NEUTRALIZATION OF MENINGOCOCCAL ENDOTOXIN BY ANTIBODY TO CORE GLYCOLIPID*

By CHARLES E. DAVIS, ELIZABETH J. ZIEGLER, and KAREN F. ARNOLD

(From the Departments of Pathology and Medicine, University of California, San Diego, California 92013)

The purpose of this study was to find an antibody that would neutralize the effects of meningococcal endotoxins from all capsular serogroups. We were interested in producing such an antibody because the capsular vaccines are serogroup-specific, and because there is no vaccine available for meningococcus (MGC)[1] B, Y, or other serogroups that may evolve into important pathogens. When our early unpublished experiments suggested that antibodies raised against the lipopolysaccharide (LPS) of one meningococcal serogroup would not be uniformly effective against the LPS of other serogroups, we turned to antibodies against *Escherichia coli* J5, the mutant of *E. coli* 0111 that is deficient in uridine 5′-diphosphate (UDP)-galactose epimerase. Because this bacterium cannot incorporate galactose into its LPS to make complete "0" side chains (1, 2), its core is accessible for stimulating antibody production to a wide range of bacteria with similar LPS cores.

Two separate lines of investigation from this laboratory encouraged us to test antibodies to this mutant *E. coli* against meningococcal endotoxemia. First, we found that purified meningococcal endotoxins were biochemically similar to enteric LPS, but were 10 times more potent for inducing the purpuric, necrotic lesions of the dermal Shwartzman phenomenon (3). This finding appeared to explain the high frequency of purpuric skin lesions in meningococcemia, and it provided a convenient way to test the effectiveness of antibodies to meningococcal LPS. Second, along with others in this laboratory, we showed that antiserum raised against *E. coli* J5 protected experimental animals against endotoxemia and bacteremia from such diverse bacteria as *E. coli*, encapsulated Klebsiella, and *Pseudomonas aeruginosa* (4–8). Finally, we reasoned that if J5 antibodies could counteract the effects of endotoxemia from diverse enteric bacilli, and if meningococcal and enteric LPS were similar both biochemically and biologically, antibodies to J5 might also neutralize meningococcal endotoxin.

Accordingly, we compared antibodies against *E. coli* J5 with antibodies against homologous and heterologous meningococcal serogroups for their capacity to prevent the dermal and general Shwartzman reactions induced by LPS from MGC A, B, and C. The superiority of antibodies to the *E. coli* mutant in these assays suggests that they will counteract the effects of meningococcal endotoxemia regardless of the capsular serogroup of the infecting strain.

Materials and Methods

Source of Microorganisms. Strains of *Neisseria meningitidis* from the collection at Walter Reed Army Institute of Research, Washington, D. C. were supplied by M. S. Artenstein. Strains

* Supported by U. S. Army contract PATH/USA/DADA 17-72-C-2040.

[1] *Abbreviations used in this paper:* i.v., intravenous; LPS, lipopolysaccharide; MGC, meningococcus; PBS, phosphate-buffered saline; TSB, tripticase soy broth; UDP, uridine 5′-diphosphate.

A_1, B_{11}, and C_{11} were originally obtained from patients with meningitis or meningococcemia, and are the prototype strains used in vaccine studies. The letter in the designation of each strain of MGC indicates its serogroup; so that A_1 is serogroup A, B_{11} is serogroup B, and C_{11} is serogroup C. *E. coli* J5, a UDP-galactose epimeraseless mutant of *E. coli* 0111, was originally described by Elbein and Heath (1). Our strain differs in that it is no longer capable of incorporating galactose into the side chains of its LPS, even when galactose is present in the culture media (7).

MGC were stored in lyophiles, rehydrated with trypticase soy broth (TSB), and grown on blood agar overnight at 37°C under CO_2. *E. coli* J5 was stored on trypticase soy agar slants and subcultured onto blood agar for 24–48 h at 37°C.

Endotoxins. Endotoxin was extracted from each strain of MGC by our modification (3) of the phenol-water method of Westphal et al. (9). Briefly, this modification involved growing the cultures for 3 days, killing the cultures with a terminal concentration of 1% formaldehyde, discarding the middle layer between the phenol and water layers, and harvesting the endotoxin from the upper layer by ultracentrifugation instead of ethanol precipitation. After resuspension of the pellet, the material was lyophilized, collected, and weighed.

