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Tumor Necrosis Factor: A Cytokine Involved in Toxic Effects of Endotoxin

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Endotoxin-induced tumor necrosis has been shown to be mediated by a factor termed *tumor necrosis factor* (TNF). The biochemical nature, source, and mode of induction of TNF have been clarified. TNF is a mediator of activated macrophages that is released into the supernatant by these cells after their stimulation with endotoxin. A number of biologic functions of TNF other than its tumoricidal activity have been demonstrated. In vivo, TNF induces reactions similar to those induced by endotoxin. Hypothermia, elevated hematocrit and plasma lactate levels, and reduced plasma glucose levels have been measured in mice injected with TNF. These reactions typically occur soon after endotoxin injection and are induced with purified recombinant TNF in mice that exhibit a low response to endotoxin. No TNF-induced production of interleukin 1 (which can induce similar effects) was detected in macrophage/monocyte cultures. Therefore, TNF appears to mediate endotoxin effects directly.

Bacterial endotoxin has been shown to exert detrimental as well as beneficial effects in various experimental models in which different means were used for compromising the host. Toxic effects, fever, tumor regression, and enhancement of nonspecific resistance to infection and lethal irradiation are some examples of endotoxin reactions. In recent years it has become apparent that some of these effects are accomplished by the interaction of endotoxin with its main target cell—the macrophage. Sensitivity to endotoxin depends on the state of activation of the host's lymphoreticular system. Endotoxin is extremely toxic for activated macrophages in BCG-infected animals [1] or for macrophages that have been activated in vitro [2]. Also, release of mediators is qualitatively and quantitatively related to the degree of macrophage activation [3]. Thus, the status of the reactivity of macrophages is of great importance to the lethal effects of endotoxin. Many investigations have addressed the role of macrophages and their release of humoral mediators in endotoxic effects.

Tumor necrosis factor (TNF) is a protein released by activated macrophages upon their stimulation with bacterial lipopolysaccharide (LPS) [2]. The molecular cloning of the cDNA for TNF and the expression of this cDNA in *Escherichia coli* [4–6] have made available large amounts of the recombinant protein for biologic research. Before the nature of the TNF molecule was known, TNF was defined by its tumor necrotizing activity in vivo and its tumor cell cytotoxic activity in vitro. Investigations of the biologic activities of purified recombinant TNF revealed that TNF not only serves as a cytotoxic molecule for tumor cells but also has an array of effects on different target cells. Comparison of the amino acid sequences of TNF and cachectin, another monokine that is secreted in response to endotoxin or other bacterial and protozoal products, revealed the identity of the two proteins [7]. The activity of cachectin leads to a decrease in lipoprotein lipase activity and to the metabolic derangements that occur during infection and cachexia [8]. Antibodies to the purified cachectin/TNF molecule inhibited the lethal activity of endotoxin, a finding indicating a role for this mediator in endotoxic effects [9, 10].

Direct analysis of the biologic effects of TNF in different test systems further demonstrated the importance of TNF in inflammatory events. Production of prostaglandin E_2 and collagenase by human synovial cells and dermal fibroblasts [11]; induction of procoagulant activity and tissue factor by the endothelium; and propagation of the coagulation pathway leading to the deposition of fibrin [12], bone resorption, and inhibition of bone formation

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[13] are effects of TNF typical for inflammatory responses.

TNF also exerts biologic effects on the neutrophil, another cell that plays a central role in inflammatory processes. Enhancement of phagocytic and antibody-dependent cytotoxic activity [14] and of adherence of neutrophils to endothelial cells [15] in response to TNF has been reported. Furthermore, TNF is an important immunologic mediator. It enhances eosinophil toxicity [16] and increases surface expression of class I major histocompatibility complex antigens on endothelial cells and dermal fibroblasts [17].

In this communication we compare some effects of recombinant TNF in vivo with typical effects of endotoxin. TNF and LPS induced similar thermoregulatory effects and changes in blood parameters when administered iv to mice. TNF induced the observed effects rather directly and not via the induction of interleukin 1 (IL-1) secretion, since TNF did not induce IL-1 production in our macrophage culture systems. These observations, therefore, further support the notion that TNF is involved in endotoxin reactions and may act as an endogenous mediator of endotoxin.

Materials, Methods, and Results

In an effort to show the connection between LPS and TNF, we measured some parameters that change significantly after iv administration of bacterial LPS. These changes were compared with the effects induced by the iv administration of purified recombinant human TNF. The TNF preparation was kindly provided by BASF (Ludwigshafen, FRG) and contained <0.03 ng of endotoxin/mg of protein, as determined in the limulus lysate assay. When TNF was injected iv into mice at a concentration of 40 μg per animal (2 mg/kg), the first symptoms resembled the toxic effects seen after endotoxin application; within hours ruffled fur, diarrhea, hypothermia, and loss of body weight were noted. The extent of the observed effects was dependent on the mouse strain used. The sensitivity of a given mouse strain (C3H/He or C3H/HeJ) differed considerably from experiment to experiment. Therefore, only values obtained in the same experiment were compared.

