Immunotoxicity of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)

and Diethylstilbestrol (DES) in the Fetal Mouse Thymus and Liver

By

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Abstract

Diethylstilbestrol (DES) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) have been identified as immunotoxicants causing thymic atrophy, thymocyte hypocellularity, phenotypic changes detected by CD4 and CD8 surface antigens, and progenitor T-cell targeting in the fetal mouse. We hypothesized that gestational exposure to these two compounds may lead to comparable histologic and gene expression alterations in the fetal mouse thymus and liver. Treatment of pregnant C57Bl/6 mice with doses of 5 or 10 μg/kg TCDD or 48 μg/kg DES by oral gavage on gestation days (gd) 14 and 16 severely depressed day 18 thymic cellularity. Histologic evaluation of day 18 fetal thymuses showed disruption of normal cortico-medullary architecture after TCDD or DES. Decreased thymocytes density was noted primarily in cortical zones where pyknotic cells were increased by either TCDD or DES treatment. Using day 18 thymocyte suspensions and flow cytometry, 7-AAD showed decreases in viable thymocytes from TCDD- or DES-treated fetal mice, and concomitant increases in thymocytes in early apoptosis. When thymoctyes were do-identified with CD4 and CD8 cell surface antigen expression, enhanced apoptosis occurred in $CD4^{+}CD8^{+}$ phenotype after TCDD treatment. After DES exposure, increased apoptosis occurred in $CD4^{-}CD8^{-}$ and $CD4^{-}CD8^{+}$ thymocytes. Both TCDD and DES increased liver to body weight ratios and decreased ratios of hematopoietic cells to hepatic cells present. Cytomegaly was seen in hepatocytes of TCDD and DES treated animals, and these cells had more variable features, such as increased cytoplasmic basophilia and more prominent nucleoli. Real time quantitative PCR demonstrated that DES decreased c-jun, bcl-2, and PKCα mRNA expression. These results suggest a shift away from proliferative activity and may reflect alterations noted predominantly in the hematopoietic population. TCDD increased c-jun mRNA expression with modest decreases in PKCα, and marked decreases in p53 also noted. Decreases in p53 suggest a pro-proliferative

status of hepatic cells, while decreases in PKCα may indicated decreases in phosphorylation of substrates required for normal cell cycle progression. The increased c-jun suggests that this gene may play a role in the hepatocyte hyperplasia, as well as the diminution of hematopoiesis.

DEDICATION

I dedicate this work to my clinical pathology residency advisor, Dr. Bernard F. Feldman, who was a constant source of reassurance and motivation; to my son, Jake, whose presence brought balance to my life; to my mother, Esther, for her words that strengthened my resolve; to Renee and Kurt, Barbara and Margaret, and to the rest of my family and friends for their support and encouragement during this final stage of my academic training.

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In addition, I would like to extend a special thanks to Joan Kalnitsky for assisting with the flow cytometry experiments; to the Histopathology Laboratory at the Veterinary Teaching Hospital, VMRCVM, for preparing all tissue samples for histologic evaluation; and to Susanne Aref, Statistical Consulting Center, for her assistance with the gene expression analysis.

DECLARATION OF WORK PERFORMED

I declare that I, Elizabeth Gayle Besteman, performed all of the work performed in this dissertation except that which is identified below.

Joan Kalnitsky operated the flow cytometer. Steve Holladay and Bonnie Smith assisted in harvesting thymus and liver tissues respectively. The Histopathology Laboratory at the Veterinary Teaching Hospital at Virginia Tech prepared the tissue samples for microscopic evaluation after formalin fixation. Susanne Aref of the Statistical Counseling Center at Virginia Tech performed the analysis of the gene expression data.

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ABBREVIATIONS

7-AAD	7-aminoactinomycinD
AhR	aryl hydrocarbon receptor
APC	antigen presenting cells
ARNT	aryl hydrocarbon nuclear translocator protein
BERKO	estrogen receptor beta knockout
cDNA	complementary deoxyribonucleic acid
CFU-GM	colony forming unit-granulocyte macrophage
CT	comparative threshold
DC	dendritic cell
DERKO	double estrogen receptor knockout
DES	diethylstilbestrol
DEX	dexamethasone
DN	double negative
DNA	deoxyribonucleic acid
DP	double positive
E2	estradiol
ER	estrogen receptor
ERKO	estrogen receptor alpha knockout
FITC	fluorescein isothiocyanate
FTOC	fetal thymus organ culture
GD	gestation day
HAH	halogenated aromatic hydrocarbon
HBSS	Hanks buffered salt solution
IL	interleukin
mRNA	messenger ribonucleic acid
NK	natural killer cell
NOAEL	no observable adverse effect
OVA	ovalbumin
PCDD	polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
PCB	polychlorinated biphenyl
PCR	polymerase chain reaction
PE	phycoerythrin
PFC	plaque forming cell
RTPCR	reverse transcription polymerase chain reaction
SP	single positive
SRBC	sheep red blood cell
TCD	3, 3’, 4, 4’-tetrachlorobiphenyl
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TcR	T cell receptor
Tdt	terminal deoxynucleotidyl transferase
TEQ	toxic equivalency factors
TG	transgenic
TUNEL	terminal dUTP nick end labeling

kg	kilogram
g	gram
mg	milligram
μg	microgram
mL	milliliter
μL	microliter