As previously described (3), each endotoxin was shown to contain less than 1% protein by the method of Folin and Ciocalteu (10), less than 1% RNA by optical density determinations at 280 and 260 nm, and to be free of detectable capsular contamination by the thiobarbituric acid technique of Warren (11) and by gas-liquid chromatography (2, 3). The presence of lipid A was confirmed by the method of Galanos et al. (12). All standards for chemical assays and gas-liquid chromatography were of the highest purity available from either Sigma Chemical Co., St. Louis, Mo., or Calbiochem, San Diego, Calif.

Immunization. Bacteria were grown in TSB under CO_2 for 48 h, harvested by centrifugation, washed three times in 0.15 M NaCl, and boiled for 2.5 h. After boiling, the cells were resuspended in 0.15 M NaCl and adjusted spectrophotometrically to a concentration of 5×10^9 cells/ml. 3-kg rabbits were exsanguinated 7 days after the last of six 1.0-ml intravenous (i.v.) injections of boiled cells given three times weekly for 2 wk. Nonimmune sera was obtained from nonimmune littermates.

Antibody Determinations. Hemagglutinating antibodies were measured in microtiter plates with human group 0 erythrocytes sensitized with the appropriate alkaline-treated endotoxin (13).

Separation and Purification of Immunoglobulins. 30- to 40-ml samples of pooled normal or immune rabbit serum were separated into 3 fractions on 5.0 × 100-cm glass columns packed with sterile Sephadex G-200 to a height of 90 cm. The eluant was phosphate-buffered saline (PBS) at pH 7.0, pumped upward from a reservoir by a peristaltic pump at 50–65 ml/h. 10-ml fractions were collected in an automatic fraction collector equipped with a 15-watt germicidal lamp. The optical density was recorded at 280 nm, and appropriate fractions were combined under sterile conditions. Chromatography of immunoglobulin classes was monitored by immunodiffusion against heavy-chain specific goat anti-rabbit IgM, IgA, and IgG. The frontal peak, which contained pure IgM by immunodiffusion, was restored to the original volume by concentration in dialysis tubing against polyethylene glycol at 4°C, and it was stored at 4°C for animal experiments. The second peak contained primarily IgG, but was contaminated with small amounts of IgA and IgM. After concentration, part of this material was stored at 4°C for animal experiments and part was further purified.

The 7S fraction was purified by chromatography over DEAE-Sephadex A-25 by the method of Hall et al. (14). 50- to 100-ml samples were dialyzed against buffer 1 and chromatographed on a 5 × 25-cm Pharmacia column (Pharmacia Inc., Piscataway, N.J.) packed with 75 g of sterile DEAE-Sephadex, also equilibrated in buffer 1. The protein peak eluted with buffer 1 (0.0175 M PBS at pH 6.3) was collected on the automatic fraction collector, pooled, and concentrated to a protein concentration equal to the original 7S peak (concentrated about 2 ×). This material was pure IgG by immunodiffusion and was retained for animal experiments. The second peak was eluted with 0.4 M PBS, pH 5.3. Before recharging the Sephadex by washing in buffer 1, it was treated with 1 M NaCl and 0.1 M NaOH.

Special precautions were taken to assure sterility as previously described (4). All sera and immunoglobulin fractions for prevention of the Shwartzman reactions were shown to be sterile and free of pyrogens.

Protection Against Meningococcal Dermal Necrosis (Local Shwartzman Phenomenon). Skin sites for the dermal Shwartzman reaction were prepared in groups of 10–20 1.0–1.5-kg rabbits by

the intradermal injection of 1.75 μg in 0.25-ml volumes of MGC A, B, or C endotoxin. The reaction was provoked 21 h later by the i.v. injection of 0.5 ml of 1.25–1.5 μg of the corresponding endotoxin. Any hemorrhage or necrosis of the skin appearing 4–18 h after the provocative dose was recorded as a positive reaction. Experimental rabbits were given either 20 ml of antiserum or 15–20 ml of immune globulin fractions 19 h after the preparatory dose (2 h before the provocative dose). Control animals received the same volume of normal rabbit serum or nonimmune globulin adjusted to an equivalent protein concentration.

Protection Against Meningococcal Renal Cortical Necrosis (Generalized Shwartzman Phenomenon). Groups of 10–20 1.0–1.5-kg rabbits were given 20 ml of either normal or immune serum 3 days before they were prepared with 12.5–20 μg of MGC A, B, or C endotoxin i.v. in 0.5-ml volumes. Renal cortical necrosis was provoked 21 h later by the i.v. injection of 10–12.5 μg of the corresponding endotoxin. The animals were sacrificed 24 h after provocation, and their kidneys were judged positive if gross hemorrhage or necrosis were present on the external surface.

