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Effect of Atrazine, Perfluorooctanoic Acid and Zearalenone on IFNγ, TNFα, and IL-5 mRNA Expression in Jurkat Cells

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Abstract − Cytokine production is a sensitive indicator for monitoring perturbations of the immune system by xenobiotics in animals and humans. In the present study, we evaluated the changes in IFNγ, IL-5 and TNFα mRNA expression after atrazine (ATZ), perfluorooctanoic acid (PFOA) or zearalenone (ZEA) exposure in Jurkat cells. The IC50 (concentration for a 50% inhibition of cell proliferation) of PFOA and ZEA after 3 days culture were 226.6 μM and 52.6 μM, respectively. The effects of ATZ on cytokine expression followed in increasing order of IFNγ > IL-5 > TNFα at 3 μM and at the lower concentrations the degree of effects on three cytokines were less clear between the cytokines when compared to control level. PFOA had marked increasing effect in order of IFNγ > TNFα > IL-5 mRNA expression at IC50, and these patterns were continued at the lower concentrations, IC50/2 and IC50/4. ZEA caused the overexpression of cytokine mRNAs in order of IL-5 > IFNγ > TNFα at both IC50 and IC50/2, and at IC50/4 the overexpression order was IL-5 > TNFα. On other hand, IFNγ was less distinct compared to the control. These data indicate that ATZ, PFOA and ZEA caused the overtranscription of IFNγ, IL-5 and TNFα mRNA, and the overproduction of these cytokines may eventually lead to immune disorders.

Keywords: Atrazine, Perfluorooctanoic acid, Zearalenone, IFNγ, TNFα, IL-5

INTRODUCTION

Endocrine disruptors (EDs) are naturally occurring or synthetic chemicals that may interfere with the production or activity of hormones of the endocrine system, leading to adverse health effects. There are multiple ways that EDs interfere with or disrupt normal hormone activity. EDs can mimic the effects of natural hormones by binding to their receptors, may block the binding of a hormone to its receptor, or they can block the synthesis of the hormone. Also EDs can interfere with the transport of a hormone or its elimination from the body. A wide and varied range of substances are thought to cause endocrine disruption (Lintelmann *et al*., 2003).

Atrazine (ATZ) is a widely-used agricultural herbicide for the control of broadleaf and grassy weeds in the cultivation of numerous crops including corn, soybean, wheat, pineapple and various grasses. Its widespread agricultural use can contaminate the ground and surface water, and can travel in rainwater; with long term environmental impacts because it breaks down very slowly (Thurman and Cromwell, 2000; Whalen *et al*., 2003). ATZ is an ED that causes abnormal reproductive development and impairs immune function in animals (Cantemir *et al.,* 1997; Pruett *et al*., 2003; Filipov *et al*., 2005; Rowe *et al*., 2006). Furthermore, ATZ exposure in rodents can make exposed animals more susceptible to allergies (Rowe *et al*., 2006).

Perfluorooctanoic acid (PFOA) is a synthetic chemical and is typically used to aid in the manufacturing of fluoropolymers. These polymers are used for non-stick cookware, stain-resistant carpets and clothing due to their fire resistance and oil and stain repellence. Continued testing has shown that this class of compounds is widely distributed in the environment and suspected to be EDs affecting the sex hormone levels (Lau *et al*., 2007; Jensen

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and Leffers, 2008). Toxicology studies have shown that exposure to PFOAs can cause cancer, growth defects, liver damage, and immune disorders (Yang *et al*., 2000; Yang *et al*., 2001; Yang *et al*., 2002; Lau *et al*., 2007; Jensen and Leffers, 2008).

Zearalenone (ZEA) is an estrogenic mycotoxin which is a toxic metabolite produced by several Fusarium fungi species, and often involved in reproductive, immunological dysfunction and carcinogenic effects. The estrogenic effects are based on the structural similarity between zearalenone and estradiol (Ouanes *et al*., 2005; Luongo *et al*., 2006).

