PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 675-678 (1975)

Mechanisms of Endotoxin Tolerance. IX. Effect of Exchange Transfusion[1] (38608)

SHELDON E. GREISMAN AND BERNARD DuBUY

From the Departments of Medicine and Physiology, University of Maryland School of Medicine, Baltimore, MD 21201

When gram-negative bacterial endotoxins are injected iv into healthy man or animal, they are cleared from the circulation in exponential fashion. The initial clearance phase is rapid, with a slower phase generally becoming evident within 30 min (1–3). The major contribution of the reticuloendothelial system (RES) to such endotoxin clearance has been documented previously (1, 2). When tolerance to the toxic activities of endotoxin is induced by prior injections of toxin, the rate, as well as total RES uptake of the injected endotoxin becomes markedly enhanced (2–5). The role of this enhanced blood clearance in the development of endotoxin tolerance remains controversial. In 1947, Beeson proposed that the enhanced RES uptake of circulating endotoxin mediates tolerance, this mechanism presumably acting by protecting other more susceptible tissues from toxin injury (5). This hypothesis, however, has been challenged by a number of subsequent studies (2, 6–25). The significance of this challenge is of more than academic interest, since it is now feasible to enhance removal of circulating endotoxin by physical means, e.g., exchange transfusion. Thus, following the introduction of a lethal dose of endotoxin into the bloodstream, augmentation of the toxin removal by early and rapid exchange transfusion can simulate the enhanced blood clearance accomplished by the RES in tolerant animals. Significant protection should now result if enhanced blood clearance per se is indeed the basis of tolerance. The present studies were designed to test this possibility.

Materials and Methods. Employing aseptic precautions, one femoral artery of 2.0–2.5 kg healthy albino New Zealand rabbits was cannulated with sterile polyethylene tubing (PE 90, Clay Adams, Inc.) previously rinsed repeatedly with pyrogen-free sterile saline. An LD_{80} dose (2500 μg) of a Boivin preparation of *Escherichia coli* endotoxin (Difco Laboratories), was then administered in 2.5 ml physiologic saline as a bolus via ear vein. Twenty minutes later exchange transfusion was carried out as follows: 10 ml blood was withdrawn via the femoral artery cannula and 10 ml freshly drawn pooled heparinized blood from healthy rabbit donors immediately returned through the same cannula by turning a three-way stopcock. The donor blood was held at room temperature (70–72°F) and filtered through sterile, pyrogen-free polyethylene screens of the type used in human transfusion sets to remove microthrombi. Additional 10 ml aliquots of blood were exchanged repeatedly until the recipient had received the equivalent of 15% body wt of donor blood (300–375 ml). This accomplished the exchange of approximately 80% of the recipients initial blood volume as determined by plasma protein labeling studies with Evans Blue dye. The entire exchange transfusion was always completed within 20 min. Randomly selected control animals were concomitantly given 2500 μg of the *E. coli* endotoxin by ear vein, and 20 min later sham exchange transfusion performed, i.e., repeated 10 ml aliquots of blood were rapidly withdrawn via a femoral artery cannula and these same blood aliquots returned to the animal after addition of sterile, pyrogen-free heparin equivalent to that used in the actual exchange transfusion (4000 U.S.P. units). Following either the exchange or sham exchange procedure, the femoral artery cannula was removed, the artery ligated, and the wound closed with sterile sutures. All animals were observed for 96-hr survival. Additional control studies were carried out to determine whether exchange transfusion per se enhanced susceptibility to endotoxin lethality. For this purpose, femoral artery cannulation was

[1] Supported by Research Grant No. AI-07052 from the U.S.P.H.S.

performed, exchange transfusion carried out, the cannula removed, the femoral artery ligated, and the femoral wound sutured with sterile precautions. The endotoxin was now injected via ear vein and 96-hr mortality compared with that of paired control animals comparably cannulated and heparinized but not exchange-transfused.

