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RECOMBINANT TUMOR NECROSIS FACTOR DEPRESSES CYTOCHROME P450-DEPENDENT MICROSOMAL DRUG METABOLISM IN MICE

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Summary - The effect of recombinant tumor necrosis factor on liver cytochrome P450 and related drug metabolism enzymes was investigated. Treatment of mice with tumor necrosis factor caused a marked depression of cytochrome P450 and some drug metabolizing enzymes (ethoxycoumarin deethylase and arylhydrocarbon hydroxylase) in the liver and many other organs. This effect was maximal 24-48 h after treatment and was dependent on the dose of tumor necrosis factor administered. Depression of liver drug metabolizing enzymes was also observed in the endotoxin-resistant C3H/HeJ strain of mice, thus ruling out that this effect may be due to minor endotoxin contamination of recombinant tumor necrosis factor. These data indicate that depression of liver drug metabolism might be an important side effect of tumor necrosis factor, and suggest a role for this macrophage product as an endogenous regulator of liver metabolism.

In 1975, Carswell and colleagues found that serum of Bacillus Calmette-Guérin-infected mice injected with endotoxin (lipopolysaccahride, LPS) contained a factor (tumor necrosis factor, TNF) which caused hemorragic necrosis of Meth A sarcoma in vivo and was cytotoxic for mouse L929 cells in vitro (1). This factor was found to be a macrophage product (2,3). Recently, different groups reported the molecular cloning of TNF and its expression in Escherichia coli (4-6), thus providing substantial amounts of pure recombinant TNF for the study of its biological activites and for clinical studies (for a review, see ref. 7). Besides its tumor necrotizing and cytotoxic properties, TNF might also have other physiological functions. In fact, it has been found to be strongly homologous and probably identical to cachectin, a macrophage product that inhibits lipoprotein lipase activity in adipocytes and was suggested to be responsible for hypertriglyceridemia and weight loss observed during infection (8). Passive immunization against TNF/cachectin has been shown to protect against the lethal effect of LPS (9). We recently observed

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that another LPS-induced macrophage product, interleukin-1 (IL-1), as well as LPS, depresses liver cytochrome P450 dependent drug metabolizing enzymes, a metabolic system involved in the metabolism and elimination of foreign compounds (10); depression of liver drug metabolism by interferon (IFN) is also reported (11). In the present paper we report that recombinant TNF depresses liver drug metabolism in mice.

MATERIALS AND METHODS

TNF - Recombinant human TNF (lot no. LYM12-101485B) was a kind gift from the Cetus Corporation, Emeryville, California. Specific activity was 10^7U/mg (by cytotoxicity on L929 cells, ref. 5). Endotoxin was <66 ng/mg (by Limulus Amebocyte Lysate assay). TNF was supplied in human serum albumin (0.5 mg TNF/10 mg albumin) and diluted in sterile pyrogen-free saline. Human serum albumin provided by Cetus Corp. (control lot. no. LYM12-101485D) was diluted the same way and used as treatment control.

Animals and treatments - Male CD1 mice of 20-25 g (Charles River Italia, Calco, Italy) were used. In some experiments, LPS-resistant C3H/HeJ mice (Jackson, Bar Harbor, Maine) were used. When indicated, CD1 mice were injected intramuscolarly with $2x10^5$ sarcoma (S 180) cells in the right paw, and were used 10-15 days later.
TNF was given in 0.2 ml of saline either intravenously (i.v.) or in the tumor site (i.t.). Mice were killed by cervical dislocation 24 h after treatment, except for time-course experiments. When microsomes were to be prepared, mice were fasted overnight before killing. Organs were quickly removed and homogenized in 4 volumes of ice-cold, 0.05 M Na-phosphate buffer, pH 7.4. Microsomes were prepared according to Kato and Takayanaghi (12).

Biochemical determinations - Ethoxycoumarin deethylase activity (ED) was measured according to Greenlee and Poland (13). Cytochrome P450 was measured according to Omura and Sato (14); Arylhydrocarbon hydroxylase (AHH) was measured as previosly described (15). Proteins were measured as previously described (16).

