INFECTION AND IMMUNITY, Nov. 1972, p. 651-656

Vol. 6, No. 5
Printed in U.S.A.

Enteric Bacteria Cross-Reactive with *Neisseria meningitidis* Groups A and C and *Diplococcus pneumoniae* Types I and III

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Received for publication 28 June 1972

Enteric bacteria of 1,335 individual strains were studied for serological cross-reactions with *Neisseria meningitidis* groups A and C and *Diplococcus pneumoniae* types I and III. Enterobacterial antigens cross-reactive with the capsular polysaccharides of these four bacteria were found. Bacteria cross-reactive with noncapsular antigens of meningococci and pneumococci were also observed. Since some enteric bacteria possess antigens with serological specificities similar to those of meningococci, the possibility that enteric bacteria cross-reactive with meningococcal antigens provide an antigenic stimulus for the observed age-related "natural" immunity to this pathogen is discussed.

his study was prompted by the observations that most children and young Army recruits in the United States have serum antibodies which react with the group A meningococcal polysaccharide, despite the rarity of this organism in this country for the past 20 years (4–6, 9, 11, 15). Several reports suggested to us that enteric bacteria could serve as the antigenic stimulus for these "natural" meningococcal group A polysaccharide antibodies. Serological cross-reactions have been described between *Escherichia coli* and the capsular polysaccharides of several pyogenic bacteria, including *E. coli* K30, K42, and K85 with *Diplococcus pneumoniae* types IV, I, and II, respectively (13), *E. coli* K1 with the meningococcal group B polysaccharide (2), and a newly described *E. coli* K antigen (f147) with the capsular polysaccharide of *Haemophilus influenzae* type b (19).

This report describes a study of a collection of *E. coli* strains and other fecal bacteria for cross-reacting antigens to the capsular polysaccharides of *N. meningitidis* group A and three other pyogenic bacteria: *N. meningitidis* group C and *D. pneumoniae* types I and III. Several *E. coli* and one *Bacillus* strain were found to possess antigens cross-reactive with the capsular polysaccharides and other antigens of these four pyogenic bacteria.

MATERIALS AND METHODS

Bacteria. Strains representative of known serotypes of *E. coli*, *Citrobacter*, *Klebsiella*, *Salmonella*, and *Arizona* were obtained from the World Health Organization Escherichia Reference Center and the Enterobacteriology Laboratory of the Center for Disease Control. In addition, strains from a previously reported collection of fecal organisms from human and animal sources were studied (19). Strains were stored on nutrient agar slants (Difco) at 4 C. *N. meningitidis* group A (ATCC 13077) and group C (ATCC 13102) were obtained from the American Type Culture Collection, Rockville, Md., and *D. pneumoniae* types I and III were provided by P. Baker and B. Prescott, National Institutes of Health, Bethesda, Md.

Antisera. Rabbit meningococcal group A and C antisera were prepared by injection of glutaraldehyde-fixed bacteria (8). Horse and rabbit anti-*D. pneumoniae* type I and type III sera were provided by B. Prescott and by K. Amiririan, New York State Department of Health, Albany. Sheep antisera against *H. influenzae* types a, b, and c and rabbit antisera against *Bacillus* (Sh 17) and *E. coli* (WHO 252) were prepared by injection of formaldehyde-fixed organisms as described (1).

Antigens. The meningococcal group A and C polysaccharides were prepared as described (10). B. Prescott provided purified samples of S I and III (14). The cross-reacting antigens from the *Bacillus* strain and the *E. coli* strain were extracted from broth supernatant fluids by ethanol precipitation and phenol extraction (19). A sonic extract of group C meningococci from an 18-hr Mueller-Hinton broth (Difco) culture was dialyzed exhaustively against water and lyophilized.

Serology. Single colonies from an overnight growth on tryptic soy agar (Difco) were inoculated into 10

ml of tryptic soy broth and incubated for 24 hr at 37 C with occasional shaking. The bacterial suspensions were allowed to react with antisera in Ouchterlony plates (2-mm wells, 6 mm apart) made of agarose (Marine Colloids, Inc., Springfield, N.J.) gel (1.2% in saline with 0.1% NaN_3) at 4 C. The gels were observed for precipitin lines at 24, 48, and 72 hr, washed in saline, air-dried, and stained with amido schwarz (19).

The effects of *N*-acetyl-D-mannosamine, D-glucuronic acid, D-glucose, D-galactose, and *N*-acetyl-D-galactosamine (Sigma Chemical Co., St. Louis, Mo.) were studied by immersion of the gels with formed precipitin bands in 10 ml of 0.15 M solutions of these monosaccharides in saline at room temperature for 15 min. Specific inhibition was defined by complete dissolution of precipitin lines or by a definite reduction in their thickness (18).

