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Bacterial lipopolysaccharides from dead bacteria have been blamed for the continuing high mortality from gram-negative infections despite antibiotic treatment. Because animal antiserum against these lipopolysaccharides has been shown to protect against several of the effects of endotoxin, we undertook the development of antiserum in human subjects. 21 men were immunized with a single injection of *Salmonclla typhimurium* or *Escherichia coli* 0:111 heat-killed cells and immune serum was collected at 2 wk. Preimmune serum was obtained as a control in all animal experiments. 1 ml antiserum given intravenously protected mice against a lethal intravenous dose of homologous endotoxin ($P < 0.005$ for both antisera). *E. coli* antiserum reduced the incidence of positive local Shwartzman reactions with *E. coli* endotoxin from 100 to 38%; *S. typhimurium* antiserum reduced the incidence from 92 to 35%. ($P < 0.0005$ for both antisera). There was no protection against heterologous endotoxin in either animal model. These experiments demonstrate for the first time that human antiserum confers exceedingly potent passive immunity to the effects of endotoxin.



# Human Antiserum for Prevention of the Local Shwartzman Reaction and Death from Bacterial Lipopolysaccharides

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ABSTRACT Bacterial lipopolysaccharides from dead bacteria have been blamed for the continuing high mortality from gram-negative infections despite antibiotic treatment. Because animal antiserum against these lipopolysaccharides has been shown to protect against several of the effects of endotoxin, we undertook the development of antiserum in human subjects. 21 men were immunized with a single injection of *Salmonella typhimurium* or *Escherichia coli* 0:111 heat-killed cells and immune serum was collected at 2 wk. Preimmune serum was obtained as a control in all animal experiments. 1 ml antiserum given intravenously protected mice against a lethal intravenous dose of homologous endotoxin ($P < 0.005$ for both antisera). *E. coli* antiserum reduced the incidence of positive local Shwartzman reactions with *E. coli* endotoxin from 100 to 38%; *S. typhimurium* antiserum reduced the incidence from 92 to 35%. ($P < 0.0005$ for both antisera). There was no protection against heterologous endotoxin in either animal model. These experiments demonstrate for the first time that human antiserum confers exceedingly potent passive immunity to the effects of endotoxin.

## INTRODUCTION

Gram-negative bacterial infections cause intractable shock and death with disturbingly high frequency despite antibiotic treatment. This failure of antimicrobial agents has been blamed partly on bacterial lipopolysaccharides from dead organisms. For this reason, we are investigating antiserum against endotoxin for treatment of these infections.

Although it had been recognized for many years that serum from patients recovering from typhoid fever could protect guinea pigs from lethal challenge with live organisms (1), Abernathy and Spink, using a Brucella model, were first to show that human convalescent serum could neutralize endotoxin (2). This observation, supported by extensive evidence that passive immunization with rabbit antiserum can protect against mouse lethality from endotoxin and the local and generalized Shwartzman reactions (3–6), has led us to study the ability of serum from immunized human subjects to protect against endotoxin.

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## METHODS

*Monovalent bacterial vaccines.* Cells of *Salmonella typhimurium* and *Escherichia coli* 0:111 B4 were boiled for 2½ h (5) and adjusted spectrophotometrically to a concentration of $1 \times 10^9$ cells/ml in physiologic saline with phenol (0.5 g/ml) as a preservative. These vaccines met the sterility and safety requirements of the Food and Drug Administration Bureau of Biologics.[1]

*Production of antiserum.* 21 healthy young men each gave a pint of preimmune blood. 2 wk later 10 subjects received $1 \times 10^9$ *S. typhimurium* cells and 11 subjects $1 \times 10^9$ *E. coli* 0:111 cells in the subcutaneous tissue of the buttock or upper arm. A pint of immune blood was obtained from each subject at the height of his antibody response. Blood was collected in anticoagulant-free bottles and allowed to clot. Serum was removed under sterile precautions and stored as individual units at 4°C without preservative. Before use antiserum was pooled to form a homogeneous sample large enough for several experiments.

*Bacterial lipopolysaccharide (endotoxin).* All the lipopolysaccharide used in this study for antibody determinations, mouse lethality experiments, and the production of the local Shwartzman reaction was prepared from the bacterial vaccine strains by the phenol-water method of Westphal (7).