RESULTS

The activity of a cytochrome P450-dependent enzyme, ED, in the liver, was measured at various times after treatment with TNF ($5x10^4$ U/mouse, i.v.). Figure 1 shows that TNFcaused a marked depression of this activity, with a peak between 8 and 24 h (-50 %). A dose-response experiment is reported in figure 2. This depression of liver drug metabolism was not confined to ED activity. In fact, as shown in Table 1, it was associated with a depression of liver cytochrome P450 levels and AHH activity. TNF at this dose also reduced total microsomal proteins but had no effect on total liver proteins or liver weight. Depression of drug metabolizing enzymes was not confined

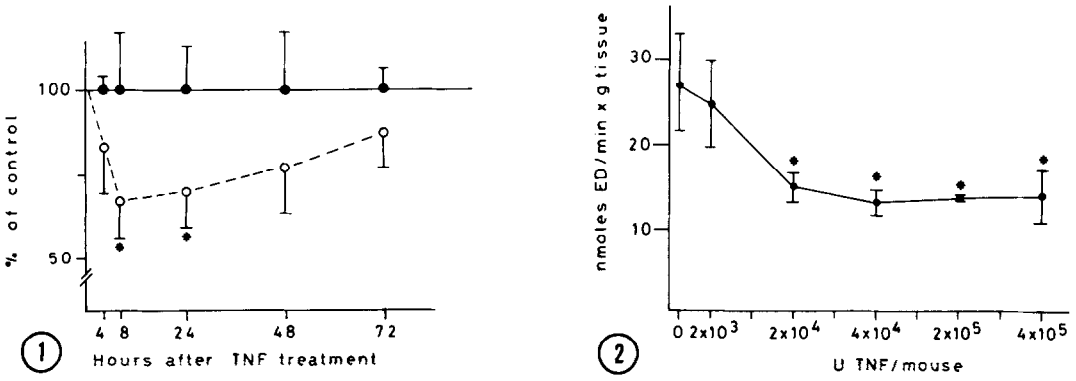

Figure 1 - Time course of liver ethoxyxoumarin deethylase (ED) activity in mice after a single injection of TNF (5×10^4 U/mouse, i.v.). Data are reported as percent of control (mean ± S.E., 4 mice per point).
Closed symbols, control; open symbols, TNF. * $p<0.05$ versus control by Student's t test.

Figure 2 - Dose-response effect of TNF on liver ED 24 h after treatment. Data are mean ± SE (4 mice per point).
* $p<0.05$ versus control by Student's t test.

to the liver but was observed in most of the organs investigated (Table 2). Since even minor LPS contamination of peptides produced by genetic engineering can cause some aspecific effects (17), we studied the effect of TNF on LPS-resistant C3H/HeJ mice; our results were reproduced in this exper-

TABLE 1

Effect of TNF on various liver enzymes

ENZYMES	CONTROL	TNF
ED (nmoles/min x g tissue)	32.49 ± 0.87	23.08 ± 0.12*
AHH (nmoles/min x g tissue)	1.61 ± 0.14	1.11 ± 0.08*
P450[a] (nmoles/mg proteins)	1.32 ± 0.09	0.89 ± 0.12*
Microsomal proteins[b] (mg/ml)	21.59 ± 0.75	17.84 ± 0.89*
Homogenate proteins[b] (mg/ml)	99.17 ± 0.32	100.18 ± 0.42

TNF was administered at the dose of 5×10^4 U/mouse, i.v., 24 h before killing. Data are mean ± S.E. (4-5 mice per group).
*$p<0.05$ versus control by Student's t test.
[a] measured on purified microsomal fractions.
[b] volume of homogenates was adjusted to 5 ml per g of liver and microsomal suspension to 2 ml per g of liver.
Data are mean ± S.E. (9 mice per group).

