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Coumestrol, Bisphenol-A, DDT, and TCDD Modulation of Interleukin-2 Expression in Activated CD+4 Jurkat T Cells

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Abstract: Endogenous estrogens are known to modulate several components of immune response, including interleukin-2 (IL-2) production. IL-2 is a cytokine that plays an important role in adaptive immune responses. These responses may be modulated by xenoestrogens such as coumestrol, bisphenol A (BPA), DDT, and TCDD. In this research, we examined the effects and potential mechanisms of action of these estrogenic compounds on IL-2 production in activated CD4+ Jurkat T cells. IL-2 production was analyzed by ELISA and Western Blot. At the transcriptional level, protein expression was examined by RT-PCR. Coumestrol, DDT and TCDD (but not BPA) significantly suppressed IL-2 production in activated CD4+ Jurkat T cells, at the transcriptional and translational levels. The transcriptional suppression of IL-2 was associated with decreased protein levels of NF-κβ, an important IL-2 positive transcription factor, without affecting the expression of Iκ–Bα protein expression, an important inhibitor of NF-κβ nuclear translocation. Although the direct mechanisms of xenoestrogens modulation of the immune system remain to be elucidated, coumestrol-, DDT- and TCDD-induced suppression of IL-2 may have ramifications for our understanding of the impact of xenoestrogens on health and disease.

Key words: Xenoestrogens, IL-2, transcription, Jurkat T cells

Introduction

Several investigations have shown that estrogens have direct effects on immune response through modulation of cytokine production, lymphocyte cell cycle distribution and transit, lymphoproliferation, and cell-mediated immune responses [1-15]. 17-β-estradiol, the most common endogenous estrogen, is an aromatized steroid hormone that binds to a cytosolic estrogen receptor (ER); this hormone-receptor complex serves as a gene transcription regulatory unit [16-18]. Recently, it has been shown that 17-β-estradiol suppresses T lymphocyte IL-2 production at the molecular level through suppression of mRNA transcription and NF-kB protein levels [19].

Derived from plant or synthetic sources, xenoestrogens are exogenous environmental compounds having the common property of exhibiting estrogenic effects on classic endocrine target organs, such as the uterus or breast [20-24]. While endocrinological effects of these compounds (ecohormones) have been widely studied and the focuses of scientific and societal debates [25-27], their immunotoxicological effects have not been thoroughly elucidated. Direct xenoestrogenic effects on

T lymphoproliferation and apoptosis have recently been described [28], suggesting that xenoestrogens influence normal lymphocyte biology. In the current study, representative xenoestrogens from major categories - phytoestrogens (coumestrol), plastic monomers (bisphenol A- BPA), herbicides/health waste incineration (TCDD), and pesticides (DDT)– were examined for their effects on Jurkat T cell IL-2 production and potential mechanisms by which modulation may occur.

Materials and Methods

Chemicals and Reagents

Coumestrol, bisphenol A - BPA, DDT, fetal calf serum, and phorbol-myristate acetate (PMA) were obtained from Sigma Chemical Co. (St. Louis, MO). 17-β-estradiol was also obtained from Sigma, and used as a positive control. TCDD was obtained from Cambridge Technologies (Cambridge, MA). RPMI medium, phenol-free RPMI, and culture supplements were purchased from Gibco/BRL (Gaithersburg, MD).

In order to detect potentially significant differences in xenoestrogen effects on IL-2 production, *in vitro* concentrations of xenoestrogens were used in a range of 10^{-9} to 10^{-6} M, based on previous studies of 17-β-estradiol required to show an effect in transformed T cell lines [19]. A23187 and antibodies for the IL-2 ELISA were obtained from Pharmingen (San Diego, CA). IL-2 RT-PCR primers were purchased from Promega (Madison, WI).

Cell Culture

The CD4+ Jurkat T cell line was obtained from American Type Culture Collection (Rockville, MD). After thorough washing, Jurkat T cells were cultured at a density of 1 x 10^6 cells/ml in phenol-free RPMI 1640 supplemented with 5% charcoal stripped (steroid free) FCS, 200 mM glutamine, and 1% penicillin/streptomycin. Cells were cultured in 24 well plates and activated with 500 nM A23187 and 50 ng/ml PMA. These concentrations were previously determined to stimulate maximal IL-2 production (data not shown). Culture supernatants were collected by centrifugation.

IL-2 ELISA

IL-2 protein concentrations in the supernatant of cell culture were determined by standard sandwich ELISA using antibodies and standards obtained from Pharmingen (San Diego, CA) according to manufacturer's instructions. Assays were performed as previously described [19] on neat and diluted samples in duplicate on 96 well plates. Absorbance was measured on a Bio-Rad spectrometer at 450 nm, and IL-2 concentrations were determined by comparison to a standard curve.

RNA Preparation and Analysis

RT-PCR was performed as previously described [19]. Total cellular RNA from Jurkat T cells was obtained by guanidium isothiocyanate lysis and centrifugation through a $CsCl_2$ cushion. Three million cells were plated at 0.5M/ml in 65 mm tissue culture dishes and stimulated with hormones. At the designated time of harvest cells were transferred to a 4 ml polypropylene tube, pelleted, washed twice in 1 X PBS and lysed in 3 ml guanidinium. Centrifugation was performed for 16 hrs at 33,000 rpm. RNA was resuspended, precipitated and quantitated spectrophotometrically.

