Passive Transfer of Protection Against Lethality of Homologous and Heterologous Endotoxins. (25296)

HENRY H. FREEDMAN (Introduced by A. S. Gordon)
Princeton Laboratories, Princeton, N. J.

The endotoxin-tolerant animal exhibits striking resistance not only to many effects of a variety of endotoxins, but to such insults as hemorrhagic or traumatic shock or infection by Gram-negative pathogens. It is believed that this tolerance is non-immunological and cannot be passively transferred by blood(1). A limited protection against lethality of endotoxins, or the contributing organism, has been described using classical immunological technics to develop high specific antibody titers in donor animals(2,3,4). The success of such procedures has been demonstrated(3, 4) to depend upon sufficiently high antibody titer in the donor to exceed a critical value for the ratio of antibody to magnitude of challenging dose of toxin in the recipient. Further, this protection was restricted to the homologous endotoxin; it did not extend to the toxin of another strain of the same organism. In a largely neglected report Creech, *et al.* (3) were able, also, to show protection by passive transfer in absence of demonstrable antibodies, this requiring relatively large amounts of gamma-globulin taken from mice but not rabbits after a single injection of endotoxin. This protection, too, was restricted to the homologous endotoxin. An apparent protection against the toxin of another strain of the same organism was reported but cannot be considered statistically significant. The present report describes protection of mice against homologous and heterologous endotoxins by passive transfer of serum of endotoxin-tolerant rabbits having low or no antibody titers to the endotoxins. Passive transfer of tolerance to pyrogenicity of endotoxin in the rabbit and studies on the mechanisms involved will be reported separately. In addition, non-specific protection against lethality afforded by serum of normal as well as tolerant rabbits when given intraperitoneally shortly before, but not simultaneously with, the *i.p.* inoculation of endotoxin will be published shortly.

Materials and methods. Endotoxins used were Difco lipopolysaccharides, *S. typhosa* 0 901 and *E. coli* 0127:B8. Rabbits of mixed breed and sex were rendered tolerant by *i.v.* injection of endotoxin for 6 days in doses of 2.5, 2.5, 5, 5, 10, 10 μg and were sacrificed 20 hours after last injection. Typical fever curves with developing tolerance were recorded. Normal rabbits from the same stock provided control serum. Precautions to avoid pyrogen contamination were observed; all glassware was heated at 180°C for 3 hours before use and solutions for dilution were proved non-pyrogenic as were the collected sera. Tests for protection against endotoxin lethality were performed in 15-18 g mice. Schedules for administration of serum are given in the Tables. Normal saline, given by various schedules, did not yield results different from those obtained in untreated mice and such results were combined for control values on lethality of challenging dose. In preliminary experiments *i.p.* LD_{50} for *S. typhosa* endotoxin was 260 μg and for *E. coli* 210 μg. The challenging doses chosen were estimated to represent the LD_{75}: 600 μg for *S. typhosa* and 500 μg for *E. coli*. In retrospect, the former proved a correct estimate but for *E. coli* the lethality was underestimated, the challenging dose representing an LD_{85}. In all experiments untreated or saline-treated groups were challenged along with experimental groups. Deaths were recorded over 72 hours; in most instances deaths occurred within 48 hours. Although serum collected 20 hours after last injection of endotoxin in the donor proved non-pyrogenic and it is known that endotoxin is rapidly cleared from blood of tolerant rabbits(5), it was thought well to include control groups of mice receiving maximum possible endotoxin content of donor blood prior to challenge. This was estimated on the (patently improbable) assumption that total endotoxin given a rabbit over 6 days was present in the serum sample collected on 7th

TABLE I. Protection by *S. typhosa* Endotoxin-Tolerant Rabbit Serum against *S. typhosa* Endotoxin Lethality in Mice.

Exp. and group	No. exp.	Dead/Total	% dead
Controls*	15	97/129	75†
A. .7 or 1 cc serum, i.p., daily, 3 days, challenged on day 4:			
Normal serum	2	13/18	72
Tolerant serum	3	3/19	16‡
Boiled tolerant serum	1	7/8	88
.8 μg endotoxin	1	9/10	90
B. 1 cc serum, i.p., challenged 2-3 hr later:			
Normal serum	2	15/20	75
Tolerant serum	3	1/29	3‡
.8 μg endotoxin	1	8/10	80
C. .5 or 1 cc, i.v., challenged 30 min. later:			
Normal serum	3	20/30	67
Tolerant serum	3	7/30	23‡
.8 μg endotoxin	1	6/10	60

* Untreated or saline inj., then challenged.
† s.e. ± 5.0%.
‡ $p < .001$.

day. The estimate is 0.8 μg/cc. An aliquot of serum was adjusted to pH 5.5 with N HCl, held in boiling water bath 5 minutes, filtered, the coagulum washed with saline and the filtrate (at original volume) adjusted to pH 7 with N NaOH; this relatively protein-free filtrate is referred to as "boiled serum" in the Tables. Antibody content was determined by ring test using undiluted serum and dilutions in saline of the endotoxin as antigen, and by precipitation following incubation. For both endotoxins the undiluted antigen was set at 1 mg/cc and serial dilutions were allowed to react with equal volumes of serum. For statistical analysis the chi squared contingency test was used(6) and significance assumed at $p \leqq 0.01$.

