INCREASED TUMOR NECROSIS FACTOR ALPHA PRODUCTION BY PERIPHERAL BLOOD LEUKOCYTES FROM TCDD-EXPOSED RHESUS MONKEYS WITH ENDOMETRIOSIS

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Introduction

Previous work has shown that exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is associated with a dose-dependent increase in the incidence and severity of endometriosis in the rhesus monkey[1]. Studies also suggest that immune mechanisms participate in TCDD-mediated toxicity and the pathogenesis of endometriosis[2,3]. Thirteen years after TCDD treatment was terminated, we characterized the phenotypic distribution of peripheral blood mononuclear cells (PBMC) from TCDD-exposed and unexposed rhesus monkeys and determined the ability of these cells to produce cytokines and exert cytolytic activity against NK and T-cell sensitive cell lines. We also determined whether elevated serum levels of TCDD, dioxinlike polyhalogenated aromatic hydrocarbon (PHAH) congeners and triglycerides correlated with changes in PBMC phenotype or function.

Methods

Detailed methods for TCDD exposure of these animals have been previously reported[1]. Control 0 PPT TCDD animals were not exposed to TCDD, animals in the low dose group were exposed to 5 parts per trillion (PPT) TCDD and monkeys in the high dose group were exposed to 25 PPT TCDD for approximately 4 years from late 1977 to early 1982. Dosing was by ingestion in the animal feed. Animals evaluated in the present study consisted of 15 live monkeys remaining in this colony and 12 additional animals of similar age with no previous TCDD exposure. The presence and severity of endometriosis in these animals was determined by diagnostic laparoscopy as described[1]. The phenotypic distribution and activational status of PB leukocytes were determined by multiparameter flow cytometric analysis. Cytokine production by rhesus PB leukocytes in response to stimulation by phytohemagglutinin (PHA; T-cell mitogen) or polyinosinic acid-polycytidylic acid (poly I:C or PIC; viral antigen) was determined by ELISA. A standard chromium-release assay was conducted simultaneously against 2 target cell lines: K562 (erythroleukemic cell) and RAJI (Epstein-Barr virus-transformed B cell).

Results and Discussion

TCDD exposure correlated with increased PBMC tumor necrosis factor alpha (TNFα) secretion in response to stimulation by T-cell mitogen (Table 1) and decreased cytolytic activity against NK-sensitive target cells ($p<0.03$; data not shown). Furthermore, increased production of this cytokine

by mitogen-stimulated leukocytes was associated with elevated serum triglyceride levels ($p<0.05$; data not shown). Leukocyte TNFα secretion in response to viral antigen and PBMC production of interferon gamma (IFNγ), interleukin (IL)-6 and IL-10 following exposure to mitogen or antigen were unaffected by previous TCDD treatment (Table 2). Although TCDD exposure was not associated with changes in PBMC surface antigen expression, elevated serum concentrations of TCDD, 1,2,3,6,7,8-hexachlorodibenzofuran (hexaCB) and 3,3',4,4',5-pentachlorobiphenyl (PnCB) correlated with increased numbers of CD3+/CD25- and CD3-/CD25+ leukocytes and enhanced secretion of TNFα by mitogen-stimulated PBMC (Table 3). These findings indicate that TCDD-exposed rhesus monkeys with endometriosis exhibit long-term alterations in sytemic immunity associated with elevated serum levels of specific PHAH congeners.

References

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Table 1. Increased production of TNFα in response to stimulation by T-cell mitogen by PBMC from TCDD-exposed animals [1]

Animal Group	TNFα pg/ml
0 PPT TCDD	ND
5 PPT TCDD	85 [a]
25 PPT TCDD	204 [a]
ALL TCDD	77 [b]
-TCDD/-EM	ND
-TCDD/+EM	ND
+TCDD/-EM	425 [c]
+TCDD/+EM	147 [d]

[1] PBMC were incubated for 48 hours in the absence or presence of PHA (TNFα-PHA). Levels of TNFα spontaneously released by cells were subtracted from total cytokine produced in the presence of mitogen. The concentration of immunoreactive cytokine in cell culture supernatants was determined by ELISA. Results are expressed as median TNFα pg/ml. PBMC production of TNFα was examined in TCDD-exposed rhesus monkeys with and without endometriosis: 0 PPT TCDD (n = 10), 5 PPT TCDD (n = 6), 25 PPT TCDD (n = 3), ALL TCDD (n = 9), No TCDD and No Endometriosis (-TCDD/-EM; n = 7), No TCDD with endometriosis (-TCDD/+EM; n = 3), TCDD and no endometriosis (+TCDD/-EM; n = 2), TCDD with endometriosis (+TCDD/+EM; n = 7). Statistical inferences reflect results of Wilcoxon signed ranks tests. Cumulative TCDD exposure vs TNFα-PHA $p < 0.04$; data not shown).

ND, not detected

[a] $p < 0.05$ 5 PPT or 25 PPT group compared with corresponding value for 0 PPT group.

[b] $p < 0.01$ ALL TCDD group compared with corresponding value for 0 PPT group.

[c] $p = 0.067$ Non-significant trend for +TCDD/-EM group versus -TCDD/-EM group.

[d] $p < 0.01$ +TCDD/+EM group compared with corresponding value for -TCDD/-EM group.

Table 2. Cytokine production by leukocytes from TCDD-exposed monkeys [a]

CYTOKINE-STIMULUS	0 PPT	ALL TCDD
TNFα–PHA	ND n = 10	128 ** n = 9
TNFα–PIC	335 n = 9	329 n = 9
IFNγ–PHA	699 n = 9	1000[b]* n = 9
IFNγ–PIC	323 n = 9	346 n = 9
IL-6 -PHA	2813* n = 11	3303 n = 9
IL-6 -PIC	4930 n = 10	5548 n = 9
IL-10-PHA	147 n = 18	308 n = 9
IL-10-PIC	183 n = 15	153 n = 9

[a] Peripheral blood leukocytes were incubated for 48 hours in the absence or presence of PHA or PIC. Levels of cytokines spontaneously released by cells were subtracted from total cytokine produced in the presence of mitogen or antigen. The concentration of immunoreactive cytokines in cell culture supernatants was determined by ELISA. Results are expressed as median cytokine levels pg/ml.

[b] Non-significant trend for PHA-stimulated IFNγ determined to be significantly different compared with corresponding value for 0 PPT TCDD group, $p = 0.056$.

** Determined to be significantly different compared with value for 0 PPT TCDD group, $p < 0.01$.

*Within subgroup PHA-stimulated cytokine determined to be significantly different compared with corresponding value for PIC-stimulated cytokine, $p < 0.05$.

Table 3. Increased serum levels of TCDD and dioxinlike congeners correlated with increased numbers of leukocyte subsets and TNFα-PHA production.

	CD3+/CD25-	CD3-/CD25+	TNFα-PHA
TCDD	$p < 0.02$	$p < 0.05$	$p < 0.03$
1,2,3,6,7,8-hexaCB	$p < 0.02$	$p < 0.05$	$p < 0.04$
3,3',4,4',5-PnCB	$p < 0.02$	$p < 0.05$	$p < 0.03$