Statistical Methods. The chi-square test was used to determine whether or not there was a significant difference in the incidence of Shwartzman reactions between the groups.

Results

Production of Antiserum. Rabbits immunized with boiled cells of *E. coli* J5 uniformly caused hemagglutination of J5-sensitized erythrocytes to a titer > 1:256 by microtiter and > 1:1,000 by the tube technique. Immunization with *E. coli* J5 also caused a rise in hemagglutinins to meningococcal LPS generally equal to that obtained by immunization with the homologous MGC. Immunization with heterologous meningococcal boiled cells resulted in production of hemagglutinins, but the response was less uniform. The reciprocals of the hemagglutination titers are shown in Tables I, II, and III. Although protective antisera always contained hemagglutinins, there was no definite correlation between the height of the hemagglutinating antibody response and the degree of protection.

Prevention of Meningococcal Dermal Necrosis

MGC A ENDOTOXIN. Antiserum to *E. coli* J5, MGC A, MGC B, and MGC C all lowered the incidence of dermal necrosis significantly below that of animals which received nonimmune serum (87% positive; Table I). Although antisera to J5 and MGC A_1 protected a larger percentage of animals (33 and 32% positive), they were not statistically more effective than antisera raised against MGC B_{11} and C_{11} (45 and 55% positive).

MGC B ENDOTOXIN. Only 28% of the rabbits given antiserum to J5 developed dermal necrosis, compared with 88% who received nonimmune serum ($P<0.0005$), and 59% who received heterologous meningococcal antiserum prepared against MGC A_1 (Table II; $P=0.01$). Antiserum to MGC B_{11} (serogroup-homologous) was also protective, but not superior to MGC A_1 antiserum. MGC C_{11} antiserum also protected all but 28% of the animals, but it was not superior to MGC A_1 or B_{11} antiserum.

MGC C ENDOTOXIN. J5 antiserum protected the largest percentage of rabbits in this experiment and was superior to nonimmune and anti-meningococcal A_1 sera (Table III). Antisera against MGC B_{11} and C_{11} were also protective.

COMPOSITE RESULTS. The results of the experiments shown in Tables I, II, and III are combined in Table IV. In this table the results of protection experiments with each antiserum against all three meningococcal endotoxins are combined, so that the protective capacity of each meningococcal antiserum

TABLE I

*Prevention of Dermal Shwartzman from MGC A_1 Endotoxin with E. coli J5 and Meningococcal Antisera**

Serum	Hemagglutination titer‡	Number and percent positive	*P* value§
Nonimmune	0	52/60 (87)	–
Anti-MGC A_1	128	12/38 (32)	<0.0005
Anti-*E. coli* J5	32	10/30 (33)	<0.0005
Anti-MGC B_{11}	8	9/20 (45)	<0.0005
Anti-MGC C_{11}	8	11/20 (55)	<0.004

* The skin of 1–1.5-kg white New Zealand rabbits was prepared with an intradermal injection of 1.75 μg of MGC A endotoxin in a 0.25-ml volume. 19 h later, 20 ml of serum was given in one lateral ear vein, 2 h before the provocative i.v. dose of 1.25 μg of MGC A endotoxin in a 0.5-ml volume was given in the other ear. Skin sites were examined at 4 and 24 h, and judged positive if hemorrhage or necrosis of the skin occurred within 24 h of the provocative dose.

‡ Reciprocal of hemagglutination titer against human group O erythrocytes sensitized with alkaline-treated MGC A_1 endotoxin.

§ *P* values are in comparison to the normal serum control and were calculated by the chi-square technique.

TABLE II

*Prevention of Dermal Shwartzman from MGC B_{11} Endotoxin with E. coli J5 and Meningococcal Antisera**

Serum	Hemagglutination titer‡	Number and percent positive	*P* value
Nonimmune	0	52/59 (88)	–
Anti-MGC B_{11}	32	14/30 (47)	<0.0005
Anti-*E. coli* J5	64	11/40 (28)	<0.0005§
Anti-MGC A_1	32	17/29 (59)	<0.002§
Anti-MGC C_{11}	16	5/18 (28)	<0.0005

* Experiments were conducted as stated in the text and in Table I except that B_{11} endotoxin was used to prepare (1.75 μg) and provoke (1.5 μg) the dermal Shwartzman reactions.