In some experiments, mRNA was harvested using a Fast Trak kit (InVitrogen, Carlsbad, CA) according to manufacturer's instructions. Aliquots of RNA were run on an ethidium stained gel to ensure good quality. Identical qualitative results were obtained with both forms mRNA and total cellular RNA. Reverse transcription (RT) of RNA was performed under standard conditions using equal µg input RNA from each condition.

The PCR reaction was performed under the following conditions in a 25 ul reaction: 30 cycles of denaturing (94^oC, 1 min), annealing (60^oC, 2 min) and extension (72^oC, 5 min). The reaction was performed in a Perkin Elmer DNA Thermal Cycler: 10 pmoles each primer, 200 (M dNTP, 1.25 U *Taq*, 1 X *Taq* reaction buffer (all Promega) as previously described [67]. PCR primers were specific for human IL-2. PCR reactions were optimized for annealing temperature and were performed at various cycle numbers to ensure that the result obtained was within linear range of the amplification curve. GAPDH was amplified from the same RT reaction as a control. Polaroid photos of ethidium stained gels are shown. The results are semi quantitative when performed in this fashion.

Western Blot Analysis

For Western analysis as previously described [19], 2 to 5 X 10^6 cells were cultured (1.0 X 10^6/ml) and stimulated. Cells were harvested and pelleted in an Eppendorf microcentrifuge (1,200 g, 5 min, 4^0C), washed in 1 X PBS and resuspended in a cell lysis buffer containing 20 mM Tris pH 8.0, 0.5% Nonidet P-40, 1 mM EDTA, 1 ug/ml leupeptin, 1 µg/ml pepstatin, 1 mM dithiothreitol, 1 mM PMSF and 0.1 M NaCl (all from Sigma). After 20 min of incubation at 4^0C, supernatants were prepared (8,000 g, 5 min, 4^0C) and total protein concentrations determined by the method of Bradford and Lowry using Bio-Rad Protein Assay reagents in a microtiter assay. Fifteen µg of total cellular protein was electrophoresed on a 12.5% SDS-PAGE gel, transferred to a polyvinylidine difluoride membrane (Amersham, Arlington Heights, IL) by electroblotting overnight (25 mM Tris pH 8.3, 192 mM glycine, 20% methanol, 15 V, 100 mA, 4^oC). Membranes were blocked with 10% electrophoresis grade biotin-depleted non-fat dry milk

(BioRad) in 1 X PBS (10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween-20), rinsed in PBS, probed with monoclonal mouse anti-human NF-kB and IkB (Pharmingen) at 1:1000 and 1:500 dilutions, respectively, and washed 3 times in PBS. The secondary antibody was HRP-conjugated goat anti-mouse whole IgG used at 1:1000 (Transduction Laboratories, San Diego,CA).

All antibodies were diluted in 1% milk in TBS. Membranes were washed three times and detection was performed by enhanced chemiluminescence with an ECL reagent kit (using 0.06 ml/cm^2 of reagent) and Hybond autoradiography film (both Amersham). Biotinylated standards were used for molecular weight determination and were detected with 1:3000 streptavidin-horseradish peroxidase (Amersham). This reagent was only used after initial detection with the secondary antibody (Transduction Laboratories) because it was found to alter the signal of some primary antibodies if used simultaneously. It also performed better than avidin-horseradish peroxidase (BioRad) provided with the biotinylated markers. Blots were stripped as per the manufacturer's instructions and probed up to three times with different primary antibodies. Incubations were performed at 37^{0}C for 30 min or RT for 1 hr. A Bio-Rad GS670 densitometer was used to determine the relative band intensity.

Statistical Analysis

Statistical analysis was performed by one-way ANOVA for multiple samples or by Student t testing with matched pairing if appropriate. Statistical significance was considered at $p < 0.05$. Data were graphically presented as means $\pm$ standard errors of the means.

Results

Coumestrol, TCDD, and DDT Suppression of IL-2 Production

The effects of coumestrol, BPA, TCDD, and DDT on the production of IL-2 were initially examined in Jurkat T cells activated with A23197/PMA for 48 hours. IL-2 was measured by ELISA as described in Materials and Methods. As shown in Figure 1, IL-2 concentrations were variably but significantly suppressed ($p < 0.05$) by 10^{-8} M to 10^{-6} M coumestrol, DDT and TCDD. Bisphenol A showed a trend towards suppression of IL-2 production that did not reach statistical significance. TCDD suppression of IL-2 production showed definite concentration dependence whereas coumestrol and DDT showed more of a threshold phenomenon at concentrations of 10^{-8} M and 10^{-6} M, reaching a statistically significant suppression without obvious concentration dependence. Concentrations of xenoestrogens at $\leq 10^{-9}$ M did not show statistically significant suppression of IL-2. 17-β-estradiol (10^{-6} M), known to suppress IL-2 production [7-9,19], was used as a positive control; non-activated cells (i.e., absent A23187, absent PMA, or both) or xenoestrogen-exposed cells without activation, did not produce detectable supernatant IL-2 (data not shown).