Table 1. Blood parameters in mice after the injection of bacterial lipopolysaccharide (LPS) or tumor necrosis factor (TNF).

Stimulus	Hematocrit (%) ± SD	Glucose (mg/100 ml) ± SD	Lactate (mg/100 ml) ± SD
Control	42.3 ± 1.7	82.3 ± 23.2	62.9 ± 12.1
LPS	56.6 ± 7.3	32.9 ± 11.5	58.6 ± 8.9
TNF	63.0 ± 1.0	14.8 ± 0.4	85.3 ± 7.9

NOTE. Each group comprised three age-, sex-, and weight-matched C3H/He mice. Animals were injected iv with LPS (*Salmonella montevideo* strain SH94, 200 μg), TNF (60 μg), or PBS (control, 100 μl). Hematocrit, plasma lactate, and plasma glucose levels were determined from blood samples taken 6 h after injection.

Changes in Blood Parameters

Blood parameters of LPS-sensitive animals (C3H/He) that had received 100 μg of LPS (*Salmonella montevideo* strain SH94 prepared according to the modified phenol-water extraction method [18]) iv changed significantly within 6 h. Animals were bled from the retroorbital plexus at intervals, and blood or plasma parameters were enzymatically determined with use of commercial kits (GlucoQuant and Monotest-Lactate kit, both from Boehringer, Mannheim, FRG). Hematocrit levels increased within 30 min (table 1). Plasma glucose levels dropped during the first 6 h after administration of LPS. Plasma lactate levels were not changed in the animals injected with LPS despite the fact that very large and almost lethal amounts of LPS are reported to enhance the lactate levels [19]. Similar results were obtained in mice injected with TNF. After TNF injection the plasma glucose levels were even lower than those after LPS injection, and the plasma lactate levels were enhanced. The contaminating amount of endotoxin in the TNF preparation (<2 pg) was not able to induce these effects (data not shown). Also, mice with a low response to LPS (C3H/HeJ strain) reacted to TNF with the same changes as C3H/He mice (data not shown), a finding that indicates these effects were due to TNF itself.

Thermoregulatory Activity of TNF

Mice react with hypothermia when injected with LPS, whereas most other species develop a fever [20]. As shown in figure 1, TNF caused a hypothermic reaction in C3H/He as well as C3H/HeJ mice. The rectal temperature of the animals was determined with an electronic temperature probe (Haake DT-10, Karlsruhe, FRG) at different times after iv injec-

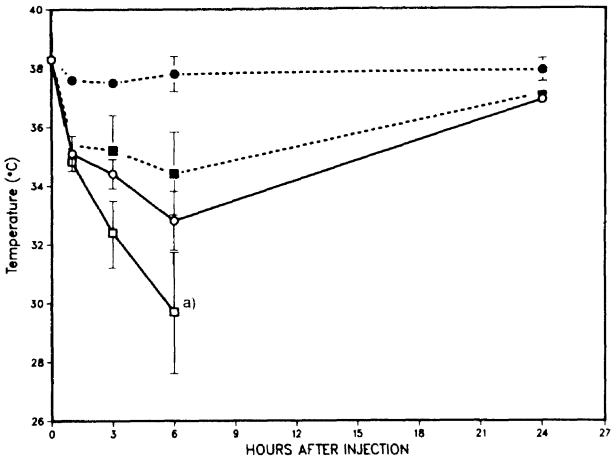

Figure 1. Hypothermia in mice after injection with bacterial lipopolysaccharide (LPS) or tumor necrosis factor (TNF). Groups of three C3H/He (———) or C3H/HeJ (- - -) mice were injected with either LPS (*Salmonella montevideo* strain SH94, 100 μg; circles) or TNF (60 μg; squares) iv and their temperatures were determined at intervals after injection. All three C3H/He mice injected with TNF died within 24 h (a).

tion of either LPS or TNF. The TNF-induced hypothermia was dose-dependent (data not shown), a finding that again indicates that TNF itself has a thermoregulatory activity similar to that of bacterial endotoxin.

Lack of IL-1 Induction by TNF

We tested whether TNF was able to induce IL-1 activity in murine or human monocyte/macrophage cultures, since the thermoregulatory activity of endotoxin has been shown to be mediated by endogenous pyrogen/IL-1. Murine peritoneal exudate cells (PEC) were induced by the injection of 1 ml of PBS ip 16 h before the collection of the exudate. The adherent fraction was cultured in the presence of either LPS or TNF or without any stimulating agent. Cell-free supernatants were tested after 24 h for their costimulator activity in a standard IL-1 assay. C3H/HeJ thymocytes (3×10^5) were cultured in the presence of suboptimal concentrations of phytohemagglutinin (50 μg/ml; PHA-M, Sigma, Deisenhofen, FRG) and with serial dilutions of the supernatants. IL-1-dependent proliferation of the thymocytes was determined by counting the incorporated radioactivity at 72 h after a 16-h pulse with 1 μCi of [^{3}H]thymidine (Amersham, Braunschweig, FRG; specific activity, 50 Ci/m*M*). Only weak IL-1 activity was determined in supernatants of TNF-stimulated C3H/He-PEC (table 2). LPS, however, induced significant IL-1 activity under these conditions. No IL-1 activity was detected when PEC of C3H/HeJ mice (low response to endotoxin) were stimulated with TNF.

