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Immunochemical Cross-Reactions Between Type III Group B Streptococcus and Type 14 *Streptococcus pneumoniae*

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Serological cross-reactions between certain streptococci and some serotypes of *Streptococcus pneumoniae* have been reported. These studies detail the serological cross-reactivity observed between hot HCl-extracted group B streptococcus type III (GBS III) antigens and *S. pneumoniae* type 14 (Pn 14) polysaccharide. Similar electrophoretic migration patterns of GBS III and Pn 14 were observed when either type-specific GBS III antisera or pneumococcal omniserum was utilized to precipitate these antigens. Both the GBS III antigen and the Pn 14 polysaccharide migrated toward the cathode, whereas all other pneumococcal polysaccharides migrated toward the anode. No cross-reactions were observed between GBS III antisera and the 11 other types of pneumococcal polysaccharides. Lines of identity were observed between type-specific GBS III antisera and monospecific Pn 14 antiserum with either GBS III antigens or purified Pn 14 polysaccharide. The cross-reacting antigens of GBS III and Pn 14 appear to be identical by immunodiffusion and immunoelectrophoresis.

Group B streptococcal disease in neonates has been well described (3, 6, 9). A similar disease state has recently been described in neonatal pneumococcal infections (7), and the question arises regarding possible interrelationships between these organisms and the disease states they produce in neonates. Type-specific antibody is required for opsonization of group B streptococci (GBS) (4, 12, 15), similar to the opsonization of pneumococci by corresponding type-specific pneumococcal antibody. Serological cross-reactivity between various serotypes of *Streptococcus pneumoniae* and other streptococci has been reported (1, 2). This paper describes the serological cross-reactivity observed between GBS III and Pn 14.

(This work was presented in part at the 78th Annual Meeting of the American Society for Microbiology [M. H. Crumrine, M. W. Balk, and G. W. Fischer, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, E111, p. 61].)

MATERIALS AND METHODS

Bacterial strains. Strain III NOR is a GBS III clinical isolate and has been used in previous studies by this laboratory (M. H. Crumrine, G. W. Fischer, and M. W. Balk, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 16th, Chicago, Ill., Abstr. no. 151, 1976). A reference strain of GBS III, SS 620, was obtained from Hazel Wilkinson, Center for Disease Control (CDC), Atlanta, Ga.

Antigens. Polysaccharide antigens from GBS III were produced by using hot HCl extraction and ethanol fractionation techniques (13).

Pneumococcal antigens used in immunoelectrophoretic procedures were contained in Lilly duodecavalent pneumococcal vaccine, 50 μg of polysaccharide per ml. The following pneumococcal polysaccharides were present in the vaccine: 1, 3, 4, 6, 7, 8, 9, 12, 14, 18, 19, and 23. The vaccine was obtained through J. C. Hill, National Institute of Allergy and Infectious Diseases, Bethesda, Md. Purified type 14 pneumococcal polysaccharide was provided by John Robbins, Bureau of Biologics, National Institutes of Health, Bethesda, Md.

Antisera. Type-specific GBS III antisera were produced according to the methods of McCarty and Lancefield (16). Pneumococcal omniserum was purchased from the Statens Seruminstitut, Copenhagen, Denmark. Type-specific Pn 14 antisera were obtained from Difco Laboratories, Detroit, Mich., and P. B. Smith, CDC.

Immunoelectrophoresis. Immunoelectrophoretic studies were performed by using a modified Scheidegger microslide technique (18). A 1% (wt/vol) agarose gel was prepared with barbital buffer at pH 8.2 and ionic strength 0.05. Wells 3 mm in diameter were utilized, and 20 μl of antigen was added to each well. Separation of antigens was accomplished at 55 V/slide, after which troughs were cut and filled with 100 μl of antiserum. The gels were held at 4°C and observed at

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24-h intervals. Photographic records were made of all precipitation lines.