‡ Reciprocal of hemagglutination titer against human group O erythrocytes sensitized with alkaline-treated MGC B_{11} endotoxin.

§ Protection by *E. coli* J5 antiserum (28% positive) was superior to that from MGC A_1 antiserum (59% positive), $P<0.01$.

is examined against heterologous as well as homologous LPS. These data show that antiserum to *E. coli* J5 was more protective (33% positive) than nonimmune serum (87% positive; $P<0.0005$), MGC A antiserum (51% positive; $P<0.015$), MGC B antiserum (49% positive, $P<0.03$), and MGC C antiserum (47% positive; $P<0.06$).

Table V compares the results of all protection experiments with *E. coli* J5 antiserum to each meningococcal antiserum tested only against the serogroup-heterologous endotoxins. In this analysis which represents a more realistic comparison of the cross-reactivity of antiserum and LPS, J5 antisera protected

TABLE III

*Prevention of Dermal Shwartzman from MGC C_{11} Endotoxin with E. coli J5 and Meningococcal Antisera**

Serum	Hemagglutination titer‡	Number and percent positive	*P* value
Nonimmune	0	35/40 (88)	–
Anti-MGC C_{11}	32	16/30 (53)	<0.002
Anti-*E. coli* J5	64	12/30 (40)	<0.0005§
Anti-MGC B_{11}	16	11/19 (58)	<0.011
Anti-MGC A_1	32	15/20 (75)	No protection§

* Experiments were conducted as stated in the text and in Table I except that C_{11} endotoxin was used to prepare (1.75 μg) and provoke (1.5 μg) the dermal Shwartzman reactions.

‡ Reciprocal of hemagglutination titer against human group O erythrocytes sensitized with alkaline-treated MGC C_{11} endotoxin.

§ Protection by *E. coli* J5 antiserum (40% positive) was superior to that from MGC A_1 antiserum (75% positive), $P<0.015$.

TABLE IV

*Prevention of Dermal Shwartzman from MGC Endotoxins—Superiority of E. coli J5 over MGC Antisera**

Serum	Number and percent positive	*P* value‡
Nonimmune	139/159 (87)	<0.0005
Anti-*E. coli* J5	33/100 (33)	–
Anti-MGC A_1	44/87 (51)	<0.015
Anti-MGC B_{11}	34/69 (49)	<0.03
Anti-MGC C_{11}	32/68 (47)	<0.06

* The results of protection experiments with each serum against endotoxins of MGC A_1, B_{11}, and C_{11} are combined in this table.

‡ *P* values compare each serum to the results obtained with *E. coli* J5 antiserum.

a higher percentage of rabbits (33% positive) than did heterologous meningococcal antisera (45% positive; $P<0.002$).

Prevention of Meningococcal Renal Cortical Necrosis. *E. coli* J5 antiserum also prevented the renal cortical necrosis of the generalized Shwartzman reaction, regardless of the serogroup of meningococcal endotoxin. Tables VI, VII, and VIII show that the protection afforded by J5 antiserum against renal cortical necrosis from MGC A_1 (18% positive), MGC B_{11} (30% positive), and MGC C_{11} (22% positive) was significant and at least as effective as that provided by homologous antisera. Heterologous meningococcal antisera did not prevent renal cortical necrosis induced by LPS from MGC B_{11} and C_{11} (Tables VII and VIII).

Protection with J5 Immunoglobulin Fractions

PURITY. The 19S fractions of whole rabbit serum chromatographed over Sephadex G-200 contained only IgM by immunodiffusion and were used in protection experiments without further purification. Because the 7S fractions of

TABLE V

*Prevention of Dermal Shwartzman from Meningococcal Endotoxins: Superiority of E. coli J5 over Heterologous Meningococcal Antisera**

Serum	Number and percent positive
All heterologous MGC antisera	68/125 (45)‡
All *E. coli* J5 antisera	33/100 (33)‡

* The results of protection experiments with each serum against only the serogroup-heterologous endotoxins are combined in this table. Thus, the results of J5 antiserum against all three meningococcal endotoxins are compared with the results of meningococcal antisera tested only against the endotoxins from the two serogroup-heterologous MGC.

‡ *E. coli* J5 antiserum is superior to heterologous MGC antisera ($P<0.002$).

