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Effects of Plasma from Normal and Endotoxin-Tolerant Humans on Endotoxin Fever in Rabbits. (29692)

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Endotoxin tolerance, the relative refractory state that follows a series of injections of endotoxin in both man and experimental animals, has been passively transferred in rabbits(1). Greisman *et al* recently reported that in humans the same dose of endotoxin administered on 2 consecutive days evoked a greater febrile response on the second day(2). Passive transfer of pooled plasma from tolerant subjects reduced the expected enhanced response of the second day to a level comparable to the first(2). This finding was interpreted as the passive transfer of endotoxin tolerance between humans.

Tolerance to the febrile effect of endotoxin has been observed following certain infectious diseases(3,4) while, in contrast, episodes of familial Mediterranean fever will diminish tolerance(5). Furthermore, administration of the pyrogenic steroid, etiocholanolone, enhances febrile reactivity to endotoxin(6). The availability of an experimental model in which the risks of human to human transfer do not exist would be of aid in investigating the factors that may play a role in these states of altered reactivity to endotoxin. The effect of the passive transfer of normal and tolerant human plasma on the febrile reactivity of rabbits to endotoxin is the subject of this report.

Materials and methods. Four normal male volunteers ranging in age from 19 to 24 years comprise the donor group. Lipexal,®* the endotoxin of *Salmonella abortus equi*, employed throughout these studies, was diluted to desired concentration with pyrogen-free physiological saline immediately prior to injection. Pyrogen-free needles, syringes and glassware were used. Two subjects (E.S. and D.D.) received 17 daily injections of endotoxin beginning with a dose of 1 mμg/kg which was increased to a daily dose of 0.25 μg given for 13 days. The other 2 volunteers (J.M. and J.D.) received 12 daily injections of 2 mμg/kg. Febrile tolerance developed in all 4 subjects.

Volunteers remained in bed for 1 hour prior to and 8 hours following each injection during which time rectal temperatures (°C) were obtained at half-hour intervals. Blood was obtained using precautions to insure sterility from each individual prior to and following the induction of tolerance. The blood was anticoagulated with heparin and the plasma separated by centrifugation and stored in sterile flasks at —20°C until used.

Recipient animals were 2 kg New Zealand albino rabbits of either sex. The day prior to testing rabbits were trained in wooden stocks with loosely fitting collars. Temperatures were monitored with thermistors inserted 4 to 6 inches into the rectum and connected to a recording telethermometer (Yellow Springs Instrument Co.). On the day of passive transfer temperatures were re-

* Kindly supplied by Dr. Fred Schultz, Jr., Dorsey Co., Omaha, Nebr.

TABLE I. Effect of Passive Transfer of Human Plasma on Febrile Reactivity of Rabbits to Endotoxin.

Subject	Normal plasma	Tolerant plasma	No plasma
E.S.	55.6 ± 6.1* (3)†	39.7 ± 1.8 (3)	—
D.D.	51.5 (1)	27.1 ± 1.2 (3)	—
J.D.	45.6 ± 2.4 (4)	32.9 ± 9.6 (4)	36.4 ± 3.3 (5)‡
J.M.	54.8 ± .4 (3)	47.7 ± 6.8 (4)	
Overall avg FI§	51.9 ± 2.2 (11)	36.9 ± 3.5 (14)	36.4 ± 3.3 (5)

* Average FI ± standard error.
† No. of recipient rabbits in parentheses.
‡ Results obtained on same day as J.D. and J.M.
§ Overall average computed by addition of means and dividing by number of subjects.

corded for 1 hour to insure a steady baseline and only rabbits with temperatures between 38.5 and 39.9°C were used. Defrosted plasma, placed in a 37°C water bath for 30 to 60 minutes, was injected into a marginal ear vein in a dose of 5 ml/kg. The pretolerant and tolerant plasma from the same subject were always administered on the same day. One hour following the plasma infusion, 0.05 μg of endotoxin was given intravenously if the recipient rabbit's temperature was stable. Normal rabbits were simultaneously tested with the same dose. The 5 hour fever response was plotted on 1 by 1 inch standard graph paper on which 1 hour and 1 degree each equalled 1 inch. The area in cm^2 was measured with a compensating polar planimeter (Keuffel and Esser 4242) and expressed as the fever index (FI).

