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ESCHERICHIA COLI K51 AND K93 CAPSULAR POLYSACCHARIDES ARE CROSSREACTIVE WITH THE GROUP A CAPSULAR POLYSACCHARIDE OF *NEISSERIA MENINGITIDIS*

Immunochemical, Biological, and Epidemiological Studies

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Serum antibodies to the capsular polysaccharide (CPS)[1] of *Neisseria meningitidis*, as well as to other encapsulated bacterial pathogens, confer immunity to invasive diseases caused by these organisms (1–6). There is an age-related development of meningococcus group A (GrA) CPS antibodies (1, 2, 4–6). During infancy and childhood, when placentally required antibodies have declined and adult levels have not yet developed, the attack rate of GrA meningitis is at its highest (1). GrA diseases have a different epidemiological pattern than the other two major pathogenic groups of meningococci (B and C). GrA diseases occur annually with high frequency in central Africa (7–9). In other parts of the world, GrA diseases occur as epidemics lasting one or two years (9–11). Yet, in both endemic and epidemic situations, asymptomatic carriage of GrA organisms is low, ~3% (10, 12, 13). In the United States, GrA organisms have been only rarely detected either in patients or in asymptomatic carriers during the past 30 years (10, 14–17). Despite the absence or low carriage rate of the homologous organism, most adults in all these countries have protective levels of GrA CPS antibodies (1, 2, 18). It has been proposed (18–26) that an important antigenic stimulus for CPS antibodies, including those of GrA, is the asymptomatic carriage of crossreacting bacteria of the respiratory and of the gastrointestinal tract (18–26). To date, two strains of bacteria have been reported to crossreact with the

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[1] *Abbreviations used in this paper:* Ab, antibody; CPS, capsular polysaccharide; GrA and GrX, *Neisseria meningitidis* serogroups A and X; Hib, *Hemophilus influenzae* type b; LT, heat-labile, cholera-like enterotoxin; PS, polysaccharide; SDS, sodium dodecyl sulfate; SP, spontaneous agglutination; ST, heat-stable enterotoxin.

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GrA CPS: a *Bacillus pumilis* species, strain Sh17, and *Streptococcus faecalis* (24–26).

The structure of the GrA CPS has been shown (27) to be a pseudo-randomly *O*-acetylated, α-1 $\rightarrow$ 6–linked linear homopolymer of manNAc-1-PO_4. A similar structure has been detected in *B. pumilis* (26). *B. pumilis* and the crossreacting strains of *S. fecalis* are not common inhabitants of the human flora and it is unlikely that these bacteria are major stimuli for the widely prevalent GrA CPS antibodies. Two crossreactive *B. pumilis* strains, however, were detected in stool cultures of skid row inhabitants of the Pacific Northwest during an outbreak of GrA meningitis (28, 29).

We report the discovery of two *Escherichia coli* CPS (K antigens), K51 and K93, that are crossreactive with GrA CPS, and describe some of their epidemiological, biological, and immunologic properties. The structure of these two *E. coli* CPS will be reported elsewhere (Guirguis, Bax, Egan, Schneerson, Robbins, submitted for publication).

Materials and Methods

Population. The crossreacting *E. coli* were discovered during a study of the etiology and some laboratory characteristics of bacterial meningitis in Egypt, 1977–1978 (30). Suspected meningitis cases, admitted on Tuesday of each week, were selected for a study of household contacts. A total of 96 families were examined. The families were visited in their homes and stool specimens were obtained from 645 members of these 96 families.

Bacteria. Crossreactive bacteria were identified by the antiserum agar technique (31). The 645 stool samples were streaked onto minimum essential medium (Difco Laboratories, Inc., Detroit, MI) containing 3% equine GrA meningococcal serum (H49) and 1.2% agarose (Marine Colloids, Rockport, ME). Controls included antiserum agar prepared with equine group B and burro group C meningococcal antisera (6, 21). The plates were incubated at 37°C for 24 h and at 4°C overnight and were then inspected for halos. The 11 *E. coli* strains were the only bacterial species that yielded immunospecific halos on the H49 antiserum agar.

E. coli strains, isolated from the urine (253 patients), tissues (3), wounds (35), and stools (29) of patients in the Clinical Center, National Institutes of Health (NIH), Bethesda, MD were kindly donated by Dr. Vee Gill. *E. coli* strains were isolated from 105 stool samples from school children in Copenhagen with diarrhea submitted to the Statens Seruminstitut. Five *E. coli* colonies, with different colonial morphology, from each stool specimen were plated onto H49 antiserum agar.

Fermentation reactions and serotyping of crossreactive *E. coli* were done at the Collaborative Center for Reference and Research on Escherichia (WHO), Copenhagen, Denmark, by established methods (32). *N. meningitidis* group X (GrX) strain S2795 was donated by Dr. Harry Feldman, State University of New York, Upstate Medical Center, Syracuse, NY.

Antisera. Hyperimmune sera against GrA strain A1; *E. coli* K93 strains 999-78, 1064-78, and 1084-77; *E. coli* K51 strain 61-78; and *B. pumilis* Sh17 were produced by multiple intravenous injections of formalinized cells into a horse and into rabbits, as described (33). Three rabbits were injected with each strain.

