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EFFECT OF ACUTE EXPOSURE TO 2,3,7,8-TETRA-CHLORODIBENZO-p-DIOXIN (TCDD) ON HUMORAL ANTIBODY PRODUCTION IN MICE*

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IN PRESS

S U M M A R Y

The effect of single dose of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD, 1.2, 6 or 30 µg/kg i.p.) on primary humoral antibody production was studied in young adult C57Bl/6J mice. TCDD profoundly suppressed the primary response to thymus-dependent (sheep erythrocytes) and independent (Type III pneumococcal polysaccharide) antigens. The inhibitory effect of TCDD was still detectable 42 days after treatment. In contrast, under these experimental conditions, in vitro lymphoproliferative responses to Concanavallin A and bacterial lypopolysaccharides and the ability to mediate graft versus host reaction were not significant affected per unit number of lymphoid cells.

INTRODUCTION

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is an extremely toxic compound found as a contaminant of some chlorinated phenols used as herbicides.

Previous work (1) on the lymphoid system of rats, mice, and guinea pigs revealed that TCDD causes profound atrophy of the thymus and thymus-dependent areas of lymphoid organs. Suppression of cell-mediated immunity and increased susceptibility to bacterial infections have also been described (1,2,3). The most dramatic immunosuppressive effects were seen after animals were exposed to TCDD in utero , or in young adults animals after chronic treatment.

Although the effect of TCDD on humoral antibody production has not been adequately tested, it has been generally assumed that the chemical does not affect humoral responses (1,4,5).

The present study, prompted by the pollution with TCDD of Seveso, a densely populated area near Milan (6,7) was made to evaluate the effects of single doses of TCDD on primary humoral antibody production against T-dependent and T-independent antigens in young adult mice, and on cell-mediated reactivities.

MATERIALS AND METHODS

Mice - C57Bl/6 and (C57Bl/6 X DBA/2)F_1 male mice obtained from Charles River, Calco, Italy, were used at 6-8 weeks of age.

TCDD - TCDD (1.2 , 6 and 30 μg/kg) obtained from Kor Isotopes, Cambridge, Mass, was given as a single i.p. injection in a volume of 0.25 ml of acetone corn-oil (1:6 v:v). Control mice received the vehicle alone. The TCDD doses used were well toletared by the animals (5,8,9).

G.V.H. assay - Graft versus host (G.V.H.) reaction was assayed by the popliteal lymph node weight gain assay (10). 5 X 10^6 splenocytes from C57Bl/6 treated mice were injected s.c. into the right hind foot-pad of (C57Bl/6 X DBA/2)F_1 mice. An equal number of formol inactivated cells was injected controlaterally. Seven days later both popliteal lymph nodes were removed and weighed. The ratio of the weight of the right to the left lymphnode (lymphnodal index L.I.) was taken as a measure of GVH reaction. Four mice per group were tested individually, injecting the splenocytes in 4 recipients mice per donor animal.

Mitogen stimulation - The splenocyte response to Concanavallin A (Con A) (Calbiochem., San Diego, California, USA) and E. coli lipopolysaccharide B (LPS, Difco Laboratories, Detroit, Mich., USA) was evaluated essentially by the method described by Kirchner (11). Briefly, $5 \cdot 10^5$ splenocytes were cultured in triplicate in RPMI 1640-10% FCS (Gibco, Bio Cult, Glasgow, Scotland) with graded mitogen doses in 0.2 ml microtiter plates (No. 76-013-05 Linbro Scientific, Hamden, Conn. USA) and incubated at 37°C in a humidified atmosphere with 5% CO_2. Forty-eight hours later 1 μCi ^{3}H-thymidine (5Ci/mmole, Amersham Radiochemical Center, Amersham, England) was added to each sample and cultures were incubated for a further 16 h. Samples were collected on a filter with an automatic harvester (Titertek, Skatron, Norway) and radioactivity was counted in a liquid scintillation spectrometer. Four mice per experimental group were inidividually tested.