EDs can interfere with normal immune functions in the body because estrogen receptors are present in the various cells of the immune system (Ouanes *et al*., 2005). Chemical mediators of immune responses include antibodies, immunoglobulins, cytokines and the complement system of plasma proteins. Among these, cytokines are secreted primarily by white blood cells, T lymphocytes, and epithelial cells (Abbas *et al*., 2007). Recent studies have suggested that the alterations of cytokine production could be a useful biomarker for environmental pollutant exposure (Camacho *et al*., 1999; Hooghe *et al*., 2000; Yang *et al*., 2000; Berek *et al*., 2001; Yang *et al*., 2001; Yang *et al*., 2002; Calemine *et al*., 2003; Devos *et al*., 2003; Devos *et al*., 2004; Luongo *et al*., 2006; Fairley *et al*., 2007). Thus, in order to evaluate the quantitative changes of cytokines after ATZ, PFOA and ZEA exposure, we investigated the changes in the interferon gamma (IFNγ, type 1 cytokine), tumor necrosis factor-alpha (TNFα, type 1 cytokine) and interleukin-5 (IL-5, type 2 cytokine) (Hooghe *et al*., 2000; Bullens *et al*., 2004; Namazi, 2004) levels after EDs exposure in Jurkat cells.

MATERIALS AND METHODS

Chemicals and its preparation

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine, CAS NO. 1912-24-9, 98.7% purity) was obtained from Supelco (Bellefonte, PA). Perfluorooctanoic acid (CAS NO. 335- 67-1, 96%), zearalenone (2,4-dihydroxy-6-(10-hydroxy-6-oxoundecyl)benzoic acid μ-lactone, CAS NO. 5975-78-0) were purchased from Sigma-Aldrich (St. Louis, MO). The chemicals were first dissolved in DMSO (dimethyl sulphoxide, Sigma-Aldrich), then diluted in PBS. The final concentration of DMSO in media was 0.1%, a concentration which did not affect cell proliferation (Minervini *et al*., 2005). Solutions were sterilized by filtration. The final volume of vehicle and chemicals in culture were 10 μl/ml and these were added at the start of culture.

Culture conditions

The human leukemic T-cell line Jurkat was cultured in Gibco RPMI 1640 medium (Invitrogen, Grand Island, NY) and supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 U/ml penicillin (Sigma-Aldrich), 100 μg/ml streptomycin (Sigma-Aldrich), 5×10^{-5} M 2-mercaptoethanol (Sigma-Aldrich), and 10 mM Hepes (Sigma-Aldrich). Cell cultures were either treated with vehicle or exposed to different concentrations of chemicals, and incubated in a humidified incubator containing 5% CO_2 at 37°C.

MTT assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was purchased from Sigma and dissolved in sterile PBS at a concentration of 5 mg/ml, filtered through a 0.22 μm filter (Gelman Science, Ann Arbor, MI) to remove any insoluble residue, and stored in the dark at 4°C for no longer than 1 week. Jurkat cells, at a concentration of 2×10^5 cells/ml, were incubated in 96-well tissue culture plates (Costar, Cambrige, MA) for 3 days with different concentrations of chemicals in the presence of 5 μg/ml phytohemagglutinin (PHA, Sigma) (Vlata *et al*., 2006). In positive control group, cells were cultured with 0.1% DMSO in the presence of 5 μg/ml PHA. At the end of the incubation, an aliquot of 10 μl MTT stock solution was added to each well and incubated for 4h in a humidified incubator containing 5% CO_2 at 37°C. After incubation, 100 μl of acidic isopropanol (0.04 N HCl in isopropanol) was added to each well and the plates were incubated at room temperature to maximize solubilization of purple formazan crystals with intermittently shacking. Within 1 h, the absorbance was measured on a *V*max Microplate Reader (Molecular Devices) at a 570 nm test wavelength and a 670 nm reference wavelength.

Cell viability

Cellular viability was determined by the trypan blue (0.4% solution in PBS, Sigma) exclusion technique (Fernandez-Botran and Větvička, 1995). Jurkat cells at 2×10^5 cells/ml with 5 μg/ml PHA were cultured with chemicals at selected concentrations in 96-well culture plates. In order to evaluate the effects of the three chemicals on cell viability, the effect value was expressed as a percentage of the PHA stimulated chemical cells in relations to the non-treated positive control value.