TABLE VI

*Prevention of Renal Cortical Necrosis from MGC A_1 Endotoxin with E. coli J5 and Meningococcal Antisera**

Serum	Number and percent positive	*P* value‡
Nonimmune	15/36 (42)	–
Anti-MGC A	7/39 (18)	0.025
Anti-*E. coli* J5	4/39 (10)	0.0025

* Rabbits received 20 ml of serum 3 days before they were prepared with 20 μg of i.v. endotoxin. The reaction was provoked 21 h later with 12.5 μg of i.v. endotoxin. The animals were sacrificed 24 h after provocation and kidneys were judged positive if gross hemorrhage and necrosis were present on the external surface.

‡ *P* values are in comparison to the normal serum control and were calculated by the chi-square technique.

TABLE VII

*Prevention of Renal Cortical Necrosis from MGC B_{11} Endotoxin with E. coli J5 and Meningococcal Antisera**

Serum	Number and percent positive	*P* value
Nonimmune	7/8 (88)	–
Anti-MGC B_{11}	3/8 (38)	<0.15
Anti-*E. coli* J5	3/10 (30)	<0.05
Anti-MGC C_{11}	6/10 (60)	No protection

* Experiments were conducted as stated in the text and in Table VI except that B_{11} endotoxin was used to prepare (12.5 μg) and provoke (10 μg) renal cortical necrosis.

both nonimmune and immune sera showed contaminating IgA, portions of each were further purified over DEAE-Sephadex A-25. The first peak eluted from the DEAE-Sephadex contained only IgG by immunodiffusion and was stored at 4°C for protection studies.

TABLE VIII
*Prevention of Renal Cortical Necrosis from MGC C_{11} Endotoxin with E. coli J5 and Meningococcal Antisera**

Serum	Number and percent positive	*P* value
Nonimmune	11/16 (69)	–
Anti-MGC C_{11}	7/19 (37)	0.06
Anti-*E. coli* J5	4/18 (22)	0.006
Anti-MGC A_1	5/10 (50)	No protection

* Experiments conducted as stated in the text and in Table VI except that C_{11} endotoxin was used to prepare (15 μg) and provoke (10 μg) renal cortical necrosis.

TABLE IX
*Prevention of Dermal Shwartzman from MGC A_1 Endotoxin with Partially Purified Immunoglobulins of E. coli J5 Antiserum**

Globulin	Number and percent positive	*P* value
Nonimmune 19S	16/19 (84)	–
Nonimmune 7S	14/20 (70)	–‡
E. coli J5 19S	14/20 (70)	No protection
E. coli J5 7S	7/19 (37)	<0.05‡

* Experiments were conducted as stated in the text and in Table I except that 15 ml of 7S or 19S immunoglobulin collected over Sephadex G-200 was tested instead of 20 ml of whole serum.
‡ *E. coli* 7S globulin was superior to nonimmune 7S globulin $P<0.05$. Immune 19S was not protective.

TABLE X
*Prevention of Dermal Shwartzman from MGC A_1 Endotoxin with Purified Immunoglobulin G of E. coli J5 Antiserum**

Serum or globulin	Number and percent positive	*P* value
Nonimmune IgG	9/10 (90)	–
E. coli J5 Serum	2/10 (20)	<0.003
E. coli J5 IgG	1/10 (10)	<0.0005

* Experiments were conducted as stated in the text and in Table I except that the 7S fractions of Sephadex G-200 were further purified over DEAE Sephadex A-25 and concentrated to protein concentrations approximately equivalent to that of the Sephadex G-200 7S fractions. Each rabbit was given 20 ml of either whole J5 serum or purified IgG 2 h before the provocative dose of endotoxin.

The 19S and 7S fractions from Sephadex G-200 were restored to the original volume of the serum before they were used in animal experiments. Normal and J5 immune IgG purified over DEAE were concentrated twofold to approximately the total protein content of the original 7S Sephadex fractions, before they were used in animal experiments.

PREVENTION OF DERMAL NECROSIS WITH IMMUNOGLOBULIN FRACTIONS. The

results of protection experiments with nonimmune and J5 immune 7S and 19S fractions from Sephadex G-200 are shown in Table IX. Immune 7S was protective ($P<0.05$) whether compared to nonimmune 7S, nonimmune 19S, or immune 19S. Immune 19S was not protective.

The next experiment shown in Table X compared purified nonimmune and J5 immune IgG from DEAE Sephadex with *E. coli* J5 antiserum. Immune IgG (10% positive) was as protective ($P<0.005$) as whole *E. coli* J5 antiserum (20% positive; $P<0.003$) when compared to nonimmune IgG (90% animals positive).