Response to sheep erythrocytes and type III pneumococcal polysaccharide - Mice (6 per experimental group) were injected i.p. with 4×10^8 sheep erythrocytes (SRBC) 7, 14, 21 or 42 days after TCDD treatment and spleen hemolytic plaque forming cells (PFC) were counted by the technique of Jerne and Nordin (12) 5 days later. For type III pneumococcal polysaccharide (SIII, a gift from Dr. P.J. Baker, N.I.A.I.D., Bethesda, Md), the optimally immunizing dose of 0.5 μg was injected i.p. 18 days after TCDD to 7 mice per experimental group and specific anti-SIII PFC were measured 5 days later as described elsewhere (13).

Statistical analysis - Results are presented as mean ± S.E. of 4-7 mice per experimental group. Statistical significance was evaluated by Dunnett's test.

R E S U L T S

Table 1 shows the effect of TCDD on primary humoral antibody production assessed by injecting the antigen (SRBC) 7 to 42 days after treatment. Exposure to TCDD resulted in a marked, dose-dependent reduction of the PFC count both per unit number of lymphoid cells (PFC/10^6 cells) and per organ (PFC/spleen). Impairment of the primary humoral response to the thymus-dependent antigen SRBC was seen throughout the 42 days observation period, although by the end partial recovery was starting.

In view of the reported selective toxicity of TCDD for the thymus and thymus-dependent areas of lymphoid organs (1,5), it was of interest to investigate the effect of this chemical on the response to the thymus-independent antigen SIII. As shown in Table 2, 18 days after treatment the humoral response to SIII was reduced to approximately 50% of the control value.

The effect of TCDD on cell-mediated reactivity has been extensively investigated, but some what different experimental conditions, including schedule of treatment and animal species, have been employed in previous studies (1,2,3,8). Therefore in a series of experiments GVH reactivity and the lymphoproliferative responses to ConA and LPS were measured at times (day 7-14) when humoral antibody production was greatly reduced. As illustrated in Tables 3, 4 and 5, no significant impairment of these cell-mediated reactions was observed on a per cell basis. However, as previously repeatedly demonstrated (1,2,5), significantly reduced (30-50%) numbers of spleen cells were recovered from mice given 6 or 30 µg/kg TCDD. Therefore the total cell-mediated reactivity per organ was presumably decreased by this chemical.

D I S C U S S I O N

The results presented here show that single doses of TCDD markedly suppress primary humoral antibody production against thymus-dependent (SRBC) and independent (SIII) antigens in 6-8 week-old mice. In contrast, under these experimental conditions, the in vitro blastogenic responses to ConA and LPS and the GVH reactivity were not significantly affected on a per cell basis, although the smaller number of spleen cells recovered from mice given TCDD (2,5) presumably results in impairment of the total capacity per organ to mediate these reactions. Suppression of the PFC response to SRBC induced by TCDD was long lasting, a dose of 6 µg/kg still inhibiting the response by more than 75 percent 42 days after treatment.

The effect of TCDD on humoral antibody production has not been adequately investigated (1,8), but it has been frequently assumed that this chemical spares humoral responses (5,14), although Vos et al. (8) reported a significant decrease in α, β, and γ-globulin in mice given non-toxic doses of TCDD.

It has been recenlty shown that in utero and neonatal exposure of rats to TCDD selectively suppresses the response to T cell mitogens without affecting antibody levels (4,14).

The different animal species (mice versus rats) or the different time of exposure in relation to the ontogenesis of the immune system might at least partially account for the apparent discrepancy between previous data and the results presented here . In this context it is noteworthy that the effect of repeated doses of TCDD on G.V.H. reaction in adult mice (1) was not confirmed by the same authors (2) in subsequent studies and that non-toxic doses of TCDD had no effect on PHA splenocyte responsiveness.

In view of the repeated selectivity of TCDD for the thymus and for thymus-dependent areas of lymphoid organs the marked inhibition we observed of the response to the thymus-independent antigen SIII is somewhat surprising. The hypocellularity of the bone marrow of TCDD-treated mice (5) might represent a cellular basis for the impairment of thymus-independent humoral responses reported here.

Mice exposed to TCDD show reduced resistance to bacterial infections (3). Inhibition of humoral antibody production might contribute to the TCDD-induced impairment of host defense mechanisms against infections.