Cell viability value (%)=(number of viable cells/number of viable cells + number of dead cells)×100
Effect value (%)=(viability value of chemical-treated group/viability value of chemical non-treated positive control)×100

Quantitative PCR using the real time system

Following the 3 day incubation, cellular RNAs were extracted from Jurkat cells using TRIzol Reagent (Invitrogen Life technologies, Carlabad, CA). cDNA was synthesized from the cellular RNAs by reverse transcription using AccuPower® RT PreMix (Bioneer, Cheongwon-gun, Chungbuk, Korea). For real-time PCR, the 20 μl PCR mixture contained a 10 μl master mix (iTag SYBR Supermix with ROX, Bio-Rad, Hercules, CA), 1 μl of each primer (10 pmol), 2 μl of cDNA (100 ng) and 6 μl of dd-H_2O. After 3 min at 95°C, the amplification conditions were 40 cycles of 10s at 95°C, 30s at 57°C and 30s at 72°C for IFNγ, and 40 cycles of 10s at 95°C, 30s at 55°C and 30s at 72°C for IL-5, TNFα and β-actin. The primer sequences and size used are shown in Table I. The amplification and detection were performed using the iCycler iQ™ real-time PCR detection system (Bio-Rad). Each PCR product has shown as a single band upon gel electrophoresis. The number of targets in the unknown samples was quantified by measuring the threshold cycle, Ct, using a standard curve. The housekeeping β-actin gene was used as a reference and the results were expressed as cytokine/β-actin mRNA ratio.

RESULTS

Effects on cell proliferation

The concentration ranges of chemical used in this initial study were chosen on the basis of the literature data (Hooghe *et al*., 2000; Yang *et al*., 2000; Luongo *et al*., 2006). The 3 day exposure time was determined to be an optimal incubation time to investigate whether chemicals affect cell proliferation based on preliminary time-dependent response experiments (data not shown) and literature (Berek *et al*., 2001; Vlata *et al*., 2006).

The dose-response relationships for Jurkat cell proliferation with ATZ, PFOA and ZEA exposure were evaluated by MTT assay. As shown in Fig. 1, PFOA and ZEA showed a marked inhibitory effect on cell proliferation in a dose-dependent manner with increasing concentrations. In contrast, ATZ showed a different pattern in its dose-response manner compared to PFOA and ZEA exposure.

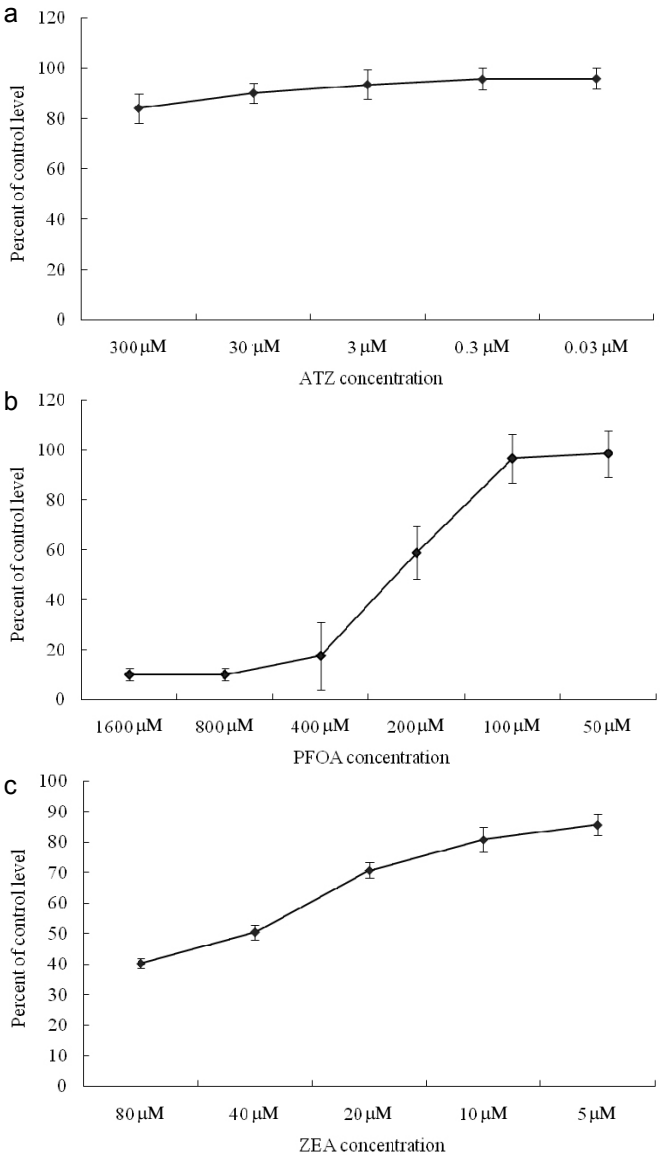

Fig. 1. Effects of ATZ (a), PFOA (b), and ZEA (c) on cell proliferation. Jurkat cells, at a concentration of 2×10^5 cells/ml, were incubated in complete medium with 5 μg/ml PHA + chemical or PHA for 3 days. The results were expressed as percent of PHA-stimulated chemical non-treated positive control levels. Values are the mean ± SD from five separated experiments.

