PROTECTION AGAINST MENINGOCOCCAL CHALLENGE AFFORDED MICE AFTER IMMUNIZATION WITH TYPHOID-PARATYPHOID VACCINE USP

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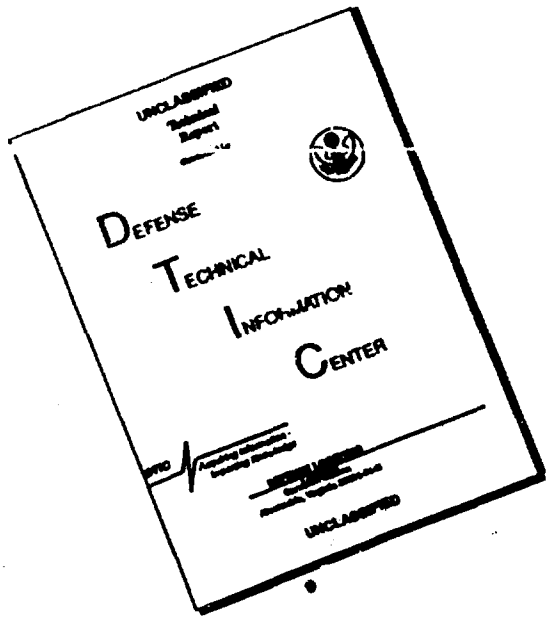

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13. ABSTRACT

Mice immunized with typhoid-paratyphoid vaccine USP were found to be protected when challenged with serological groups A, B, and C of *Neisseria meningitidis*. Mice immunized with this vaccine both 14 and 7 days prior to challenge were protected against mean lethal doses (LD_{50}) of 5,220 and 1,151 of groups A and B, respectively, and in two separate experiments they were protected against 3,568 and 268 LD_{50} of group C. Mice immunized four or more times on successive days prior to challenge were also protected. They demonstrated tolerance and survived a much larger LD_{50} challenge than mice immunized by any other immunization schedule. Mice immunized once or twice anytime within 7 days prior to challenge were not protected and thus failed to exhibit nonspecific resistance. Serum antibody probably was responsible for the protection afforded mice immunized 14 and 7 days prior to challenge. However, this was not demonstrated conclusively.

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Protection Against Meningococcal Challenge Afforded Mice After Immunization with Typhoid-Paratyphoid Vaccine USP

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Mice immunized with typhoid-paratyphoid vaccine USP were found to be protected when challenged with serological groups A, B, and C of *Neisseria meningitidis*. Mice immunized with this vaccine both 14 and 7 days prior to challenge were protected against mean lethal doses (LD_{50}) of 5,220 and 1,151 of groups A and B, respectively, and in two separate experiments they were protected against 3,568 and 268 LD_{50} of group C. Mice immunized four or more times on successive days prior to challenge were also protected. They demonstrated tolerance and survived a much larger LD_{50} challenge than mice immunized by any other immunization schedule. Mice immunized once or twice anytime within 7 days prior to challenge were not protected and thus failed to exhibit nonspecific resistance. Serum antibody probably was responsible for the protection afforded mice immunized 14 and 7 days prior to challenge. However, this was not demonstrated conclusively.

Shilo, in 1959, exhaustively reviewed the literature on nonspecific resistance to infections (21). Numerous references were cited on reports of increased resistance to infections by heterologous genera and species of bacteria and viruses after immunization with lipopolysaccharides or cellular components of gram-negative bacteria. Rowley (18) reported that immunization with the cell walls of various strains of *Salmonella typhimurium* produce good protection against *Escherichia coli* infection. This nonspecific resistance reached a maximum level at 24 h and receded to normal after 5 days. The same author later reported that endotoxin was the active component (19).

