Increase in Nonspecific Resistance to Infection in Mice Following Administration of Staphylococcal Extracts. (26533)

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The enhanced nonspecific resistance to experimental infections induced by endotoxins derived from Gram-negative bacteria is a well documented phenomenon(1). The biphasic febrile response, Shwartzman reaction, and leucopenia in rabbits produced by these lipopolysaccharides are examples of their other manifold biological activities(2,3). Recently, extracts derived from Gram-positive cocci also have been reported to manifest endotoxin-like properties including the aforementioned and the dermal reactivity of the rabbit to epinephrine(4,5,6).

The experiments described here demonstrate that extracts derived from staphylococci can protect mice against an ordinarily lethal dose of *Escherichia coli*, and substantially increase survival time of mice subjected to lethal doses of *Salmonella typhimurium*. Furthermore, these extracts elicit a pyrogenic response in rabbits; however, the leucopenic response was found to differ qualitatively from that produced by Gram-negative endotoxins.

Materials and methods. Two staphylococcal extracts designated Staphylococcus A lipopolysaccharide control #902761 and control #446423, obtained from Difco Laboratories, are referred to by us as *Staphylococcus* A-1 and *Staphylococcus* A-2, respectively. *S. typhosa* 0-901 lipopolysaccharide (Difco) was used as a representative Gram-negative endotoxin. Mice of the ICR strain (Bellewood Farm), weighing 17 to 18 g, were employed routinely one day after receipt. The culture of *E. coli* was originally obtained from Dr. W. L. Mallmann, Michigan State University, East Lansing. The culture of *S. typhimurium* from our collection was serotyped by the Bureau of Bacteriology, N. J. Dept. of Health. Both cultures were maintained on nutrient agar slants (Difco), transferred to brain heart infusion broth (Difco) and grown at 35°C for 18 hr. The *E. coli* culture was appropriately diluted in sterile broth to give an approximate viable count of 2.1×10^8 cells per ml. Mice received 0.25 ml *i.p.* of this dilution as a challenge inoculum. The 18 hr viable count of *S. typhimurium* approximated 1.3×10^9 organisms per ml. A challenge inoculum of this culture consisted of 0.25 ml *i.p.* of a 10^{-1} or 10^{-3} dilution. Under these conditions, the majority of deaths occurred within 3 days in untreated mice inoculated with *E. coli*. Mice were observed for a period of one week. Depending on the challenge dose of *S. typhimurium*, about half the deaths occurred either by one or 3 days in untreated mice. Animals so infected were observed for 2 weeks.

Pretreatment schedule: Stock solutions of each material were made in saline at 1 mg/ml and kept at −20°C. Mice, in groups of 10, received 1 ml *i.p.* of the endotoxin or staphylococcal extract at 1 or 10 μg/ml, 24 hr prior to infection.

Male rabbits of mixed breed weighing 2 to 2.5 kg were used for determination of febrile and leucopenic responses to *i.v.* pyrogens. Preparation of animals for, and handling during testing were as previously described(7).

Throughout all of this work, rigid precautions were taken to avoid contamination by any extraneous pyrogenic materials. Nonpyrogenic saline was used for control or dilution purposes and all needles, syringes, and glassware were heated at 180°C for 2 to 3 hours.

Results. Preliminary experiments demonstrated that the degree of protection afforded our mice against a challenge of *E. coli* or *S. typhimurium* after the mice were pretreated with *S. typhosa* endotoxin was time and dose dependent as reported by Landy, *et al.*(8). Thus, a model system of endotoxin pretreatment which repeatedly demonstrated significant increased resistance to such infections was chosen to explore the protective effects of extracts of staphylococci.

Both staphylococcal extracts provided at least equivalent protection to a challenge of

TABLE I. Results in Mice Pretreated with Staphylococcal Extracts and *S. typhosa* Endotoxin and Challenged with *E. coli*.

Treatment	Dose, μg, i.p.	No. of mice	% mortality*
Saline	—	30	80
Staph. A-1 extract	10	30	10
S. typhosa endotoxin	10	29	13
Saline	—	20	75
Staph. A-2 extract	10	20	15
S. typhosa endotoxin	10	20	25

* No. of deaths observed 3 days after challenge. $p < .01$.