Table I. Primer sequences and sizes

IL-5	Sense	5'-CAGCCAAAGATTTTGGAGGA-3'	142 bp
	Antisense	5'-TGCTTTCTGGCAAAGTGTCA-3'	
IFNγ	Sense	5'-GCATCCAAAAGAGTGTGGAGA-3'	105 bp
	Antisense	5'-CCGAATAATTAGTCAGCTTTTCG-3'	
TNFα	Sense	5'-AACCTCCTCTCTGCCATCAA-3'	100 bp
	Antisense	5'-GGAAGACCCCTCCCAGATAG-3'	
β-actin	Sense	5'-CACAGGGGAGGTGATAGCAT-3'	112 bp
	Antisense	5'-CACGAAGGCTCATCATTCAA-3'	

ATZ had less inhibiting effect on Jurkat cell proliferation at all five tested concentrations. Indeed, at the maximum tested concentration (300 μM/ml) in this study, the inhibitory effect of ATZ was 15.9%, and the inhibitory effect at less than 3 μM concentrations were less than 10%.

For the next step, the concentrations of PFOA and ZEA causing 50% inhibition of cell proliferation (IC50%, inhibiting concentration) from these data were calculated to be 226.6 μM and 52.6 μM using the WinNonlin program version 2.1, respectively. These data indicate that ZEA showed most powerful in inhibiting effect on Jurkat cell proliferation when compared on the basis of the IC50 values, and that ATZ was less effective on it when considered that inhibitory effect of ATZ at the maximum tested concentration (300 μM/ml) in this study was 15.9%.

Effects on cell viability

To investigate whether the inhibition of proliferation after chemical exposure was associated with cell death, cell viability was determined after 3 days exposure to a select concentration of chemicals. The exposure doses of PFOA and ZEA were chosen on the basis of the IC50 values. PFOA was added to the culture at three concentrations: IC50=226.6 μM, IC50/2=113.3 μM and IC50/4=56.6 μM. ZEA was IC50=52.6 μM, IC50/2=26.3 μM, and IC50/4= 13.2 μM. The exposure dose of ATZ (3 μM, 0.3 μM and 0.03 μM) was chosen on the basis of the literature data (Hooghe *et al*., 2000) and our findings.

As shown in Fig. 2a, the cell viability percentages after ATZ exposure, were 83.1 ± 1.7% at 3 μM, 93.5 ± 3.3% at 0.3 μM and 98.9 ± 4.2% at 0.03 μM. These data suggest that the effect on cell death at these concentrations were less distinct. PFOA and ZEA exposure caused a marked decrease of cell viability at IC50 concentrations (59.1 ± 5.2% and 54.3 ± 8.4% of control levels, respectively), and the effect at low doses were not clear (Figs. 2b, c). In addition, the exposure to IC50/4 of PFOA and ZEA did not affect the effect values of cell viability (97.1 ± 4 and 94.3 ± 3% of control levels, respectively) (Figs. 2b, c).

