Immunological Relationship between the Capsular Polysaccharides of 
Neisseria meningitidis Serogroups Z and 29E

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The immunological relationship between serogroups 29E and Z of Neisseria meningitidis was investigated using bacterial agglutination, precipitin-in-gel, primary antigen binding and immune lysis assays. The two capsular polysaccharides were both cross immunogenic and cross reactive. Demonstration of the relationship using group Z antisera depended on which assay was used. It was most readily apparent in assays of immune lysis, less apparent when precipitins were sought in gel and inapparent when bacterial agglutination, the standard assay for determining serogroup, was employed. The cross reacting epitope was expressed 10-fold more within the group 29E polysaccharide than within the group Z polysaccharide. These findings imply that inclusion of either polysaccharide in a polyvalent meningococcal vaccine may obviate the need to include the other polysaccharide.

INTRODUCTION

Since its initial description (Evans et al., 1968), the exact relationship of serogroup (Gp) 29E to the other serogroups of Neisseria meningitidis has been confused by its 'one way' cross reaction with serogroup Z. Slaterus et al. (1963) designated strains that did not react with Gp Z antisera but that induced antisera that agglutinated Gp Z strains as Z'; an identical relationship between Gp 29E and Gp Z strains was described by Devine & Haggerman (1970). Fallon (1976) showed that Gp Z' and Gp 29E strains were serologically identical. The continued use of the two appellations, one with priority, the other in common usage, has not been helpful.

The Gp 29E capsular polysaccharide (29Esss) consists of alternating d-galactosamine and 2-keto-3-deoxyxulosonate (KDO) residues, linked α-D at C-3 and β-D at C-7, respectively. It is O-acetylated at C-4 and C-5 of the KDO residues (Bhattacharjee et al., 1978). The Gp Z capsular polysaccharide (Zsss) consists of alternating d-galactosamine and glycerol residues, linked through phosphate diester groups (Jennings et al., 1979). Despite knowledge of the chemical structure of their respective capsular polysaccharides, the immunochemical basis for the cross reaction between the two serogroups has not been investigated. With the development of vaccines against meningococcal serogroups other than A and C (Farquhar et al., 1978; Zollinger et al., 1979; Griffiss et al., 1981), the formulation of a polyvalent meningococcal vaccine has become feasible. If the 29Esss induces antibody which lyses Gp Z strains, its inclusion in a polyvalent preparation should provide protection against the latter serogroup (Griffiss, 1982), thus reducing the number of required components. The studies reported here were undertaken to more fully characterize the immunological basis of the one way cross reaction between 29Esss and Zsss.
METHODS

Organisms. Gp 29E strain 106, from the Walter Reed Army Institute of Research collection, was a nasopharyngeal isolate; Gp Z strain S-355 was provided by Dr Harry Feldman, Syracuse, New York. They were characterized as Neisseria meningitidis by Gram stained morphology, oxidase reaction and carbohydrate utilization. They were serogrouped by the slide-agglutination technique (Evans et al., 1968), serotyped by the modified Gold system (Griffiss et al., 1977) and stored as lyophilized cultures.

Serological tests. Group-specific capsular polysaccharides were prepared from 16 h liquid cultures by the method of Brandt et al. (1972). Briefly, polysaccharides were precipitated from culture supernates with 0.1% (w/v) Cetavlon (Eastman Kodak), followed by dissociation of the detergent–polysaccharide complex by extraction with 0.9 M CaCl₂. Nucleic acids were removed by fractionation with 25% (v/v) absolute alcohol and the polysaccharides were removed by fractionation with 75% (v/v) absolute alcohol. The precipitates, dissolved in distilled water, contained < 1% protein, as estimated by the Lowry method, and 5% nucleic acids, as estimated by absorbance at 260 and 280 nm (Layne, 1957). For use in the radioactive antigen binding assay (RABA), capsular polysaccharide was intrinsically labelled by the addition of [¹⁴C]acetate to the culture medium (Brandt et al., 1972). Hyperimmune antisera were raised in rabbits against the Gp 29E and Z strains by immunizing three and two animals, respectively, with formalin-killed organisms (Evans et al., 1968). Purifying antibodies were detected by double diffusion in gels, using the Ouchterlony technique described by Artenstein et al. (1971), with 0.9% (w/v) ion agar no. 2 (Colab Laboratories, Chicago Heights, Ill., U.S.A.). Binding antibodies specific for each capsular polysaccharide were quantified using the RABA of Brandt et al. (1973). Results are expressed as ng polysaccharide bound by 0.05 ml of undiluted serum (antigen binding capacity). The weight of 29Esss was calculated as twice the content of KDO, as determined by the method of Osborn (1963); the weight of Zsss was calculated from its hexosamine content as determined by the modified Morgan–Elson method (Reissig et al., 1955). The reproducibility of the assay is ±4% (Brandt et al., 1972).