Rowley, later in 1964, discussed the evidence for nonspecific immunity and numerous reports on the evidence for cellular and humoral immunity and presented a synthesis of the current opinions on the subject at that time (20). Landy and Pillemer (9) reported that the nonspecific resistance lasted for about 72 h when mice were immunized with *S. typhosa* lipopolysaccharide and challenged with heterologous gram-negative pathogens. Recently an endotoxin-free component, protodyne, of bacterial protoplasm of *E. coli*, has been reported to increase nonspecific resistance (1, 17). In 1969, Nowotny reviewed the molecular aspects of endotoxin reactions (14) which included a table of references on the enhancement of nonspecific resistance and the development of tolerance, an induced resistance to endotoxins. Petersdorf (15) described tolerance as the diminution of several of the toxic properties of bacterial endotoxins which are associated with their frequent administration, as opposed to nonspecific resistance conferred upon the host by a single challenge or immunity associated with a rise in specific antibody. Miller and Boor (13) reported on cross-precipitin reactions between antimeningococcal antisera and pneumococcal polysaccharides. Heidelberger and Cordoba (7) reported on cross-reactions of antityphoid and antiparatyphoid horse antisera with various polysaccharides. Jackson and Jenkins (8) protected mice with acetylated galactan from gum arabic.

McCabe (12) reported that R antigens of the basal core of lipopolysaccharides afforded heterologous protection to mice when used in either active or passive immunization.

This study concerns the resistance of mice immunized with typhoid and paratyphoid vaccine USP (Lederle) to challenge with *Neisseria meningitidis* groups A, B, and C.

MATERIALS AND METHODS

Animals. Mice of the HA/ICR strain were purchased from the A. R. Schmidt Co., Madison, Wis.

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The mice weighed 16 to 18 g at time of challenge.

Microorganisms used for challenge. *N. meningitidis* group A (strain MK01), group B (strain WILL-B), and group C (strain PTS5), as defined by Devine et al. (3), were used in this study. The challenge organisms were stored in a lyophilized state or in defibrinated sheep blood at −70 C. Prior to use in the mouse protection test (2), the organisms were plated on Mueller-Hinton agar (BBL) containing 25 U of polymyxin B and 10 mg of ristocetin and incubated for 18 h. A platinum loop (2 mm) was used, and four loopsful of the 18-h culture were placed in 2.0 ml of 0.15 M NaCl solution and mixed well. Six milliliters of 4% suspension of hog gastric mucin (Wilson Laboratories), in water with the pH adjusted to 7.2 to 7.4, was added to the saline mixture. Each of six mice was infected intraperitoneally (i.p.) with 1 ml of the suspension of cells. Five hours later the mice were bled from the retro-orbital sinus (22). Approximately 2 ml of blood was pooled in the presence of 0.025 ml of heparin (1,000 U/ml). Serial log dilutions of the blood were made in suspensions of hog gastric mucin and used as the challenge inoculum for the mouse protection test. The number of meningococci used to challenge the mice was determined by plating these dilutions on Mueller-Hinton agar.

Immunizing antigen. Typhoid and paratyphoid vaccine USP (Lederle) containing 10^9 organisms of *S. typhosa* per ml, 2.5×10^8 organisms of *S. paratyphi* A per ml, and 2.5×10^8 organisms of *S. paratyphi* B per ml were used to immunize the mice. Immunization consisted of 0.1 ml of the vaccine given i.p. or subcutaneously (s.c.), according to the various schedules shown in the tables for each of the experiments.

Statistical determinations. Estimates of mean lethal dose (LD_{50}) were determined by using the method of Reed and Muench (16).

RESULTS

Demonstration of heterologous protection of immunized mice. Mice immunized i.p. with typhoid-paratyphoid vaccine USP at 14 days and again s.c. at 7 days prior to i.p. challenge with strains of serological groups A, B, and C of *N. meningitidis* were protected (Table 1). Fifty percent of the immunized mice survived challenges of 5,220, 1,151, and 3,568 LD_{50} of meningococcal strains of serological groups A, B, and C, respectively.

Failure to demonstrate nonspecific resistance with a single immunization. Mice in eight groups of 20 mice each were given one injection prior to challenge, as shown in Table 2. One group of mice was immunized twice by the same schedule, as shown in Table 1. Twenty unimmunized mice were used as controls. All mice were challenged with 268 LD_{50} of group C meningococci. Protection was afforded to only that group of mice immunized at 14 and 7 days prior to challenge ($P < 0.05$).