Antisera were absorbed with the homologous capsular polysaccharide adsorbed to alum (1 mg of polysaccharide/ml of serum). Absorption of sera with the cross-reacting antigens was done by addition of 0.1 ml of a 1% (w/v) solution of the antigen to 1 ml of serum. These sera were incubated at 37 C for 1 hr and at 4 C for 24 hr, and the immune precipitate or alum was removed by centrifugation at 10,000 × *g* at 4 C for 20 min.

Serotyping of *E. coli* possessing cross-reacting antigens was done by George Hermann, Enterobacteriology Laboratory, Center for Disease Control, Atlanta, Ga. Ruth Gordon, Institute of Microbiology, Rutgers University, kindly identified the *B. pumilis* strain.

RESULTS

Among the 1,335 strains of enteric bacteria studied, 13 strains (1%) with cross-reacting antigens were detected and are listed in Table 1.

Strains reactive with meningococcal antisera. Three strains, Sh 17 (*Bacillus pumilis*), C 32, and WHO 290 yielded precipitin lines with meningococcal group A antiserum (Fig. 1). The Sh 17 antigen yielded a partial identity reaction with the meningococcal group A polysaccharide. Absorption of the group A antiserum with the group A polysaccharide removed precipitin activity to Sh 17 but did not affect precipitation with C 32 and WHO 290. Group A antiserum absorbed with Sh 17 reacted less intensely with group A polysaccharide, but its reactivity with C 32 and WHO 290 was unaffected. Absorption with WHO 290 or C 32 removed reactivity with both of these antigens but did not detectably alter reactivity with the Sh 17 or meningococcus group A polysaccharides. Of the five monosaccharides tested, only *N*-acetyl-D-mannosamine dissolved the precipitin lines with Sh 17 and group A polysaccharides. The reaction with C 32 and WHO 290 was unaffected by any of the four monosaccharides. Sh 17 antiserum yielded a complete identity reaction with meningococcal group A polysaccharide and the Sh 17 antigen (Fig. 2).

Two of the bacteria, C 32 and WHO 290, reactive with the meningococcal group A antiserum, also precipitated with meningococcal group C antiserum (Fig. 3). Reactions of identity were observed with each of these *E. coli* antigens when tested with the meningococcal group A and C antisera. A third strain, FEC 12975, also precipitated with group C antiserum, although not with group A antiserum. The precipitin reactions with C 32, WHO 290, and FEC 12975 were not removed after absorption of the group C antiserum

TABLE 1. *Characteristics of enteric bacteria with cross-reactive antigens*

Strain designation	Species	Serotype	Cross-reaction	Specificity	
				Capsular	Other
Sh 17	*B. pumilis*	—	*N. meningitidis* group A	+	
C 32	*E. coli*	O 10:H4	*N. menginitidis* groups A and C		+
WHO[a] 290	*E. coli*	O 10:H4	*N. meningitidis* groups A and C		+
12975 FEC	*E. coli*	O undet[b]	*N. meningitidis* group C		+
BOS 12	*E. coli*	O 16:NM	*N. meningitidis* group C	+	
WHO 11	*E. coli*	O 48:NM	*D. pneumoniae* type I	+	
WHO 42	*E. coli*	O 113:H21:K75	*D. pneumoniae* type I		+
WHO 30	*E. coli*	O 7:H4:K7	*D. pneumoniae* type III	+	
WHO 252	*E. coli*	O 14:NM	*D. pneumoniae* type III	+	
WHO 165	*E. coli*	O 50:H7:K56	*D. pneumoniae* type III	+	
BOS Y2	*E. coli*	O 150:NM	*D. pneumoniae* type III	+	
ISB 1353	*E. coli*	O 50:H1	*D. pneumoniae* type III	+	
LH	*E. coli*	O 14:NM	*D. pneumoniae* type III	+	

[a] Organisms designated WHO were provided by F. Ørskov from the reference collection of the Escherichia Center, World Health Organization.

[b] O group undetermined owing to roughness.