IgM and IgG were purified from the serum of a single rabbit (no. 114) immunized with the Gp Z strain by the previously described GV-2 method of continuous, in-line chromatography over molecular sieve and ion-exchange gels (Griffiss et al., 1978).

Bactericidal activity of whole sera and of the purified IgM and IgG was assayed against the two strains in the radioactive bactericidal test of Kasper & Wyle (1972). Human serum without bactericidal activity against either strain served as a source of exogenous complement. Details of the test procedure and calculation of results have been reported previously (Griffiss, 1975). The titre of bactericidal activity of whole sera was that dilution which lysed 50% of the test inoculum of 10⁷ meningococci. The titre of IgM and IgG was computed similarly using the assumption that 40% of each Ig was recovered from whole serum (Griffiss et al., 1978).

Bactericidal activity against the Gp 29E strain of Gp Z antisera no. 114 was inhibited by each capsular polysaccharide. Antiserum (0.1 ml) diluted in Geys’ balanced salt solution to the highest dilution that gave 100% lysis was mixed with increasing quantities of the respective polysaccharides (0.6–80 μg) in 0.2 ml of the same buffer. The reaction mixtures were incubated, with shaking (150 r.p.m.) at ambient temperature, for 1 h prior to addition of 0.1 ml of exogenous complement and 0.1 ml bacterial suspension (10⁷ organisms). Results are expressed as the weight of polysaccharide effecting a 50% reduction in lysis compared with the uninhibited control.

RESULTS

Serotyping

Gp 29E strain 106 and Gp Z strain S-355 were of types IV and VII, respectively. Common minor serotype factors were not observed (Griffiss et al., 1977).

Agglutinating and precipitating antibodies

As expected, antisera from all three rabbits immunized with the Gp 29E strain agglutinated both Gp 29E and Gp Z organisms; antiserum from neither rabbit immunized with the Gp Z strain agglutinated Gp 29E bacteria, while both agglutinated the Gp Z strain. When reacted in gel, the three Gp 29E antisera formed precipitin lines of partial identity with 29Esss and Zsss (Fig. 1 a). Differences were not seen among the sera.

When similarly reacted, the precipitation of Zsss by the two Gp Z antisera was identical to that by the Gp 29E antisera and partially identical to that of 29Esss by the Gp 29E antisera (Fig. 1 b). A fortuitous separation of the immunoprecipitates (Fig. 1 b) clearly demonstrated the dual determinants on the 29Esss, one common to Zsss and one unique to 29Esss.
With the proportions used in Fig. 1, no precipitin reaction was seen between Gp Z antisera and 29Esss. However, a weak precipitin reaction could be obtained by varying these proportions. The equivalence zone for this reaction was quite narrow as compared with that between Gp Z antisera and Zsss or Gp 29E antisera and either polysaccharide.

There was no evidence for a second, unique determinant on Zsss (absence of spur toward 29E antisera). Thus, the relationship between the antigenic determinants of 29Esss and Zsss can be described as A + B : A, which is in agreement with their containing a common chemical constituent (galactosamine), as well as unique constituents.

**Binding antibody**

The binding capacities of antisera for the two polysaccharides are listed in Table 1. Each organism induced antibody which bound both polysaccharides; in each case the homologous response was about ten times greater than the heterologous response. None of the preimmune sera had antibody capable of binding either polysaccharide. Both IgM and IgG from serum no. 114 bound 29Esss (Table 2).

**Lytic antibody**

The bactericidal activity of antiserum from each high responder rabbit in the two immunization groups (nos 110 and 114) was determined for each strain (Table 2). Each antiserum lysed both bacteria. At equivalent lytic titre, the Gp Z antiserum bound 10-fold less 29Esss than Zsss, while the Gp 29E antiserum bound roughly equivalent amounts of both polysaccharides.

Although IgG in serum no. 114 (group Z) bound 29Esss, it was unable to initiate immune lysis of Gp 29E organisms. IgM both bound 29Esss and initiated lysis of the Gp 29E strain. Taking account of volume changes, all of the bactericidal activity of antiserum no. 114 against the Gp 29E strain was accounted for by IgM. IgG initiated immune lysis of its homologous Gp Z strain.