Human peripheral blood mononuclear lympho-

Table 2. Induction of interleukin 1 (IL-1) in murine peritoneal exudate cell (PEC) cultures.

Mouse strain, stimulus (μg/ml)*	IL-1 (U/ml)†
C3H/He	
LPS, 10	24
TNF	
10	6
1	<4
0.1	<4
None	<4
C3H/HeJ	
TNF	
10	<4
1	<4
0.1	<4
None	<4

* Adherent PEC (2×10^6) from either C3H/He or C3H/HeJ mice were cultured in the presence of either lipopolysaccharide (LPS; *Salmonella montevideo* strain SH94, 10 μg/ml), tumor necrosis factor (TNF; 0.1-10 μg/ml), or no stimulus in a volume of 1 ml for 24 h.

† IL-1 units were determined as the reciprocal dilution of the cell-free culture supernatant that induced [^{3}H]thymidine incorporation 2.5-fold higher than the background.

Table 3. Induction of interleukin 1 (IL-1) by bacterial lipopolysaccharide (LPS) or tumor necrosis factor (TNF) in human monocyte cultures.

Stimulus*	IL-1 (U/ml)†
LPS, 10 ng/ml	64
TNF	
10 μg/ml	<4
1 μg/ml	<4
100 ng/ml	<4
10 ng/ml	<4
1 ng/ml	<4
None	<4

* Adherent human mononuclear cells (3×10^5) were cultured in the presence of LPS (*Salmonella typhimurium* strain 0901, Difco, Detroit), of TNF at the indicated concentrations, or with no stimulus in a volume of 1 ml for 24 h.

† IL-1 units were determined as indicated in table 2.

cytes (consisting of ~95% monocytes) that had been purified by centrifugation over ficoll-Hypaque [21] and adherence to plastic did not produce IL-1 activity when stimulated with TNF at concentrations ranging from 1 ng/ml to 10 μg/ml (table 3). In further experiments we tested whether TNF was able to modulate LPS-induced IL-1 activity and whether inhibitory activities were generated that could interfere with the IL-1 test system. However, TNF neither modulated the induction of IL-1 production nor interfered with the thymocyte costimulator test system (data not shown). From these results we concluded that TNF per se is an endogenous thermoregulatory mediator similar to IL-1 and that it acts on the thermoregulatory system without the prior induction of IL-1 production by monocytes.

Conclusions

The effect of macrophages in sepsis and in inflammatory responses is mediated by a large array of secreted products. The macrophage is the most important target cell for endotoxin, and the lethal effects of endotoxin are dependent on the stage of activation of the macrophages. This indicates the involvement of endotoxin-induced monokines in the toxic effects of endotoxin. There is strong evidence that TNF, like IL-1, is an important mediator of endotoxic effects. (*1*) It was demonstrated by Beutler et al. [9] that reduction of endotoxin-caused lethality in mice can be achieved with antibodies to TNF. (*2*) In this paper we discuss some endotoxin-like reactions in vivo (changes in blood parameters and induction of hypothermia) that can be evoked by injection of TNF alone. (*3*) It was shown that TNF production was not induced in endotoxin-tolerant mice [3]. In endotoxin-tolerant mice other mediators, such as IL-1, were generated in lower amounts than in nontolerant controls, but no TNF activity could be detected. (*4*) Preliminary data by Aderka et al. [22] demonstrated that peripheral blood monocytes of patients with cancer spontaneously produced larger amounts of TNF than did cells of controls, a finding indicating that TNF may also mediate cachexia in cancer.

The induction of IL-1 activity by TNF in endothelial cells has been described by several investigators [23, 24]. Highest levels of IL-1 activity were detected when endothelial cells were exposed to TNF for >20 h. However, we did not detect any costimulator activity generated in murine or human monocyte/macrophage cultures after 24 h when the influence of endotoxin was excluded by the use of PEC from C3H/HeJ mice or by the selection of culture medium and fetal calf serum lacking IL-1-inducing activity [25]. Whether IL-1 activity in macrophages can be induced by TNF under modified experimental conditions or is induced in amounts smaller than those detectable in the costimulator assay needs to be clarified. Hybridization experiments for the detection of TNF-induced IL-1 mRNA are currently being done. From the results obtained in this investigation, it became obvious that TNF – in contrast to LPS – did not induce the production of significant titers of IL-1 in cultures of murine adherent PECs or human monocytes. Therefore, TNF seems to be directly responsible for the thermoregulatory changes measured 2–3 h after TNF application.

From the literature and from the data presented in this report, it becomes clear that the tumor-necrotizing action describes an important function of TNF. However, the name *tumor necrosis factor* is historical and may be misleading since it falls short of indicating the potential importance of this monocyte-derived mediator. Considering all the biologic activities attributed to TNF, one can rank TNF among mediators such as IL-1 and interferon-γ in its ability to modulate the immune system and affect the host organism.

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