Polysaccharides. GrA CPS, obtained from strain A1, was donated by Drs. Carl Frasch and Chou Ming Tsai, Office of Drugs and Biologics, FDA, Bethesda, MD. GrX CPS was kindly donated by Dr. Harold Jennings, Medical Research Council, Ottawa, Canada. *E. coli* K93 and K51 and *B. pumilis* Sh17 polysaccharides (PS) were prepared by transferring 2.0 liters of a 6 h growth to a 50 liter fermenter containing minimum essential medium, supplemented with 0.1% dialyzed yeast extract (21). After 16 h incubation at 37°C with vigorous aeration, the culture was centrifuged and an equal volume of 0.2% (wt/vol) cetyltrimethylammonium bromide (Cetavalon; Sigma Chemical Co., St. Louis, MO) in

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water was added to the supernatant. The resulting precipitate was separated by centrifugation and thoroughly suspended in 0.9 M $CaCl_2$. The polysaccharides were purified by extraction with DNase, RNase, cold phenol, and ultracentrifugation as described (34–36), and contained <1% wt/wt of protein, nucleic acids, and lipopolysaccharide. Gel filtration of the purified *E. coli* CPS used CL-4B Sepharose (Pharmacia, Inc., Piscataway, NJ) equilibrated in 0.2 M NaCl (35). The K93 CPS was de-*O*-acetylated by heating in 0.1 M ammonium hydroxide at 37°C for 1 h.

Serology. Double immunodiffusion and rocket immunoelectrophoresis were performed as described (22, 37, 38). Overnight cultures of the K93 and K51 strains were adjusted to OD 1.4 at 540 nm. The CPS concentrations of these supernatants were compared with standard solutions of 5–300 μg/ml of either K93 or K51 CPS. The plates were dried, washed, and stained with 1% Coomassie Brilliant Blue.

Quantitative precipitation analysis was performed by adding 0.5 ml of 25–300 μg/ml polysaccharide solutions to 0.5 ml of antiserum. The tubes were mixed and then incubated at 37°C for 1 h and at 4°C for 48 h, with occasional agitation. The precipitates were separated by centrifugation, washed three times with 0.15 M NaCl, and dissolved in 0.8% sodium dodecyl sulfate (SDS). Their protein content was measured by absorption at 280 nm, assuming an antibody extinction coefficient of 14.0 (22). *Hemophilus influenzae* type b (Hib) CPS was used as a control.

Bactericidal Reactions. The complement-dependent bactericidal assay was performed with GrA strain A1 organisms and *E. coli* K51 and K93 rabbit antisera before and after absorption with either GrA, K93, or K51 CPS (22, 39). Pre-colostral calf serum was used as the source of complement. Heat-inactivated H49 antiserum and fetal calf serum were used as controls.

Antimicrobial Sensitivity Tests. The following antimicrobial disks were used: penicillin (10 μg/disk), ampicillin (10 μg/disk), cephalothin (30 μg/disk), neomycin (30 μg/disk), erythromycin (15 μg/disk), gentamycin (10 μg/disk), kanamycin (30 μg/disk), streptomycin (10 μg/disk), chloramphenicol (30 μg/disk), tetracycline (30 μg/disk), sulfisoxazole (0.25 mg/disk), and tobramycin (10 μg/disk). Mueller-Hinton agar, 4 mm deep, was used. *Staphylococcus aureus* ATCC 25923, *E. coli* 25923, and *Pseudomonas aeruginosa* ATCC 27853 were obtained from the Centers for Disease Control (CDC), Atlanta, GA and used as reference strains.

Toxin Production. Enterotoxin production was examined by the Chinese hamster ovary cell assay for the detection of heat-labile, cholera-like enterotoxin (LT) and by the suckling mouse assay for the production of heat-stable enterotoxin (ST) as described (40, 41).

Invasiveness. The 11 crossreactive strains from Egypt were tested for invasiveness in the guinea pig conjunctivitis model as described by Sereny (42). Positive and negative *E. coli* strains for LT, ST, and invasiveness were kindly provided by Dr. R. L. Geurrant, University of Virginia Medical School, Charlottesville, VA.

Results

Prevalence and Characterization of Escherichia Coli Crossreactive with GrA CPS. Halo-positive organisms, cultivated on the H49 antiserum agar plates, were found in 11 of 645 (1.7%) stool specimens obtained in Egypt (Table I). These isolates gave fermentation reactions characteristic of *E. coli*; 10 of the 11 strains showed an unusual marker that was adonitol positive and late or rhamnose negative. These 10 strains belonged to the sero-biotype *O*107:K93:H27 or the flagellar, negative SP type, and may be considered to be progeny of a single *E. coli* strain or a clone. Eight of these 10 isolates gave spontaneous agglutination (SP) when tested against H antisera. All K93 strains were crossreactive with K53 antiserum; this serological reaction was shown to be due to their CPS (43). The remaining crossreactive *E. coli* strain was typed as *O*7:K51:H18 and lacked the adonitol marker. WHO Reference *E. coli* strains of K51 and K93, although