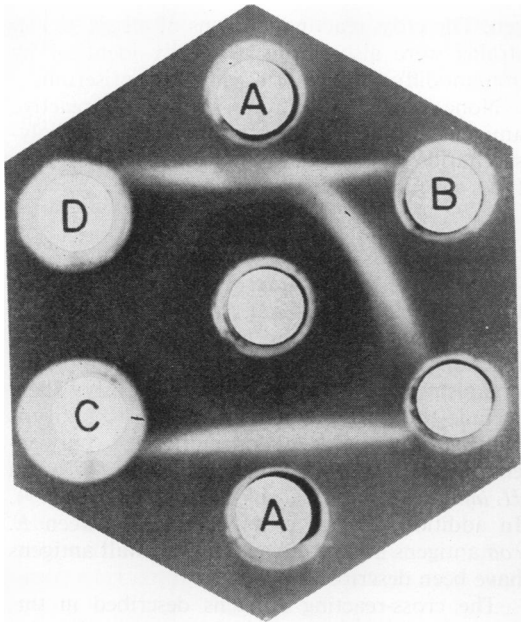

FIG. 1. *Immunodiffusion analysis with meningococcal group A antiserum (10 μliters) in center well. Samples in peripheral wells (10 μliters) are antigen solutions (concentrations expressed as grams [dry weight]/100 ml). Samples include: (A) group A meningococcal polysaccharide (0.01%), (B) Sh 17 (1%), (C) C32 (1%) and (D) WHO 290 (1%). Note partial identity of Sh 17 with group A polysaccharide.*

with the group C polysaccharide. Absorption o the group C antiserum with C 32, WHO 290, or FEC 12975 removed activity with all three *E. coli* preparations, suggesting immunological identity; reactivity with group C polysaccharide was unaffected by these absorptions.

The reactivity of the meningococcal group C antiserum with the three *E. coli* strains reacting with noncapsular antibodies (C 32, WHO 290, FEC 12975) could not be removed by absorption with group C meningococci as boiled or Formalin-fixed whole bacteria or as lyophilized, sonically disrupted bacteria. These crude antigen preparations of meningococci apparently do not contain sufficient amounts of the material cross-reactive with the three *E. coli* strains for absorption of the antibody. The specificity of these noncapsular reactions was verified by the absence of precipitins in the sheep *H. influenzae* antisera and in 16 normal adult rabbit sera.

E. coli strain BOS 12 also reacted with meningococcal group C antiserum, yielding a partial identity reaction to the group C polysaccharide (Fig. 3). Absorption of group C antiserum with the group C polysaccharide removed the reactivity toward BOS 12. Absorption of the group C antiserum with BOS 12 reduced the reactivity toward the group C polysaccharide but did not detectably alter the precipitin reaction with C 32, WHO 290, and FEC 12975.

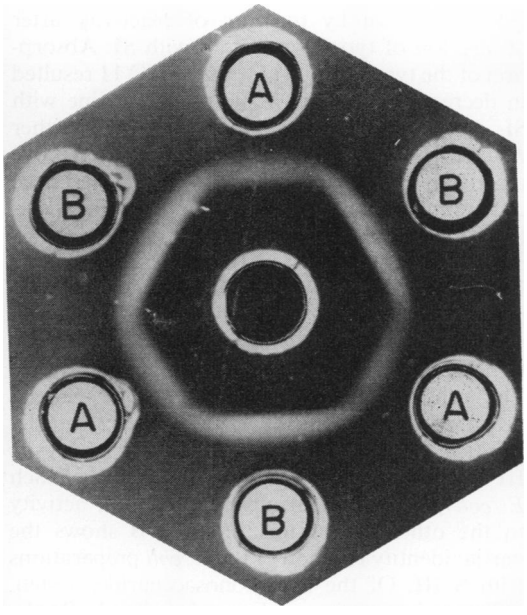

FIG. 2. *Immunodiffusion analysis with Bacillus (Sh 17) antiserum in center well. Samples include: (A) group A meningococcal polysaccharide (0.01%) and (B) Sh 17 (0.01%).*

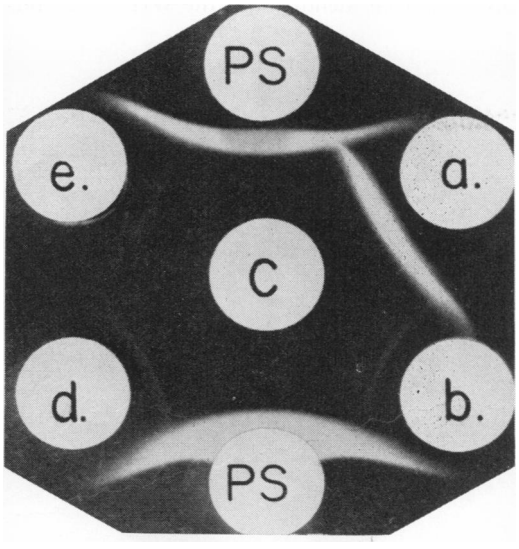

FIG. 3. *Immunodiffusion analysis with meningococcal group C antiserum in center well. Samples include: (PS) group C capsular polysaccharide (0.01%), (a) BOS 12 (1%), (b) FEC 12975 (4%), (d) C 32 (1%), and (e) WHO 290 (1%).*

