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Increased Toxicity of Endotoxin for Tumor-Bearing Mice and Mice Responding to Bacterial Pathogens: Macrophage Activation as a Common Denominator

MICHAEL J. BERENDT,* MICHAEL F. NEWBORG, AND ROBERT J. NORTH
Trudeau Institute, Inc., Saranac Lake, New York 12983

Mice bearing the syngeneic SA-1 sarcoma or treated with live *Mycobacterium bovis* BCG or Formalin-killed *Corynebacterium parvum* acquired a greatly increased susceptibility to the lethal effects of endotoxin. In all three experimental models, the acquisition of increased sensitivity to endotoxin was concordant with the generation of a systemically activated macrophage system.

It was observed during the course of a study of endotoxin-induced tumor regression (2, 3) that increasing the therapeutic intravenous (i.v.) dose of bacterial endotoxin from 50 to 100 μg was lethal for mice bearing endotoxin-susceptible syngeneic tumors. This observation was in agreement with much earlier findings by others (5, 12) that occasionally tumor-bearing animals treated with microgram quantities of endotoxin rapidly succumbed. Moreover, our previous studies (2, 3) also showed that immunogenic tumor growth was associated with the generation by the host of a highly activated macrophage population, as evidenced by an increased capacity to destroy the bacterial parasite, *Listeria monocytogenes*.

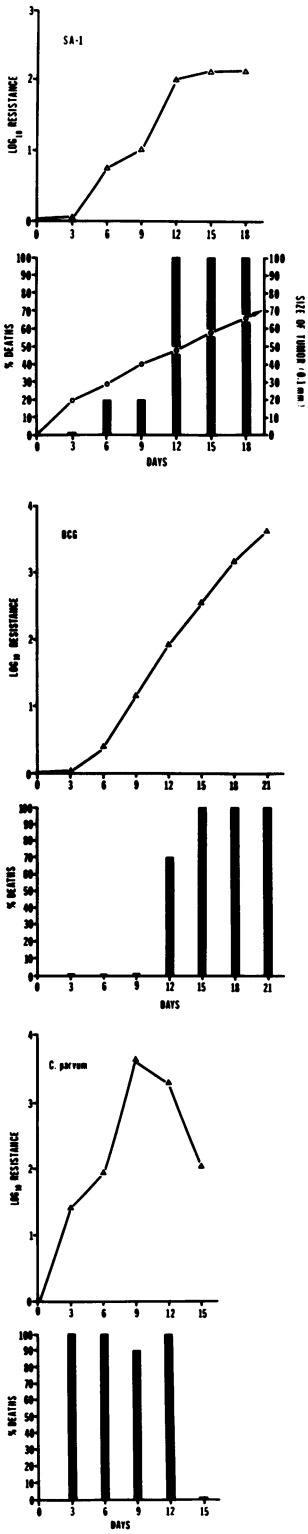
The numerous reports (1, 4, 6, 7, 10, 11, 14–16) documenting the enhanced lethal effect of endotoxin for mice infected with microbial pathogens that cause systemic macrophage activation suggest that macrophage activation is the common underlying requirement for the increased sensitivity to endotoxin. If so, tumor-bearing mice and mice infected with bacterial pathogens should show a close temporal correlation between the development of increased susceptibility to endotoxin and the generation of an activated macrophage system. This study showed that this was the case in mice bearing the SA-1 sarcoma infected with BCG or treated with Formalin-killed *Corynebacterium parvum*.

Adult male and female $AB6F_1$ hybrid mice (A × C57BL/6) were used throughout this study. *Salmonella enteritidis* endotoxin (Difco, boivin extraction, lot no. 648750) was suspended in phosphate-buffered saline (PBS); 100 μg was injected i.v. in a volume of 0.2 ml for all experiments. The procedures for maintaining and handling the SA-1 sarcoma (strain A origin) were described in a previous publication (2). Primary tumors were initiated in the right-hind footpad with 10^6 viable tumor cells suspended in PBS (0.05 ml), and tumor growth was measured with dial calipers. *C. parvum* (Burroughs-Wellcome Co., lot. no. CA 582A) was suspended in PBS, and 175 μg was injected i.v. in a volume of 0.2 ml. *Mycobacterium bovis*, strain BCG Pasteur (Trudeau Institute Mycobacterial Culture Collection), was diluted in 10% Tween 80-saline and subjected to 8 s of ultrasound to dissociate clumps. This preparation was diluted appropriately in phosphate-buffered saline, and 5×10^6 viable colony-forming units were inoculated i.v. in a volume of 0.2 ml.

