In Vivo Microscopic Observations of the Responses of Kupffer Cells and the Hepatic Microcirculation to *Mycobacterium bovis* BCG Alone and in Combination with Endotoxin

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Kupffer cell function and hepatic microvascular hemodynamics were studied by in vivo microscopy in *Mycobacterium bovis* BCG-infected NMRI mice before and after treatment with minute (0.01 μ g) tolerance-producing doses and doses causing 70% lethality (0.5 μ g) of *Escherichia coli* 0111:B5 endotoxin alone and in combination. BCG-induced granulomas distorted the hepatic microvasculature and impeded blood flow in many sinusoids; flow also was altered further by leukocytes adhering to the sinusoidal walls and by enlarged Kupffer cells that bulged into the lumen. Nevertheless, in BCG-infected mice, the ratio of Kupffer cells which phagocytosed latex to sinusoids containing blood flow and capable of delivering these particulates to Kupffer cells was significantly greater than that in uninfected mice. The phagocytosis of single latex particles by individual Kupffer cells also was more rapid. This indicated an expansion of the numbers and activation of Kupffer cells. In this hyperreactive state, the tolerance-inducing dose of endotoxin produced no change in the rate of phagocytosis after 2 h. In contrast, the 70% lethal dose reduced the rate by 123%, unless tolerance was induced, in which case there was no reduction in the rate of phagocytosis. Twenty-four hours after injection of the tolerance-inducing dose, however, the rate of phagocytosis was accelerated slightly (17%). This suggested that the Kupffer cells had been activated and perhaps were more effective in clearing subsequent endotoxin from the blood but without sufficient release of toxic substances to be lethal. That some mediators were released, however, was suggested by the microvascular alterations that accompanied the above phagocytic responses. These results further support the concept of a central role for Kupffer cells in endotoxin-mediated, nonspecific host defense mechanisms.

Kupffer cells are phagocytic cells and comprise one of the cellular components of hepatic sinusoids. At this site they play a key role in the removal of gut-derived endotoxins from the blood and thereby prevent these toxic substances from entering the systemic circulation (12, 13, 21, 22, 25). Like other macrophages, Kupffer cells are thought to play a key role in host defense mechanisms, including nonspecific resistance (24). The latter has been attributed to the release of various mediators from these cells, e.g., colony stimulating factor, interferon, interleukin 1, and tumor necrotizing factor (3-5, 9, 10). Other substances also can be released such as lysosomal enzymes, prostaglandins, and oxygen-derived, reactive free radicals (2, 3, 5, 11). Many of these substances, if released in

sufficient quantities, can be inflammatory, vasoactive, and toxic. The release of all these substances is influenced by endotoxins (1, 5, 11, 14, 20, 22, 24). Thus, although large doses of endotoxin usually precipitate a toxic response, low doses have been shown to produce an increased tolerance to normally lethal doses of endotoxins. As a result, the low levels of gut-derived endotoxin normally found in portal blood may contribute to nonspecific resistance (29).

In an attempt to improve understanding of the role of the liver in protecting the animal against endotoxemia and in contributing to nonspecific resistance, a series of studies were initiated to evaluate the effects of endotoxin on Kupffer cell function and hepatic microvascular hemodynamics by direct in vivo microscopic study (15-

FIG. 1. In vivo video image of a dilated sinusoid adjacent to a granuloma in the liver of a BCG-infected mouse. Note the adherence of numerous lymphocytes (L) to the sinusoidal lining (arrows).

cles to the Kupffer cells, was significantly greater in the BCG-infected animals (Fig. 2D).

Two hours after the administration of an LD₇₀ dose (in BCG-infected animals) of ET, the rate of phagocytosis of single latex particles by individual Kupffer cells was significantly lengthened from 23 to 49 sec (Fig. 3). Concomitantly, the

numbers of phagocytic Kupffer cells and sinusoids containing blood flow also was reduced by half (Fig 4) due to plugging of the sinusoids with leukocytes, platelets, fibrin, and swollen Kupffer and endothelial cells. In contrast, a LD₁₀ dose (in BCG-infected animals) of ET did not change the rate of phagocytosis, which was

FIG. 2. Effect of BCG on Kupffer cells and hepatic microcirculation. (A) Rates of phagocytosis of single latex particles by individual Kupffer cells. (B) Numbers of phagocytic Kupffer cells per microscopic field; these were not significantly different. (C) Numbers of perfused sinusoids per microscopic field. (D) Ratio of phagocytic Kupffer cells to perfused sinusoids. Values are the mean $\pm$ the standard error.