Strains reactive with pneumococcal antisera. Two *E. coli* strains, WHO 42 and WHO 11, yielded precipitin reactions with pneumococcal type I antiserum (Fig. 4). Only WHO 11 cross-reacted with the type I capsular polysaccharide (SI), as shown by the loss of reactivity after absorption of type I antiserum with SI. Absorption of the type I antiserum with WHO 11 resulted in decreased intensity of the precipitin line with SI. Absorption of type I antiserum with either *E. coli* WHO 11 or WHO 42 failed to remove reactivity toward the other *E. coli* preparation, confirming their individual immunological specificity.

Six *E. coli* strains (WHO 30, WHO 252, WHO 165, BOS Y2, ISB 1353, and LH) reacted with pneumococcal type III antiserum. Absorption with SIII removed the precipitin activity of the type III antiserum with all six *E. coli* preparations. The cross-reacting moiety of the six *E. coli* preparations was shown to be immunologically identical by immunodiffusion analysis with type III antiserum (Fig. 5A) and by the ability of each *E. coli* preparation to absorb precipitin activity to the other five strains. Figure 5B shows the partial identity reaction of the *E. coli* preparations with S III. Of the five monosaccharides tested, only D-glucuronic acid partly dissolved the precipitin line of S III and the cross-reacting *E. coli* preparations with the type III antiserum. Antiserum raised in a rabbit with one *E. coli* strain (WHO 252) precipitated with S III which showed partial identity with the WHO 252 antigen. The cross-reacting antigens of all six *E. coli* strains were also immunologically identical by immunodiffusion analysis with this antiserum.

None of the 13 strains with cross-reactive antigens and none of the purified capsular polysaccharide preparations reacted with *H. influenzae* types a, b, and c antisera raised in sheep.

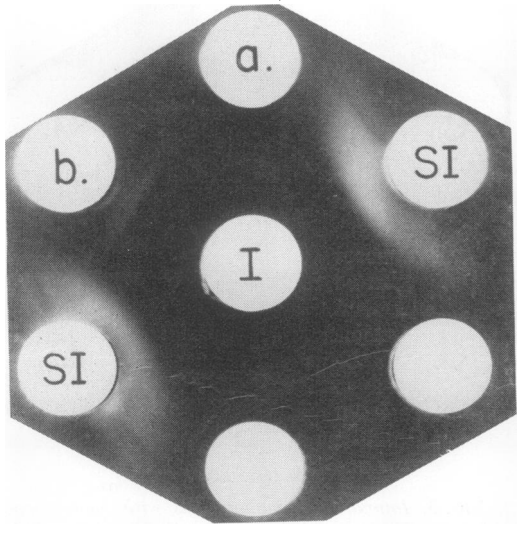

FIG. 4. *Immunodiffusion analysis with pneumococcal type I antiserum in center well. Samples include: (a) WHO 42 (1%), (b) WHO 11 (1%), and (SI) type I capsular polysaccharide (0.01%).*

DISCUSSION

Thirteen strains of enteric bacteria were found to contain antigens which reacted with pneumococcal or meningococcal antisera. Several cross-reactions between enteric bacteria and pathogenic organisms have been described previously. These include the cross-reaction of *E. coli* K antigens with various types of pneumococcal polysaccharides (13), group B meningococcus (12), and *H. influenzae* type b capsular polysaccharide (19). In addition, several cross-reactions between *E. coli* antigens and mycobacterial cell wall antigens have been described (17).

The cross-reacting antigens described in this study are extractable with phenol and precipitable with ethanol. Furthermore, the extracted antigens cross-react with purified pneumococcal and meningococcal polysaccharides, and the precipitation of two of the cross-reacting preparations (Sh 17 and WHO 252) was inhibited with monosaccharides. Thus, it appears that the cross-reacting antigens are polysaccharides.

