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Tumor Necrosis Factor Mediates Endotoxic Effects in Mice

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Endotoxic reactions induced in mice by recombinant human tumor necrosis factor (TNF) were examined. Mice showed a dose-dependent hypothermia after intravenous TNF injection which was similar to a reaction to lipopolysaccharide injection. Plasma glucose levels were decreased, and plasma lactate levels were increased. Blood hematocrit levels were increased after TNF injection. No interleukin-1 activity was detected in the plasma of TNF-treated animals. The number of leukocytes was reduced 30 min after TNF injection and returned to normal within 24 h. Thus, the data demonstrate that the pathophysiological effects induced by TNF were similar to the effects induced by bacterial endotoxin. Since lipopolysaccharide is a very potent agent for eliciting TNF release from activated macrophages, these results suggest that TNF could act as an endogenous mediator of endotoxin effects.

Tumor necrosis factor (TNF) is a well-documented monocyte and macrophage product (6, 16, 22, 23, 30) that causes tumor necrosis in vivo. TNF produced by different macrophagelike tumor cells or promyelocytic cell lines has been purified (1, 15, 26, 28, 33). With the use of purified TNF, it became clear that the molecule induced a variety of physiological effects in addition to tumoricidal activity, such as suppression of lipoprotein lipase activity (5), stimulation of collagenase and prostaglandin E_2 production by synovial and dermal fibroblasts (7), stimulation of bone resorption and inhibition of bone formation (2), enhancement of blood coagulation (24), and radioprotection (R. Urbaschek, manuscript submitted). These effects are classical inflammatory reactions.

In addition, Beutler and co-workers (4) proposed the involvement of TNF in the pathological effects provoked by lipopolysaccharides (LPS). Passive immunization with polyclonal antibodies against cachectin/TNF protected mice from the lethal effects of LPS. Also, induction of endotoxic shock symptoms have been reported (3). In this study, we investigated whether TNF injected into mice could induce other effects which are typical of reactions to bacterial endotoxin. Therefore, thermoregulation and changes in plasma enzyme levels were monitored after TNF injection. Although TNF was originally defined as the endogenous mediator specifically responsible for endotoxin-induced tumor necrosis, it has not been possible to dissociate the tumor-necrotic activities of the TNF molecule from other endotoxin activities. Therefore, effects described for bacterial endotoxins may be effects of the induced TNF.

MATERIALS AND METHODS

Mice. Male C3H/He mice 5 to 7 weeks of age were obtained from the Zentralinstitut für Versuchstierkunde GmbH, Hannover, Federal Republic of Germany. C3H/HeJ mice were purchased from Bomholdgard Ltd., Ry, Denmark, or from Jackson Laboratory, Bar Harbor, Maine.

Reagents. Bacterial LPS was isolated from *Salmonella montevideo* SH94 by the phenol-chloroform-petrol ether procedure (10). Recombinant human TNF was kindly supplied by BASF AG, Ludwigshafen, Federal Republic of Germany. It had an endotoxin content of less than 1.3 ng/mg of protein.

Determination of body temperature. The rectal temperature of mice was measured just before TNF injection and at various intervals thereafter with a temperature probe (DT-10; Haake, Karlsruhe, Federal Republic of Germany).

Plasma preparation. The animals were bled via the retroorbital sinus under ether anesthesia; blood was collected into Eppendorf tubes containing 5 μl of Heparin-Natrium (5,000 U/ml; Braun, Melsungen, Federal Republic of Germany). After centrifugation, the supernatant was used for examination of plasma.

Plasma enzyme level determination. Plasma glucose and plasma L-lactate levels were determined enzymatically without previous deproteinization by using commercial kits (Gluco-Quant and Lactat-Monotest; Boehringer GmbH, Mannheim, Federal Republic of Germany).

Interleukin-1 (IL-1) assay. Single-cell suspensions of C3H/HeJ mouse thymocytes (5×10^5) were cultured in flat-bottom 96-well plates. The total volume was 0.2 ml, containing the indicated plasma samples and 50 μg of phytohemagglutinin M (Sigma Chemical Co., St. Louis, Mo.) per ml. The cultures were pulsed for 16 h with 1 μCi of [6-^{3}H]thymidine (specific activity, 50 Ci/mmol [185 GBq/mmol]; Amersham Corp., Arlington Heights, Ill.) per well after 3 days of culture in 5% CO_2 at 90% relative humidity at 37°C. The DNA was then precipitated onto glass fiber filters by using a cell harvester, and the radioactivity was measured in a liquid scintillation counter.