TABLE 2

Effect of TNF on ethoxycoumarin deethylase (ED) in various organs

ORGAN	ED (nmoles/min x g)	
	CONTROL	TNF
Liver	27.98 ± 0.98	15.34 ± 1.16 (-46%)*
Heart	0.15 ± 0.03	0.12 ± 0.01 (-12%)
Intestine	0.94 ± 0.09	0.62 ± 0.05 (-34%)*
Kidney	2.58 ± 0.23	1.77 ± 0.16 (-31%)*
Lung	1.87 ± 0.25	1.16 ± 0.16 (-38%)*
Adrenal	0.30 ± 0.01	0.10 ± 0.01 (-67%)*
Spleen	0.14 ± 0.01	0.11 ± 0.02 (-23%)

TNF was administered at the dose of 5 x 10^4 U/mouse, i.v., 24 h before killing.
Data are mean ± S.E. from 4-5 mice. In parentheses, percentage reduction compared to control.
* $p<0.05$ by Student's t test.

imental model (data not shown). Since TNF was also reported to be active even when injected directly in the tumor, we have investigated the effect of TNF in tumor (S 180)-bearing mice. This tumor was chosen because it is TNF-sensitive (18). Table 3 shows that liver drug metabolism was also depressed when TNF was injected into the tumor.

TABLE 3

Depression of liver ethoxycoumarin deethylase (ED) by TNF in normal and tumor-bearing mice after systemic (i.v.) and intratumor (i.t.) administration

EXPERIMENTAL GROUP	ED (nmoles/min x g liver)	
	CONTROLS	TNF
Normal mice, TNF i.v.	34.93 ± 2.22	25.03 ± 1.14 (-29%)*
Tumor-bearing mice, TNF i.v.	25.01 ± 1.17	12.64 ± 1.39 (-48%)*
Tumor-bearing mice, TNF i.t.	25.01 ± 1.17	12.64 ± 0.64 (-48%)*

TNF was administered at the dose of 5 x 10^4 U/mouse, i.v., 24 h before killing.
Data are mean ± S.E. from 4-5 mice. In parentheses, percentage reduction compared to control.
* $p<0.05$ by Student's t test.

DISCUSSION

We have shown that TNF depressed the liver microsomal drug metabolizing system in mice. This has obvious relevance as a possible side effect of TNF therapy, and should be kept in mind when TNF is administered in association with other drugs. In fact, depression of cytochrome P450 might result either in diminished ability to eliminate a drug (therefore leading to increased toxicity) or in reduced efficacy of drugs that require cytochrome P450 dependent metabolic activation (e.g. cyclophosphamide). On the other hand, the finding that TNF and cachectin are the same (8) suggests that TNF might be one of the LPS-induced mediators responsible for some of the *in vivo* effects of LPS (19). In our particular case TNF, together with IFN and IL-1, might contribute to the regulation of the liver drug metabolism by the immune system, thus augmenting the list of non-immunological effects of these mediators. Interestinglv enough, receptors for recombinant TNF were also reported in some normal cells (20), and receptors for cachectin/TNF were reported in cells other than adipocytes, including liver cells (21).

The possible mechanism(s) of this effect of TNF on liver drug metabolism remain to be elucidated, and further studies will be necessary to determine whether the loss of cytochrome P450 is due to increased degradation or decreased synthesis. In this respect, it is important to note that TNF also reduces the protein content of liver microsomes, although this effect alone could not account for the lowering of cytochrome P450 levels. A second point is whether this TNF's effect is direct or involves some other mediator. For instance, TNF was reported to induce IL-1 in human monocytes (22); since IL-1 was reported to depress liver drug metabolism (10), it may, to some extent at least, mediate this effect of TNF. Preliminary experiments from this laboratory show that, while TNF markedly depresses liver drug metabolism *in vivo*, it is not active on isolated rat hepatocytes *in vitro*, thus suggesting that induction of a second mediator might be required. Also, administration of TNF caused an increase in plasma fibrinogen, an effect that is considered an *in vivo* marker for IL-1 activity (23). Studies are in progress to determine the role of IL-1 in the depression of liver drug metabolism by TNF, and the biochemical mechanism of this effect.

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