Immunodiffusion. Immunodiffusion studies were carried out on microscope slides in the same 1% barbital-buffered agarose gel as in the electrophoretic studies. Antigen or antiserum, 20 μl, was placed in the 3-mm-diameter wells, and the slides were held at 4°C and examined at 24-h intervals. Photographic records were made as in immunoelectrophoretic studies.

RESULTS

Figure 1 illustrates the reactions observed when pneumococcal antigens, upper well, and GBS III SS 620 antigens, lower well, were electrophoresed and GBS III NOR antiserum was added to the trough. The crude extract of GBS III, lower well, had two mobility patterns. The group B antigen migrated toward the anode, and the type III antigen migrated toward the cathode. The upper pattern in Fig. 1 shows the cathodal migration of the cross-reactive antigen from the pneumococcal vaccine.

The precipitation bands in Fig. 2 are those of the electrophoresed pneumococcal antigen, center well, precipitated by omniserum from the upper trough and GBS III NOR antiserum from the lower trough. Again the cathodal migration of the cross-reactive antigens was observed. The upper trough contained omniserum and the lower trough contained GBS III NOR antiserum. As previously described, the cathodal migration of the cross-reactive antigens was observed.

Cross-reactions observed with the immunoelectrophoretic technique were also observed in the immunodiffusion gels (Fig. 3 and 4).

Each of the three antisera, GBS III NOR, GBS III SS 620, and CDC Pn 14, formed a precipitin band with both Pn 14 polysaccharide and alcohol-fractionated GBS III NOR type III antigens. A line of identity was observed among GBS III NOR, GBS III SS 620, and CDC Pn 14 antisera against Pn 14 polysaccharides. Similarly, a line of identity was formed between the three antisera and GBS III NOR alcohol-fractionated antigen which had been absorbed with group B-specific antisera.

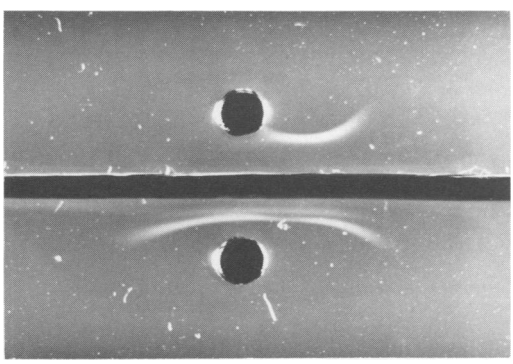

FIG. 1. *Immunoelectrophoresis of GBS III SS 620 antigens in the bottom well and S. pneumoniae antigens 1, 3, 4, 6, 7, 8, 9, 12, 14, 18, 19, and 23 in the upper well and antiserum against GBS III in the trough. Anode is on the left.*

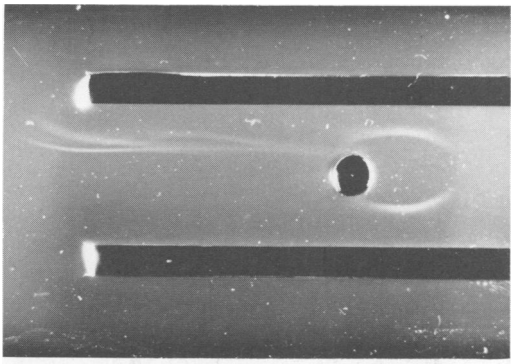

FIG. 2. *Immunoelectrophoresis of S. pneumoniae antigens 1, 3, 4, 6, 7, 8, 9, 12, 14, 18, 19, and 23 in the well and pneumococcal omniserum in the upper trough and GBS III antiserum in the lower trough. Anode is on the left.*