RESULTS

TNF was injected into mice intravenously in order to determine TNF effects in vivo. Hunched back, ruffled fur, and diarrhea were the immediately obvious symptoms after TNF injection. The body temperature of the animals decreased from normal (37°C) within 6 h after injection in a dose-dependent manner (Fig. 1). Some animals died when higher doses (more than 60 μg per animal) were given. The body temperature of surviving animals returned to normal (37°C) within 48 h (Table 1). Injection of LPS (0.4 to 8.9 mg/kg of body weight) into mice also resulted in a dose-dependent body temperature decrease with similar kinetics, whereas LPS contamination as found in the TNF prepara-

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TABLE 1. Time course of the changes in body temperature

Control or endotoxin (concn[a])	Mean temp change (°C) ± SD (no. of survivors/total no. treated) at the following time postinjection of strain[b]:							
	C3H/He				C3H/HeJ			
	3 h	6 h	24 h	48 h	3 h	6 h	24 h	48 h
PBS	−1.0 ± 0.9 (6/6)	−1.8 ± 0.1 (6/6)	−0.4 ± 0.7 (6/6)	−0.1 ± 0.7 (6/6)	−0.3 ± 1.7 (6/6)	−0.0 ± 1.7 (6/6)	−0.7 ± 1.5 (6/6)	−0.4 ± 1.6 (5/6)
TNF (4.3–4.5 mg)	−6.3 ± 1.4 (6/6)	−10.2 ± 2.9 (6/6)	−5.1 (2/6)	−4.0 (2/6)	−6.2 ± 2.7 (6/6)	−7.7 ± 4.8 (5/6)	−12.3 (1/6)	(0/6)
LPS (8.6–8.9 mg)	−3.4 ± 1.4 (6/6)	−3.0 ± 1.6 (6/6)	−1.5 ± 0.5 (6/6)	−1.7 ± 0.4 (6/6)	−0.4 ± 0.9 (6/6)	−1.3 ± 1.0 (6/6)	−1.3 ± 0.6 (5/6)	−0.6 ± 0.7 (5/6)
LPS (5.8–5.9 ng)	−1.1 ± 0.8 (6/6)	−1.7 ± 0.5 (6/6)	−0.3 ± 0.3 (6/6)	−0.4 ± 0.5 (5/6)	−1.6 ± 1.6 (6/6)	−1.3 ± 1.4 (6/6)	−1.5 ± 0.9 (5/6)	−0.5 ± 1.4 (4/6)

[a] Per kilogram of mouse body weight.
[b] The body temperature of the untreated mice was 37.4 ± 0.30°C at the beginning of the experiment.

tion (5.8 to 5.9 ng of LPS per nanogram of TNF) had no effect on the body temperature.

As expected, C3H/HeJ mice which were unresponsive to LPS (11, 31, 32) did not show hypothermic reactions after LPS injection, in contrast to C3H/He mice. However, they responded with a significant decrease in body temperature after TNF injection (Table 1). The body weights of animals of both strains dropped by about 10% within 48 h after TNF injection.

Blood and plasma parameters which are affected by LPS treatment were measured in these mice after TNF injection. C3H/He mice showed a significant increase in hematocrit levels 3 to 6 h after injection with either LPS or TNF (Fig. 2a). Plasma glucose levels dropped below 50% of normal in animals which had received either LPS or TNF (Fig. 2b). Plasma L-lactate levels were significantly increased with TNF (Fig. 2c). Similar hematocrit and plasma glucose levels were obtained with TNF in non-LPS-responsive C3H/HeJ mice (Fig. 2d and e, respectively), but these animals did not respond to the same amount of LPS as was given to C3H/He animals. Plasma L-lactate levels in the non-LPS-responsive mice were not affected by the injected dose of TNF (reduced dose in comparison to the dose given to C3H/He mice) (Fig. 2f).

Bacterial endotoxins cause a reduction in the leukocyte number and an increase in the erythrocyte number (9). The peripheral blood was tested for changes in cell composition after TNF injection. The number of peripheral blood leukocytes in C3H/HeJ mice dropped to about 50% of normal within 1 h after intravenous injection with 40 μg of TNF (1.69 ± 0.07 mg/kg of body weight) and returned to normal values within 24 h (Table 2). The number of erythrocytes and the hematocrit levels increased within 1 h after TNF injection and also returned to normal within 24 h (Table 2).

Since IL-1 could be responsible for the hypothermic reaction after injection of LPS or TNF into mice (18, 27), plasma from hypothermic mice was tested for IL-1 activity. Plasma from C3H/He mice was tested for IL-1 activity 30 min and 6 h after intravenous injection of LPS, TNF, or phosphate-buffered saline. No IL-1 activity was detected after TNF or phosphate-buffered saline injection. At 30 min after LPS injection, no IL-1 activity was measured; however, significant IL-1 activity was measured in the plasma 6 h after injection (data not shown).