Coumestrol, BPA, TCDD, and DDT Suppression of IL-2 mRNA Transcripts

Since steroid hormones act as transcription factors [17, 18], it was postulated that xenoestrogens modulate IL-2 production through suppression of mRNA. Using qualitative reverse transcription polymerase chain reaction (RT-PCR), and estrogen as a positive control [19], coumestrol, BPA, TCDD and DDT (10^{-6} M) were shown to suppress IL-2 mRNA transcripts at 18 hours of exposure, as indicated in the representative experiment (Figure 2). Similar results were seen in two additional experiments. In comparison to the ELISA results, in which BPA trended towards IL-2 suppression but did not reach statistical significance, all of the selected xenoestrogens variably suppressed IL-2 mRNA transcripts as assessed by RT-PCR.

Coumestrol, TCDD, and DDT Suppression of NF-κB Binding

IL-2 transcription is dependent on the binding and cooperation of several positive regulatory transcription factors including NF-κB [29,30]. Coumestrol, TCDD, and DDT appeared to inhibit the transcription of IL-2 in Jurkat T lymphocytes through the suppression of nuclear transcription. While multiple transcription factors are involved in the regulation of IL-2 production, the initial focus was on xenoestrogenic modulation of NFκB as being quantitatively more important in Jurkat T lymphocytes [29].

As shown in the representative experiment (n = 3), coumestrol, DDT and TCDD but not BPA, inhibited NF-kB expression at 8 hrs in activated Jurkat T cells at 10^{-6} M, as determined by Western blotting (Figure 3). While coumestrol, TCDD and DDT exposure of Jurkat cells reduced nuclear NF-κB protein levels, these xenoestrogens had no effect on IκBα, the cytoplasmic inhibitor that binds NF-κB and prevents its translocation to the nucleus [31, 32].

As it can be seen in the representative results (n = 3; Figure 3), coumestrol, DDT, bisphenol A and TCDD had no effect on Iκ-B after 8 hrs of exposure; compared to suppression of NF-κB protein levels by coumestrol, TCDD, and DDT. Similar results were seen in two additional experiments (not shown).