Failure to demonstrate nonspecific resistance with one or two immunizations immediately prior to challenge. Table 3 shows that protection against challenge with group C meningococci was not observed when mice were immunized with a single injection of vaccine given by the s.c. or i.p. route at either 1 or 2 days

TABLE 1. *Heterologous protection of mice immunized twice with typhoid-paratyphoid vaccine USP on the 14th and 7th day prior to challenge with strains of groups A, B, and C Neisseria meningitidis*[a]

Organism dilution	Serogroup of challenge					
	A		B		C	
	Con	Vac	Con	Vac	Con	Vac
10^{-2}	10	17	10	16	10	10
10^{-3}	10	12	9	8	10	14
10^{-4}	9	10	8	9	9	17
10^{-5}	9	7	7	7	10	2
10^{-6}	9	3	6	0	8	1
10^{-7}	6	ND	4	ND	7	ND
10^{-8}	5	ND	6	ND	6	ND
10^{-9}	4	ND	0	ND	0	ND
Mucin control	0	ND	0	ND	0	ND
LD_{50}	7.67	3.95	6.50	3.44	7.60	4.03
Organisms LD_{50}	4.90		79.70		5.00	
Protection[b]	5,220	5,220	1,151	1,151	3,568	3,568

[a] Abbreviations: Con, number controls dead of 10; Vac, number vaccinates dead of 20; ND, not done.
[b] Protection in LD_{50}.

TABLE 2. *Failure of a single immunization with typhoid-paratyphoid vaccine USP, given at various times before challenge, to protect mice against challenge with 1.4 × 10³ (ca. 268 LD_{50}) group C meningococci*

Day of immunization[a]	Route of immunization[b]	No. mice surviving/no. mice challenged	Survival (%)
14	s.c.	5/20	25
7	i.p.	6/20	30
6	i.p.	7/20	35
5	i.p.	4/20	20
4	i.p.	5/20	25
3	i.p.	6/20	30
2	i.p.	5/20	25
1	i.p.	6/20	30
14, 7[c]	s.c., i.p.	10/20	50

[a] Day of immunization prior to challenge with meningococci.

[b] Abbreviations: s.c., subcutaneous; i.p., intraperitoneal.

[c] $P < 0.05$ for this group vs. all other groups in this study.

TABLE 3. *Failure of mice immunized with typhoid-paratyphoid vaccine USP, either 1 or 2 days prior to challenge, to resist a challenge of 1.4 × 10³ (ca. 268 LD_{50}) group C meningococci*

Days of immunization[a]	Route of immunization[b]	No. mice surviving/no. mice challenged	Survival (%)
2	i.p.	5/20	25[c]
2	s.c.	7/20	35
1	i.p.	6/20	30[c]
1	s.c.	1/20	5
2, 1	i.p., s.c.	6/20	30
2, 1	s.c., s.c.	6/20	30
14, 7[d]	i.p., s.c.	10/20	50[c]

[a] Day of immunization prior to challenge with meningococci.

[b] Abbreviations: s.c., subcutaneous; i.p., intraperitoneal.

[c] These data are also included in Table 2.

[d] $P < 0.05$ for this group vs. other groups in this study.

prior to challenge. Likewise, no protection was afforded when mice received two successive daily injections, either i.p.–s.c. or s.c.–s.c. on the 2nd and 1st day prior to challenge. Also, mice immunized 1 h before challenge were not protected. However, a group of 20 mice immunized by the regimen shown in Table 1 were protected ($P < 0.05$). The experiments shown in Tables 2 and 3 were designed so as to be able to challenge all of the mice in both experiments with the same meningococcal culture on the same day. This allows for more valid comparison by elimination of any variation in the LD_{50} of meningococci that inevitably occur when challenges are conducted on separate days. Thus, some of the data shown in Table 3 are also shown in Table 2, as indicated in a footnote to Table 3.

Demonstration of tolerance in mice receiving successive multiple immunization. Four groups of mice were immunized three or more times on consecutive days and challenged either 1 or 2 days after the last immunization, as shown in Table 4. Another group of mice was immunized at 14 and 7 days prior to challenge, as previously shown in the experiment in Table 1. These mice were not protected against a challenge of 1,905 LD_{50} in this experiment. Only those mice immunized four or more times and challenged within 2 days of the last injection were protected ($P < 0.001$).

DISCUSSION

Mice immunized with typhoid-paratyphoid vaccine have been shown to be resistant to challenge by meningococci. This type of resistance may be due to any one of three principle phenomena or combinations thereof. The three are: (i) nonspecific resistance; (ii) tolerance; and (iii) production of humoral antibodies.