FIG. 3. Effect of minute and toxic doses of endotoxin alone and in combination on the rate of phagocytosis of single latex particles by individual Kupffer cells in BCG-infected mice. Values are the mean $\pm$ the standard error.

still 22 sec 2 h after this tolerance-producing dose of ET was administered (Fig. 3). This dose, however, did reduce the total numbers of phagocytic Kupffer cells and, to a lesser extent, sinusoids containing blood flow (Fig. 4).

Twenty-four hours after administration of the LD₁₀ dose of ET, the rate of phagocytosis was slightly accelerated (17%) when compared with saline-treated, BCG-infected animals ($P < 0.05$) (Fig. 3). Although the number of sinusoids containing blood flow returned to control levels, the number of phagocytic Kupffer cells remained less than the number of control cells but was not statistically different from the number seen 2 h after injection (Fig. 4). In contrast, little or no blood flow and phagocytic activity was seen in the livers of the few animals surviving 24 h after receiving the LD₇₀ dose of ET. Tolerance to this LD₇₀ dose of ET was induced, however, by the administration of an LD₁₀ dose of ET 24 h before challenge with a normal LD₇₀ dose (in BCG-infected animals) of ET. Under these conditions, the rate of phagocytosis remained unaltered 2 h after the challenge dose of ET (Fig. 3). However, the number of phagocytic Kupffer cells increased, whereas the number of sinusoids containing blood flow decreased slightly when compared with animals given the LD₁₀ dose alone (Fig. 4).

DISCUSSION

The present studies provide further support for the important role played by Kupffer cells in endotoxin-mediated pathophysiological reactions and host defense mechanisms. The results directly demonstrate in vivo some of the alterations in the hepatic microcirculation and Kupffer cell function that occur in BCG-infected mice which are hyperreactive to ET (26) and how the livers of such mice respond to doses of endotoxin that are lethal or produce tolerance to subsequent challenges of usually lethal doses of ET.

The effect of BCG in activating the mononuclear phagocyte and lymphoid systems is clearly reflected in these studies by the increased rate of phagocytosis of latex by enlarged Kupffer cells, as previously reported (18, 19), the increased numbers of Kupffer cells in the sinusoids, and the association of lymphocytes with the sinusoidal wall. The latter probably reflects the well-known lymphocyte-macrophage interaction that is invoked in host defense. The exclusion of blood flow from granulomas may explain the reported failure of phagocytes in these areas to sequester colloidal material (8).

Kupffer cells are the principal cells responsible for clearing endotoxin from the blood (13, 25), and during this process, they release a variety of toxic and beneficial mediators (1, 20, 22, 24). The accelerated rate of phagocytosis and the increased numbers of phagocytic cells demonstrated in the livers of BCG-infected mice is

FIG. 4. Effect of minute and toxic doses of endotoxin alone and in combination on the numbers of phagocytic Kupffer cells and perfused sinusoids per microscopic field in BCG-infected mice. Values are the mean $\pm$ the standard error.

consistent with the central role of the Kupffer cells in endotoxin-mediated events since an accelerated rate of endotoxin clearance (29) and elevated levels of mediators in the posthepatic blood (23, 29, 30) have been reported in BCG-infected animals.

The results demonstrate that the blood concentration of endotoxin and the functional state of the Kupffer cell are important determinants in the response of the host to endotoxin. Doses of ET which induce tolerance and increase nonspecific host resistance in normal mice become lethal in BCG-infected mice (1, 26). Nevertheless, minute doses of ET can induce tolerance in the latter animals (29). Interestingly, endotoxin injected into such tolerant animals still elicits elevated levels of mediators thought to be beneficial in promoting nonspecific host resistance (29). In the present study, minute tolerance-producing doses of endotoxin did not inhibit phagocytosis 2 h after injection which is what occurs with higher doses (18, 19). That mediators are released by these doses, however, is suggested by the presence of microvascular responses including reduced sinusoidal blood flow due to plugging of these vessels with adherent leukocytes and, concomitantly, reduced numbers of Kupffer cells that have access to latex. Similar responses are seen when larger, but nonlethal, doses of ET are given to normal mice (18, 19).