Antibodies to *H. influenzae* type b and to meningococci have been demonstrated in the sera of adult humans and of several laboratory animals (7–9, 20). The development of these "natural" antibodies is age-related and has been proposed to confer immunity to meningitis caused by these organisms (3, 9). In the case of the meningococcus, it has been shown that these "natural" antibodies can arise as a result of the asymptomatic carriage of the homologous bacterium (9). However, it is unlikely that this stimulus is the only mechanism for the production of these antibodies. The low nasopharyngeal carrier rate of *H. influenzae* type b (22) and the recent report of bacteria from normal flora of humans and animals that cross-react with the *H. influenzae* type b capsular polysaccharide (3) suggest that "natural" antibodies may arise by stimulation with cross-reactive structures. The evidence is stronger for cross-reactive antigens as the stimulus for "natural" antibodies to group A meningococcal polysaccharide. Epidemiological surveys have demonstrated the almost complete absence of group A meningococci in the United States since 1953 (2, 4–6, 16). Thus the identification of an antigen in *B. pumilis*, a bacterium closely related to *B. subtilis* (21), cross-reacting

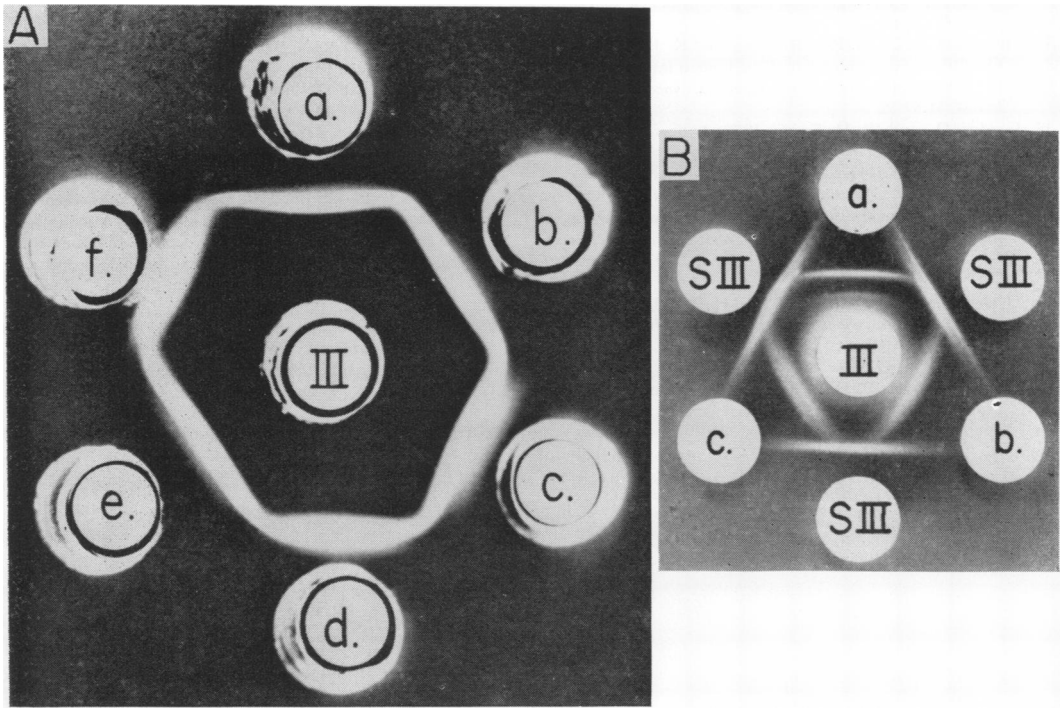

FIG. 5. *Immunodiffusion analysis with pneumoccocal type III antiserum in center wells. Samples include: (a) WHO 30 (1%), (b) WHO 252 (1%), (c) WHO 165 (1%), (d) BOS Y2 (1%), (e) ISB 1353 (1%), (f) LH (0.5%), and (SIII) type III capsular polysaccharide (0.01%). The cross-reacting antigenic determinants of the six cross-reactive strains are serologically identical with each other (part A) and partially identical with SIII (part B).*

with the group A meningococcal polysaccharide may provide an explanation for the prevalence of antibodies to this antigen in children and young adults. Further studies utilizing an antiserum agar technique (3) to identify bacteria cross-reacting with the meningococcal group A polysaccharide from mixed bacterial throat and stool cultures may provide a more accurate evaluation of their prevalence.

Hyperimmune sera raised against cross-reactive *E. coli* strains have been shown to precipitate the *H. influenzae* type b capsular polysaccharide. In addition, these *E. coli* antisera possess bactericidal and mouse protective activity toward *H. influenzae* type b similar to the homologous antiserum (19). We have shown that antiserum raised with the cross-reactive *Bacillus* (Sh 17) strain precipitates with the group A polysaccharide and antiserum raised with *E. coli* strain WHO 252 precipitates with SIII. Further studies of the immunogenicity of these cross-reactive antigens are in progress. These include experiments to define whether gastrointestinal colonization of neonatal animals by these nonpathogenic bacteria will induce protective immunity to the cross-reacting pathogenic bacterium.

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