Brief Definitive Report

ADRENALECTOMY SENSITIZES MICE TO THE LETHAL EFFECTS OF INTERLEUKIN 1 AND TUMOR NECROSIS FACTOR

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The protective effect of corticosteroids against the lethal effect of high doses of bacterial endotoxins (LPS) has been known since 1954 (1–3), and it is also acknowledged that adrenalectomized animals are more sensitive to the lethal effect of LPS (3, 4). In this respect, the protective effect of corticosteroids could be exerted at many sites. It has been suggested that inhibition of the activity of the reticuloendothelial system by corticosteroids might be important in their pharmacological effect (5). Recent studies have indicated that most of the in vivo effects of LPS are probably mediated by LPS-induced monocyte products, the attention having been focused on two of them in particular, IL-1 and TNF. IL-1 was originally described as the LPS-induced leukocytic pyrogen (6), and TNF as the LPS-induced mediator responsible for the antitumor and toxic (7) effects of LPS. Tracy et al. (8) recently suggested that TNF might have an important role in LPS-induced shock, and showed that rTNF can induce shock in rats, while passive immunization against TNF protected against the lethal effect of LPS (9). More recently, rIL-1 was also reported to induce hemodynamic shock in rabbits (10). The finding that corticosteroids are potent inhibitors of the synthesis of IL-1 and TNF in macrophages (11–13) has cast fresh light on the mechanisms underlying the protective effect of corticosteroids against LPS, suggesting that inhibition of synthesis of these toxic mediators might be important in the anti-LPS properties of corticosteroids. In addition to this general inhibitory effect of corticosteroids on monokine synthesis, other interregulatory relationships between corticosteroids and monokines were reported. For instance, IL-1 was shown to influence glucocorticoid-regulated metabolism (14), to induce release of corticosteroids in plasma by stimulating the release of adrenocorticotrophic hormone (15), and to potentiate the synthesis of some acute-phase proteins (16). Therefore, we have investigated the effect of adrenalectomy on the susceptibility to the lethal effects of LPS, TNF, and IL-1.

Materials and Methods

Male, adult (25–30 g) CD-1 mice (Charles River Breeding Laboratories, Calco, Italy) were used. Adrenalectomy was performed under ether anesthesia 10 d before the experiment. Adrenalectomized mice were given 1% (wt/vol) sodium chloride in drinking water.

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Animals were housed five per cage in air-conditioned quarters (60% relative humidity, 22°C) with a 12-h light/dark cycle, and were given standard laboratory chow (Altromin, Rieper, Bolzano, Italy). The endotoxin used was *Escherichia coli* LPS, serotype 055:B5. (Sigma Chemical Co., St. Louis, MO).

Human rIL-1α was a kind gift from Dr. Peter T. Lomedico, Hoffmann-La Roche, Inc., Nutley, NJ. Human rTNF-α was a kind gift from Dr. Leo S. Lin, Cetus Corp., Emeryville, CA. Murine rTNF (17) was prepared as previously described (18) and obtained through the courtesy of Dr. J. Tavernier, Biogent, Belgium. Dexamethasone phosphate (Soldesam) was from Farmacologico Milanese s.p.a., Milano, Italy. Ibuprofen (Artrene) was from IRBI, Pomezia, Roma, Italy.

Results

Table I shows the lethality of LPS, human and murine TNF, and IL-1 in adrenalectomized mice. No mortality was observed when the same doses of LPS, TNF, or IL-1 were administered to control or sham-operated mice (data not shown), or when adrenalectomized mice were given heat-inactivated (90°C, 30 min) TNF or IL-1 (data not shown), indicating that lethality was not due to endotoxin contamination of the genetically engineered proteins. It can be noted that TNF (both human and murine) proved to be consistently less toxic, on a weight basis, than IL-1 or LPS.