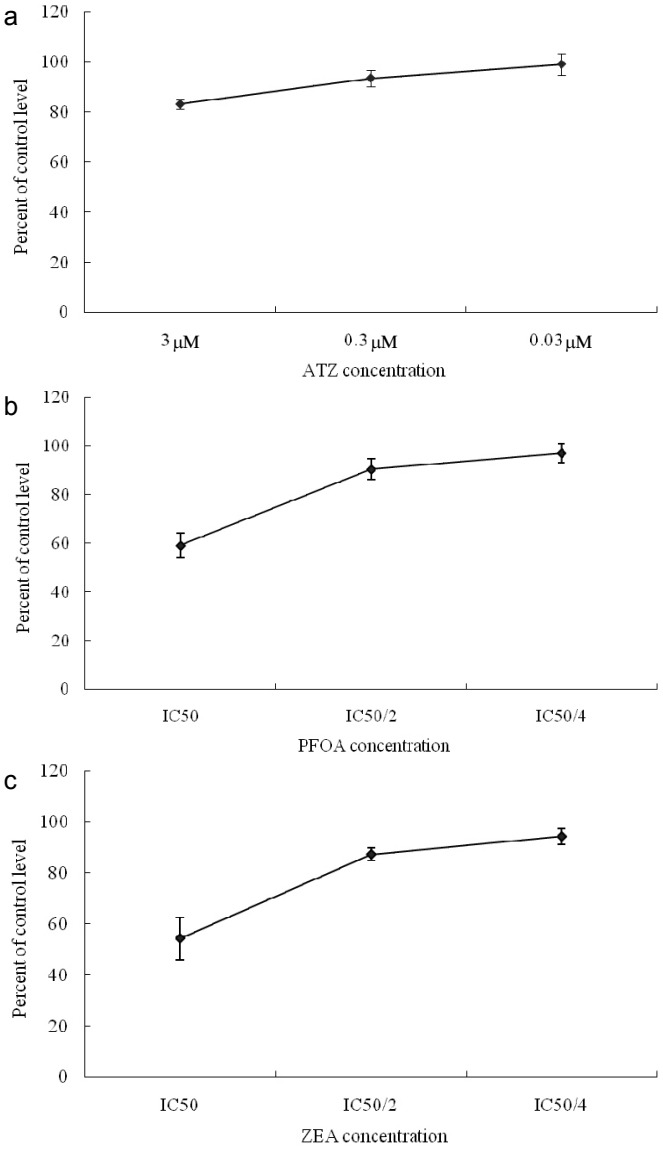

Fig. 2. Effects of ATZ (a), PFOA (b), and ZEA (c) on cell viability. Jurkat cells at 2×10^5 cells/ml were incubated in complete medium with 5 μg/ml PHA+chemical or PHA for 3 days. PFOA was IC50 (concentration for a 50% inhibition of cell proliferation)=226.6 μM/ml, IC50/2=113.3 μM/ml, and IC50/4= 56.6 μM/ml. ZEA was IC50=52.6 μM/ml, IC50/2=26.3 μM/ml, and IC50/4=13.2 μM/ml. The results were expressed as percent of PHA-stimulated chemical non-treated positive control levels. Values are the mean ± SD from five separated experiments.

Effects on cytokine mRNA expression

Recent studies showed that cytokine production was a sensitive indicator for monitoring perturbation of the immune system by xenobiotics in animals and humans (Hooghe *et al*., 2000). In order to more definitively demonstrate the effect of ATZ, PFOA, and ZEA on immune function, this study quantified the changes in the cytokine mRNA expressions after chemical exposures. Jurkat cells were treated with PHA+vehicle or PHA + different concentrations of chemicals for 3 days and assayed by real-time PCR.

As shown in Fig. 3a, ATZ at 3 μM had increased IFNγ, IL-5 and TNFα mRNA expression levels (306.9%, 225.0% and 156.5% of control level, respectively). At 0.3 μM, the levels decreased (144.6%, 124.7% and 138.0%, respectively), and seem to be within the control level range at