Nonspecific resistance was not demonstrated to be present in mice immunized and challenged, as shown in Tables 2 and 3. If nonspecific resistance were the mechanism affording protection, the mice immunized up to 5 days prior to challenge would have been protected. Likewise, those groups of mice immunized consecutively on the 2nd and 1st day prior to challenge were not protected, i.e., they too failed to exhibit nonspecific resistance.

Tolerance, produced by the frequent administration of the antigen, was exhibited in two groups of mice (Table 4). The data in Table 4 suggest that tolerance may be more effective than humoral immunity, since only those mice receiving consecutive daily doses survived the meningococcal challenge. There appears to be an enhanced susceptibility to heterologous challenge, similar to that described by Fukui, at 24 h compared to 48 h after immunization by the s.c. route, but not after the i.p. immunization, as shown in Table 3 (4) ($P < 0.025$). However, Fukui reported the disappearance of enhanced susceptibility at 24 h. The tolerance described by Petersdorf referred to the administration of endotoxin, and this vaccine was a whole-cell product which also contained some endotoxin.

Immunity produced by the administration of antigens 14 and 7 days prior to challenge probably

TABLE 4. *Protection afforded mice immunized with three or more consecutive daily doses of typhoid-paratyphoid vaccine USP when followed in either 1 or 2 days by challenge with 1.35 × 10⁸ (ca. 1,905 LD_{50}) virulent group C meningococci*

Days immunized prior to challenge	Route of immunization[a]	No. mice surviving/no. mice challenged	Survival (%)
6, 5, 4, 3, 2[b]	s.c., i.p., i.p., i.p., i.p.	11/20	55
5, 4, 3, 2[b]	s.c., i.p., i.p., i.p.	12/20	60
4, 3, 2	s.c., i.p., i.p.	4/20	20
3, 2, 1	s.c., i.p., i.p.	6/20	30
14, 7	i.p., s.c.	1/20	5

[a] Abbreviations: s.c., subcutaneous; i.p., intraperitoneal.
[b] $P < 0.001$ for these groups vs. other groups in this study.

was due to antibody production. Mice immunized by using this regimen were protected against meningococcal challenge, as shown in Tables 1–3. These antibodies probably resulted from immunization with one or more salmonella antigens immunologically related to one or more of the antigens in the meningococci of the three serogroups.

Antigens with only one immunodominant sugar in common will not produce cross-reacting antibodies in rabbits but will in the horse, goat, or hen (10). Salmonella group E_4 polysaccharide containing O factors 1, 3, and 19 have been shown by Lüderitz et al. (10, 11) to have four immunodominant monosaccharides for the horse but only two for the rabbit. Although there is no evidence to support this hypothesis, mice may also respond to salmonella polysaccharides as does the goat, horse, or hen. This would increase the possibility of mice producing cross-reacting protective antibodies against meningococci.

There are numerous possibilities for immunological cross-reactions among different genera of bacteria. Miller and Boor (13) reported on the serological cross-reactions between pneumococcal polysaccharides and meningococcal antiserum. Heidelberger and Cordoba (7) observed numerous serological cross-reactions between antityphoid and antiparatyphoid B horse serum with naturally occurring polysaccharides. Artificial protective antigens have been produced against pneumococci (5, 6) and *S. typhimurium* (8). An artificial antigen, colitose, linked to bovine serum albumin and injected into rabbits, failed to agglutinate *E. coli* O111, but, when injected into goats, produced antibacterial agglutinins to this strain (11).

The heterologous protection observed in this study may also have resulted from antigenic similarities in the basal portion of the lipopolysaccharides of the meningococci and the salmonella. Significant heterologous protection of this kind has recently been demonstrated against *K. pneumoniae* in active and passive immunization using R mutants containing only the basal core antigens of *S. minnesota* (12). Investigations of possible antigenic relationships of meningococci and the salmonellae are currently under study.

