absorption studies with sera from patients with natural meningococcal infections revealed that these polysaccharides are the major antiphagocytic determinants for group A and group C meningococci. These studies are consistent with previous reports suggesting that immunization with group A and group C polysaccharides may well provide group-specific protection against meningococcal infections.

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Fig. 7. (a): The interaction between strain C-11 and granulocytes incubated for 1 hr in the presence of preimmunization serum. Most organisms stained with Wright-Giemsa remain extracellular. The cytoplasmic granules of leukocytes appear intact. × 1760. (b): The interaction between strain C-11 and granulocytes incubated for 1 hr in the presence of group C polysaccharide antiserum. Organisms are observed either on or in phagocytic cells and degranulation has occurred in cells containing many bacteria. × 1760.