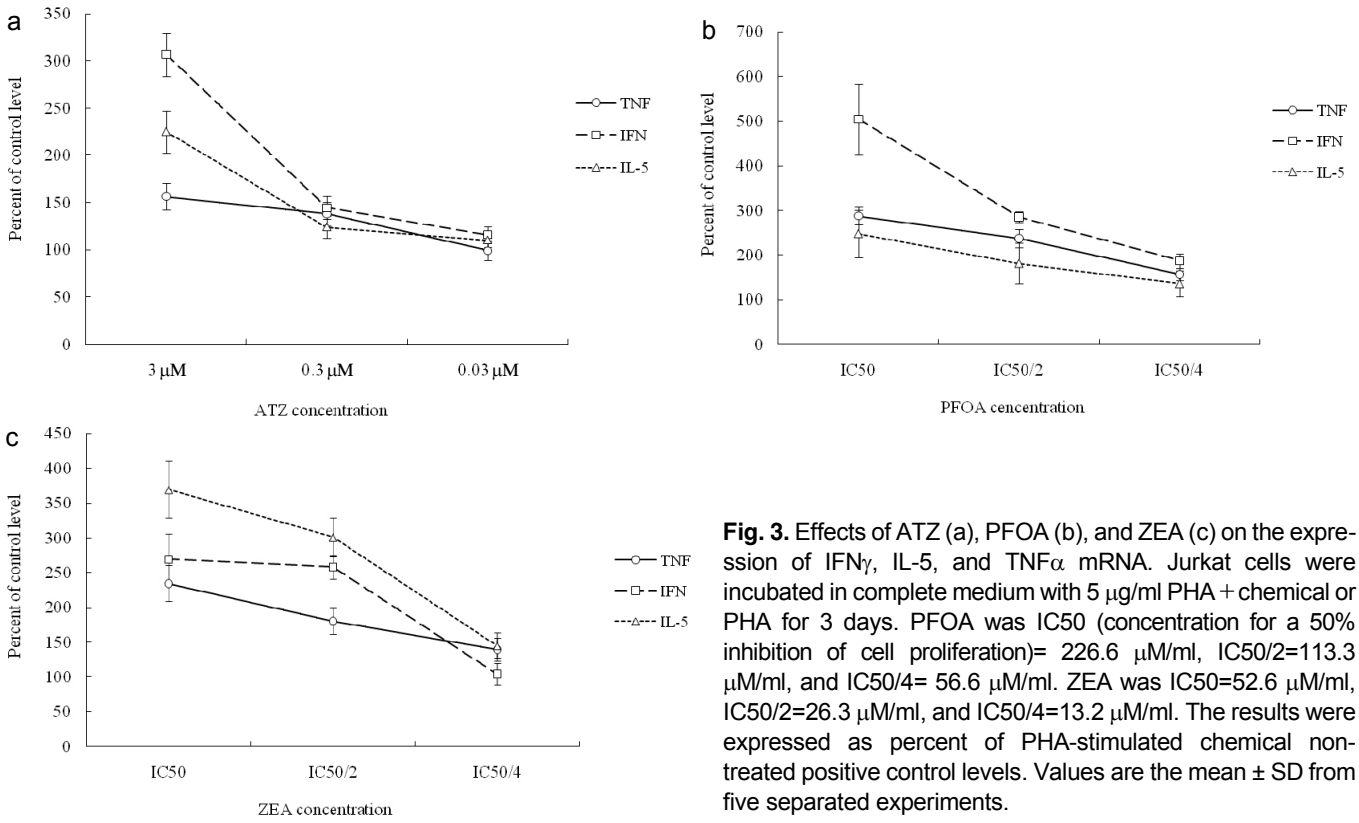

Fig. 3. Effects of ATZ (a), PFOA (b), and ZEA (c) on the expression of IFNγ, IL-5, and TNFα mRNA. Jurkat cells were incubated in complete medium with 5 μg/ml PHA + chemical or PHA for 3 days. PFOA was IC50 (concentration for a 50% inhibition of cell proliferation)= 226.6 μM/ml, IC50/2=113.3 μM/ml, and IC50/4= 56.6 μM/ml. ZEA was IC50=52.6 μM/ml, IC50/2=26.3 μM/ml, and IC50/4=13.2 μM/ml. The results were expressed as percent of PHA-stimulated chemical non-treated positive control levels. Values are the mean ± SD from five separated experiments.

0.03 μM (115.8%, 110.1% and 99.0%, respectively).

PFAO at IC50 caused a marked overexpression of IFNγ, IL-5 and TNFα (504.3%, 248.1% and 287.8% of control level, respectively). At IC50/2 their levels began to decline (285.5%, 181.8% and 237.4%, respectively), with the overexpression pattern persisting continuously at IC50/4 (187.2%, 136.4% and 157.4%, respectively) (Fig. 3b).

ZEA at IC50 also induced overexpression of IFNγ, IL-5 and TNFα (269.3%, 369.5%, 234.5% of control level, respectively), but at IC50/2 their levels were starting to decline (258.3%, 301.0%, 180.5%, respectively). At IC50/4, the expression patterns were similar to those of PFOA at the same concentration except IFNγ (104.4%, 145.0%, 139.7%, respectively) (Fig. 3c).

DISCUSSION

Jurkat cell was chosen to observe the effects of ATZ, PFOA, and ZEA on immune responses because it has been used for long time as a useful T cell model for different immunological studies and sensitive to estrogen treatment (Luongo *et al*., 2006).

In the MTT assay, PFOA and ZEA showed a marked inhibitory effect on cell proliferation in a dose-dependent manner, and ATZ has been to have less inhibiting effect on Jurkat cell proliferation at the tested concentrations. These results were a similar pattern to previous reports using different cell lines, different incubation time, or different assays (Berek *et al*., 2001; Devos *et al*., 2003; Minervini *et al*., 2005; Luongo *et al*., 2006; Vlata *et al*., 2006). When peripheral blood mononuclear cells (PBMCs) were cultured with ATZ for 3 days, ATZ exposure at 0.3 μM, 3 μM, and 30 μM concentrations caused the inhibition of cell proliferation by 78 ± 9, 57 ± 21 and 57 ± 24%, respectively, and less than 0.03 μM of ATZ had no effects at all (Devos *et al*., 2003). However, the present data showed that ATZ was less effective to the inhibition of Jurkat cell proliferation in the same range of concentrations or none at all. These discrepancies may due to the differences in the types of cells examined, the assay method, and in sensitivity (Luongo *et al*., 2006).