Confirmation of the specificity of the Gp 29E lytic antibody in Gp Z antiserum was obtained in inhibition tests. Both Zsss and 29Esss inhibited lysis; on a weight basis, 29Esss was 10-fold more effective (13.5 μg 29Esss was required to give 50% inhibition of lysis by comparison with 155.4 μg Zsss). This ratio is consistent with the ratio of binding capacities for the two polysaccharides at equivalent bactericidal titre for each target strain (Table 2).
Table 1. *Radioactive antigen binding assay of Gp 29E and Gp Z capsular polysaccharide (29Esss and Zsss) antibody in antisera raised to Gp 29E and Gp Z strains of N. meningitidis*

<table>
<thead>
<tr>
<th>Rabbit no.</th>
<th>Immunizing organism</th>
<th>29Esss binding capacity</th>
<th>Zsss binding capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>29E</td>
<td>$4.1 \times 10^4$</td>
<td>$2.4 \times 10^3$</td>
</tr>
<tr>
<td>112</td>
<td>29E</td>
<td>$2.4 \times 10^4$</td>
<td>$1.9 \times 10^3$</td>
</tr>
<tr>
<td>113</td>
<td>Z</td>
<td>$3.4 \times 10^3$</td>
<td>ND</td>
</tr>
<tr>
<td>114</td>
<td>Z</td>
<td>$3.0 \times 10^2$</td>
<td>$2.4 \times 10^3$</td>
</tr>
</tbody>
</table>

ND, Not determined.
*Expressed as ng of antigen bound by 0.05 ml serum. Reproducibility of the assay is ± 4%.

Table 2. *Antibody capable of lysing Gp 29E and Gp Z N. meningitidis in antisera raised to each strain*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Immunizing strain</th>
<th>Lytic titre*</th>
<th>Lytic binding capacity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole serum no. 110</td>
<td>29E</td>
<td>1:2419</td>
<td>33.8</td>
</tr>
<tr>
<td>Whole serum no. 114</td>
<td>Z</td>
<td>1:377</td>
<td>1:6</td>
</tr>
<tr>
<td>IgG</td>
<td>Z</td>
<td>&lt;1:2</td>
<td>246.0</td>
</tr>
<tr>
<td>IgG</td>
<td>Z</td>
<td>1:102.5</td>
<td>2:3</td>
</tr>
</tbody>
</table>

ND, Not determined.
*Determined for killing of $5 \times 10^6$ meningococci; the mean of three estimates.
†Expressed as ng of corresponding capsular polysaccharide bound at a dilution corresponding to indicated lytic titre.

DISCUSSION

It is clear from these data that the group 29E and Z capsular polysaccharides of *N. meningitidis* show cross immunological reactivity, but that the demonstration of this relationship in Gp Z antisera depends on the assay employed and, to a lesser degree, on the serum sample tested. Cross reactivity was most readily apparent when the assay was the extremely sensitive bactericidal test (Griffiss, 1979), less apparent when precipitating antibodies were sought and inapparent when agglutination was used. This serological variation could not be accounted for by differences in the Ig isotypes induced. Gp Z antisera contained IgM (usually an efficient agglutinating antibody) that bound 29Esss and initiated immune lysis of Gp 29E strains, but failed to agglutinate these same strains. Gp Z IgG bound both capsular polysaccharides, precipitated 29Esss weakly, if at all, and was unable to initiate immune lysis of group 29E strains. The latter cannot be explained by the introduction of anticomplementary substances, since the same IgG initiates immune lysis of group Z strains.

While the data are consistent with a shared epitope, perhaps involving the galactosamine residues common to both polysaccharides, they are equally consistent with a bi-clonal response to the 29Esss, with one clone being highly specific for 29Esss, the second for Zsss. The weak reaction of the product of the second clone with 29Esss suggests similarity but not identity between the inducing epitope within 29Esss and the specific epitope within Zsss. The latter explanation is more consistent with the two different antibody populations seen when the immunoprecipitates are separated as in Fig. 1 and with differences in antibody binding capacity (Table 1), since a single antibody population would bind similar quantities of identical or nearly identical antigens, at saturation.

That the cross reacting epitope is not equally distributed within the two polysaccharide chains is suggested by the fact that Gp Z antiserum bound 10-fold more Zsss than 29Esss, at equivalent bactericidal titre, and the corresponding 10-fold difference in inhibitory capacity of the two
polysaccharides for lysis of Gp 29E strains by Gp Z antiserum. A relative paucity of expression of the cross immunogenic epitope within Zss, leading to meagre 29Esss antibody induction, might explain the difficulty in demonstrating the immunological relationship in Gp Z antisera. Inferences from these data must be treated with caution as the structure of the two polysaccharides may be more complex than previously reported.

Regardless of the basis of the immunological relationship, data from experiments with lytic antibody suggest that inclusion of either polysaccharide in a polyvalent meningococcal vaccine would preclude the need for the other. In the absence of a major epidemiological shift, the inclusion of neither polysaccharide would be necessary. However, the recent emergence of Gp Y and Gp W135 \textit{N. meningitidis} as real and fairly common causes of disease (Griffiss et al., 1981), emphasizes the need to develop the capsular polysaccharides of the epidemiologically ‘minor’ serogroups as potential vaccines. The optimal formulation of a polyvalent meningococcal vaccine may become clearer with the results of human safety and immunogenicity testing of serogroup B, Y, W135 and 29E capsular polysaccharide vaccines currently underway (Farquhar et al., 1978; Zollinger et al., 1979; Griffiss et al., 1981).

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