DOSE-DEPENDENT INCREASE IN TUMOR NECROSIS FACTOR-ALPHA PRODUCTION IN TCDD EXPOSED MICE IS AH RECEPTOR DEPENDENT

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ABSTRACT: Tumor necrosis factor-alpha (TNF-α), originally termed cachectin, is an immune system hormone and endogenous pyrogen produced by macrophages in response to endotoxin. 2,3,7,8,-Tetrachlorodibenzo-*p*-dioxin (TCDD) treatment of C57BL/6J (Ahbb) mice resulted in a dose-related increase in TNF-α released into serum following endotoxin exposure, with a significant increase being observed at a dose of 10 µg/kg TCDD. At a dose of 500 µg/kg TCDD Ahbb mice demonstrated a 46-fold increase over the control response. In contrast, a congenic Ah receptor deficient mouse (Ahdd), did not show a significant increase in TNF-α production until exposed to 150 µg/kg TCDD and the maximum response was an 8-fold increase over control . The pyrogenic response to endotoxin increased to a greater extent in TCDD-treated Ahbb mice. These data suggest that the Ah receptor mediates TCDD-induced increases in the production of TNF-α following endotoxin exposure, and that this increase may be responsible for endotoxin hypersensitivity in TCDD- treated animals.

INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is one of the most toxic chemicals known with an LD_{50} of 0.6 µg/kg in the guinea pig (1). The toxic and biochemical responses to TCDD are mediated through a cytoplasmic binding protein, the Ah receptor (2,3). C57BL/6J mice congenic at the Ah locus have been bred (Ahbb, TCDD-responsive; Ahdd, TCDD-nonresponsive) and these mice demonstrate differential sensitivities to the toxic effects of TCDD (4,5). The most common characteristic of TCDD toxicity is a "wasting syndrome" in which weight loss occurs due to a reduction in feed intake and depletion of adipose stores (6). Tumor necrosis factor-alpha (TNF-α), originally known as cachectin, induces a similar pathology. This polypeptide is produced by macrophages in response to endotoxin and is the principal mediator of endotoxin toxicity (7). TCDD treatment increases endotoxin toxicity in TCDD-responsive mice (8,9,10). Based upon the similar pathologies of TCDD and TNF-α, and the importance of TNF-α in the manifestation of endotoxin toxicity, we investigated the effects of TCDD on TNF-α production in Ahbb and Ahdd mice.

METHODS

Congenic C57BL/6J mice (Ahbb and Ahdd, 5 mice/dose group) were treated p.o. with the indicated concentrations of TCDD , and 5 d after exposure injected ip with 313 µg/kg of endotoxin. Ninety minutes after endotoxin exposure, the time corresponding to peak serum TNF-α, the animals were bled and serum

frozen until assay. Serum samples were assayed for TNF-α activity by the standard L929 cytotoxicity assay with one unit of activity resulting in 50% lysis of the cells (11).

RESULTS AND DISCUSSION

Table 1 includes results from two experiments. Experiment one assessed TCDD dose levels ranging from 1-30 μg/kg and in experiment two, dose levels of 150 and 500 μg/kg were evaluated. Serum TNF-α levels are presented as units/ml and percent of control.

Table 1. TNF-α in serum of TCDD-treated mice after exposure to endotoxin.

	Ahbb mice		Ahdd mice	
DoseTCDD (μg/kg)	TNF-α (units/ml)	% cont	TNF-α (units/ml)	% cont
Exp.1				
control	1400 ± 120	-	570 ± 140	-
1	1430 ± 120	102	240 ± 160	52
3	1590 ± 310	114	690 ± 70	152
10	3840 ± 590	274	850 ± 250	188
30	4310 ±1140	308	80 ± 40	17
Exp. 2				
control	314 ±143	-	453 ± 120	-
150	3177 ± 747	1012	4915 ± 1425	862
500	14658 ±2451	4668	2269 ± 632	398

Values represent mean ± S.E.M.