Complete protection against the lethal effect of LPS, TNF, or IL-1 was achieved when dexamethasone (DEX) was administered at the dose of 30 mg/Kg, intraperitoneally, 30 min before LPS, TNF, or IL-1 (Table II). In an attempt to define whether a high dose of DEX was required for the protective effect, we gave adrenalectomized mice different doses (30, 3, 0.3, and 0.03 mg/Kg) 30 min before injection of 75 μg/Kg of LPS. The lowest effective dose that completely prevented the lethal effect of LPS was 0.3 mg/Kg (data not shown). No protection was observed when adrenalectomized mice were pretreated with the

TABLE I
Lethality of LPS, TNF, and IL-1 in Adrenalectomized Mice

Treatment	Dose (i.v.)	Mortality* (%)
	μg/Kg	
LPS	75	10/10 (100)
	30	10/10 (100)
	3	1/5 (20)
	0.3	0/5 (0)
Human TNF	75	6/10 (60)
	30	5/19 (26)
	7.5	0/10 (0)
Murine TNF	150	6/10 (60)
	75	2/12 (17)
IL-1	30	7/10 (70)
	7.5	3/5 (60)
	3.0	0/5 (0)

* Number dead/number treated; all deaths occurred within 24 h. Mice were monitored for 1 wk before terminating the experiment.

TABLE II

Protective Effect of Dexamethasone Against the Lethal Effect of LPS, TNF, or IL-1 in Adrenalectomized Mice

Treatment‡	Mortality*	
	Without DEX (%)	With DEX§ (%)
LPS	5/5 (100)	0/5 (0)
Human TNF	2/5 (40)	0/5 (0)
IL-1	4/5 (80)	0/5 (0)

* Number dead/number treated.
‡ × 30 μg/Kg i.v.
§ DEX was given at the dose of 30 mg/Kg i.p., 30 min before treatment.

cyclooxygenase inhibitor ibuprofen (30 mg/Kg, i.p., data not shown), which was previously reported to protect against the lethality of high-dose LPS (20 mg/Kg) in normal (nonadrenalectomized) rats and against IL-1-induced hemodynamic shock, suggesting that ibuprofen is not as potent as DEX.

Discussion

While the increased sensitivity of adrenalectomized animals to the lethal effect of LPS has already been described, this is the first report on the sensitizing effect of adrenalectomy on the lethality of rTNF or rIL-1 at doses that are normally well tolerated in control or sham-operated mice. This extends the list of the effects of LPS that can be mimicked by TNF or IL-1. With respect to the lower toxicity of TNF (both human and murine), when compared with IL-1 or LPS, in adrenalectomized mice, it is important to remember that in judging the relative "potency" of a protein in an in vivo system, many factors might influence the results, including pharmacokinetics and disposition of the protein administered.

The protective effect of DEX in this experimental model is not surprising in view of previous data on LPS toxicity. On the other hand, the fact that adrenalectomy increases the toxicity of IL-1, not only of TNF, clearly indicates that the protective effect of corticosteroids on endotoxic shock cannot be explained merely by their inhibition of LPS induction of these monokines usually considered as the mediators of LPS toxicity. In fact, while corticosteroids antagonize the toxic effect of LPS and the synthesis of LPS-induced monokines, they potentiate the synthesis of many acute-phase proteins (16, 19), and this might be important in their pharmacological action, in view of the fact that most of the acute-phase proteins induced by LPS have detoxifying and anti-LPS (or anti-shock) activities. For instance, angiotensinogen, which is important in the maintenance of blood pressure and electrolyte homeostasis, was reported to be increased in vivo, after LPS and glucocorticoids, to be required for its induction, not observed in adrenalectomized mice (19). Therefore, the protective effect of dexamethasone, and the increased sensitivity of adrenalectomized mice on IL-1 and TNF toxicity might be due either to a direct hemodynamic effect

of glucocorticoids or to their "permissive" action on the induction of protective acute-phase proteins.

While it was known that high-dose TNF can be lethal, to our knowledge, no lethality has ever been reported with IL-1, and our finding in adrenalectomized mice supports the report by Okusawa et al. (10) that IL-1 induces hemodinamic shock and that, therefore, IL-1, not only TNF, might be important in the lethality of LPS.

Summary

To clarify the possible role of TNF and IL-1 in endotoxic shock, the lethality of rTNF (human and murine) and IL-1 in adrenalectomized mice was studied. Adrenalectomy, which has long been known to increase the susceptibility to endotoxin, rendered mice susceptible to TNF and IL-1 in terms of mortality. The lethality of endotoxin, TNF, or IL-1 was totally prevented by pretreatment with dexamethasone (minimal effective dose, 0.3 mg/Kg) but not by ibuprofen (10 mg/Kg).

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