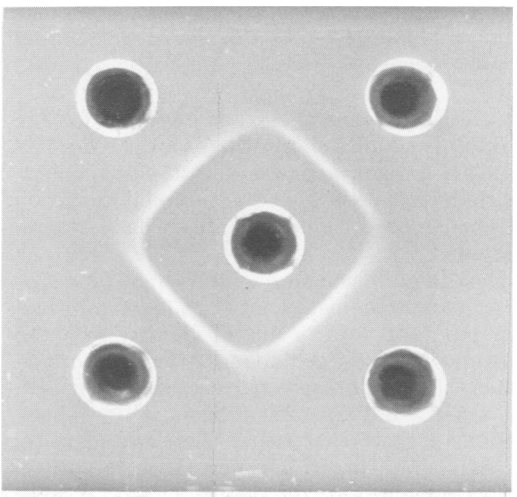

FIG. 3. *Immunodiffusion in agarose of Pn 14 polysaccharide in the center well, and clockwise from the upper left, antisera to the following organisms: CDC Pn 14, GBS III NOR, CDC Pn 14, and GBS III SS 620.*

DISCUSSION

The serological cross-reactions between GBS III and Pn 14 initially observed by immunoelectrophoresis were studied using various other in vitro techniques. In each situation, the cross-reactivity was observed and reproduced. The electrophoretic mobility of the cross-reactive Pn

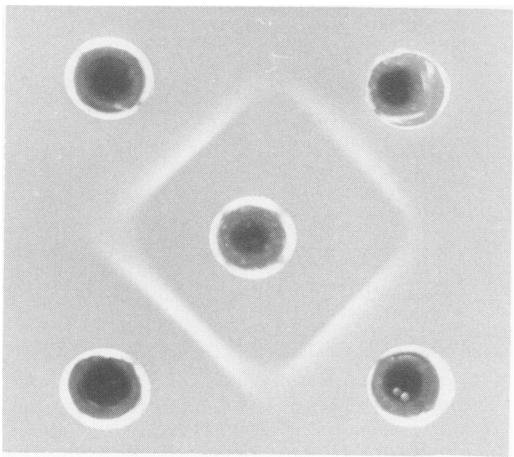

FIG. 4. *Immunodiffusion in agarose of GBS III NOR antigens in the center well, and, clockwise from the upper left, antisera to the following organisms: CDC Pn 14, GBS III NOR, CDC Pn 14, and GBS III SS 620.*

14 antigen is similar to that observed for the GBS type III antigen by others (5, 17). The GBS type III antigen migrates due to electroendosmotic forces in the gel. The cathodal migration of pneumococcal type 14 antigen has been reported (11).

Immunodiffusion studies show that type-specific antisera against type 14 *S. pneumoniae* and type III GBS form a line of identity with the pneumococcal antigen. Identity lines are formed when the same antisera are reacted against GBS III ethanol-fractionated antigen. These observations suggest that either there are identical determinants shared between GBS III and Pn 14 carbohydrate or the determinants are quite similar and may differ slightly in polysaccharide composition. The report of Lindberg et al. (14) describing the structure and composition of the Pn 14 polysaccharide and the work of Baker et al. (5) and Russell and Norcross (17) describing the composition of the type III GBS antigen show similarities in monosaccharide composition between the two antigens. The recent report of Kasper et al. (10) confirms our previous observation (Crumrine et al., Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 16th, Chicago, Ill., Abstr. no. 151, 1976) that cross-reactions between GBS III polysaccharide and Pn 14 polysaccharide occur.

Studies by Fischer et al. (8) clearly demonstrate the ability of Pn 14 antisera to opsonize GBS III. In their studies, Pn 14 antisera opsonized GBS III NOR but did not opsonize Pn 3 or GBS III O90R, which has no type antigens. Their in vivo studies also demonstrated a significant protective effect of Pn 14 antisera in a neonatal rat model of GBS III sepsis and meningitis. Data from Kasper's laboratory (10), however, appear to conflict with those of Fischer et al. (8). Kasper et al. (10) report poor opsonic responses for GBS in humans immunized with multivalent pneumococcal vaccine.

The data presented in this paper and the initial report from our laboratory clearly establish the fact that similar antigenic determinants are shared between GBS III and type 14 pneumococcus. Studies by Fischer et al. (8) and Kasper et al. (10) give further credence to these findings. However, further investigations are necessary to detail the role of opsonizing antiserum against GBS III antigen and Pn 14 antigen, and the protection they may afford in view of the present conflicting data.

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