Discussion

We have shown in these studies that antibodies to *E. coli* J5, a rough mutant of *E. coli* 0111, can protect against the two most dramatic manifestations of meningococcal endotoxemia: dermal purpura and renal cortical necrosis. Because this mutant is deficient in UDP-galactose epimerase, it cannot build the complex "0" antigenic side chains that mask the LPS core of most endotoxins, including *E. coli* 0111 and MGC A_1, B_{11}, and C_{11}. This enzymatic block, which is equivalent to that of the Rc forms of Salmonella (15), exposes the core so that it can stimulate antibodies capable of cross-reactions with all antigenically similar endotoxin cores. It is this structural property of *E. coli* J5 that is responsible for stimulating antibodies that protect against endotoxemia and bacteremia due to *E. coli* (4–7), Salmonella (4–6), Klebsiella (7), *P. aeruginosa* (8), and meningococcal LPS, regardless of serogroup. We have shown previously that the "0" antigenic side chains, responsible for serogrouping enteric bacilli, interfere with antibody production to the antigenically similar LPS core. In these studies, antibodies to the parental form of J5, *E. coli* 0111, unlike J5 antibodies, would not protect against diverse gram-negative bacilli such as Klebsiella (7) and *P. aeruginosa* (8).

The current study with meningococcal LPS shows that the oligosaccharide side chains of meningococcal LPS also interfere with serogroup heterologous protection. Whereas antibodies to *E. coli* J5 protected against dermal and renal necrosis induced by LPS from MGC A_1, B_{11}, and C_{11}, antibodies to MGC A_1 failed to protect against dermal necrosis or renal cortical necrosis induced by MGC C_{11} LPS (Tables III and VIII). Similarly, MGC C_{11} failed to protect against renal cortical necrosis induced by MGC B_{11} (Table VII). Although antiserum to MGC A_1 prevented dermal necrosis from B_{11} LPS, this protection was inferior to that from J5 antibodies (Table II). Finally, the protection by J5 antibodies against dermal necrosis induced by LPS from all three meningococcal serogroups was better than anti-meningococcal sera, even when the homologous endotoxin was included in the calculations (Table IV). The complete absence of cross protection in some experiments shows that meningococcal endotoxins are antigenically different. Because antibodies to the common core of LPS (*E. coli* J5) are completely cross-reactive, this antigenic variability must reside elsewhere in the LPS complex, most likely in the "0" antigenic units. Antigenic variability of "0" side chains is well known among enteric bacilli, but has not been generally appreciated in meningococcal LPS, although Zollinger et al. (16) showed incomplete serological cross-reactivity of antisera raised against MGC B and C.

The experiments with immunoglobulin fractions (Table IX and X) indicate

that the protective factor in J5 antiserum is antibody. These studies, which show that purified IgG is as effective as whole antiserum, are important because a satisfactory in vitro assay of cross-reactivity between J5 antiserum and meningococcal endotoxins is not yet available. Our hemagglutinin results indicate that cross-reactive antibodies were stimulated by immunization with J5, but there was no definite correlation between the height of antibody response and the degree of protection. More promising results have been obtained with the solid-phase radioimmunoassay. Sadoff et al. (17), have shown that J5 antibodies react strongly with all MGC tested in this assay.

The failure of isolated IgM to prevent dermal necrosis is puzzling. It is possible that IgM reacts with whole bacteria rather than purified LPS, or that IgM antibodies can react only with certain endotoxins. In previous studies of immunoglobulin fractions directed against the homologous endotoxin, both anti-*E. coli* 0111 7S and 19S fractions were protective against renal cortical necrosis induced by 0111 endotoxin (6). Some antibodies of the IgM class should be present at 21 days when immunized rabbits were exsanguinated, but it is possible that the concentration of protective antibodies is too low to detect in animal assays.