E. coli as *S. typhosa* endotoxin (Table I).

The data demonstrating increased survival times provided by *Staphylococcus* A-1 and *S. typhosa* endotoxin over the saline controls in infection studies employing *S. typhimurium* are summarized in Table II. With a challenge dose of approximately 1.3×10^8 cells per ml, about 60% mortality occurred within 24 hr in the control groups, while the same number of deaths in those groups of mice pretreated with *Staphylococcus* A-1 extract or *S. typhosa* endotoxin occurred by 72 hr. A more sensitive indication of increased resistance to *S. typhimurium* was found when a challenge inoculum of 1.3×10^6 cells per ml was employed. While the time to reach about 50% mortality in the saline controls was 3 days, the experimental groups reached this same endpoint at about 8 days. A small percentage of the treated mice still survived at 14 days, but were obviously ill. In other experiments using 1 μg of each material and the larger challenge dose of *S. typhimurium*, increased survival times were evident to about the same extent as shown in Table II.

These infection studies clearly demonstrate significant protecion in mice against *E. coli* and *S. typhimurium* by staphylococcal extracts comparable to that observed with a representative Gram-negative endotoxin.

Both staphylococcal extracts also produced endotoxin-like biphasic fevers, but differed in their effects upon circulating leucocytes and in their relative lack of toxicity as measured by mouse lethality tests. Results of a representative experiment are described in Fig. 1

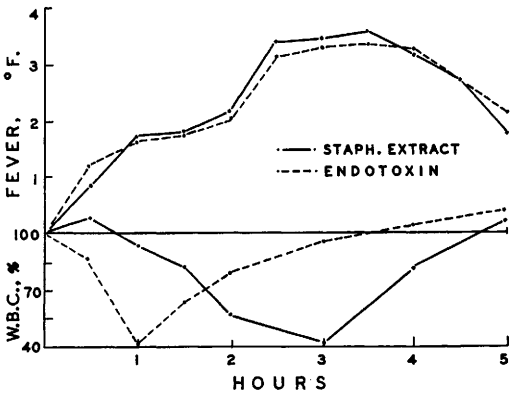

FIG. 1. Differentiation of leucopenic responses to *Staphylococcus* A-1 extract and to *S. typhosa* endotoxin at equivalent febrile reactions.

showing the mean febrile and peripheral leucocyte responses in 5 rabbits given 5 μg of *Staphylococcus* A-1, and for comparison, responses of 3 rabbits injected with an equivalent pyrogenic dose of *S. typhosa* endotoxin (2.5 μg). The early leucopenia produced by the latter is well established and has been described by many workers using preparations derived from a number of genera (2,3,9). The delayed leucopenia in the animals given *Staphylococcus* A-1, without change in num-

TABLE II. Results in Mice Pretreated with Staphylococcal Extracts and *S. typhosa* Endotoxin and Challenged with *S. typhimurium*.

Treatment	No. of organisms	No. of mice	% cumulative mortality — Days after infection													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
Saline	3.3×10^7	30	60	70	83	93	97	97	97	—	—	—	—	—	—	—
Staph. A-1* extract	"	30	3	17	57	77	90	97	97	—	—	—	—	—	—	—
*S. typhosa** endotoxin	"	28	7	11	50	64	71	89	96	—	—	—	—	—	—	—
Saline	3.3×10^1	20	0	0	50	85	85	95	95	100	—	—	—	—	—	—
Staph. A-1* extract	"	20	0	0	10	10	25	35	45	50	65	65	70	70	70	70
*S. typhosa** endotoxin	"	20	0	0	5	10	25	45	55	75	80	85	85	85	85	85

* Dose of 10 μg i.p.

bers of leucocytes at 1 hr post-treatment, stands in strong contrast.

Cross-over experiments also demonstrated failure of this extract to elicit the typical 1 hr endotoxin leucopenia. Eleven rabbits were given the extract on day 1 and *S. typhosa* endotoxin on day 2, both at 2.5 μg *i.v.* The mean preinjection and 1 hr postinjection counts were: day 1, 12,320 and 14,850 per mm^3; day 2, 16,840 and 9,800 per mm^3. For 3 rabbits given the pyrogens in reverse order the counts were: day 1, 11,330 and 4,520 per mm^3; day 2, 12,370 and 12,120 per mm^3.

The *Staphylococcus* A-2 extract gave a typical endotoxin-like time course of leucopenia with a maximum drop at 1 hr. However, both extracts differed from Gram-negative endotoxins in that refractoriness to the leucopenic response developed after a first injection. For 8 rabbits given 5 μg of *Staphylococcus* A-2 on day 1, the mean preinjection and 1 hr postinjection leucocyte counts were 10,975 and 6,685 per mm^3. On day 2, 6 of these were given a second equal dose, the counts being 13,540 and 15,720 per mm^3. Two of the 8, and 2 fresh rabbits were given 5 μg of *S. typhosa* endotoxin and for these preinjection and postinjection counts were 11,700 and 6,650 per mm^3, and 10,600 and 6,950 per mm^3, respectively. In other experiments involving daily administration of either *Staphylococcus* extract, counts done on days 4, 5, or 9 revealed neither characteristic time course of leucopenia, unlike results obtained with Gram-negative endotoxins.