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TABLE I

Identification, Serotype Source, and Reactivity with Equine Group A Meningococcal Antiserum-agar of Bacteria

Strain designation	Serotype			Source	Halos on GrA meningococcal (H49) antiserum agar
	0:	K:	H:		
Survey No. 1. Egypt, 1977–1978 (n = 645)					
61-78	7	51	18	Human stool	+
163-78	107	93	27		+
464-78	107	93	27		+
999-78	107	93	SP.		+
1014-78	107	93	SP.*		+
1064-77	107	93	SP.		+
1064-78	107	93	SP.		+
1084-77	107	93	SP.		+
1084-78	107	93	SP.		+
1109-77	107	93	SP.		+
1139-77	107	93	SP.		+
Survey No. 2. Clinical Center, NIH, 1984 (n = 320)					
51-6-84	1	51	7	Human stool	+
4-5-84	107	93	27	Human urine	+
19-5-84	107	93	27	Human urine	+
188-10-84	17	93	18	Human spleen	+
314-12-84	17	93	18	Human sacrum wound	+
Survey No. 3. School Children, Copenhagen, Denmark, 1985 (n = 105)					
F39950.85	17	93	18	Human stool	+
F39229.85	17	93	18		+
F39830.85	17	93	18		+
WHO Reference *E. coli* strains:					
A183a	1	51	—	Human, appendicitis	+
1935	150	93	6	Chicken, septicemia	+
PA236	6	53	—‡	Human, peritonitis	−
B. pumilis (Sh17)				Human stool	+
N. meningitidis GrA (A1)				Human CSF‖	+
N. meningitidis GrX (S2795)§				Human CSF	+

* Spontaneous agglutination.
‡ The *E. coli* K53 PS is crossreactive with K93.
§ From the collection of Dr. Harry Feldman, SUNY, Syracuse, NY.
‖ Cerebrospinal fluid.

differing in their *O* antigens, gave strong halos on H49 antiserum agar. The WHO K53 test strain, known to crossreact with K93, did not yield detectable halos with H49 antiserum agar (44).

Four K93 strains and one K51 strain were found among 320 *E. coli* samples (1.6%) from patients at the NIH Clinical Center. The serotypes of these strains

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were as follows: (*a*) two from urine were *O*107:K93:H27; (*b*) one from spleen taken during an autopsy was *O*17:K93:H18; (*c*) one from a wound infection was *O*77:K93:H18, and (*d*) one from stool was *O*1:K51:H7. Three K93 strains were found among the 105 (2.9%) stool samples from children in Copenhagen; all were of the *O*17:K93:H18 serotype; no K51 strains were found in this survey (Table I).

Antibiotic Sensitivities. The 11 crossreactive strains from Egypt varied in their susceptibility to antimicrobial drugs; most of them were sensitive to cephalothin, erythromycin, gentamycin, and tobramycin and were resistant to penicillin, ampicillin, neomycin, kanamycin, streptomycin, chloramphenicol, tetracycline, and sulfisoxazole.

Invasiveness and Enterotoxigenicity. None of the crossreactive isolates from Egypt were invasive in the guinea pig corneal test nor did they secret ST or LT.

Rocket Immunoelectrophoresis. All 11 overnight culture supernatants of the *E. coli* K93 and K51 strains from Egypt exhibited rapid anodal migration (data not shown). The intensity of the *E. coli* CPS rockets was less than that of the GrA CPS. The rockets formed by the K93 and K51 CPS were higher but less intense than those formed by the GrA CPS at comparable concentrations. The amount of K93 CPS produced by the 10 strains varied between 12 and 25 μg/ml culture supernatant.

Serological. Fig. 1 shows halos of immunoprecipitation surrounding colonies of a representative strain of *E. coli* K93 (999-78) on H49 agar. Similar halos were observed with GrA, GrX, and *E. coli* K51 organisms. No halos were observed with these or the other K51 or K93 strains grown on antiserum agar prepared with either group B or group C meningococcal antiserum or with H49 antiserum absorbed with GrA CPS.

Fig. 2 shows double immunodiffusion of the supernatants of *E. coli* K93 and K51 overnight cultures with H49 antiserum. All nine K93 strains exhibited an identical reaction with each other and a partial identity with GrA CPS. The K51

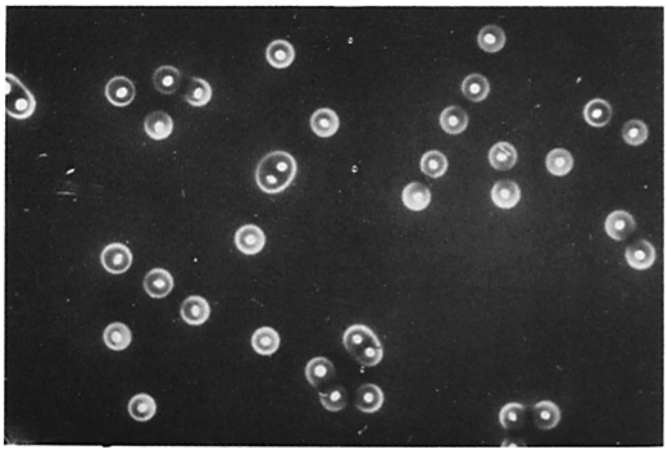

FIGURE 1. Halo formation surrounding colonies of *Escherichia coli*, strain 999-78, serotype *O*107:K93:SP, cultivated on H49 equine GrA meningococcal antiserum agar. Halos, indistinguishable from those formed by this organism, were formed by all *E. coli* K51 and K93 and GrA *Neisseria meningitidis* strains.