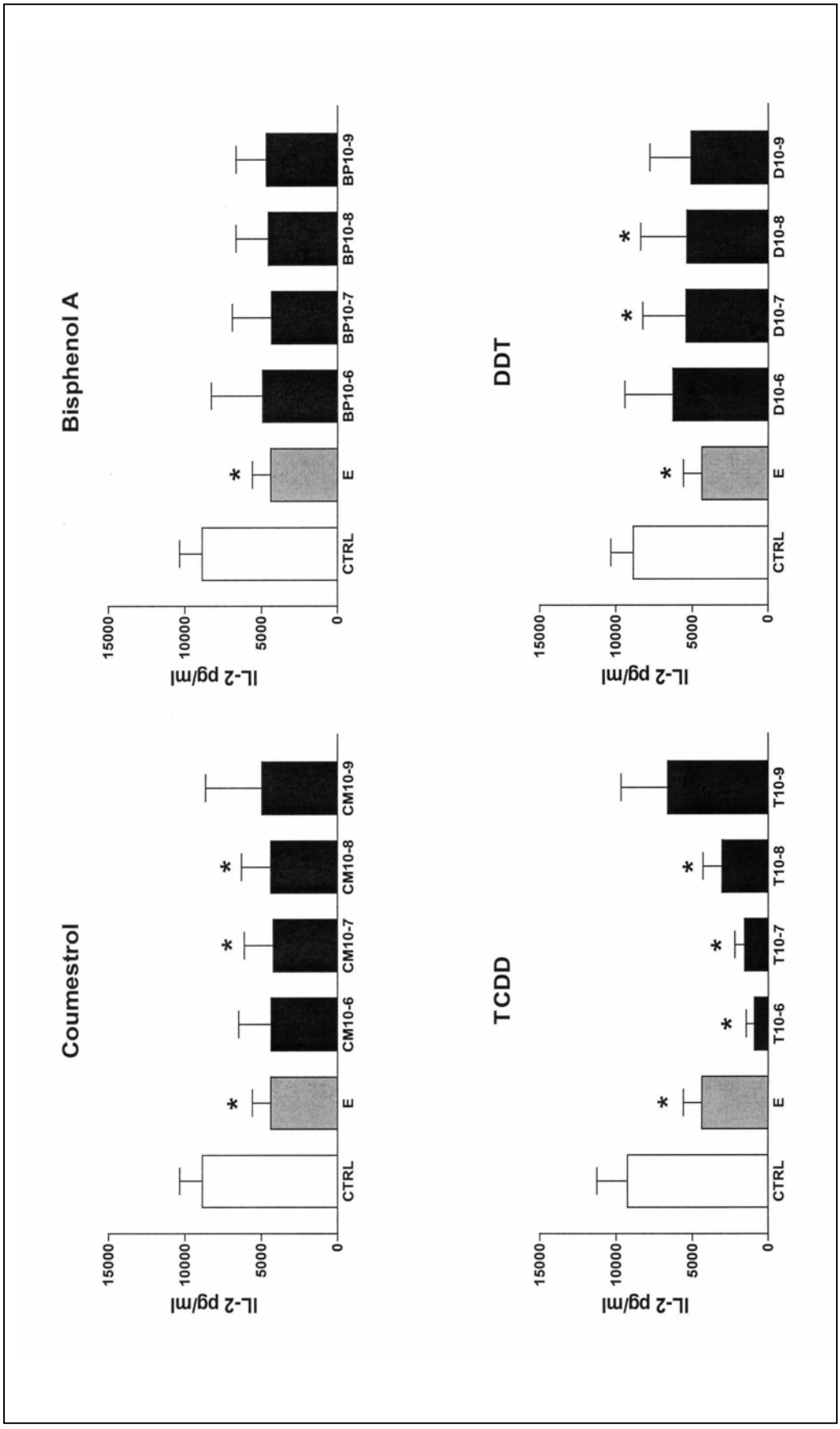

Figure 1. ELISA data on the effects of BPA, coumestrol, TCCD, and DDT on IL-2 production in activated Jurkat T cells after 48 hrs of exposure (E-17-β-estradiol (10^{-6} M); *Significant reduction at $p < 0.05$). Coumestrol, TCDD, and DDT (but not BPA) exposure significantly suppress IL-2 production.

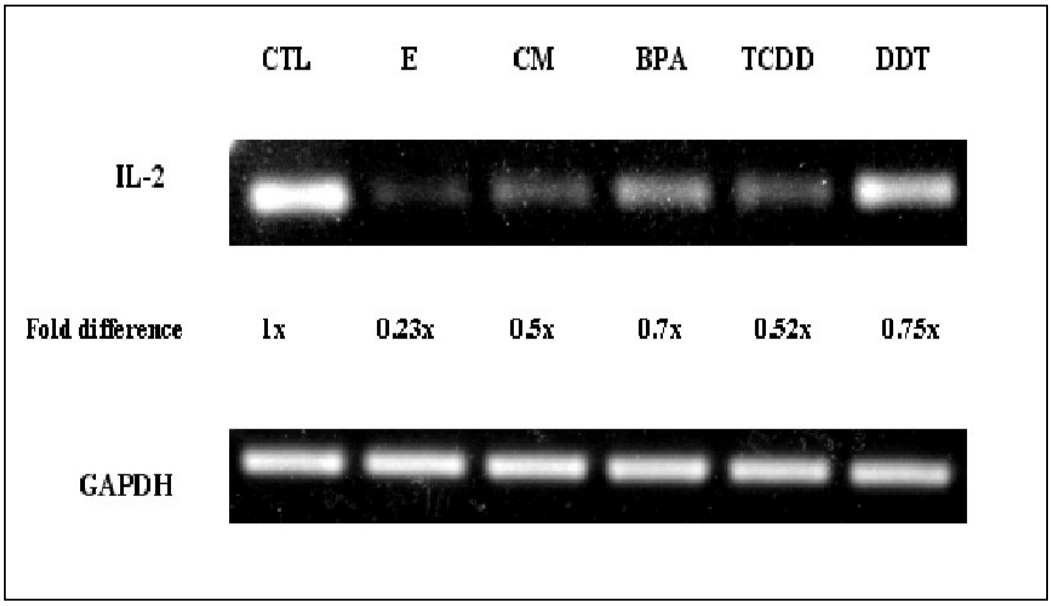

Figure 2. Semi quantitative RT-PCR of IL-2 mRNA transcripts from activated Jurkat T cells at 18 hrs in control (C) vs. 17-β-estradiol, coumestrol, bisphenol A, TCDD, and DDT exposed cells (10^{-6}M). GAPDH is used as a housekeeping gene. One representative experiment (n = 3) is shown. MRNA transcripts were variably suppressed (0.2-0.75x of control) in 17-β-estradiol, coumestrol, BPA, TCDD, and DDT exposed Jurkat T cells compared to control (1x).