When tested for lethality in 24 to 27 g mice, the *Staphylococcus* A-2 extract proved nonlethal at a dose which for *S. typhosa* endotoxin is 100% lethal (Table III); this is in contrast to their equivalent potency as pyrogens.

Discussion. The type and degree of resistance to infection in mice as well as type and degree of febrile response in rabbits produced by the staphylococcal extracts are strikingly parallel to that demonstrated by a representative Gram-negative endotoxin. On the other hand, a leucopenia either of the delayed or 1 hr type is produced by one or the other of the staphylococcal extracts. Over a period of months both materials were tested in several dozen rabbits, comparing each directly or with *S. typhosa* lipopolysaccharide and the peculiar time course of leucopenia has been consistently reproduced. Parenthetically, we are advised by Dr. C. W. Christensen that the Difco Staphylococcus A lipopolysaccharide control #446423 was packaged at a later date from the same bulk of lipopolysaccharides as the control #902761 we originally received. The delayed leucopenia observed cannot be a function of dose, for at lesser doses of endotoxin the early leucopenia still occurs until a dose too small to elicit the response is reached, and at higher doses the leucopenia is simply prolonged from the initial drop. Regarding the prompt leucopenic tolerance to subsequent injections of both staphylococcal extracts, it has been shown that daily injections of endotoxin continue to elicit a leucopenia of constant magnitude(9) and Herion *et al.*(10) have recently extended this describing a decrease in duration but not in degree of leucopenia under similar conditions.

TABLE III. Lethality in Mice of *S. typhosa* Endotoxin and *Staphylococcus* A-2 Extract.

Dose, mg, i.p.	Deaths/Total	
	S. typhosa endotoxin	*Staph.* A-2 extract
.3	4/10	—
.6	10/17	0/10
.9	10/10	0/10

One important consideration is the possibility that these extracts from a Gram-positive organism were contaminated in preparation or subsequent handling during the experimental work by Gram-negative bacteria or their endotoxic components. Several points suggest that this did not occur. First, the effects reported here were all obtained at levels of 10 μg or less, equivalent to a highly potent Gram-negative endotoxin, indicating an initial gross contamination would have been necessary. Secondly, such contamination seems highly unlikely in view of (a), the differentiation in type of leucopenic response discussed above, and (b), the finding that these extracts are nonlethal at corresponding lethal doses of *S. typhosa* endotoxin. Third, Boivin-type preparations from *Brucella abor-*

tus and *Streptococcus* Gr. A. (Difco) were found to be inactive in terms of fever, toxicity, and protection against infection. Fourth, numerous control experiments have demonstrated none of the endotoxic effects, indicating contamination by the diluent, syringes, needles, or glassware was highly improbable. Altogether, these observations strongly suggest that contamination by Gram-negative endotoxins can be excluded.

Several findings of other workers are pertinent to our work. Fruhman has reported mobilization of neutrophils in the peritoneal fluid of the rat and mouse following intraperitoneal injection of a *Staphylococcus* A lipopolysaccharide(11). The directed migration of polymorphonuclear leucocytes and mononuclear phagocytes has been found to reside in a polysaccharide fraction of staphylococci as well as other Gram-positive and Gram-negative organisms(12). A strain of *Alcaligenes* has produced precipitation in agar gel with staphylococcal rabbit antiserum and the erythrocyte sensitizing antigen common to *S. aureus* is apparently present in at least one *Pseudomonas* strain(13). Cross reactivity, then, may be a factor, as well as a peritoneal neutrophilia somewhat akin to the dual mechanism proposed by Rowley for stimulation of nonspecific immunity provoked by endotoxins(14).

Further studies are in progress to implement these preliminary findings and to clarify some of the mechanisms involved.

Summary. Pretreatment of mice by the intraperitoneal route with staphylococcal extracts significantly protects the host against a subsequent lethal challenge of *E. coli* and prolongs survival time of mice infected with *S. typhimurium.* The same extracts were also found to produce a biphasic fever and a leucopenic response in rabbits to which refractoriness was rapidly developed.

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Immunologic Identification of Coxsackie A21 Virus with Coe Virus.* (26534)

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By means of reciprocal neutralization tests, the recently described Coxsackie group A, type 21 virus prototype strain was found to be immunologically identical to the Coe virus, recovered from individuals with mild respiratory illness.

The Coe virus, described by Lennette *et al.* (1), was isolated from the throat washings of 4 military recruits with mild respiratory illness. Subsequently, isolations of the Coe virus were reported from patients with respiratory illness in Great Britain(2), the Nether-

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