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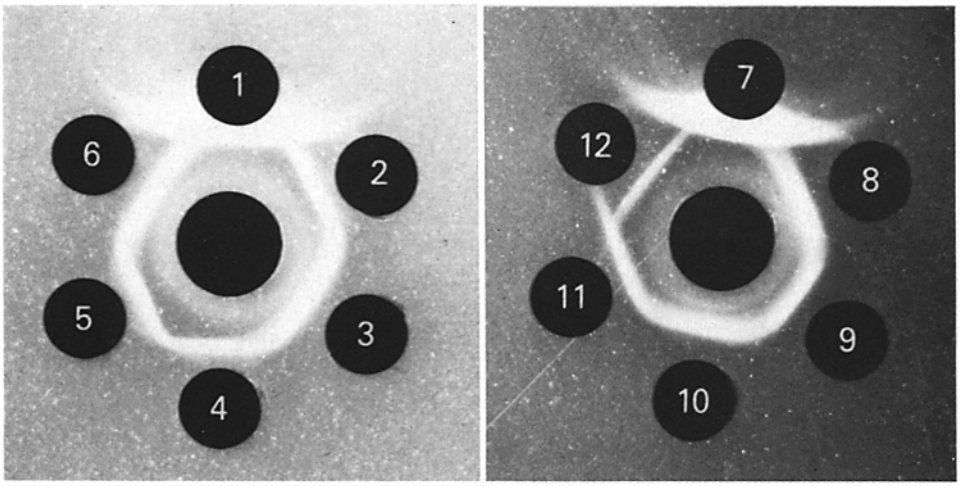

FIGURE 2. Double immunodiffusion of the supernatants from overnight cultures of *E. coli* K93 and K51 strains with equine GrA meningococcal antiserum (H49). Wells 1 and 7 contain GrA meningococcal CPS, 100 μg/ml. The following wells contained the supernatants from *E. coli* K93 strains: (2) 1139-77, (*3*) 1109-77, (*4*) 163-78, (*5*) 464-78, (*6*) 999-78, (*8*) 1014-78, (*9*) 1064-78, (*10*) 1084-77, (*11*) 1084-78; (*12*) the supernatant from K51 61-78. All supernatants from *E. coli* K93 strains exhibited an identical reaction with each other and a partial identity with GrA CPS. The K51 supernatant in well 12 yielded a partial identity with both GrA CPS and K93 CPS.

CPS yielded a partial identity with both K93 and GrA CPS. Identical results were obtained with the purified K93 and K51 polysaccharide (data not shown).

Fig. 3 shows the antigenic relations between the four PS and four antisera. The *B. pumilis* Sh17 PS yielded a nonidentical reaction with both K93 and K51 CPS and a partial identity reaction with the GrA CPS when examined against the H49 antiserum (Fig. 3*A*).

None of the rabbits immunized with the K93 strains, the K51 strain, or with the Sh17 strain had preexisting precipitating antibodies, as measured by double immunodiffusion, to any of the four PS. The rabbits injected with the K93 and K51 strains responded in a similar fashion after the first course of immunization; all the sera showed homologous but not heterologous precipitating antibodies. In contrast, the antisera obtained from the rabbits immunized with the Sh17 strain showed both homologous and GrA CPS reactions after the first immunization. The antisera taken after the second course of immunization with the K93 and K51 strains precipitated with the homologous as well as with the GrA CPS. The K93 antiserum obtained after the second course of immunization also precipitated with the K51 CPS. The hyperimmune K51 antisera, in contrast, did not react with the K93 CPS or Sh17 PS.

After the second immunization, the K93 sera (Fig. 3*B*) yielded a dense precipitin band with K93 CPS and a partial identity reaction with GrA CPS. Fig. 3*B* fails to show the faint reaction between the K93 serum and the K51 CPS. No reaction was observed between K93 antiserum and the Sh17 PS.

Similarly, the K51 serum yielded (Fig. 3*C*) a dense precipitin line with the K51 CPS and a partial identity reaction with the GrA CPS. No reaction was observed between the K51 antiserum and the K93 CPS and Sh17 PS.