The demonstration that purified antibodies raised against an *E. coli* mutant can prevent the manifestations of meningococcal endotoxemia suggests that the core of meningococcal endotoxin is the toxic moiety, and underscores the antigenic similarity of LPS from otherwise unrelated bacteria. Moreover, this antibody may provide an additional immunological weapon against meningococcemia. The pioneering work of Goldschneider et al. (18) and Gotschlich et al. (19, 20) at Walter Reed has provided capsular vaccines that prevent meningococcal disease caused by MGC of groups A and C (21–23). Unfortunately, this protection is serogroup-specific. Group B capsule is not immunogenic in man (24), and there is no vaccine available for serogroup Y or other groups that may evolve into important pathogens. Because of the serogroup specificity of the capsular vaccines, the nonimmunogenicity of group B capsule, and the pathogenic potential of other meningococcal serogroups, investigators have turned to studies of outer membrane antigens that might cross-react between capsular groups (16, 25–27). These studies have resulted in the identification of a number of different outer membrane serotypes that cross serogroup lines. The protective capacity of serotype antibodies against group B strains tested in chick embryos, however, appears to be serogroup-specific (28); in other words, antisera raised against whole MGC B of different serotypes showed primarily group-specific protection. Serogroup and serotype antibodies did appear to provide synergistic protection against the homologous serogroup.

On the other hand, antiserum raised against *E. coli* J5 protects rabbits from meningococcal endotoxemia regardless of serogroup. Similarly, gamma globulin prepared from these broad-spectrum antibodies should counteract the devastating effects of meningococcal endotoxemia in patients infected with any capsular serogroup.

Summary

Antibodies to *Escherichia coli* J5, a uridine 5′-diphosphate-galactose epimerase-less mutant of *E. coli* 0111, neutralized meningococcal endotoxemia from all

three major capsular serogroups. We chose the dermal necrosis of the local Shwartzman phenomenon and the renal cortical necrosis of the general Shwartzman phenomenon as assays because these are the hallmarks of meningococcemia, and because meningococcal lipopolysaccharide (LPS) is a uniquely potent cause of dermal purpura and necrosis. Meningococcal antisera raised against LPS from MGC A, B, and C also provided good protection against endotoxemia from the homologous capsular groups, but it was inconsistent against the heterologous serogroups. The superiority of J5 antibodies (purified IgG as well as antiserum) is probably due to the fact that J5 LPS contains only the endotoxin core. Consequently, immunization with this mutant stimulates production of antibodies to core LPS without interference by the "0" antigenic determinants of the side chains.

These observations indicate that the endotoxin core is the toxic moiety of meningococcal LPS, that the core LPS of meningococcus (MGC) is immunologically similar to enteric LPS, and that the antigenically variable "0" side chains of MGC LPS interfere with antibody production against the common core. They also suggest that antibodies prepared against this *E. coli* mutant could interrupt the devastating course of meningococcal endotoxemia in man, regardless of the capsular serogroup of the infecting strain.

Received for publication 30 November 1977.

References

1. Elbein, A. D., and E. C. Heath. 1965. The biosynthesis of cell wall lipopolysaccharides in *Escherichia coli*. I. The biochemical properties of uridine diphosphate galactose 4-epimeraseless mutant. *J. Biol. Chem.* 240:1919.
2. Davis, C. E., S. D. Freedman, H. Douglas, and A. I. Braude. 1969. Analysis of sugars in bacterial endotoxins by gas-liquid chromatography. *Anal. Biochem.* 28:243.
3. Davis, C. E., and K. Arnold. 1974. Role of meningococcal endotoxin in meningococcal purpura. *J. Exp. Med.* 140:159.
4. Davis, C. E., K. R. Brown, H. Douglas, W. J. Tate, III, and A. I. Braude. 1969. Prevention of death from endotoxin with antisera. I. The risk of fatal anaphylaxis. *J. Immunol.* 102:563.
5. Braude, A. I., and H. Douglas. 1972. Passive immunization against the local Shwartzman reaction. *J. Immunol.* 108:505.
6. Braude, A. I., H. Douglas, and C. E. Davis. 1973. Treatment and prevention of intravascular coagulation with antiserum to endotoxin. *J. Infect. Dis.* 128(Suppl.):S157.
7. Ziegler, E., H. Douglas, C. E. Davis, and A. I. Braude. 1973. Treatment of *E. coli* and Klebsiella bacteremia in agranulocytic animals with antiserum to a UDP-GAL epimerase deficient mutant. *J. Immunol.* 111:433.
8. Ziegler, E. J., J. A. McCutchan, H. Douglas, and A. I. Braude. 1975. Prevention of lethal Pseudomonas bacteremia with epimerase deficient *E. coli* antiserum. *Trans. Assoc. Am. Phys.* 88:101.
9. Westphal, O., O. Luderitz, and E. Bister. 1952. Uber die extraction von bacterien mot Phenol/wasser. *Z. Naturforsch. Sect. Teil B Anorg. Chem. Org. Chem. Biochem. Biophys. Biol.* 7:148.
10. Folin, O., and V. Ciocalteu. 1927. Tyrosine and tryptophane determinations in proteins. *J. Biol. Chem.* 73:627.