For blood clearance studies, the *E. coli* endotoxin was labelled with $NaCr^{51}O_4$ (Abbott Laboratories) by the method of Braude and coworkers (26). Control solutions of $NaCr^{51}O_4$ were treated identically except for omission of endotoxin to determine the quantity of $NaCr^{51}O_4$ that became nondialyzable as a result of spontaneous aggregation under the conditions of labelling. It was found that a maximum of only 1% of the labelled *E. coli* endotoxin preparation could be contaminated with unbound $Cr^{51}O_4$. Clearance of labelled endotoxin was performed in three groups of rabbits—normal, exchange-transfused, and tolerant. Tolerant animals were studied on day 8 following seven daily iv injections of 100 μg unlabelled *E. coli* endotoxin. In preliminary studies, 10 animals thus pretreated were found highly tolerant, i.e., exhibited no mortality after ear vein administration of 2500 μg *E. coli* endotoxin, the LD_{80} dose for nontolerant rabbits. The femoral artery of each test animal was cannulated, and 2500 μg Cr^{51}-tagged *E. coli* endotoxin injected via ear vein. At carefully timed intervals, 1 ml blood samples were removed from the femoral artery cannula and discarded (washout), and a second 1 ml sample removed and placed in plastic tubes of uniform size. Radioactivity was determined by counting in an automatic gamma well counter for sufficient time to permit reproducibility to within 5%. The amount of circulating endotoxin was expressed as percentage of administered dose of radioactivity calculated to be present at each time interval in the total blood volume of each animal.

Results. Ear vein administration of 2500 μg Cr^{51}-tagged *E. coli* endotoxin into healthy nontolerant rabbits resulted in typical blood clearance patterns, i.e., an initial rapid phase followed within 30 min by the slower phase. This latter phase was characterized by prolonged circulation of appreciable quantities of the initially injected dose of toxin, between 20 to 30%, Fig. 1 (curve 1). In contrast, when the tagged toxin was injected into endotoxin tolerant animals, the expected marked enhancement in blood clearance was seen, Fig. 1 (curve 3). When exchange transfusion was carried out in nontolerant animals, the circulating endotoxin was rapidly and permanently reduced to levels closely approximating those in tolerant animals, Fig. 1 (curve 2).

Despite the rapid and marked reductions in circulating endotoxin levels achievable by exchange transfusion, no significant protection occurred against the endotoxin challenge. Thus, in 23 animals given 2500 μg *E. coli* endotoxin and exchange-transfused 20 min later, 96-hr mortality was only slightly, and not statistically significantly reduced, being 70% compared to 83% mortality in 23 control animals sham exchanged with their own blood.[2] Additional control studies indicated that exchange transfusion did not per se significantly enhance susceptibility to endotoxin lethality. When an LD_{20} (500 μg) of the *E. coli* endotoxin was administered iv to a group of 10 rabbits *after* completion of exchange transfusion, the mortality rate was identical to that in paired cannulated and heparinized but nonexchanged controls.

Discussion. By means of exchange transfusion begun 20 min following iv injection of an LD_{80} bolus of *E. coli* endotoxin into healthy nontolerant rabbits, it was possible to simulate the tolerant state by minimizing the high levels of endotoxin that otherwise continue to circulate for hours. The 20-min interval between endotoxin injection and exchange transfusion was carefully selected as the earliest time that permitted sufficient RES uptake of endotoxin such that the exchange would not remove more than 50% of the administered dose.[3] It is emphasized

[2] The observed difference in percentage mortality (13%) is less than twice its standard error (12.4).

[3] The maximum effect on mortality that would then be expected if exchange transfusion acted simply to decrease the effective administered endotoxin dose, as judged from dose-response data obtained with the same endotoxin preparation (27), would be to shift the LD_{80} to an LD_{50}.