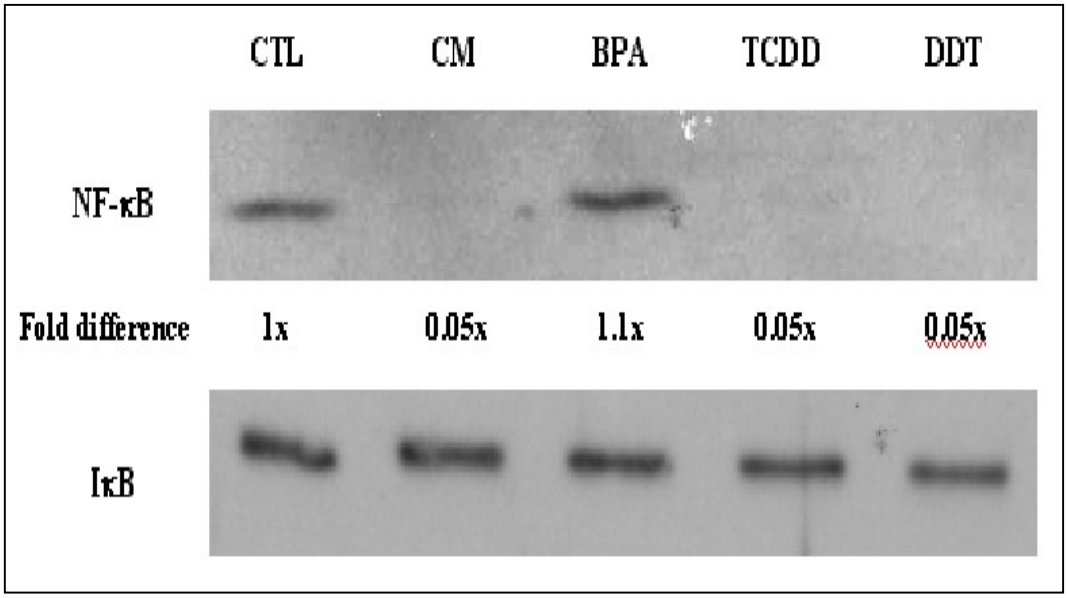

Figure 3. Western blot analysis of coumestrol, bisphenol A, TCDD and DDT effects on NF-kB in Jurkat T cell. Coumestrol, TCDD and DDT but not bisphenol A suppressed (0.05x of control) NF-kB protein levels in Jurkat T cells as assessed by Western blotting at 8 hrs (10^{-6} M). Coumestrol, DDT, bisphenol A and TCDD induced had no effect on IkB protein in Jurkat T cells. Similar results were obtained in two additional experiments.

Discussion

Xenoestrogens are environmental hormones (ecohormones) or compounds that exhibit estrogenic activity. They may interact with or disrupt endogenous estrogenic activity, and, as suggested by some investigations, may have implications for health and disease [20-27]. In the present study, coumestrol, TCDD, and DDT, but not bisphenol A, were shown to variably but significantly suppress IL-2 production in Jurkat CD4+ T cells. Bisphenol A did not achieve statistically significant suppression of IL-2, although there was a trend towards suppression. Coumestrol and DDT suppressed IL-2 at defined concentrations (10^{-7} M and 10^{-8} M), whereas TCDD showed a clear concentration- dependent suppression of IL-2. The variability is likely due to xenoestrogenic potency with respect to estrogenic activity [21, 22], as well as possible differences in mechanisms of action [23-25]. IL-2 suppression appeared to occur at the level of transcription and was associated with inhibition of NF-κB by coumestrol, TCDD, and DDT. Discrepancies between IL-2 ELISA data (no statistically significant suppression for BPA), IL-2 mRNA transcript assessment (RT-PCR), and NF-κB protein levels (BPA not suppressed) are not completely unexpected. While having the common action of estrogen mimicry, they are unlikely to act (as discussed below) via identical mechanisms, and are likely to have pleiotropic effects that are time-, concentration-, and tissue-dependent. Nevertheless, our experimental data suggest that these specific xenoestrogens, at high concentrations, have specific effects on T lymphocyte IL-2 production and its transcriptional mechanisms.

IL-2 is an essential growth factor for T cells [29, 33-36] and is important not only for T cell survival but impacts proliferation and differentiation of T cells into TH1 or TH2 predominance [35-38]. Inadequate IL-2 production may lead to T cell death or induction of clonal anergy [39,40], and deficient IL-2 production has been associated with autoimmune and inflammatory states [41,42]. Hence, the finding of xenoestrogenic modulation of IL-2 production may have ramifications for immunodeviation as well as manifestations of health or disease. Suppression of IL-2 production has been found in humans with xenoestrogen exposure [43]. Animal studies have shown that TCDD exposure is associated with suppressed IL-2 production and altered lymphocyte biology [44-46]. Coumestrol, a phytoestrogen, and DDT, a pesticide, appear to also share this property. Hence, further studies may be needed to confirm our results in lymphocytes. Jurkat T cells are useful models to examine transcriptional mechanisms of IL-2 production [33-35], as they share many IL-2 signaling characteristics of normal T cells [29-34].

While concentrations of xenoestrogens used in this study may exceed those detected in the environment and the general population, chronic, low level of exposure is known to have biological effects [22, 26, 47]. The purpose of this investigation was to identify possible mechanisms of immunomodulation and not necessarily establish environmental exposure-based cause-and-effect evidence. Furthermore, acute *in vitro* effects cannot be adequately extrapolated to chronic, low dose exposure *in vivo* effects, so results in the current study should be interpreted with utmost caution. Observed effects also occurred only in select xenoestrogens, implying that effects may be compound-specific and that broad generalization for individual compounds are not appropriate. As shown by the variability in degree of mRNA transcript or NF-kB protein levels, imunotoxicological effects are likely time-, concentration- or compound-specific, so that this preliminary study merely establishes the possibility that ecohormones may have an effect on IL-2 production and not that specific compounds used in this study have any direct relationship to effects on human health and disease. Nevertheless, xenoestrogens exhibit bioaccumulation such that immunomodulatory concentrations may be reached in exposed individuals over time; furthermore, these compounds may interact with each other or with endogenous estrogens, in either an additive, synergistic, or antagonistic manner [21, 23, 27].