11. Warren, L. 1959. The thiobarbituric acid assay of sialic acids. *J. Biol. Chem.* 234:1971.
12. Galanos, C., O. Luderitz, and O. Westphal. 1971. Preparations and properties of antisera against the lipid-A component of bacterial lipopolysaccharides. *Eur. J. Biochem.* 24:116.
13. Neter, E., O. Westphal, O. Luderitz, E. A. Gorzynski, and E. Eichenberger. 1956. Studies of enterobacterial lipopolysaccharides. Effects of heat and chemicals on erythrocyte-modifying, antigenic, toxic, and pyrogenic properties. *J. Immunol.* 76:377.
14. Hall, W. H., R. E. Manion, and H. H. Zinneman. 1971. Blocking serum lysis of brucella abortus by hyperimmune rabbit immunoglobulin A. *J. Immunol.* 106:181.
15. Luderitz, V., A. M. Staub, and O. Westphal. 1966. Immunochemistry of O and R antigens of Salmonella and related enterobacteriaceae. *Bacteriol. Rev.* 30:192.
16. Zollinger, W. D., C. L. Pennington, and M. S. Artenstein. 1974. Human antibody response to three meningococcal outer membrane antigens: comparison by specific hemagglutination assays. *Infect. Immun.* 10:975.
17. Sadoff, J. C., E. J. Ziegler, and W. D. Zollinger. 1976. Immunochemical relationships between lipopolysaccharides of gram-negative bacteria. *16th International Conference on Antimicrobial Agents and Chemotherapy*. 92.
18. Goldschneider, I., E. C. Gotschlich, and M. S. Artenstein. 1969. Human immunity to the meningococcus. I. The role of humoral antibodies. *J. Exp. Med.* 129:1307.
19. Gotschlich, E. C., T. Y. Liu, and M. S. Artenstein. 1969. Human immunity to the meningococcus. III. Preparation and immunochemical properties of the group A, group B, and group C meningococcal polysaccharides. *J. Exp. Med.* 129:1349.
20. Gotschlich, E. C., I. Goldschneider, and M. S. Artenstein. 1969. Human immunity to the meningococcus. IV. Immunogenicity of group A and group C meningococcal polysaccharides in human volunteers. *J. Exp. Med.* 129:1367.
21. Gold, R., and M. S. Artenstein. 1971. Meningococcal infections. II. Field trial of group C meningococcal polysaccharide vaccine in 1969–1970. *Bull. W. H. O.* 45:283.
22. Wahdan, M. W., F. Rizk, A. M. El-Akkad, A. El Ghoroury, R. Hablas, N. I. Girgis, A. Amer, W. Boctar, J. E. Sippel, E. C. Gotschlich, R. Triau, W. R. Sanborn, and B. Cvjetanovic. 1973. A controlled field trial of a serogroup A meningococcal polysaccharide vaccine. *Bull. W. H. O.* 48:667.
23. Artenstein, M. S. 1975. Control of meningococcal meningitis with meningococcal vaccines. *Yale J. Biol. Med.* 48:197.
24. Wyle, F. A., M. S. Artenstein, B. L. Brandt, E. C. Tramont, D. L. Kasper, P. L. Altieri, S. L. Berman, and J. P. Lowenthal. 1972. Immunologic response of man to group B meningococcal polysaccharides. *J. Infect. Dis.* 126:514.
25. Zollinger, W. D., D. L. Kasper, B. J. Veltri, and M. S. Artenstein. 1972. Isolation and characterization of a native cell wall complex from *Neisseria meningitidis*. *Infect. Immun.* 6:835.
26. Gold, R., and F. A. Wyle. 1970. New classification of *Neisseria meningitidis* by means of bactericidal reactions. *Infect. Immun.* 1:474.
27. Frasch, C. E., and S. Chapman. 1972. Classification of *Neisseria meningitidis* group B into distinct serotypes. I. Serological typing by a microbactericidal method. *Infect. Immun.* 5:98.
28. Frasch, C. E., L. Parkes, R. M. McNelis, and E. C. Gotschlich. 1976. Protection against group B meningococcal disease. I. Comparison of group-specific and type-specific protection in the chick embryo model. *J. Exp. Med.* 144:319.