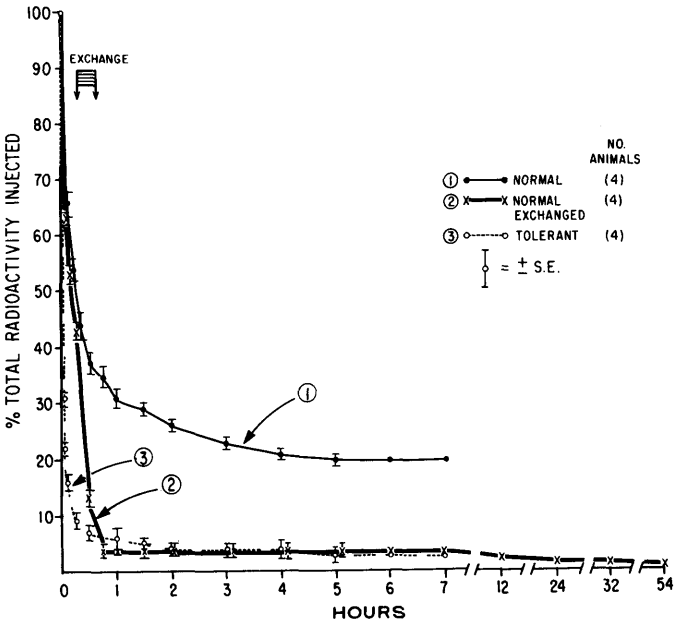

FIG. 1. Blood clearance of 2500 μg Cr^{51}-tagged *E. coli* endotoxin in normal (curve 1), normal exchange-transfused (curve 2), and tolerant (curve 3) rabbits. Note the prolonged circulation of high concentrations of endotoxin in normal animals and the ability of exchange transfusion to simulate the endotoxin clearance curve of the tolerant animal.

that the enhanced blood clearance of endotoxin achieved by exchange transfusion did not precisely duplicate that of the tolerant animal, since the latter cleared the toxin more rapidly during the initial 20-min waiting period. Nevertheless, the resulting final endotoxin blood levels, as well as the time at which such levels attained minimal values after exchange transfusion, were virtually identical to that in the tolerant animal. Despite this ability of exchange transfusion to elicit rapid and sustained reduction of endotoxemia, only slight and statistically insignificant reduction in the subsequent 96 hr mortality ensued. This contrasted with the zero mortality in the tolerant animals. This minimal protection afforded by exchange transfusion could not be related to enhanced endotoxin susceptibility secondary to the exchange procedure *per se*.

Previous studies have demonstrated that the endotoxin tolerance mechanisms continue to function despite RES "blockade" (6, 7), that enhanced rather than reduced endotoxin susceptibility occurs after stimulation of RES phagocytic activity with zymosan, triolein, glucan, or graft versus host reactions (8–13), that decreased rather than augmented endotoxin susceptibility occurs after depression of RES phagocytic activity with ethyl stearate or methyl palmitate (10, 13), that tolerance wanes following discontinuance of endotoxin injections despite persistance of accelerated blood clearance of toxin (14), that certain endotoxins are highly toxic despite their rapid blood clearance (15), that certain antisera to endotoxin can protect despite minimal increases in blood clearance (16), and that diversion of endotoxin into hepatic reticuloendothelium by portal vein administration does not result in pyrogenic tolerance (17). Considered collectively, these observations strongly suggest that mechanisms other than enhanced blood clearance are key determinants of the endotoxin tolerant state. The present findings directly corroborate this thesis and support the alternative concept developed during studies on pyrogenic tolerance to endotoxin, i.e., that tolerance is based primarily upon increased resistance of susceptible reticuloendothelial cells to endotoxin injury, and that augmented blood clearance represents only an ancillary pro-

tective mechanism in that toxin is brought more efficiently into these resistant cells (18, 19). This concept is also consistent with *in vitro* studies on endotoxin induced macrophage cytotoxicity (20, 21), detoxification kinetics (22, 23), and hepatic lysosomal labilization (24, 25).