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LITERATURE CITED

1. Berger, F. M., G. M. Fukui, B. J. Ludwig, and J. P. Rosselet. 1968. Increase of nonspecific resistance to infection by protodyne, a protein component derived from bacterial protoplasm. Proc. Soc. Exp. Biol. Med. **127**:556–559.
2. Branham, S. E., and M. Pitman. 1940. A recommended procedure for the mouse protection test in evaluation of antimeningococcus serum. Pub. Health Rep. **55**:2340–2346.
3. Devine, L. F., and C. R. Hagerman. 1970. Relationship of serological groups of *Neisseria meningitidis*. I. Microagglutination, gel diffusion, and slide agglutination studies of meningococcal antisera before and after absorption with RAS-10 strain of meningococci. Infect. Immunity **1**:226–231.
4. Fukui, G. M. 1964. Some factors affecting endotoxin-induced "nonspecific" resistance, p. 373–381. *In* M. Landy and W. Braun (ed.), Bacterial endotoxins. Rutgers University Press, New Brunswick, N.J.
5. Goebel, W. F. 1939. Studies on antibacterial immunity induced by artificial antigens. I. Immunity to experimental pneumococcal infection with an antigen containing cellobiuronic acid. J. Exp. Med. **69**:353.
6. Goebel, W. F. 1940. Studies on antibacterial immunity induced by artificial antigens. II. Immunity to experimental pneumococcal infection with antigens containing saccharides of synthetic origin. J. Exp. Med. **72**:33.
7. Heidelberger, M., and F. Cordoba. 1956. Cross-reactions of antityphoid and antiparatyphoid B horse sera with various polysaccharides. J. Exp. Med. **104**:375–382.

8. Jackson, G. D. F., and C. R. Jenkins. 1969. Further studies on artificial antigens and immunity to mouse typhoid. II. The ability of galactans containing various acyl groups to immunize mice against mouse typhoid. Aust. J. Exp. Biol. Med. Sci. **47**:91–96.
9. Landy, M., and F. Pillemer. 1956. Increased resistance to infection and accompanying alteration in properdin levels following administration of bacterial lipopolysaccharides. J. Exp. Med. **104**:383–409.
10. Lüderitz, O., A. M. Staub, and O. Westphal. 1966. Immunochemistry of O and R antigens of *Salmonella* and related *Enterobacteriaceae*. Bacteriol. Rev. **30**:192–255.
11. Lüderitz, O., O. Westphal, A. M. Staub, and L. LeMinor. 1960. Preparation and immunological properties of an artificial antigen with colitose (3-deoxy-L-fucose) as determinant group. Nature (London) **188**:556–558.
12. McCabe, W. R. 1972. Immunization with R mutants of *S. minnesota*. I. Protection against challenge with heterologous gram-negative bacilli. J. Immunol. **108**:601–610.
13. Miller, C. P., and A. K. Boor. 1931. The immunological characteristics of some substances obtained from the gonococcus. Transactions of the Association of American Physicians. **46**:223.
14. Nowotny, A. 1969. Molecular aspects of endotoxic reactions. Bacteriol. Rev. **33**:72–98.
15. Petersdorf, R. G., and J. A. Shulman. 1964. The role of tolerance in the action of bacterial endotoxins, p. 482–499. *In* M. Landy and W. Braun (ed.), Bacterial endotoxins. Rutgers University Press, New Brunswick, N.J.
16. Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty percent endpoints. Amer. J. Hyg. **27**:493–497.
17. Rosselet, J. P., S. G. Murphy, R. J. Adamski, M. J. Fletcher, and B. J. Ludwig. 1969. Endotoxin-free biologically active component of *Escherichia coli*. J. Bacteriol. **98**:434–436.
18. Rowley, D. 1055. Stimulation of natural immunity to *Escherichia coli* infections. Lancet **1**:232–234.
19. Rowley, D. 1956. Rapidly induced changes in the level of nonspecific immunity in laboratory animals. Brit. J. Exp. Pathol. **37**:223–234.
20. Rowley, D. 1964. Endotoxin induced changes in susceptibility to infections, p. 359–372. *In* M. Landy and W. Braun (ed.), Bacterial endotoxins. Rutgers University Press, New Brunswick, N.J.
21. Shilo, M. 1959. Nonspecific resistance to infections. Annu. Rev. Microbiol. **13**:255–278.
22. Stone, S. H. 1954. Method for obtaining venous blood from the orbital sinus of the rat or mouse. Science **119**:100.