*Antibody determinations.* Antibody titers of serum were measured by hemagglutination of human group 0 red cells sensitized with alkali-treated endotoxin (8).

*Mouse lethality.* 32 CF1 mice[2] weighing 20–25 g were given 0.5 or 1.0 ml human antiserum or preimmune serum

[1] Code of Federal Regulations, Title 21, Part 273.

[2] Carworth Div., Becton, Dickinson & Co., New City, N. Y.

TABLE I

*Prevention of Mouse Lethality from Endotoxin by Passive Immunization with Homologous Human Antiserum*

| Serum Route | Serum Amount | | Deaths at 72 h | $LD_{50}$ | *P* value |
|---|---|---|---|---|---|
| | *ml* | | | *μg* | |
| *S. typhimurium* endotoxin | | | | | |
| IV | 1.0 | Immune | 14/32 | 213 | <0.005 |
| | | Preimmune | 24/32 | 86 | |
| IV | 1.0 | Immune | 9/32 | 337 | <0.0001 |
| | | Preimmune | 25/32 | 84 | |
| IV | 0.5 | Immune | 18/32 | 160 | >0.05 |
| | | Preimmune | 19/32 | 144 | |
| IV | 0.5 | Immune | 10/32 | 320 | <0.05 |
| | | Preimmune | 18/32 | 157 | |
| *E. coli* 0:111 endotoxin | | | | | |
| IV | 1.0 | Immune | 7/32 | 333 | <0.002 |
| | | Preimmune | 28/32 | 125 | |
| IV | 0.5 | Immune | 10/32 | 315 | >0.05 |
| | | Preimmune | 10/32 | 312 | |

In each experiment 32 mice were given antiserum and 32 mice control serum i.v. 6 h later each group of 32 received graded doses (eight mice/dose) of i.v. endotoxin ranging from 63 to 500 μg in 0.25 ml saline. Total deaths were recorded at 72 h.

i.v. All serum was heated at 56°C for 30 min to minimize the risk of anaphylaxis to endotoxin (9). 6 h later the mice received graded doses (eight mice/dose) of i.v. endotoxin ranging from 63 to 500 μg in 0.25 ml physiologic saline and total deaths were recorded at 72 h. The $LD_{50}$ was calculated by the Reed-Muench technique (10). The significance of differences in survival between antiserum-treated and control groups was determined by the normal approximation to the binomial distribution (9).

*Local Shwartzman reaction.* Skin sites in 1 kg New Zealand White rabbits[3] were prepared by intradermal injection of endotoxin in 0.25 ml physiologic saline. The reaction was provoked 24 h later by injection of 0.25 ml endotoxin into the marginal ear vein. The preparative dose of *S. typhimurium* endotoxin was 30 μg and the provocative dose was 15 μg. With *E. coli* 0:111 endotoxin the preparative dose was 250 μg and the provocative dose 60 μg. Any hemorrhage or necrosis of the skin occurring after the provocative dose was recorded as a positive reaction. Antiserum in volumes ranging from 5–20 ml was injected i.v. 2 h before the provocative dose of endotoxin. Control rabbits received equal volumes of preimmune serum. The chi-square test was used to determine if the incidence of Shwartzman reactions in antiserum-treated groups was significantly less than in the controls.

## RESULTS

*Production of antiserum.* The subjects who received *S. typhimurium* vaccine responded with a 3- to 11-fold rise (average 6-fold) in hemagglutinating antibody titer to homologous endotoxin. Final titers ranged from 1:512 to 1:8,000 (geometric mean 1:1,550). Men who were given *E. coli* cells had a 6- to 10-fold titer rise (average 8-fold), with final titers of from 1:4,000 to 1:16,000 (geometric mean 1:4,700). Peak responses in both groups occurred from 6 to 14 days and averaged 10 days. Both vaccines elicited mild local erythema, induration, and moderate tenderness with no disability. Occasional mild febrile reactions were controlled with aspirin.