Results. The results are given in Table I. When the endotoxin injection was preceded by infusion of normal (*i.e.* nontolerant) plasma there was significant enhancement of febrile response (F.I. 51.9 *vs* 36.4, $p<0.01$, two sided t test). However, when the endotoxin was preceded by tolerant plasma, this enhancement was not noted (FI = 36.9 *vs* 36.4). When the fever indices elicited by endotoxin in animals previously infused with either nontolerant or tolerant plasma were compared, significant suppression was observed in animals that had received tolerant plasma ($p<0.01$). Tolerant plasma was also available from 2 other subjects from whom normal plasma had not been obtained. A mean FI of 27.8 ± 5.8 was elicited by injection of endotoxin into 3 rabbits which had previously received plasma from one volunteer, while a mean FI of 50.9 ± 5.6 was elicited in 4 rabbits which had received plasma from the other. In the absence of control plasma from these donors no definite conclusions can be drawn. However, based on observations obtained using other normal plasma (Table I), it would appear that suppressed febrile reactivity was passively transferred with the first of these samples.

None of the animals died following the infusion of plasma. However, 2 of 3 animals receiving normal plasma from D.D. and one of 4 receiving plasma from J.M. died after endotoxin injection. Following the infusion of J.D.'s tolerant plasma and endotoxin, one of 5 rabbits died.

Discussion. The mixing of normal human plasma with endotoxin *in vitro* will enhance both the pyrogenic(7) and lethal properties of endotoxin in rabbits(8). Administration of the plasma before endotoxin will also significantly increase lethality in rabbits(8). The rare occurrence of death in the experimental animals in the present studies is probably due to the small dose of endotoxin employed(8).

The enhancement of pyrogenicity from endotoxin by prior administration of human plasma, to our knowledge, has not been previously reported. It has been proposed that *in vitro* mixing of plasma and endotoxin leads to alterations in the physical state (but not in chemical characteristics) of the pyrogen (9). Since the present studies have demonstrated passive transfer of enhanced febrile reactivity to endotoxin, then the postulated changes in physical state must be able to occur *in vivo* as well. Another possible mechanism for the observed enhancement of fever is that macromolecular constituents of normal human plasma "blockade" the reticuloendothelial system of the recipient rabbits, thus

leading to increased febrile reactivity. If this hypothesis were correct, a similar effect would be expected following the infusion of tolerant plasma as well; this did not occur.

Incubation of endotoxin with plasma from tolerant rabbits *in vitro* does not result in the expected enhancement of fever noted when endotoxin is incubated with normal plasma(10). These results are similar to those of Griesman *et al* who reported a diminution in the expected enhanced second day febrile response to endotoxin in normal humans who had received tolerant plasma(2). In the latter report and the present studies the failure to transfer enhancement with tolerant plasma may represent the passive transfer of endotoxin tolerance. The fact that administration of tolerant plasma resulted in suppression of expected enhanced responses, but not to a level below that of normals, would argue against these phenomena representing the passive transfer of endotoxin tolerance. However, it has been reported that tolerant rabbit serum contains a gamma globulin inhibitor antagonistic to the fever augmenting effect of normal serum(7), and this may also be the case with tolerant human plasma.

The role of humoral factors, especially of antibody, in endotoxin tolerance is currently the subject of extensive investigation. Using the bentonite flocculation technique(11), we have been unable to measure any serologic response in humans following administration of this lipopolysaccharide(12). It is difficult, therefore, to assign a prominent role to classical antibody in the effect noted after transfer of tolerant plasma. Under proper experimental conditions an "endotoxin detoxifying component" (EDC), distinct from antibody, can be found in normal human serum or citrated plasma(13). Increased quantities of EDC may be another explanation for the failure to transfer enhancement of fever with tolerant plasma.

Passive transfer techniques, as employed in these studies, might lead to a better understanding of some of the altered biologic properties of endotoxin induced by interaction with plasma. Present studies in our laboratory are directed toward elucidation of humoral factors involved in the various states of altered endotoxin reactivity in man.

Summary. Passive transfer of normal human plasma one hour before administration of endotoxin, enhanced the febrile response of rabbits. Plasma from endotoxin tolerant humans, similarly transferred, did not elicit enhanced pyrogenic reactivity. The described techniques may provide a convenient model for further characterization of the role of humoral factors in various states of altered endotoxin reactivity in man.

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