THE RELATIONSHIP BETWEEN *NEISSERIA MENINGITIDIS* OF SEROGROUPS Z1 AND 29E

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PLATE XIX

Slaterus (1961) described three new serogroups of meningococci that were not typable with agglutinating antisera to groups A, B, C, and D. He provisionally designated these strains as types X, Y, and Z. In the course of another study of meningococci from carriers and patients during a non-epidemic period in Holland (Slaterus, Ruys and Sieberg, 1963) strains were isolated that did not react with antisera to groups A, B, C, D, X, Y, or Z. However, antisera to these untypable strains agglutinated and precipitated with group-Z strains. These untypable strains were designated Z1. Evans, Artenstein and Hunter (1968) described three new serogroups of meningococci among strains isolated from patients in the USA and Europe. These new serogroups were designated Bo, 29E, and 135. Bo appeared to be identical to the Y strain of Slaterus. Devine and Hagerman (1970) found that antisera to group 29E always agglutinated group-Z strains but that group-Z serum did not agglutinate 29E strains—a relationship that they recognised as identical with the description by Slaterus et al. (1963) of the serological relationship of Z1 to Z. The present study was prompted by the observation that groups Z1 and 29E gave similar reactions when examined by the micro-agar-gel precipitation technique described by Slaterus (1961).

MATERIALS AND METHODS

*Meningococci* of groups A, B, C, and D were from the National Collection of Type Cultures, London, groups X, Y, Z, and Z1 from Dr K. W. Slaterus, groups 29E and 135 from Dr M. S. Artenstein, and the RAS-10 strain from Commander L. F. Devine.

*Capsular-polysaccharide antigens* from strains Z1 and 29E were prepared by the method of Kasper et al. (1973) except that the volumes of culture media were less, cultures were treated with a final concentration of 1% β-propiolactone before processing to reduce the hazards of centrifuging, and concentration of the culture supernate was by dialysis against polyethylene glycol instead of by membrane ultrafiltration. The resulting polysaccharide preparations did not contain protein detectable by the method of Lowry et al. (1951).

*Antisera* were prepared in rabbits by intravenous inoculation of heat-killed suspensions of meningococci followed by live suspensions (method modified from Slaterus, 1961) or by the method of Edwards and Devine (1968). Antisera were checked for specificity by slide agglutination and counterimmunoelectrophoresis (CIE) against all the strains noted above.

*Sera were absorbed* by adding an equal volume of meningococci suspended in 0.06M NaCl and incubation at 56°C for 30 min., then at 37°C for 2 h; the meningococci were then removed by centrifugation.

*Slide-agglutination tests* were performed with live organisms under examination with a low-power microscope (Devine and Hagerman, 1970).

*Tube-agglutination tests* were performed by the technique of Branham (1958).

*Gel-diffusion tests.* Whole-bacterial antigens were prepared by making a thick, creamy suspension, in 0.06M NaCl, of meningococci harvested from horse-blood agar or Mueller-Hinton agar plates that had been incubated overnight in an atmosphere of 10% CO2. The gels, consisting of 1% Ionagar (Oxoid) in distilled water or 0.06M NaCl, were poured on

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TABLE I

Results of tube-agglutination tests with antisera prepared against meningococci of groups Z1 and 29E before and after absorption with meningococci of either group Z1 or group 29E

<table>
<thead>
<tr>
<th>Antiserum prepared against group</th>
<th>Absorbing strain</th>
<th>Agglutinating titre with meningococci of group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Z1</td>
</tr>
<tr>
<td>Z1</td>
<td>Nil</td>
<td>40</td>
</tr>
<tr>
<td>Z1 absorbed with Z1 supernate</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>Z1 absorbed with 29E supernate</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>29E</td>
<td>Nil</td>
<td>80</td>
</tr>
<tr>
<td>29E absorbed with Z1 supernate</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>29E absorbed with 29E supernate</td>
<td>&lt; 10</td>
<td>&lt; 10</td>
</tr>
</tbody>
</table>

8.2 × 8.2-cm glass lantern slides. The cups were 4 mm in diameter and the cup centres were 1 cm apart. Tests were read after 1 and 2 days at room temperature.

Haemagglutination-inhibition tests were performed by the method of Cohen and Artenstein (1972).

Counterimmunoelectrophoresis tests were performed by the method of Edwards, Muehl and Peckinpaugh (1972). The antigens were suspensions of meningococci, either live or heated at 100°C for 20 min.