*Protection against mouse lethality.* 1 ml of human antiserum i.v. gave excellent protection against death from homologous endotoxin, exceeding the 0.5% level of significance in all cases (Table I). With 0.5 ml antiserum i.v. protection was no longer evident. However, 0.5 ml of *S. typhimurium* antiserum given i.p. produced a small but significant reduction in mortality. Attempts to demonstrate heterologous protection by using *E. coli* antiserum against *S. typhimurium* endotoxin and vice versa, were not successful. Anaphylactic deaths occurred only with *S. typhimurium* antiserum and homologous endotoxin; there were 5/32 deaths at 1 h with 1.0 ml and 1/32 with 0.5 ml antiserum.

*Prevention of the local Shwartzman reaction.* Antiserum from subjects immunized with *S. typhimurium* strikingly reduced the frequency of Shwartzman reactions (Table II) with *S. typhimurium* endotoxin from 92 to 35%. Antiserum from those immunized with *E. coli* 0:111 endotoxin lowered the frequency of Shwartzman reactions with *E. coli* 0:111 from 100 to 38% ($P < 0.0005$ for both sets of experiments). Protection correlated to some extent with both titer and volume of antiserum. We occasionally encountered preprovocative-positive reactions with *S. typhimurium* endotoxin and discarded those animals from the series. *S. typhimurium* antiserum provided no heterologous protection against *E. coli* endotoxin.

TABLE II

*Prevention of Local Shwartzman Reaction by Passive Immunization with Homologous Human Antiserum*

| Endotoxin | Amount of serum | Titer of antiserum | Number of positive reactions: Antiserum | Number of positive reactions: Control serum |
|---|---|---|---|---|
| | *ml* | | % | % |
| *S. typhimurium** | 15 | 1:1,000 | 8/12 (67) | 11/12 (92) |
| | 10 | 1:8,000 | 1/5 (20) | 10/12 (83) |
| | 10 | 1:4,000 | 4/11 (36) | 11/11 (100) |
| | 5 | 1:8,000 | 1/12 (8) | 11/12 (92) |
| | | | 14/40 (35) | 43/47 (92) |
| | | | ($P < 0.0005$) | |
| *E. coli* 0:111‡ | 20 | 1:4,000 | 8/26 (30) | 29/29 (100) |
| | 10 | 1:2,000 | 6/11 (55) | 11/11 (100) |
| | | | 14/32 (38) | 40/40 (100) |
| | | | ($P < 0.0005$) | |

* Preparative dose 30 μg; provocative dose 15 μg.
‡ Preparative dose 250 μg; provocative dose 60 μg.

[3] Rancho de Conejo, Vista, Calif.

## DISCUSSION

These experiments demonstrate for the first time that men immunized with killed gram-negative bacteria raise antibody which confers on animals highly potent passive immunity to homologous lipopolysaccharide.

The immunization schedule is simple, side effects are minimal, and "0" antibody response compares favorably with that achieved after two injections of typhoid vaccine a month apart (11, 12). Immunoglobulin fractionation studies were not performed because it has been shown that both 7S and 19S fractions contain protective "0" antibody (6).

1 ml of i.v. human antiserum prevented death in mice challenged suddenly with a lethal dose of homologous endotoxin i.v., a most rigorous stress of the protective capabilities of antiserum. Homologous protection against the dermal Shwartzman reaction was comparable to results with rabbit antiserum. Rare positive reactions after antiserum, before the provocative dose of endotoxin, were felt to be another manifestation of hypersensitivity to bacterial lipopolysaccharide induced by serum from a foreign species (9). Such reactions would not be expected in human subjects given human antiserum.

In contrast to the excellent protection against homologous endotoxins, these human antisera against smooth organisms were of no value in preventing the Shwartzman reaction or death from heterologous endotoxins. This failure of heterologous protection with smooth antisera has been reported earlier against both endotoxins and viable bacteria and can be explained by the concept that "0" determinants conceal the common core of endotoxin. Using antiserum from animals immunized with "0"-deficient mutants whose "core" is unencumbered by "0" side chains, we have shown excellent heterologous protection against death from endotoxin (4), the local Shwartzman reaction (5), disseminated intravascular coagulation (6), and lethal bacteremia (13). Chedid, Parant, Parant, and Boyer (14) and McCabe (15) have obtained similar protection against mouse bacteremia. Encouraged by these results we are now immunizing human subjects with "0"-deficient mutant vaccines.

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