Summary. Healthy New Zealand rabbits were injected iv with an LD_{80} dose of *E. coli* endotoxin. Twenty minutes later, after removal of over 50% of the endotoxin by the RES, exchange transfusion was performed, accomplishing a rapid and sustained reduction in the level of endotoxemia simulating that seen in animals rendered highly tolerant by seven prior sublethal injections of toxin. Despite such reduction in endotoxemia, 96-hr mortality was only slightly, and not significantly, reduced compared to sham exchanged controls (70 vs 83% respectively). Additional control studies indicated that exchange transfusion *per se* did not enhance endotoxin mortality. The findings directly support the concept that endotoxin tolerance is based primarily upon enhanced RES resistance to endotoxin toxicity rather than upon enhanced RES clearance of circulating endotoxin.

1. Braude, A. I., Carey, F. J., and Zalesky, M., J. Clin. Invest. **34,** 858 (1955).
2. DiLuzio, N. R., and Crafton, C. G., Proc. Soc. Exp. Biol. Med. **132,** 686 (1969).
3. Greisman, S. E., Hornick, R. B., Wagner, H. N. Jr., Woodward, W. E., and Woodward, T. E., J. Clin. Invest. **48,** 613 (1969).
4. Carey, F. J., Braude, A. I., and Zalesky, M., J. Clin. Invest. **37,** 441 (1958).
5. Beeson, P. B., J. Exp. Med. **86,** 39 (1947).
6. Greisman, S. E., Carozza, F. A. Jr., and Hills, J. D., J. Exp. Med. **117,** 663 (1963).
7. Wolff, S. M., Mulholland, J. M., and Ward, S. B., J. Lab. Clin. Med. **65,** 268 (1965).
8. Benacerraf, B. G., Thorbecke, J., and Jacoby, D., Proc. Soc. Exp. Biol. Med. **100,** 796 (1959).
9. Suter, E., Ullman, E. G., and Hoffman, R. G., Proc. Soc. Exp. Biol. Med. **99,** 167 (1958).
10. Stuart, A. E. and Cooper, G. N., J. Pathol. Bact. **83,** 245 (1962).
11. Lemperle, G., Proc. Soc. Exp. Biol. Med. **122,** 1012 (1966).
12. Howard, J. G., Nature (London) **190,** 1122 (1961).
13. DiLuzio, N. R., and Crafton, C. G., Amer. J. Physiol. **217,** 736 (1969).
14. Atkins, E., and Wood, W. B. Jr., J. Exp. Med. **101,** 519 (1955).
15. Chedid, L., Parant, F., Parant, M., and Boyer, F., N. Y. Acad. Sci. **133,** 712 (1966).
16. Braude, A. I., and Douglas, H., J. Immunol. **108,** 505 (1972).
17. Greisman, S. E., and Woodward, C. L., J. Immunol. **105,** 1468 (1970).
18. Dinarello, C. A., Bodel, P. T., and Atkins, E., Trans. Ass. Amer. Phys. **81,** 334 (1968).
19. Greisman, S. E., and Hornick, R. B., J. Infect. Dis. **128** (Supplement), 265 (1973).
20. Heilman, D. H., Int. Arch. Allerg. Appl. Immunol. **26,** 63 (1965).
21. Kessel, R. W. I., and Braun, W., Aust. J. Exp. Biol. Med. Sci. **43,** 511 (1965).
22. Rutenburg, S. H., Rutenburg, A. M., Smith, E. E., and Fine, J., Proc. Soc. Exp. Biol. Med. **118,** 620 (1965).
23. Trejo, R. A., and DiLuzio, N. R., Proc. Soc. Exp. Biol. Med. **141,** 501 (1972).
24. Janoff, A., Weissmann, G., Zweifach, B. W., and Thomas, L., J. Exp. Med. **116,** 451 (1962).
25. Janoff, A., and Zweifach, B. W., Proc. Soc. Exp. Biol. Med. **114,** 695 (1963).
26. Braude, A. I., Carey, F. J., Sutherland, D., and Zalesky, M., J. Clin. Invest. **34,** 850 (1955).
27. Greisman, S. E., J. Exp. Med. **112,** 257 (1960).

Received October 7, 1974. P.S.E.B.M. 1975, Vol. 148.