Effect of Common Enterobacterial Antiserum on Experimental *Salmonella typhimurium* Infection of Mice[1] (35757)

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The precise mechanisms leading to immunity to salmonellosis have not yet been fully elucidated. It has been established that living, attenuated, smooth strains of *Salmonella* provide protection superior to that achieved with killed, virulent strains (1, 2). Even certain living, rough strains are effective; Germanier (3) reported that an epimeraseless mutant of a rough strain of *Salmonella typhimurium* stimulated protection equal to that engendered by a smooth, virulent strain. It is generally agreed that increased phagocytosis and intracellular killing play major roles, cell hyperfunction, as shown *in vivo* (4) and *in vitro* (5), contributing to the resulting immunity. The contribution made by antibodies, including cytophilic antibodies (6), remains a matter of speculation, and the analysis is rendered particularly complex, since the protective effect may depend upon the route of infection. Blanden *et al.* (7) showed that the protective effect of immune serum against *S. typhimurium* becomes manifest upon intraperitoneal, but not intravenous, challenge. Antibodies other than those directed against the well known O, H, and K antigens may conceivably contribute to immunity to infection by salmonellae and other enterobacteriaceae. It has been shown that antibodies against the common enterobacterial antigen (CA), first described by Kunin *et al.* (8), opsonize for phagocytosis various enteric bacteria possessing this antigenic determinant (9). In addition, Domingue *et al.* (10) recently reported that immunization with this antigen or administration of the corresponding antibody protects rabbits against experimentally induced *Proteus* pyelonephritis. In the present investigation, it is shown that antiserum against this antigen provides slight, transient protection in Swiss albino and C57BL/6Ha mice against *S. typhimurium* (C5S) infection.

Materials and Methods. Smooth strains of *Escherichia coli* serogroups O111 and O14 were selected from our stock culture collection; *S. typhimurium* (C5S), used for challenge, was obtained through the courtesy of Dr. G. B. Mackaness (Trudeau Institute, Saranac Lake, New York). The methods for the preparation of bacterial antigens, including ethanol-soluble CA of *E. coli* O111, were described previously (11).

CA antisera were prepared in New Zealand white rabbits (female, 1.8 to 2.2 kg). Antisera 225 and 226 were obtained from animals injected intravenously with the ethanol-soluble CA from *E. coli* O111 in the following amounts and schedule: 1.0 ml on day 0 and 2.0 ml on days 3, 6, 9, and 13. Antisera 227 and 228 were obtained from animals injected intravenously with the heat-killed *E. coli* O14 suspension as follows: 0.1 ml on days 0 and 3; 0.2 ml on day 6; 0.4 ml on day 9; and 0.6 ml on day 13. Blood was collected from each rabbit on day 0, prior to injection of antigen, and on day 21, 8 days after the completion of immunization. Serum samples obtained prior to immunization, normal rabbit serum (NRS), were used for control purposes. CA antibody titers were determined by the hemagglutination technique, described previously (11). All sera were stored at −20°.

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TABLE I. Effect of CA Antiserum on Survival of Mice Challenged with *S. typhimurium* (C5S).

Source of immunogen	Antiserum no.	No. of mice	% comparisons in which immunized mice lived longer than mice given normal serum			
			10 LD_{50} *Salmonella*		10^4 LD_{50} *Salmonella*	
			Swiss[a]	C57[b]	Swiss	C57
Ethanol-soluble	225	48	53	67	54	60
fraction of	226	48	62	64	59	57
E. coli O111	Pooled data	96	56[c]	66[c]	57[c]	58[c]
Suspension	227	48	57	57	49	61
of	228	48	67	59	62	58
E. coli O14	Pooled data	96	59[c]	58[c]	59[c]	59[c]
Combined data		192	58[c]	62[c]	60[c]	59[c]

[a] Swiss albino mice.
[b] C57BL/6Ha mice.
[c] Significantly different from 50 at the 95% confidence level.

C57BL/6Ha mice (male, 4 to 7 weeks old), which had been continuously inbred by brother–sister matings for more than 20 generations, were used. Outbred Swiss albino mice were obtained from a local dealer. The mice were housed in groups of 10 and given water and food *ad libitum*. Twenty-four hr before infectious challenge, groups of mice were injected intraperitoneally with 0.5 ml of CA antiserum (1:10) or 0.5 ml of NRS (1:10) obtained from the same rabbit prior to immunization.

Suspensions of *S. typhimurium* (C5S) for infectious challenge were prepared from log-phase growth in brain heart infusion broth (Difco). Plate counts were performed on brain veal agar (Difco). Mice were injected intraperitoneally (0.5 ml) and, by means of the Reed and Muench method (12), the LD_{50} for both mouse strains by day 4 was determined to be approximately 8 viable bacteria. Groups of mice injected with CA antiserum or NRS were divided into two subgroups; one group received 10 LD_{50} and the other 1×10^4 LD_{50} doses.