Data in the current study suggest a direct suppressive effect of coumestrol, TCDD and DDT on NF-kB. Moreover, coumestrol, TCDD and DDT did not affect IkB protein levels in Jurkat T cells. Normally IkB sequesters NF-kB and prevents its translocation to the nucleus, thus preventing nuclear transcription, especially during the progression of the inflammatory cascade [30-32]. While verification and extension of our results is needed, the changes induced in NF-kB by coumestrol, TCDD, and DDT suggests at least one of potentially several mechanisms, by which the transcription of IL-2 is suppressed in xenoestrogens-treated Jurkat T lymphocytes.

The effects observed in the current study may be estrogen receptor dependent or independent [48]. Jeon and Esser [49], in contrast to the findings of the current study, have shown that TCDD upregulated IL-2 promoter activity and IL-2 production through binding of the AHR to distal DNA IL-2 promoter motifs. However, xenoestrogens may also have AHR receptor independent effects on T lymphocytes [44] and xenoestrogen mechanisms of action are likely pleiotropic [50-52]. Delineating xenoestrogen-mediated effects through the ER or AHR is pivotal to understanding molecular mechanisms of xenoestrogens, but is beyond the scope of this initial study.

T-cell exposure to selective xenoestrogens at the time of activation may lead to variable, but less than optimal production of IL-2. Since the quality and quantity of an immune response may be determined by the quantity of IL-2 produced [35-40], coumestrol, TCDD and DDT may act as immunomodulators, and, in key situations requiring an IL-2 response, a primary

immunosuppressant. While TCDD has been best studied and shown previously to suppress IL-2 production [45, 46], the current study expands the finding of IL-2 suppression to other xenoestrogens (coumestrol and DDT) and further demonstrates their effects on transcription and the transcription factor NF-KB, an important signal transducer for inflammatory signaling [32]. Not unexpectedly, xenoestrogen effects on IL-2 production are not uniform at equimolar concentrations, compatible with their structural and estrogenic potency heterogeneity [20-23]. Given the protean actions of NF-kB [32, 54], its suppression by coumestrol, TCDD, and DDT expands the possible influence of ecohormones on lymphocyte biology. Verification and expansion of current results is required; nevertheless, these data identify potential mechanisms by which accumulated ecohormones may influence immune or autoimmune responses.