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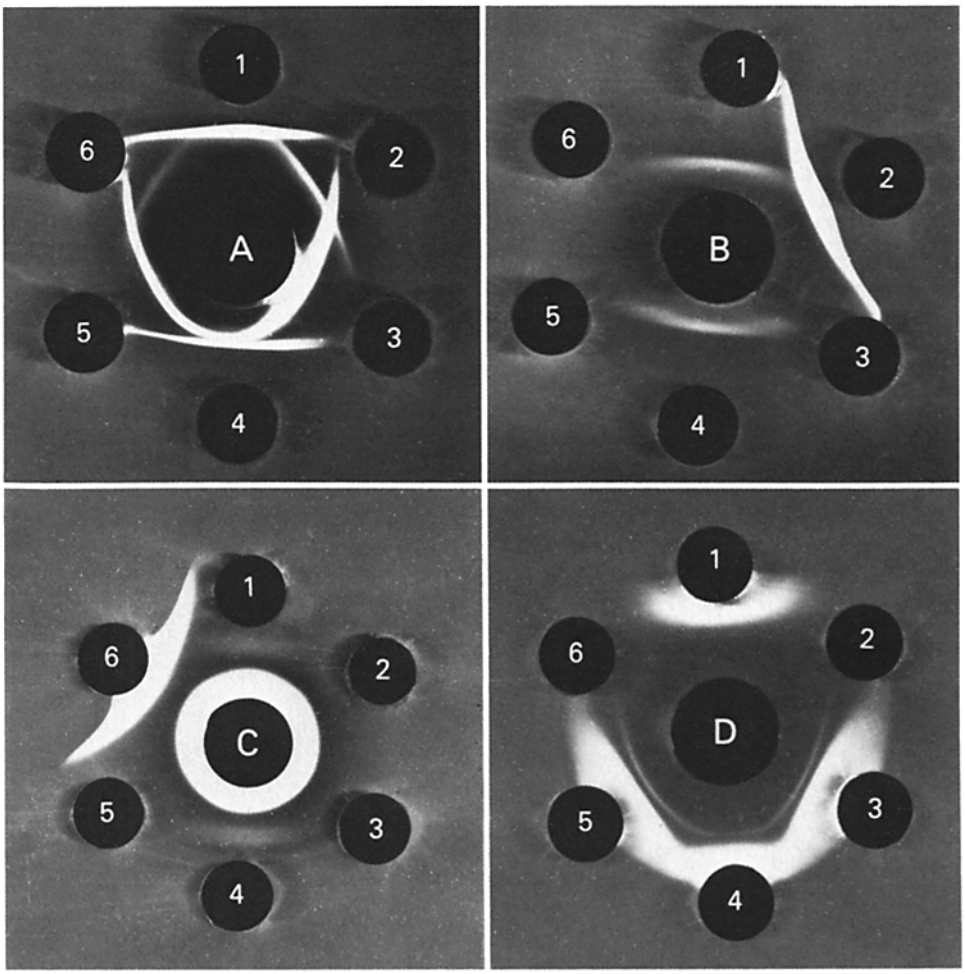

FIGURE 3. Double immunodiffusion of hyperimmune antisera with purified PS. The center wells contain the following: (*A*) equine meningococcal GrA antiserum (H49), (*B*) rabbit *E. coli* K93 antiserum, (*C*) rabbit *E. coli* K51, (*D*) rabbit *B. pumilis* Sh17. Outer wells contained the following PS at 100 μg/ml: (*1* and *4*) meningococcal GrA CPS, (*2*) *E. coli* K93 CPS, (*3* and *5*) *B. pumilis* Sh17 PS, (*6*) *E. coli* K51 CPS.

Fig. 3*D* shows an identity reaction between the Sh17 antiserum and the Sh17 PS and GrA CPS. No reaction was observed between the Sh17 antiserum and either the K93 and K51 CPS.

De-*O*-acetylation of the K93 CPS resulted in complete loss of its reactivity with H49 antiserum and in a partial identity reaction, compared with the native K93 CPS when reacted against the rabbit K93 antiserum (data not shown). All K93 preparations gave an identical reaction with the WHO *E. coli* K53 reference serum (data not shown).

Absorption Experiments. Fig. 4 shows the effect of absorption with the four polysaccharides upon the precipitating antibodies of the H49 antiserum. Absorption of H49 antiserum with the GrA CPS removed its precipitin activity against