In TCDD-responsive mice there was a dose-related increase in serum TNF-α levels following endotoxin injection. This is in contrast to TCDD-nonresponsive mice, which did not respond in a dose-related manner. Treatment with 500 μg/kg TCDD resulted in 4668 and 398% increases of serum TNF-α in TCDD-responsive and nonresponsive mice, respectively. It is known that TCDD increases endotoxin sensitivity, and that this effect is Ah related (8-10), though the mechanism of this response has not been described. TNF-α is a principal mediator of endotoxin toxicity, and is itself a toxic cytokine, with a reported LD_{50} of 0.6 mg/kg in rats (7). When TCDD-responsive mice were treated with 500 μg/kg TCDD, serum TNF-α levels increased to approximately 0.7 mg/kg, which, based upon a reported LD_{50} of 0.6 mg/kg in rats (7), is a

toxic level. We have determined that alteration of endotoxin disposition does not account for the observed changes in serum TNF-α levels in TCDD exposed animals. Also TNF-α elimination from the serum is not affected by TCDD treatment (data not shown).

TNF-α is one of several endogenous pyrogens, produced in response to the exogenous pyrogen endotoxin. If mice are maintained at room temperature, exposure to endotoxin results in decreased body temperature. The body temperatures of mice were not affected by TCDD treatment alone. Endotoxin treatment (313 μg/kg, ip) of both Ahbb and Ahdd mice decreased body temperature (Fig. 1). The onset of the pyrogenic response to endotoxin, in both control mice, occurred between 2 and 3 h after endotoxin treatment. In TCDD-pretreated mice, the time-to-response interval following endotoxin treatment decreased for both Ahbb and Ahdd mice to 1 hour. The pyrogenic response to endotoxin in TCDD-treated Ahbb mice was greater then that observed in the vehicle-treated, LPS-injected control at 3 h. This did not occur for Ahdd mice, as there were essentially no differences in the maximum responses to endotoxin in either vehicle or TCDD-treated mice.

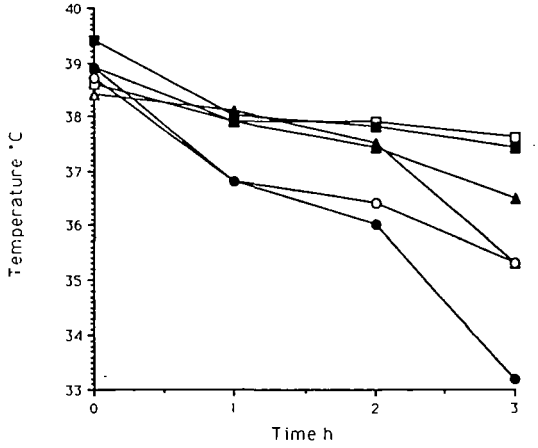

Figure 1. Ahbb and Ahdd mice were exposed to 50 μg/kg TCDD by oral gavage and 7 days later injected ip with 313 μg/kg of endotoxin (E. coli LPS 055:B5) and rectal temperatures recorded. Data shown are the means of 5 individual animals per treatment group. Treatments are vehicle treated Ahbb without endotoxin (■); vehicle treated Ahdd without endotoxin (□); vehicle treated Ahbb with endotoxin (▲); vehicle treated Ahdd with endotoxin (△); TCDD treated Ahbb with endotoxin (●); and TCDD treated Ahdd with endotoxin (○). TCDD treated Ahbb and Ahdd mice that did not receive endotoxin responded similarly to vehicle-treated, endotoxin unexposed controls (data not shown).

In conclusion, these data suggest that exposure to TCDD increases the production of TNF-α following endotoxin exposure, and that this is an Ah receptor mediated effect. In current studies, we are attempting to understand the mechanistic relationship between acute toxicity of TCDD and elevated TNF-α concentrations.

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