Tolerance was induced rapidly (within 24 h) with a minute dose of endotoxin as reported previously (29). At this time, the Kupffer cells exhibited a slight, but significant, acceleration in the rate of phagocytosis when compared with control, BCG-infected animals. This suggested that the tolerance-producing dose of ET elicited further activation of the Kupffer cells. The rate of phagocytosis and the hepatic microcirculation were not significantly altered by a subsequent, normally lethal dose of endotoxin. These responses were similar to those seen 24 h after injecting detoxified endotoxin into normal mice (18, 19) to induce tolerance to normally lethal doses of endotoxin (18, 19).

The mechanisms involved in producing tolerance are not clear. However, the tolerance that was rapidly induced within 24 h is not likely due to the presence of specific immunoglobulins since their formation requires a considerably longer period of time (7, 27, 28). Thus, the results in the current study suggest that tolerance-inducing doses of endotoxin modify the ability of Kupffer cells to produce and/or release toxic mediators but not beneficial mediators (29). Concomitantly, the normal inhibitory action of endotoxin on phagocytosis initially seen after administration is abolished. These results further support the concept of a central role of

Kupffer cells in endotoxin-mediated, nonspecific host defense mechanisms.

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LITERATURE CITED

- Berry, L. J. 1977. Bacterial toxins. *Crit. Rev. Toxicol.* 5:239-318.
- Bhatnagar, R., R. Schrimmer, M. Ernst, and K. Decker. 1981. Superoxide release by zymosan-stimulated rat Kupffer cells *in vitro*. *Eur. J. Biochem.* 119:171-175.
- Brouwer, A., R. J. Barelds, A. M. De Leeuw, and D. L. Knook. 1982. Maintenance cultures of Kupffer cells as a tool in experimental liver research, p. 327-334. *In* D. L. Knook and E. Wisse (ed.), *Sinusoidal lining cells*. Elsevier/North-Holland Press, Amsterdam.
- Cohen, S. A., D. Salazar, J. Wicher, and J. P. Nolan. 1982. Natural cytotoxicity of isolated murine nonparenchymal liver cells, p. 461-462. *In* D. L. Knook and E. Wisse (ed.), *Sinusoidal lining cells*. Elsevier/North-Holland Press, Amsterdam.
- Decker, K., M. Birmelin, R. Bhatnagar, and H. Rieder. 1982. The role of Ca^{2+} and prostaglandins in the response of rat Kupffer cells to external stimuli, p. 361-368. *In* D. L. Knook and E. Wisse (ed.), *Sinusoidal lining cells*. Elsevier/North-Holland Press, Amsterdam.
- Dubos, R. J., and R. W. Schaedler. 1957. Effects of cellular constituents of mycobacteria on the resistance of mice to heterologous infections. I. Protective effects. *J. Exp. Med.* 106:703-715.
- Greisman, S. E., and R. B. Hornick. 1976. Endotoxin tolerance, p. 43-50. *In* R. F. Beers, Jr. and E. G. Basset (ed.), *The role of immunological factors in infectious, allergic, and autoimmune processes*. Raven Press, New York.
- Hardonk, M. J., F. W. J. Dijkhuis, A. Feringa, and J. Koudstaal. 1982. The development of granulomas in rat livers after i.v. injection of particulate pig intestinal alkaline phosphatase, p. 185-192. *In* D. L. Knook and E. Wisse (ed.), *Sinusoidal lining cells*. Elsevier/North-Holland Press, Amsterdam.
- Howard, J. G., G. Bizzio, B. N. Halpern, C. Stiffel, and D. Mouton. 1959. The effect of *Mycobacterium tuberculosis* (BCG) infection on the resistance to bacterial endotoxin and *Salmonella enteritidis*. *Br. J. Exp. Pathol.* 40:281-290.
- Kirn, A., F. Koehren, and A. M. Steffan. 1982. Interferon synthesis in primary culture of Kupffer and endothelial cells from the rat liver. *Hepatology* 2:676.
- Leser, H. G., K. M. Debatin, H. Northoff, and D. Gerns. 1982. Kupffer-cell-enriched nonparenchymal cells and peritoneal macrophages differ in prostaglandin E release production of interleukin 1 and tumor cytotoxicity, p. 369-376. *In* D. L. Knook and E. Wisse (ed.), *Sinusoidal lining cells*. Elsevier/North-Holland Press, Amsterdam.
- Maier, R. V., and F. J. Ulevitch. 1981. The response of isolated rabbit hepatic macrophages (H-M0) to lipopolysaccharide (LPS). *Circ. Shock* 8:165-181.
- Mathison, J. C., and R. Ulevitch. 1979. The clearance, tissue distribution, and cellular localization of intravenously injected lipopolysaccharide in rabbits. *J. Immunol.* 123:2133-2143.
- McCabe W. R. 1980. Endotoxin: microbiological, chemical, pathophysiologic and clinical correlations. *Sem. Infect. Dis.* 3:38-88.