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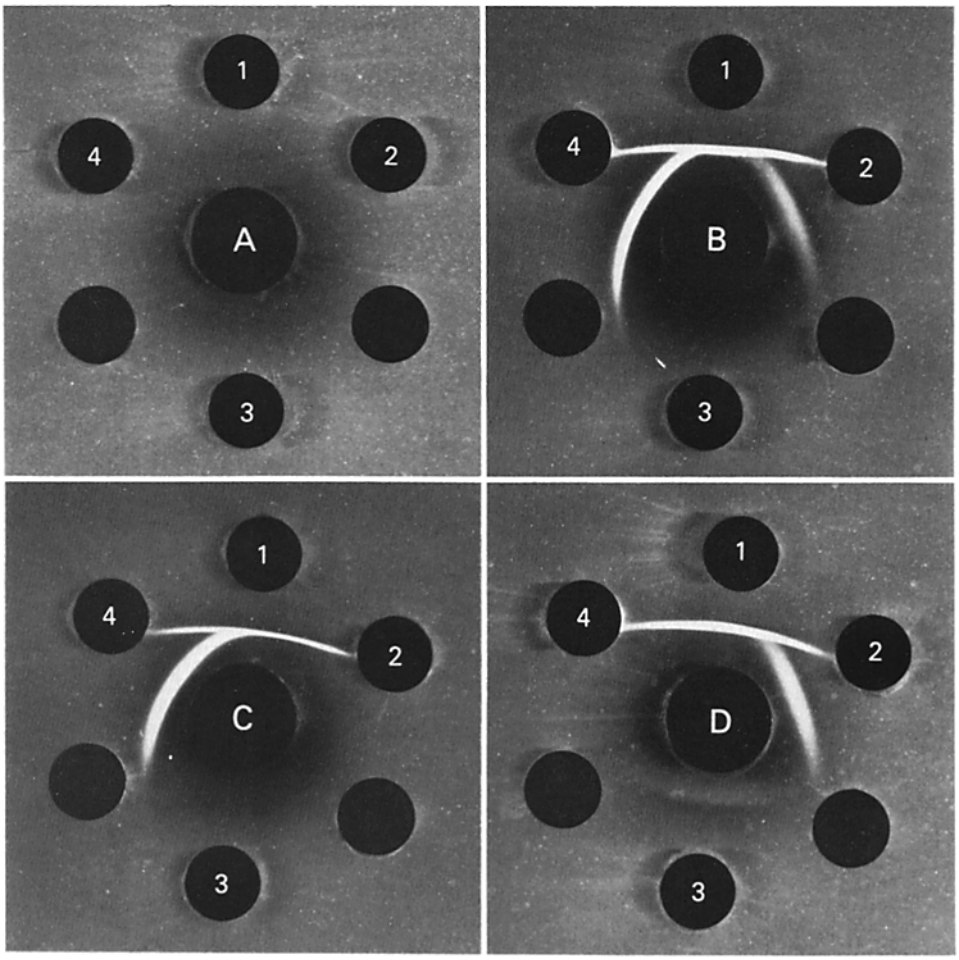

FIGURE 4. The effect of absorption with the homologous and crossreacting PS upon the reactivity of equine GrA meningococcal antiserum (H49). Central wells contained the following antisera. (*A*) H49 absorbed with GrA CPS. (*B*) H49 absorbed with *E. coli* K51 CPS. (*C*) H49 absorbed with *E. coli* K93 CPS. (*D*) H49 absorbed with *B. pumilis* Sh17 PS. Peripheral wells contained the following PS at 100 μg/ml: (*1*) GrA CPS. (*2*) *E. coli* K93 CPS, (*3*) *E. coli* K51 CPS, (*4*) *B. pumilis* Sh17 PS.

all four polysaccharides (Fig. 4*A*). Absorption of H49 antiserum with the K51 CPS removed only the homologous reaction; the absorbed serum still precipitated with K93 and Sh17 PS (Fig. 4*B*). Absorption of H49 antiserum with the K93 CPS removed its reaction with the K93 and the K51 CPS (Fig. 4*C*). The K93-absorbed H49 antiserum still precipitated with Sh17 PS. Absorption of H49 antiserum with Sh17 removed only the Sh17 PS reaction (Fig. 4, *C–D*).

Quantitative Precipitation Analysis. Fig. 5 shows that all four PS gave a symmetrical precipitation curve, typical of a single antigen, with H49 antiserum. The H49 GrA antiserum contained 5.1 mg GrA CPS antibody (Ab)/ml. The K51, K93, and Sh17 PS precipitated 25, 46.8, and 50% of H49 antibodies, respectively. Hib CPS (control) did not precipitate with H49 antiserum.

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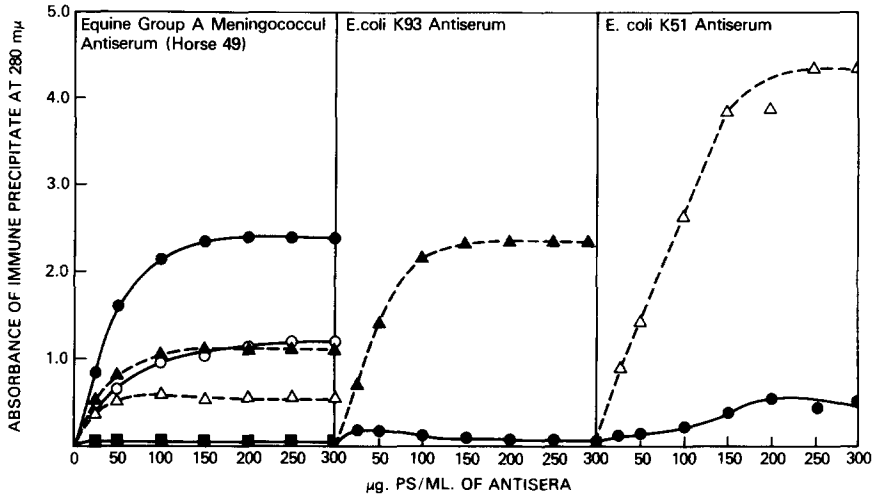

FIGURE 5. Quantitative precipitation analyses of the reaction between GrA meningococcal *E. coli* K93 and K51, *B. pumilis* Sh17, and Hib PS and equine GrA meningococcal (H49), rabbit *E. coli* K93, and rabbit *E. coli* K51 antisera. PS (25–300 μg) in 0.5 ml were added to 0.5 ml of each antisera, mixed, and incubated at 37°C for 1 h and at 4°C for 48 h, with occasional agitation. The precipitates were separated by centrifugation, washed with saline, dissolved in 0.8% SDS solution and their protein content measured by absorbance at 280 nm.