The comparative effects of CA antiserum and NRS were measured following a procedure employed by Gehan (13). Accordingly, mice infected with 10 LD_{50} *S. typhimurium* (C5S) were observed at the same time each day for 11 days and mice infected with 1×10^4 LD_{50} for 7 days. Numbers of dead mice at each inspection were recorded. *S. typhimurium* was the etiologic agent of death; blood samples, randomly obtained from dead or dying mice, consistently yielded this microorganism in pure culture. The percentages of comparisons in which immunized mice lived longer than mice given normal rabbit serum were calculated; hereafter, this computation will be referred to as %C. Statistical analysis of differences in death rates between immunized and control mice was performed by the chi-square test.

Phosphate hemagglutination buffer (pH 7.3; Difco) was employed as diluent throughout.

Results. The effect of CA antiserum on experimental *S. typhimurium* (C5S) infection in two strains of mice was investigated by comparing death rates with those of control mice injected with the preimmunization serum from the same rabbit. Percentages of comparisons (%C) in which mice treated with 4 CA antisera survived mice treated with NRS are recorded in Table I. In 15 of 16 comparisons, mice treated with a CA antiserum lived longer than did the respective NRS controls (%C, 53 to 67). Pooled data of paired CA antisera and NRS consistently yielded findings significantly different from 50 at the 95% confidence level, identified by *c* in Table I. No significant difference in protection was noted between the CA antisera to *E. coli* O111 and O14 nor were differences observed between the two mouse strains or

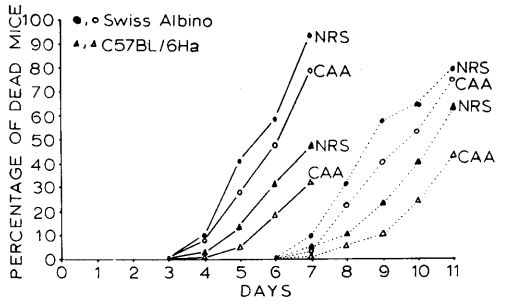

FIG. 1. Effects of CA antiserum (CAA) and normal rabbit serum (NRS) on survival of 192 Swiss albino and 192 C57BL/6Ha mice to infectious challenge with *S. typhimurium* (C5S). Sera were administered 1 day prior to challenge: (—) 1×10^4 LD_{50} doses; (---) 10 LD_{50} doses.

between the infectious doses employed. Collective data of the four CA antisera and respective NRS controls are shown graphically in Fig. 1. Although protection was slight or moderate and temporary, this protection was significant ($p<0.05$). The specificity is suggested by the following observations: NRS had no CA hemagglutinins in the dilution employed (1:10) and the antisera had a mean CA antibody titer of 3200 (range, 1600–6400). Further, *S. typhimurium* O antibodies were not detected in any of the sera (1:10).

Discussion. The present study has shown that CA antiserum offers slight or moderate, albeit transient, protection to Swiss albino and C57BL/6Ha mice against infection by the highly virulent *S. typhimurium* (C5S) strain. It is likely that the noted protection is specific, because CA, but not O antibodies (group B), were found in high titer in the CA antisera employed. Moreover, the *E. coli* serogroups used to prepare the CA antisera do not share with *S. typhimurium* O antigenic determinants.

The finding that CA antisera of *E. coli* O14 and *E. coli* O111 are similarly effective is supported by the fact that CA hemagglutinins are of identical specificity, independent of enterobacterial stimulus (14). Although CA antibodies are not bactericidal (16), they do opsonize for phagocytosis heterologous enteric bacteria possessing CA (9) and thus may contribute toward protection against certain enterobacterial infections. Recently, Domingue *et al.* (10) observed that immunization with CA or administration of CA antibodies provides protection against pyelonephritis in rabbits. Although these antibodies have been characterized as IgM (16), it is conceivable that IgG is present in CA antiserum and participates in the noted protection. This conjecture is supported by the recent report (17) that IgG is more protective than IgM in mice against *Pseudomonas aeruginosa*. It would be of interest to determine whether or not CA antibodies are cytophilic and, by this mechanism, promote cellular resistance. Results of such studies in the mouse and other experimental models are desirable, preliminary to employing CA or CA antiserum for immunization of subjects against certain enterobacterial infections.

Summary. The protection afforded against *Salmonella typhimurium* (C5S) infection by antibodies to the common enterobacterial antigen (CA) was determined in Swiss albino and C57BL/6Ha mice; animals inoculated with the preimmunization sera from the identical rabbits served as controls. Comparison of death rates revealed that CA antisera, obtained from rabbits immunized with either the ethanol-soluble CA of *Escherichia coli* O111 or CA of *E. coli* O14, provided slight to moderate, but only temporary, protection against 10 LD_{50} or 1×10^4 LD_{50} challenge.

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