IC50 values following ZEA exposure were somewhat different according to the research groups. In human PBMCs using [^{3}H]thymidine, IC50 of ZEA was 9.5 μg/ml (Vlata *et al*., 2006). However, in C5-O and Caco-2 cell lines using MTT assay, the IC50 were 24.0 μg and 43.7 μg/ml, respectively, and higher than 100 μg/ml in HepG2, V79 and CHO-K1 cell lines (Cetin and Bullerman, 2005). These pre-

vious results and our IC50 at 52.6 μM/ml indicate that the Jurkat cell line was relatively sensitive to ZEA. Whereas, the present data also showed that Jurkat cell was comparatively less sensitive to PFOA with IC50=226.6 μM/ml when compared to ZEA.

Previous studies on the effect of ATZ on cell viability reported that ATZ did not show any apoptotic or necrotic activity at concentrations from 0.04 μg/ml to 4.7 μg/ml (=21.7 μM/ml) in human lymphocytes (Zeljezic *et al*., 2006) and 10 μM of ATZ did not affect the cell viability of NK cells (Whalen *et al*., 2003). These previous reports were consistent with our data, in which ATZ had less distinct effects on the cell viability of Jurkat cells at 0.03 μM to 3 μM concentrations, which may account for the lack of a clear inhibitory effect on cell proliferation. 15 and 30 mg/L (=72.4 μM/ml) of PFOA caused a decrease of cell viability to 77.1%, 73.6%, respectively, in freshwater tilapia hepatocytes (Liu *et al*., 2007). Although the exposure time, dose and tested cell line were different from our work, the pattern of the results were similar to our data, in which a dramatic decrease of cell viability (59.1% of control level) was observed after exposure to IC50 (226.6 μM/ml) and there was no significant decrease in the lower exposure groups. The effect of ZEA on cell viability was recently studied. In bovine lymphocytes, the inhibitory effect was observed at least in 4 μM/ml treatment (Lioi *et al*., 2004). In human PBMCs, ZEA caused a distinct decrease on cell viability at 30 μg/ml, whereas at 1 μg/ml did not affect on it (Vlata *et al*., 2006). The present study used Jurkat cell lines and ZEA at IC50 (52.6 μM/ml=93.6 μg/ml) caused a marked decrease of cell viability, with the ZEA-induced cell death possibly related to the necrosis (Lioi *et al*., 2004; Vlata *et al*., 2006). On the basis of the results, we thought that the dose-dependent decrease of cell proliferation after PFOA and ZEA exposure were related to the decrease in cellular viability.

Activated T cells proliferate, differentiate, and produce type 1 cytokines including IL-2, IL-12, IFNγ, and TNFα or type 2 cytokines (IL-4, IL-5, IL-6, IL-10, IL-13) (Bullens *et al*., 2004; Namazi, 2004; Luongo *et al*., 2006). IFNα is involved in the regulation of nearly all phases of the immune and inflammatory responses, including the activation and differentiation of lymphocytes, macrophages, and others (Gattoni *et al*., 2006). TNFα, which is a proinflammatory cytokine involved in the cytokine cascade and a proimmune cytokine required for IL-12 and IFNγ production (Zganiacz *et al*., 2004), is a principal mediator in the pathogenesis of infection, tissue injury, and inflammation, and functions to stimulate the recruitment of neutrophils and monocytes to site of infection and to activate these cells to eradicate microbes (Odeh, 1990; Bianchi *et al*., 1996; Abbas *et al*., 2007). IL-5, which was initially identified by its activity as a growth factor of B cells and eosinophils, is one of the key cytokines for fate-determination of terminal B cell differentiation to antibody-secreting plasma cells (Nagai *et al*., 1999). Based on these previous reports, IFNγ, TNFα and IL-5 were chosen to examine the changes of cytokine profiles after EDs exposure.