RESULTS

Slide agglutination. Antisera to groups Z1 and 29E agglutinated meningococci of groups Z, Z1, and 29E but of no other serogroup. Absorption of either serum with either group Z1 or 29E meningococci removed all agglutinating activity.

Tube agglutination. The titres of the antisera before and after absorption are shown in table I. Both antisera had the same agglutination titre for the homologous and for the heterologous strains. Absorbed antisera exhibited no agglutinating activity.

Haemagglutination-inhibition tests. Supernates from suspensions of meningococci of

TABLE II

Results of haemagglutination tests with antisera to meningococci of groups Z1 and 29E before and after absorption with culture supernates from meningococci of groups Z1 or 29E

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Agglutination of erythrocytes coated with crude polysaccharide from meningococci of group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Z1</td>
</tr>
<tr>
<td>Z1</td>
<td>+</td>
</tr>
<tr>
<td>Z1 absorbed with Z1 supernate</td>
<td>-</td>
</tr>
<tr>
<td>Z1 absorbed with 29E supernate</td>
<td>-</td>
</tr>
<tr>
<td>29E</td>
<td>+</td>
</tr>
<tr>
<td>29E absorbed with 29E supernate</td>
<td>-</td>
</tr>
<tr>
<td>29E absorbed with Z1 supernate</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = Agglutination; – = no agglutination.
groups Z1 and 29E were tested for their ability to inhibit the agglutination of polysaccharide-sensitised red cells by homologous antisera. The results are shown in table II. Antisera prepared against meningococci of either serogroup agglutinated erythrocytes sensitised with crude polysaccharide of either group. Absorption of antisera with supernate from cultures of either serogroup abolished all haemagglutinating activity of the serum.

Counterimmunoelectrophoresis. The results of CIE of antisera to groups Z1 and 29E against suspensions of live or heated meningococci of groups Z1 and 29E are shown in table III. It is of interest that the bacterial component reacting in the CIE is heat-stable.

Gel diffusion. Reactions were performed in gels containing 1% Ionagar in either water or 0.06M NaCl because of the observation (Fallon and Jackson, 1967) that some antigen-antibody reactions developed better in water agar than in saline agar. The reactions seen in water-agar gels were similar to but stronger than those seen in agar in 0.06M saline. Antisera to Z1 and to 29E gave identical reactions with bacterial suspensions of groups 29E and Z1. Hence, both sera gave two lines of precipitate with Z1 and 29E meningococcal antigen.

To establish the nature of the antigen precipitating in agar gel, polysaccharide preparations were substituted for whole-bacterial antigens in the gel-diffusion test. There was a reaction of identity between the precipitates formed by the reaction of antisera to meningococci of groups 29E or Z1 and polysaccharide antigens prepared from these strains (figure). Other tests showed a reaction of identity between one of the two lines between either antiserum and the bacterial suspension and the line between the antiserum and purified polysaccharide, showing that one of the antigens demonstrated from the bacterial suspension was indeed the group-specific polysaccharide. There was no reaction between the polysaccharides extracted from groups Z1 and 29E meningococci and antisera to any other group of meningococci.

**DISCUSSION**

These experiments show that in agglutination reactions and in reactions that depend more certainly on the polysaccharide antigen of *N. meningitidis*, antisera prepared against
meningococci of groups Z1 and 29E reacted identically with antigens prepared from either strain. Absorption of antiserum to either strain with organisms of either strain abolished the reactivity of the antiserum. Hence, these two groups of *N. meningitidis* are identical. Their relationship with group-Z meningococci remains to be elucidated, but preliminary observations show that there is no cross relationship demonstrable by the haemagglutination-inhibition test.

Although the designation 29E is in common use, Z1 has priority. It is, however, important that the relationship between group 29E-Z1 and group Z be elucidated before the designations of the "new" meningococcal serogroups are decided.

**SUMMARY**

Meningococci of serogroups Z1 and 29E were examined serologically and shown to be identical. These meningococci should be designated either as group Z1, which has priority, or preferably by a new designation forming a logical sequence with the currently accepted serogroups.

I wish to acknowledge the technical assistance of Mr W. Brown and Mr W. Abraham, the help of the Department of Audio-Visual Services, Stobhill General Hospital, Glasgow, and the secretarial assistance of Miss E. P. Laird.

**REFERENCES**


Figure.—Gel-diffusion reaction between antiserum to *Neisseria meningitidis* group 29E (1) and group Z$^+$ (2) and polysaccharides from *N. meningitidis* group 29E (3) and Z$^+$ (4).