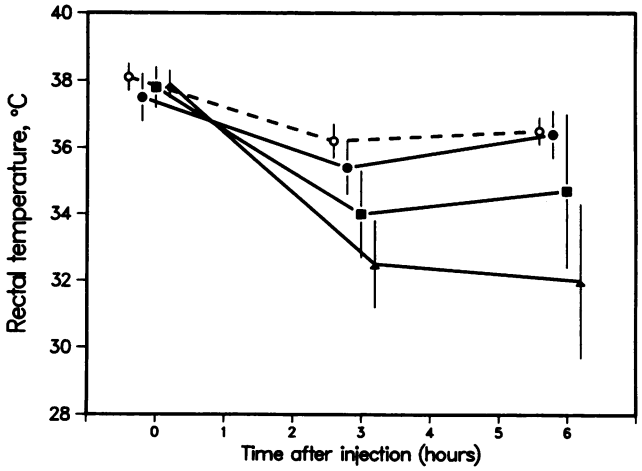

FIG. 1. Body temperature of C3H/He mice before and at different times after intravenous injection with phosphate-buffered saline (○) or various doses of TNF (10 μg per animal, equivalent to 0.35 mg/kg of body weight [●]; 50 μg per animal, equivalent to 1.6 mg/kg of body weight [■]; and 100 μg per animal, equivalent to 3.2 mg/kg of body weight [▲]). Data are expressed as the means ± standard deviations of groups of five to seven mice.

DISCUSSION

LPS has been found to induce the release of TNF into the serum of mice with an activated mononuclear phagocyte system (6, 22). Therefore, it was interesting to determine whether TNF had physiological effects similar to those of LPS and whether TNF could serve as an endogenous mediator of LPS. To answer this question, we compared the effects of TNF and LPS on hypothermic response and on changes in blood parameters, such as plasma D-glucose, plasma L-lactate, IL-1 activity, hematocrit, and leukocytes. The thermoregulatory effect of LPS in mice (13, 14, 27) could be mimicked with TNF (Fig. 1). To eliminate the possibility that endotoxin contamination of the TNF preparation was responsible for this hypothermia, non-LPS-responsive animals were used for these experiments. Endotoxin concentrations as found in the TNF preparation also had no effect on the body temperature. Thus, the hypothermia induced by TNF injection was not due to LPS but was an effect of TNF itself.

TNF has been shown to exert effects similar to those of IL-1. Prostaglandin E_2 production (7), tumor cytocidal activity (19, 21, 25), bone resorption (2, 12, 17), and induction of hypothermia in rats (18) are a few examples of effects which are elicited by both mediators. In addition, TNF has been shown to be an endogenous pyrogen in rabbits (8). To