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TABLE 1

EFFECT OF TCDD ON PRIMARY HUMORAL RESPONSE TO SRBC

Day after treatment	TCDD (μg/kg)	PFC/10^6 splenocytes	PFC/spleen
7	0	204 (173 - 240)	27,334 (24,106 - 30,990)
	1.2	94 (77 - 114)**	10,514 (8,698 - 12,708)**
	6	29 (21 - 41)**	3,723 (2,666 - 5,199)**
	30	11 (9 - 12)**	915 (765 - 1,095)**
14	0	266 (236 - 306)	33,871 (30,844 - 37,194)
	1.2	154 (114 - 208) *	19,600 (13,869 - 27,700)*
	6	79 (52 - 121) *	9,064 (5,967 - 13,768)**
	30	30 (19 - 48)**	3,381 (2,065 - 5,536)**
21	0	509 (452 - 572)	88,583 (82,041 - 95,647)
	1.2	202 (173 - 237)**	30,609 (26,046 - 35,971)**
	6	119 (96 - 147)**	14,230 (11,476 - 17,643)**
	30	58 (40 - 85)**	5,890 (4,274 - 8,117)**
42	0	1,028(938 -1,127)	83,086 (73,215 - 94,287)
	1.2	458(367 - 572)*	51,844 (45,153 - 59,562)
	6	152(115 - 201)**	16,795 (11,865 - 23,774)**
	30	128(98 - 167)**	12,226 (10,247 - 14,586)**

Results are the means ± (1.S.E.)after logarithmic transformation of the data

* $P < 0.05$

** $P < 0.01$

TABLE 2

EFFECT OF TCDD ON PRIMARY RESPONSE TO SIII

Day after treatment	TCDD (μg/kg)	PFC/10^6 splenocytes	PFC/spleen
18	0	127 (108 - 150)	19,561 (15,746 - 24,300)
	1.2	60* (49 - 74)	11,339 (9,441 - 13,619)
	30	69* (58 - 83)	8,159* (6,751 - 10,130)

Results are the means ± (1 S.E.) after logarithmic transformation of the data.

* $p < 0.05$

TABLE 3

EFFECT OF TCDD ON THE MITOGENIC RESPONSE TO ConA

Day after treatment	TCDD (μg/kg)	Con A (μg/sample)				
		0	0.1	0.8	1.6	3.2
7	0	2,304[+] (1,209)	9,209 (3,271)	59,400 (15,267)	28,593 (7,465)	3,404 (2,445)
	1.2	1,142 (587)	8,222 (1,318)	52,654 (18,434)	23,549 (9,280)	4,404 (1,663)
	6	5,148 (2,478)	10,416 (2,875)	72,484 (26,386)	76,140 (31,561)	10,477 (7,866)
	30	4,991 (166)	3,721 (671)	92,710 (15,319)	121,340* (15,020)	44,589* (14,087)
14	0	5,929 (981)	19,619 (1,789)	331,256 (58,585)	306,293 (62,486)	68,693 (21,406)
	1.2	4,430 (1,340)	18,624 (7,072)	258,133 (60,329)	249,430 (67,545)	91,642 (33,658)
	6	9,012 (1,582)	29,054 (10,111)	295,477 (55,390)	291,792 (47,099)	122,720 (30,622)
	30	5,129 (613)	10,415 (2,771)	309,645 (61,953)	368,125 (52,192)	103,837 (27,788)

[+]CPM = counts per minute (± 1 S.E.)

* $p < 0.05$

TABLE 4

EFFECT OF TCDD ON THE MITOGENIC RESPONSE TO LPS

Day after treatment	TCDD (μg/kg)	LPS (μg/sample)			
		0	0.05	5	120
7	0	10,620[+] (1,428)	15,185 (2,104)	43,069 (2,542)	1,478 (283)
	30	12,380 (3,451)	13,706 (3,085)	36,230 (10,231)	3,189 (833)
14	0	6,287 (1,396)	15,853 (3,270)	21,077 (5,315)	1,738 (281)
	30	10,541 (3,060)	20,736 (4,078)	23,861 (2,987)	1,693 (341)

[+]CPM = counts per minute (± 1 S.E.)

TABLE 5

EFFECT OF TCDD ON G.V.H. REACTION

Day after treatment	TCDD (µg/kg)	L.I. +
7	0	2.50 ± 0.31
	30	2.07 ± 0.10
14	0	2.31 ± 0.05
	30	2.48 ± 0.26

+Lymphnodal index.

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