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References

1. Whitacre, C. C.; Reingold, S. C.; O'Looney, P. A.; and the Task Force on Gender, Multiple Sclerosis and Autoimmunity. A gender gap in autoimmunity, *Science* **1999**, *283*, 1277-1278. Supplemental material online at www.sciencemag.org/feature/data/983519.shl.
2. Olsen, N. J.; Kovacs, W. J. Gonadal steroids and immunity. *Endocrine Rev.* **1996**, *17*, 369-384.
3. Fox, H. S. 1995. Sex steroids and the immune system. *Ciba Foundation Symposium* **1995**, *191*, 203-211.
4. Ahmed, S. A.; Penhale, P. J.; Talal, N. Sex hormones, immune responses, and autoimmune diseases: Mechanisms of sex hormone action. *Am. J. Pathol.* **1978**, *121*, 531-551.
5. Giron-Gonzalex, J. A.; Moral, F. J.; Elvira, J.; Garcia, G. D.; Guerrero, F.; Gavilan, I.; Escobar, L. Consistent production of a higher TH1:TH2 cytokine ratio by stimulated T cells in men compared to women. *Eur. J. Endocrinol.* **2000**, *143*, 31-36.
6. Ahmed, S.A.; Dauphinee, M.J.; Talal, N. 1984. Effects of short-term administration of sex hormones on normal and autoimmune mice. *J. Immunol.* **1984**, *134*, 204-210.
7. Pung, O. J.; Tucker, A. N.; Vore, S. J.; Luster, M. I. Influence of Estrogen on Host Resistance: Increased Susceptibility of Mice to *Listeria Monocytogenes* Correlates with Depressed Production of Interleukin 2. *Infect. Immunity* **1985**, *50*, 91-96.
8. Elbourne, K.; Keisler, D.; McMurray, R. W. 1998. Differential effects of estrogen and prolactin on autoimmune disease in the NZB x NZW F_1 mouse model of systemic lupus erythematosus. *Lupus* **1998**, *7*, 420-427.
9. Pozsonyi, T.; Jakab, L.; Jakob, L.; Onody, K.; Cseh, K.; Kalabay, L. Effect of estrogen on the blast transformation of lymphocytes and interleukin 2 production in lupus erythematosus. *Orv Hetil* **1992**, *133*, 1167-1171.
10. Stein, B.; Yang, M. X. Repression of the Interleukin-6 Promoter by Estrogen Receptor Is Mediated by NF-κB and C/EBPβ. *Mol. Cell. Bio.* **1995**, *15[9],* 4971-4979.
11. Ray, A.; Ray, P. Down modulation of interleukin 6 gene expression by 17B estradiol in the absence of high affinity DNA binding by the estrogen receptor. *J. Biol. Chem.* **1994**, *269*, 12940-12946.
12. Herrera, L. A.; Montero, R.; leon-Cazares, J. M.; Rojas, E.; Gonsebatt, M. E.; Ostroskyu-Wegman, P. 1992 Effects of progesterone and estradiol on the proliferation of phytohemagglutinin-stimulated human lymphocytes. *Mutat. Res.* **1992**, *270*, 211-218.
13. Blagosklonny, M. V.; Neckers, L. M. 1994. Cytostatic and cytotoxic activity of sex steroids against human leukemia cell lines. *Cancer Letters* **1994**, *76*, 81-86.
14. Kincade, P. W.; Medina, K. L.; Smithson, G.. 1994. Sex hormones as negative regulators of lymphopoiesis. *Immunol. Rev.* **1994**, *137*, 119-149.
15. Athreya, B. H.; Pletcher, J.; Zulian, F.; Weiner, D.B.; Williams, W. V. 1993. Subset specific effects of sex hormones and pituitary gonadotropins on human lymphocyte proliferation in vitro. *Clin. Immunol. Immunopathol.* **1993**, *66*, 201-1211.
16. Wilson, J. D.; Foster, D. W. (eds.). Williams Textbook of Endocrinology 8th edition; Philadelphia, PA: WB Saunders Company, **1992**.
17. Beato, M., et al. Gene regulation by hormones. *Cell* **1990**, *56*, 335-375.
18. Tsai, M.; O'Malley, B. W. 1994. Molecular mechanisms of action of steroid/thyroid receptor superfamily member. *Annu. Rev. Biochem.* **1994**, *63*, 451-486.
19. McMurray, R. W.; Ndebele, K.; Jenkins, J. 17-β-estradiol suppresses IL-2 and IL-2 receptor. *Cytokine* **2001**, *14*, 324-333.
20. Wolff, M. S. Environmental estrogens. *Environ. Health Perspect.* **1995**, *103(9),* 784-785.
21. Safe, S. H. Endocrine disrupters and human health--is there a problem? An update. *Environ. Health Perspect.* **2000**, *108(6),* 487-93.
22. Kaiser, J. Endocrine disrupters. Panel cautiously confirms low-dose effects. *Science* **2000**, *290(5492),* 695-697.
23. Neubert, D. Vulnerability of the endocrine system to xenobiotic influence. *Reg. Toxicol. Pharmacol.* **1997,** *26*, 9-29.
24. Ahmed, S. A.; Hissong, B. D.; Verthelyi, D.; Donner, K.; Becker, K.; Karpuzoglu-Sahin, E.