It is well known that cytokine production is a sensitive indicator for monitoring perturbation of the immune system by xenobiotics in animals and man (Hooghe *et al*., 2000). Previous studies reported mostly that EDs, we used in our study, had the immunosuppressive effects. For example, after 2 days of ATZ exposure (0.3-3 μM) in human PBMCs, the production of IFNγ, IL-5 and TNFα were impaired by up to 70, 50 and 70% respectively, in a concentration-dependent manner (Hooghe *et al*., 2000). Similar *in vitro* experimental data also reported that the production of IFNγ, IL-5 and TNFα in human PBMCs was greatly reduced in the presence of 3 μM ATZ (Devos *et al*., 2003; Devos *et al*., 2004). PFOA given in the diet to mice caused thymic and splenic atrophy, decreasing the number of thymocytes and splenocytes. In addition, PFOA inhibited the evocation of IgG and IgM after immunization (Yang *et al*., 2000; Yang *et al*., 2001; Yang *et al*., 2002). Recently PFOA was shown to be immunotoxic and its exposure augmented IgE response to environmental allergens (Fairley *et al*., 2007). α-zearalenol (α-ZEA), which is an alcohol metabolites of ZEA, treatment on Jurkat cells caused the inhibition of IL-2 (at 40 and 80 μM concentrations) and IFNγ mRNA expression (at 20, 40 and 80 μM concentrations) (Luongo *et al*., 2006). In contrast, ZEA on human T cells did not substantially alter the production of IL-5, IFNγ and TNFα at micromolar concentrations of less than 10^{-2} nM (Camacho *et al*., 1999). In other words, only a high concentration of ZEA yielded immunosuppressive effects (Berek *et al*., 2001). The Con-A stimulated splenocytes from mice given α-ZEA in the diet secreted significantly decreased levels of IFNγ (Calemine *et al*., 2003).

Until recently, one research group reported that these immunosuppressive effects of ATZ, PFOA and ZEA were related to an estrogen-like activity (Luongo *et al*., 2006). EDs are known to interact with estrogen receptor (Rawlins *et al*., 1999) and these receptors are expressed in a variety of immunocompetent cells, including $CD4^+$ and $CD8^+$ T cells and macrophages (Salem, 2004). In addition, estrogen had been reported to have some anti-inflammatory effects, including the inhibition of cytokine production (Rawlins *et al*., 1999).

By contrast, in our *in vitro* study using quantitative real

time PCR, we observed that more than 0.3 μM ATZ cause the overexpression of IFNγ, IL-5 and TNFα mRNA, and lower doses did not affect cytokine production. PFOA marked increased IFNγ, IL-5 and TNFα mRNA expressions at the tested concentrations. Also, ZEA markedly inducted cytokine mRNA expressions except IFNγ, which was less distinct at low concentration when compared to the control. These results supported by another recent *in vivo* study showing that ATZ caused the immunopotentiation, which in turn may potentiate clinical diseases, such as autoimmune disease and hypersensitivity (Rowe *et al*., 2006). As considered about the effect values of cell viability at IC50 PFOA and ZEA, the present data suggest that the macromolar concentrations of PFOA and ZEA may cause a marked enhancement of cytokine production.

Indeed, the other research group has clearly shown that estrogen had increased IFNγ, IL-6, and TNFα synthesis (Ahmed, 2000; Carruba *et al*., 2003; Karpuzoglu and Ahmed, 2006). From these, we thought that EDs, which had estrogen-like activity, could cause the overexpression of IFNγ, IL-5, and TNFα mRNA. The discrepancies among the results of various research groups suggest that EDs-induced changes in cytokine production may be due to differences in cell types, exposed dose, levels and types of estrogen receptors, and culture time (Carruba *et al*., 2003; Karpuzoglu and Ahmed, 2006).

Recent studies showed that increased TNFα synthesis may eventually lead to systemic clinical and pathological abnormalities including fever, septic shock, cachexia, hemorrhagic shock, and other inflammatory states (Odeh, 1990; Bianchi *et al*., 1996; Carruba *et al*., 2003), that overproduced IFNγ is associated with autoimmune diseases (Ahmed, 2000), and IL-5 plays important roles in pathogenesis of asthma, hypereosinophilic syndromes and eosiniphil-dependent inflammatory diseases (Nagai *et al*., 1999; Takatsu *et al*., 2009). In this study, we demonstrated that ATZ, PFOA and ZEA caused the overtranscription of IFNγ, IL-5 and TNFα mRNA in Jurkat cells. Taken together, it suggested that the overproduction of these cytokines after EDs exposure may cause a number of immune disorders.

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