15. McCuskey, R. S. 1981. *In vivo* microscopy of internal organs. Prog. Clin. Biol. Res. **59B**:79–87.
16. McCuskey, R. S. 1981. *In vivo* microscopy of the liver and phagocytosis of latex particles by Kupffer cells. Anat. Rec. **199**:167A.
17. McCuskey, R. S., F. D. Reilly, P. A. McCuskey, and R. V. W. Dimlich. 1979. *In vivo* microscopic studies of the hepatic microvascular system. Bibl. Anat. **18**:73–76.
18. McCuskey, R. S., R. Urbaschek, P. A. McCuskey, and B. Urbaschek. 1982. *In vivo* microscopic responses of the liver to endotoxin. Klin. Wochenschr. **60**:749–751.
19. McCuskey, R. S., R. Urbaschek, P. A. McCuskey, and B. Urbaschek. 1982. Studies of Kupffer cells in mice sensitive or tolerant to endotoxin, p. 387–392. In D. L. Knook and E. Wisse (ed.), Sinusoidal liver cells. Elsevier/North-Holland Press, Amsterdam.
20. Morrison, D. C., and R. J. Ulevitch. 1978. The effects of bacterial endotoxins on host mediation systems. Am. J. Pathol. **93**:525–618.
21. Munford, R. S. 1978. Endotoxins and the liver. Gastroenterology **75**:532–535.
22. Nolan, J. P. 1981. Endotoxin, reticuloendothelial function, and liver injury. Hepatology **1**:458–465.
23. Parant, M. A., F. J. Parant, and L. A. Chedid. 1980. Enhancement of resistance to infections by endotoxin-induced serum factor from *Mycobacterium bovis* BCG-infected mice. Infect. Immun. **28**:654–659.
24. Rosenstreich, D. L., and S. N. Vogel. 1980. The central role of macrophages in the host response to endotoxin, p. 11–15. In D. Schlessinger (ed.), Microbiology—1980. American Society for Microbiology, Washington, D.C.
25. Ruiter, D. J., J. van der Maulen, A. Brouwer, M. J. R. Hummel, B. J. Mauer, J. C. M. van der Ploeg, and E. Wisse. 1981. Uptake by liver cells of endotoxin following its intravenous injection. Lab. Invest. **45**:38–45.
26. Suter, E., and E. M. Kirsanov. 1961. Hyperreactivity to endotoxin in mice infected with *Mycobacterium*. Induction and elicitation of the reactions. Immunology **4**:354–365.
27. Urbaschek, B., and A. Nowotny. 1968. Endotoxin tolerance induced by detoxified endotoxin (endotoxoid). Proc. Soc. Exp. Biol. Med. **127**:650–652.
28. Urbaschek, B., and R. Urbaschek. 1977. Some aspects of microcirculatory and metabolic changes in endotoxemia and in endotoxin tolerance, p. 286–292. In D. Schlessinger (ed.), Microbiology—1977. American Society for Microbiology, Washington, D.C.
29. Urbaschek, R., and B. Urbaschek. 1982. Aspects of beneficial endotoxin-mediated effects. Klin. Wochenschr. **60**:746–748.
30. Urbaschek, R., and B. Urbaschek. 1983. Ability of post-endotoxin serum from BCG-infected mice to induce non-specific resistance and stimulation of granulopoiesis. Infect. Immun. **39**:1488–1490.