The generation of macrophage-mediated, nonspecific resistance acquired in response to tumor growth, BCG infection, or *C. parvum* treatment was determined by measuring changes against time in the capacity of the host to resist a lethal 10^5 i.v. challenge infection with *L. monocytogenes*. Resistance was recorded as the $\log_{10}$ difference between the 48-h growth of *L. monocytogenes* in the livers of control and experimental mice. The preparation of the *Listeria* inoculum, the technique for monitoring the growth of the organism in the liver, and the meaning of 48-h $\log_{10}$ resistance are discussed in previous publications (8, 9).

In all experiments, concurrent measurements were made of macrophage-mediated, nonspecific resistance and susceptibility to the lethal effects of a 100-μg dose of endotoxin given i.v. This involved sampling populations of experimental animals at the time intervals indicated. Groups of five mice were used to measure antibacterial resistance in the liver, and groups of 10 were used to determine the percentage that died during a 48-h period after endotoxin injection.

The results obtained with the SA-1 sarcoma, BCG, and *C. parvum* are shown in Fig. 1, 2, and 3, respectively. The host response to all agents resulted in increased macrophage-mediated an-



tibacterial resistance which was concordant with the development of lethal susceptibility to a dose of endotoxin easily tolerated by normal mice. The closest temporal correlation was seen with the more-rapid response of mice treated with *C. parvum*. In this case, the decline in macrophage activation was associated with a rapid loss of susceptibility to endotoxin. In tumor-bearing and BCG-infected mice, the development of susceptibility to endotoxin toxicity lagged behind the generation of antibacterial resistance. It should be pointed out in this connection, however, that the measurements of antibacterial resistance are plotted against the time that the test organism was injected, rather than against the time that it was enumerated in the livers 48 h later. The reason for recording the results in this way is discussed in a previous publication (9) which shows that macrophage-mediated resistance is expressed mainly within the first 8 to 12 h of the challenge infection. It is obvious from the present data, moreover, that bacterial resistance was increasing during the 48-h period of the assay. Consequently, part of the lag between increased antibacterial resistance and increased sensitivity to endotoxin is more apparent than real. Lower levels of macrophage activation, although not associated with endotoxin-induced death, were nevertheless associated with greatly increased toxic effects of endotoxin. For instance, in contrast to normal mice, all mice injected with endotoxin on day 6 or 9 of growth of the SA-1 sarcoma or on day 9 of BCG infection exhibited diarrhea, ruffled fur, and lethargy.

On the basis of the foregoing results, it is clear that a systemically activated macrophage system was a common denominator during the development of increased susceptibility to the lethal effect of endotoxin in mice bearing the SA-1 sarcoma, infected with BCG, or stimulated with *C. parvum*. This study provides no direct evidence, however, that macrophage activation was the direct cause of increased susceptibility to endotoxin. This possibility is being considered by others on the basis of the finding that endo-

FIG. 1–3. *Concurrent measurements of changes against time in the level of macrophage-mediated antibacterial resistance in the liver and the percentage of animals that died from a single i.v. 100-μg dose of endotoxin during the response to subcutaneous growth of the SA-1 sarcoma (Fig. 1), i.v. infection with BCG (Fig. 2), or i.v. injection of Formalin-killed C. parvum (Fig. 3). Fig. 1 includes a curve showing the rate of tumor growth (bottom line graph). Five mice were employed at the times indicated to measure antibacterial resistance, and 10 were used to measure endotoxin-induced death.*

toxin triggers increased prostaglandin production by activated macrophages (18) and that prostaglandins mimic some of the toxic effects of endotoxin (13). It remains possible, however, that some other mechanism is responsible for increased susceptibility to endotoxin and that macrophage activation is merely a reflection of its presence. Whatever the underlying pharmacological basis for increased sensitivity to endotoxin, it seems reasonable to conclude that the mechanism acquired in response to immunogenic tumor growth is the same as that acquired in response to infectious agents.

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