Results. Sera of rabbits rendered tolerant to *S. typhosa* endotoxin yielded maximum titers to homologous endotoxin of 1:16. No demonstrable antibody to heterologous *E. coli* endotoxin was found. Examination of sera of *E. coli*-tolerant rabbits yielded results consistent with those for *S. typhosa*.

The data for lethality in the mouse of *S. typhosa* endotoxin with *S. typhosa*-tolerant rabbit serum are given in Table I. Neither normal rabbit serum nor maximum amount of endotoxin that could possibly be present in the tolerant serum altered the response to the endotoxin challenge. Tolerant serum, by all schedules, effected highly significant protection, which was lost on heating. Protection following intravenous administration rules out donor-serum inactivation of the endotoxin challenge intraperitoneally.

In Table II are given data showing significant protection against challenge with the heterologous *E. coli* endotoxin. Whether the *S. typhosa*-tolerant serum is less potent in protecting against *E. coli* than against *S. typhosa* cannot be determined from these data since a relatively more lethal challenge of *E. coli* endotoxin was used. In limited experiments with *E. coli*-tolerant rabbit serum given *i.p.* to mice 2.5 hours before challenge with *E. coli* endotoxin, saline-injected controls suffered 8/10 deaths, normal serum 8/10, and the tolerant serum 4/10. Using the same serum and schedule and testing with *S. typhosa* endotoxin the results were: saline 12/20, normal serum 7/10, tolerant serum 5/20. It appears then that the difference in degree of protection is based upon the difference in potency of challenge.

Discussion. Low antibody titers to endotoxin would be expected from the weak antigenicity of lipopolysaccharides and from the brief schedule of *i.v.* injections used to develop tolerance. It is clear that the protection described does not depend upon classical immunological mechanisms.

A highly significant degree of protection

TABLE II. Protection by *S. typhosa* Endotoxin-Tolerant Rabbit Serum against *E. coli* Endotoxin Lethality in Mice.

Exp. and group	No. exp.	Dead/Total	% dead
Controls*	8	58/68	85†
B. 1 cc serum, i.p., challenged 2-3 hr later:			
Normal serum	2	18/20	90
Tolerant serum	3	13/29	45‡
C. .5 or 1 cc, i.v., challenged 30 min. later:			
Normal serum	2	16/20	80
Tolerant serum	2	8/20	40‡

* Untreated or saline inj., then challenged.
† s.e. ± 5.1%.
‡ $p < .001$.

against lethality of both homologous and heterologous endotoxins is afforded by passive transfer of serum of a tolerant animal of another species. The success with the *i.v.* route shortly before the *i.p.* challenge rules out direct neutralization of the endotoxin by residual serum, or inactivating factor, in the peritoneum. (It is known(1) that a mixture of endotoxin and antiserum retains the toxicity of the former.) Preliminary experiments indicate that the protective serum of tolerant donors stimulates the reticuloendothelial system of the recipient, as measured by carbon-clearance in the latter. Whether there is equivalent protection against homologous and heterologous challenge cannot be answered unequivocally; the data do not require an assumption of non-equivalence. While it is true that tolerance to, *e.g.*, pyrogenicity by treatment with one endotoxin confers tolerance to pyrogenicity of other endotoxins, whether there is quantitative equivalence of tolerance is far from proved.

Summary. Serum of rabbits rendered tolerant by a brief schedule of endotoxin administration protects mice against the lethality of homologous or heterologous endotoxin and this protective effect is not attributable to antibody content of the serum.

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Effects of Steroid Induced Potassium Depletion on Renal Control of Sodium.* (25297)

JOHN W. POUTSIAKA, MILTON NASVESCHUK AND LLOYD G. MILLSTEIN
(Introduced by B. N. Craver)
Squibb Inst. for Medical Research, New Brunswick, N. J.

Sodium retention is a common side effect of continuous therapy with corticoids. Another potential effect of prolonged dosage with cortical hormones is potassium depletion. Since potassium deficiency is known to produce significant alterations in renal function (1), we hypothesized that renal management of sodium during steroid treatment might be influenced by this known change in potassium balance. The present experiments compared renal responses of intact dogs to intravenous administration of sodium after they had received large repeated daily doses of nine-alpha-fluorohydrocortisone (FF) with and without potassium supplements.

Materials and method. The experiments were performed on 12 mongrel dogs weighing between 8.1 and 12.7 kg, divided according to weight into 1 control and 2 test groups, each containing 2 males and 2 females. They were housed individually in metabolism cages and offered a daily food ration of 280 g of Gaines meal wetted with 300 ml of distilled water. Their daily dietary intakes of sodium and potassium were 42 and 62 mEq respectively. Dogs in Group A were injected intramuscularly once daily for 3 weeks with FF alcohol (1 mg/kg), suspended in an aqueous vehicle. This regimen usually elicits a negative potassium balance, manifested by a drop in serum potassium and mild muscular weakness(2).

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