Gender and risk of autoimmune diseases: possible role of estrogenic compounds. *Environ. Health Perspect.* **1999**, *107(l 5),* 681-686.
25. Crinnion, W. J. Environmental medicine, part one: the human burden of environmental toxins and their common health effects. *Altern. Med. Rev.* **2000**, *5(1),* 52-63.
26. Ashby, J. Testing for endocrine disruption post-EDSTAC: extrapolation of low dose rodent effects to humans. *Toxicol. Lett.* **2001**, *120(1-3),* 233-242.
27. Ziegler, J. Environmental "endocrine disrupters" get a global look. *J. Natl. Cancer Inst.* **1997**, *89(16),* 1184-7.
28. Ndebele, K.; Tchounwou, P. B.; McMurray, R. W. Effects of xenoestrogens on T lymphocytes: Modulation of Bcl2, p53, and apoptosis. *Int. J. Mol. Sci.* **2003***, 4,* 45-61.
29. Hughes, C. W.; Pober, J. S. Transcriptional regulation of the interleukin-2 gene in normal human peripheral blood T cells. *J. Biol. Chem.* **1996**, *271*, 5369-5377.
30. Arima, N.; Kuziel, W. A.; Grdina, T. A.; Greene, W. C. 1992. IL-2 induced signal transduction involves the activation of nuclear NF-kappa B expression. *J. Immunol.* **1992**, 149:83-91.
31. Whiteside, S. T.; Israel, A. I kappa B proteins: structure, function, and regulation. *Semin. Cancer Biol.* **1997**, *8*, 75-82.
32. Karin, M.; Delhase, M. The I kappa B kinase and NF-kB: key elements of proinflammatory signalling. Se*min. Immunol.* **2000**, *12*, 85-98.
33. Weiss, A.; Wiskocil, R.L.; Stobo, J. D. 1984. The role of T3 surface molecules in the activation of human T cells: A two-stimulus requirement for IL-2 production reflects events occurring at a pre-translational level. J. *Immunol.* **1984**, *133*, 123-128.
34. Landegren, U.; Andersson, J.; Wigzell, H. 1985. Analysis of human T lymphocyte activation in a T cell tumor model system. *European J. Immunol.* **1985**, *15*, 308-311.
35. Weiss, A., and Littman, D., R. 1994. Signal transduction by lymphocyte antigen receptors. *Cell* **1994**, *76*, 263-274.
36. Smith, K. Interleukin 2: inception, impact, implications. *Science* **1988**, *240*, 1169-1176
37. Mosmann, T. R.; Sad, S. 1996. The expanding universe of T cell subsets: Th1, Th2 and more, *Immunol. Today* **1996**, *17*, 138-146.
38. Scott, P. 1993. Selective differentiation of CD4+ T helper cell subsets. *Curr. Opinion Immunol.* **1993**, 5, 391-397.
39. Schwartz, R. H. A cell culture model for T lymphocyte clonal anergy. *Science* **1990**, 1349-1356.
40. Go, C.; Miller, J. 1992. Differential induction of transcriptional factors that regulate the interleukin-2 gene during anergy induction and restimulation. *J. Exp. Med.* **1992**, *175*, 1327-1336.
41. Huang Y. P.; Perrin, L.; Miescher, P.; Zubler, R. H. 1988. Correlation of T and B cell activities *in vitro* and serum IL-2 levels in systemic lupus erythematosus. *J. Immunol.* **1992**, 141, 827-833.
42. Schimpl A, Berberich I, Kneitz B, Kramer S, Santner-Nanan B, Wagner S, Wolf M, Hunig T. IL-2 and autoimmune disease. *Cytokine Growth Factor Rev*. **2002** Aug-Oct; 13(4-5): 369-78.
43. Daniel, V.; Huber, W.; Bauer, K.; Suesal, C.; Conradt, C.; Opelz, G. Associations of blood levels of PCB, HCHS, and HCB with numbers of lymphocyte subpopulations, in vitro lymphocyte response, plasma cytokine levels, and immunoglobulin autoantibodies. *Environ. Health Perspect.* **2001**, *109(2),* 173-178.
44. Hossain, A.; Tsuchiya, S.; Minegishi, M.; Osada, M.; Ikawa, S.; Tezuka, F. A.; Kaji, M.; Konno, T.; Watanabe, M.; Kikuchi, H. The Ah receptor is not involved in 2,3,7,8-tetrachlorodibenzo-p-dioxin-mediated apoptosis in human leukemic T cell lines. *J. Biol. Chem.* **1998***, 273(31),* 19853-19858.
45. Nohara, K.; Fujimaki, H.; Tsukumo, S.; Inouye, K.; Sone, H.; Tohyama, C. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on T cell-derived cytokine production in ovalbumin (OVA)-immunized C57Bl/6 mice. *Toxicology* **2002**, *172(1),* 49-58.
46. Prell, R. A.; Dearstyne, E.; Steppan, L. G.; Vella, A. T.; Kerkvliet, N. I. CTL hyporesponsiveness induced by 2,3,7, 8-tetrachlorodibenzo-p-dioxin: role of cytokines and apoptosis. *Toxicol. Appl. Pharmacol.* **2000**, *166(3),* 214-221.
47. Ulrich, E. M.; Caperell-Grant, A.; Jung, S. H.; Hites, R. A.; Bigsby, R. M. Environmentally relevant xenoestrogen tissue concentrations correlated to biological responses in mice. *Environ. Health Perspect.* **2000**, *108(10),* 973-977.
48. Frigo, D. E.; Burow, M. E.; Mitchell, K. A.; Chiang, T. C.; McLachlan, J. A. DDT and its metabolites alter gene expression in human uterine cell lines through estrogen receptor-independent mechanisms. *Environ. Health Perspect.* **2002,** *110(12),* 1239-45.
49. Jeon, M. S.; Esser, C. The murine IL-2 promoter contains distal regulatory elements responsive to the Ah receptor, a member of the evolutionarily conserved bHLH-PAS transcription factor family. *J. Immunol.* **2000***, 165(12),* 6975-6983.
50. Kharat, I.; Saatcioglu, F. Antiestrogenic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin are mediated by direct transcriptional interference with the liganded estrogen receptor. Cross talk between aryl hydrocarbon- and estrogen-mediated signaling. *J. Biol. Chem.* **1996***, 271(18),* 10533-10537.
51. Umbreit, T. H.; Gallo, M. A. Physiological implications of estrogen receptor modulation by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicol. Lett.* **1988***, 42(1),* 5-14.
52. DeVito, M. J.; Thomas, T.; Umbreit, T. H.; Gallo,

M. A. Multi-site regulation of estrogen receptor by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Prog. Clin. Biol. Res.* **1992**, *374, 321*-336.

53. Silverstone, A. E.; Frazier, D. E. Jr.; Fiore, N. C.; Soults, J. A.; Gasiewicz, T. A. Dexamethasone, beta-estradiol, and 2,3,7,8-tetrachlorodibenzo-p-dioxin elicit thymic atrophy through different cellular targets. *Toxicol. Appl. Pharmacol.* **1994**, *126(2),* 248-259.
54. Cerillo, G.; Rees, A.; Manchanda, Reilly, C.; Brogan, I.; White, A.; Needham, M. 1998. The oestrogen receptor regulates NF-kB and AP-1 activity in a cell specific manner. *J. Steroid Biochem.* **1998**, *67*, 9-88.