K93 rabbit antiserum (strain 999-78), after the first immunization, had 4.1 mg K93 CPS Ab/ml; there was no detectable precipitate with the GrA, K51, or Sh17 PS. After the second immunization, the K93 antiserum contained 4.6 mg K93 CPS Ab/ml; GrA CPS precipitated 7% of these antibodies (Fig. 5).

Similarly, K51 rabbit antiserum had 4.5 mg K51 CPS Ab/ml and had no precipitating GrA CPS antibodies after the first immunization. After the second immunization, however, there were 9.0 mg K51 CPS Ab/ml; GrA CPS precipitated 25% of these antibodies. Sh17 antiserum had 4.9 mg Ab/ml to the homologous CPS antigens after the first immunization. Both GrA CPS and Sh17 PS precipitated 4.9 mg Ab/ml from this antiserum (data not shown), which is consistent with the immunodiffusion data (Fig. 3*D*).

Bactericidal Activity of Escherichia Coli K93 and K51 Rabbit Antisera Against GrA Meningococci. Preimmunization sera of the rabbits had low levels of bactericidal activity to GrA strain A1 (Table II). A progressive increase in bactericidal titer was observed with all the sera taken after the first and second immunizations with the K51 and K93 *E. coli*. Absorption of both the K93 and K51 sera, taken after the second immunization, with GrA or with their homologous CPS reduced their titers to preimmunization levels. Absorption of the K93 serum with K51 CPS did not result in a detectable loss of bactericidal activity against GrA organisms. In contrast, absorption of the K51 antiserum with the K93 CPS resulted in a fourfold reduction in bactericidal activity against the GrA strain.

K93 and K51 CPS. Gel filtration on Sepharose CL-4B showed a homogenous component of 0.30 Kd (partition coefficient) for the K93 CPS and 0.34 Kd for the K51 CPS (data not shown). The eluted fractions precipitated with H49 antiserum by double immunodiffusion.

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TABLE II

Complement-dependent Bactericidal Activity against Group A Meningococcus Strain A1 of Rabbit Antisera to Escherichia coli K93 and K51 Before and After Absorption with Group A Meningococcal and E. Coli K51 and K93 Capsular Polysaccharides

Rabbit sera	Reciprocal bactericidal titers			
	Unabsorbed	Absorbed with CPS		
		GrA	K93	K51
K93 (999-78)				
Preimmunization	16	ND	ND	ND
Post 1st immunization	512	ND	ND	ND
Post 2nd immunization	4,096	8	32	4,096
K51 (61-78)				
Preimmunization	4	ND	ND	ND
Post 1st immunization	64	ND	ND	ND
Post 2nd immunization	1,024	4	256	4

ND, not determined.

Discussion

Serum GrA CPS antibodies have been demonstrated in most children and adult humans in the United States despite the rarity of this organism in this country during the past thirty years (1, 6, 18, 45–51). The development of GrA CPS "natural" antibodies is age related and has been reported to confer immunity to meningitis caused by this organism (1, 2, 6, 18). CPS antibodies can be elicited during convalescence from disease or from asymptomatic carriage of the homologous organism (6); it is unlikely, however, that this is the only or the major stimulus for the production of GrA CPS antibodies. The most probable antigenic source for GrA CPS antibodies is crossreactive bacteria from gastrointestinal flora (19–26). Despite their structural and serological similarities, it is unlikely that the *B. pumilis* Sh17 PS is an important stimulus for the widely prevalent GrA CPS antibodies. *E. coli* strains of the K51 and K93 capsular types have a prevalence rate of ~2% in surveys from three continents, including the U.S., and could be a likely source of this antigenic stimulus.

The K93 isolates from Egypt and the U.S. were of the *O*107:K93:H27 serotype. The consistent pattern of serotype, antibiotic sensitivity, and metabolic properties among the K93 strains from Egypt is consistent with the hypothesis that these organisms are descendents of a single bacterium and may be considered a "clone" (44). The WHO reference *E. coli* K51 and K93 strains also showed this cross reactivity with GrA CPS. The CPS purified from the WHO reference K93 and K51 and our isolates were indistinguishable. The *E. coli* K53 CPS, which is crossreactive with K93, did not react with H49 antiserum (43).

E. coli K51, K53, and K93 are common capsular antigens found in association with several different *O*-antigens. Both Vahlne (52) and Sjostedt (53) described common serotypes of *E. coli* with K51 and K53 CPS isolated from human extraintestinal disease and from normal feces. Strains with K51, K53, and K93 CPS were all represented in *E. coli* blood isolates from patients in the U.S. and Denmark (54, 55). It is likely that some of the earlier described K53 strains in

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these studies were actually K93 strains. It also appears from the files at the Escherichia Centre in Copenhagen that these K CPS are represented not infrequently in strains from both man and animals. K93 strains are found in isolates from the urinary tract. Strains with K51 and K93 antigens should, therefore, be regarded as belonging to that group of opportunistic pathogenic *E. coli* that are commonly found in the human intestine and which may cause extraintestinal disease (59). Such strains are well suited to stimulate GrA CPS antibodies without causing disease in the healthy host. K93 strains were more frequent than K51 strains in our surveys and were more crossreactive with the GrA CPS than the K51 CPS. It is likely, therefore, that the K93 is a more important stimulus for GrA CPS antibodies than either the K51 CPS or Sh17 PS.