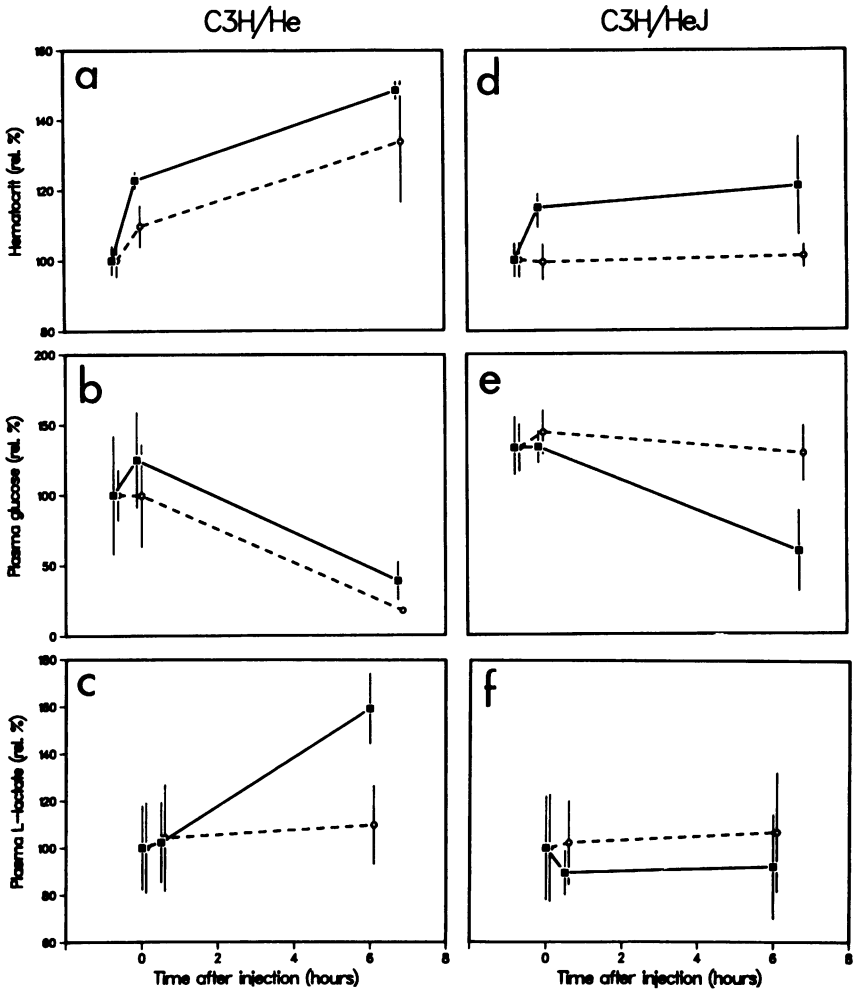

FIG. 2. Time course of changes in hematocrit, plasma D-glucose levels, and plasma L-lactate levels after intravenous injections of LPS or TNF into LPS-responsive mice (strain C3H/He, panels a to c) or into non-LPS-responsive mice (strain C3H/HeJ, panels d to f). The data are presented as the ratio of treated animals to control animals (mean percent ± standard deviation). Control animals were injected with phosphate-buffered saline (100 μl). Groups of five to seven mice were bled at different times after injection with LPS (○) (9.4 to 10.4 mg/kg of body weight in both strains) or TNF (■) (3.0 mg/kg of body weight in C3H/He mice; 2.4 mg/kg of body weight in C3H/HeJ mice).

clarify whether the hypothermic reaction occurring after TNF injection was mediated via IL-1 production, we measured the IL-1 activity in the plasma of both LPS- and TNF-treated mice. IL-1 activity could be demonstrated in the plasma of LPS-treated mice concomitant with the hypothermia induced by LPS. However, no IL-1 was detected in plasma of mice injected with TNF. Therefore, it seems unlikely that IL-1 causes TNF-induced hypothermia.

TABLE 2. Time course of changes in blood parameters after intravenous injection of TNF into C3H/HeJ mice[a]

Time (h) postinjection	Body temp (°C)	No. of leukocytes/μl	No. of erythrocytes (10^6/μl)	Hematocrit (%)
0	36.5 ± 0.4	5,830 ± 700	9.66 ± 1.41	44.0 ± 0.0
0.5	35.3 ± 0.2	2,690 ± 670	9.78 ± 0.27	51.3 ± 0.5
1	34.1 ± 0.5	2,600 ± 290	10.41 ± 1.79	53.3 ± 1.1
6	33.1 ± 2.4	4,300 ± 1,690	10.03 ± 0.98	50.6 ± 5.5
24	36.6 ± 0.1	4,580 ± 510	9.49 ± 0.73	47.6 ± 1.5

[a] Values are expressed as means ± standard deviations of groups of four animals (TNF dose, 40 μg/100 μl; 1.69 ± 0.07 mg/kg of body weight).

LPS or TNF was injected into non-LPS-responsive mice, as well as into LPS-responsive mice, in order to determine further TNF-specific effects. Changes in hematocrit and in plasma glucose levels were similar after injection of LPS and TNF into LPS-responsive mice and LPS-nonresponsive mice, respectively (Fig. 2). In conclusion, changes in blood and plasma parameters which are typical effects of bacterial endotoxin (9, 20, 29) were observed after TNF injection: hematocrit levels were significantly increased, plasma glucose levels were decreased, and plasma lactate levels were increased (Fig. 2). Such an increase in plasma lactate levels was observed only when large amounts of TNF were injected into the animals, a treatment which led to symptoms of shock. Leucopenia, which is a typical effect of LPS administration (9) or sepsis, was also observed after injection of TNF into C3H/HeJ mice (Table 2).

In summary, these data demonstrate that the pathophysiological effects of intravenous injection of TNF into mice could not be distinguished from those produced by LPS. The endotoxic effects occurring after injection of TNF may indicate that some of the observed effects of LPS may be mediated via the subsequent production of TNF. This may

be the general physiologic pathway of bacterial endotoxin effects. It remains to be studied whether TNF can be used safely in therapy by eliminating the endotoxic side effects of the molecule.

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ADDENDUM

The conclusion that cachectin/TNF is capable of mediating many of the deleterious effects of endotoxin has also been drawn recently from data obtained in a rat model (K. J. Tracey, B. Beutler, S. F. Lowry, J. Merryweather, S. Wolpe, I. W. Milsark, R. J. Harriri, T. J. Fahey III, A. Zantella, J. D. Albert, G. Tom Shires, and A. Cerami, Science **234**:470–474, 1986).

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