The absorption experiments with H49 antiserum and the K93, K51, or Sh17 PS showed the antigenic relations between these crossreactive antigens. GrA CPS absorbed both the homologous and crossreactive antibodies from H49 antiserum. Sh17 PS, reported to be composed of a linear homopolymer of → 6)-manNAc-1-(PO_4 → , glycerol phosphate teichoic acid–containing *N*-acetylglucosamine, alkali-labile alanine esters, and a mucopeptide, is closest of the three PS in its structure to GrA CPS (26). The crossreactivity of Sh17 PS with GrA CPS can be explained by its content of poly → 6)-manNAc-1-(PO_4 →. Sh17 PS precipitated 50% of GrA CPS antibodies when tested by the quantitative precipitation analysis. K51 was found to be a polymer of → 3)-GlcNAc-1-(PO_4 →. Yet, this apparently closely related structure was the least crossreactive of the three PS, removing only 25% of the GrA CPS antibodies from the H49 serum. Although *N*-acetylglucosamine and *N*-acetylmannosamine are epimers, the linkages and *O*-acetylation between the two CPS are different.

The surprising finding was that K93 CPS, capable of precipitating 46.8% of GrA CPS antibodies from H49 antiserum, had no obvious structural resemblance to GrA CPS. K93 CPS is composed of an unusual disaccharide-repeating unit, galactofuranose and glucuronic acid, and contains no mannosamine or phosphate group. The unexpected crossreaction presumably derives from the three-dimensional similarity between the K93 CPS and the GrA CPS, a point we plan to pursue. The importance of the three-dimensional similarity in conferring this crossreactivity was shown by the change in reactivity between the de-*O*-acetylated and native K93 CPS with both the H49 and K93 antisera. A similar relationship, between the three-dimensional structure of pyruvylated derivatives of D-galactose and D-glucose and their reactivity with human monoclonal antibodies, has been reported (56).

The immune rabbit sera elicited by intravenous injection of both *E. coli* K93 and K51 strains had bactericidal activity against GrA organisms. These *E. coli*–induced bactericidal antibodies were removed by absorption with GrA CPS. Cross-absorption experiments showed that K93 CPS was capable of absorbing most of the bactericidal effect of K51 antiserum on GrA organisms; the reverse was not demonstrable. These findings suggest that K51 and K93 strains are an antigenic stimulus for the widely prevalent GrA CPS bactericidal antibodies and the immunity in areas with few or no GrA organisms. It is also possible that these crossreactive *E. coli* strains in the gastrointestinal tract could stimulate blocking GrA CPS antibodies of the IgA isotype, as Griffis has suggested (57). The use of

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the purified *E. coli* K51 and K93 CPS as immunoadsorbents in human sera, as has been done with the crossreacting *E. coli* K100 CPS and Hib CPS, should provide further information on these possibilities (23, 58).

Summary

Eleven *Escherichia coli* strains, crossreactive with the capsular polysaccharide (CPS) of *Neisseria meningitidis* group A (GrA), were detected among 645 stool isolates from healthy families in Cairo, Egypt. 10 of these strains were of the *O*107:K93:H27 or *O*107:K93:SP serotypes and may be considered descendents of a single bacterium or as a clone. The remaining crossreactive strain was of the *O*7:K51:H18 serotype. None of the 11 strains produced enterotoxins and none were enteroinvasive. The purified CPS of these *E. coli* strains, as well as a polysaccharide (*PS*) from *B. pumilis*, strain Sh17, precipitated with equine GrA (H49) antiserum. A partial identity between the *E. coli* K93, K51 and Sh17 PS on the one hand and the GrA CPS on the other was observed by double immunodiffusion when reacted against the H49 antiserum. Four K93 strains and one K51 strain were found among 320 *E. coli* strains from patients at the Clinical Center, National Institutes of Health, and three K93 strains were found in 105 stool samples from children in Copenhagen. The data from these three surveys suggest that these crossreactive *E. coli* are common organisms and could serve as a stimulus for "natural" GrA CPS antibodies.

Quantitative precipitation analysis showed that K51, K93, and Sh17 PS precipitated 25, 46.8, and 50% of H49 antibodies, respectively. Absorption of H49 antiserum with the GrA CPS removed its precipitating activity with the *E. coli* K93, K51, and Sh17 PS. Absorption of H49 antiserum with either K51 CPS or Sh17 PS removed the homologous crossreactivity only, whereas K93 CPS absorbed both K93 and K51 reactivities. Antibodies, raised by intravenous injection of formalinized *E. coli* K93 or K51 cells into rabbits, precipitated with GrA CPS and were bactericidal against GrA meningococci. The crossreaction between the *E. coli* K93 and the GrA CPS was unexpected since these two CPS are compositionally so dissimilar.

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