Effects of Various Chemicals Including Endocrine Disruptors and Analogs on the Secretion of Th1 and Th2 Cytokines from Anti CD3-Stimulated Mouse Spleen Cells

Kazuma Yano,[a] Shuji Ohno,[a] Yonako Nakajima,[a] Satoshi Toyoshima,[b] and Shizuo Nakajin*,[a]

[a]Department of Biochemistry, Hoshi University School of Pharmacy and Pharmaceutical Sciences, 2–4–41 Ebara, Shinagawa-ku, Tokyo 142–8501, Japan and [b]Pharmaceuticals and Medical Devices Evaluation Center, National Institute of Health Science, 3–8–21 Toranomon, Minato-ku, Tokyo 105–8409, Japan

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The *in vitro* effects of various chemicals, such as styrene dimers, styrene trimers, alkylphenols, phthalate esters, phytoestrogens, and organotin compounds, on the production of interferon-gamma (IFN-γ), a type 1 helper T-cells (Th1) specific cytokine, and interleukin-4 (IL-4), a type 2 helper T-cells (Th2) specific cytokine, which are secreted from anti CD3-stimulated mouse spleen cells, were examined. These chemicals suspected of having an endocrine disruptor function and to which humans may become exposed *via* ingestion through food, food containers, and food packaging. It was found the organotin compounds bis(tributyltin) oxide, tributyltin chloride, and dibutyltin dichloride at concentrations which did not elicit any cytotoxicity inhibited secretion of the Th1 and Th2 specific cytokines IFN-γ and IL-4 at concentrations (0.01–0.03, 0.07–0.1, and 0.096–0.132 μM for IC_{50}, respectively) that were much lower than those of the other chemicals. However, these butyltin compounds exhibited similar degrees of inhibitory effects on IFN-γ and IL-4 secretion and did not selectively inhibit the secretion of one or the other cytokine. However, diphenyltin dichloride and phenyltin trichloride enhanced the secretion of IL-4 at the comparatively low concentrations of 0.3 μM and 1.0 μM, respectively, although these compounds significantly inhibited IFN-γ secretion at the same concentrations. In addition, 4-*t*-pentylphenol enhanced IL-4 secretion although it inhibited IFN-γ secretion at the comparatively high concentration of 30 μM. It was also found that some styrene trimers, phthalate esters and flavonoids as well as the alkyl phenols octylphenols and nonylphenol among others, inhibited the secretion of both cytokines at comparatively high concentrations (< 30 μM).

Key words —— spleen cells, type 1 helper T-cells, type 2 helper T-cells, cytokines, endocrine disruptors

INTRODUCTION

Ever since the classification, due to the different cytokines produced, of two helper T-cell populations established from mice, namely type 1 and type 2 helper T-cells (Th1 and Th2), a wide variety of research has been conducted, and it has now become clear that various immunoreactions are controlled by these Th1 and Th2 cells.[1] It has also been found that the same types of T-cell subsets exist in humans as well, and there have been many reports indicating that these cells play important roles in the onset of various diseases.[2] Th1 cells are involved in cell-mediated immunity *via* their secretion of interleukin-2 (IL-2) and interferon-gamma (IFN-γ), while Th2 cells are involved in humoral immunity *via* their secretion of IL-4, IL-5, and other interleukins.[3] These two cell populations, reflected by the differences in the cytokines produced, possess different functions in immunoreactions, and control immunoreactions in phylaxis and immune diseases through a reciprocal balancing mechanism.[4] Furthermore, it has been suggested control of the balance between these Th1 and Th2 cells is mediated by cytokines.[5] Therefore, it is thought the failure or breakdown of the Th1-Th2 balance control mechanism is the cause of the onset of many immune diseases. In particular, diseases which are Type I reaction allergies such as atopic dermatitis and pollinosis are believed to be caused by an imbalance in the Th1-Th2 balance, in the direction of Th2,

*To whom correspondence should be addressed: Department of Biochemistry, Hoshi University School of Pharmacy and Pharmaceutical Sciences, 2–4–41 Ebara, Shinagawa-ku, Tokyo 142–8501, Japan. Tel.: +81-3-5498-5775; Fax: +81-3-5498-5776; E-mail: nakajin@hoshi.ac.jp

which is due to the increased secretion of cytokines such as IL-4 and IL-5 by Th2 cells.[6,7]

On the other hand, it has been pointed out in recent years there is a strong likelihood that chemicals present in the environment, termed endocrine disruptors, may adversely affect the reproduction of wild animals and cause the feminization of male organisms. It is also feared these chemicals may have deleterious effects on human health.[8] However, we are concerned not only about the risks and dangers on reproduction and the feminization of males associated with these endocrine disruptors, but also the causal relation with Type I allergic immune diseases, which have been increasing dramatically in recent years. Therefore, in the present paper, we have conducted the following experiment as part of our effort to elucidate the effects of various chemicals, including typical endocrine disruptors and their analogs, on the homeostasis of biological immunoreactions. Of various chemicals suspected of having an endocrine disruptor function, we examined the *in vitro* effects of various chemicals to which humans may be exposed *via* ingestion through food, food containers, and food packaging, on the production of IFN-γ, a Th1 specific cytokine, and IL-4, a Th2 specific cytokine, that are secreted from anti CD3-stimulated mouse spleen cells.

MATERIALS AND METHODS

Chemicals —— 2,4-Diphenyl-1-butene, *trans*-1,2-diphenylcyclobutane, *cis*-1,2-diphenyl-cyclobutane, 1,3-diphenylpropane, 2,4,6-triphenyl-1-hexene, 1α-phenyl-4a-(1-phenylethyl)-1,2,3,4 tetra hydro-naphthalene, 1,3,5-triphenylcyclohexane, 4-*n*-nonylphenol (4*n*NP), 4-*n*-octylphenol (4*n*OP), 4-*t*-octylphenol (4*t*OP), 4-*n*-heptylphenol, 4-*n*-hexylphenol, 4-*n*-pentylphenol, 4-*t*-pentylphenol, 2-*t*-butylphenol, 3-*t*-butylphenol, 4-*t*-butylphenol, phthalic acid, bisphenol A, and monooctyltin oxide were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Butyl benzyl phthalate, di(2-ethylhexyl) phthalate (DEHP), dicyclohexyl phthalate (DCHP), diethylhexyl adipate (DEHA), diethyl phthalate, di-*n*-butyl phthalate (DBP), di-*n*-hexyl phthalate, di-*n*-pentyl phthalate, dipropyl phthalate, 4-*br*-nonylphenol (4*br*NP: mixture of 4-nonyl phenol isomers having different nonyl chain branches), 4-*n*-butylphenol, 4-dodecylphenol, and trimethyltin chloride were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Methyltin trichloride, butyltin trichloride, dibutyltin dichloride (DBT), tributyltin chloride (TBT), tetrabutyltin, bis(tributyltin) oxide (TBTO), dioctyltin dichloride, phenyltin trichloride, diphenyltin dichloride, triphenyltin chloride (TPT), and tetraphenyltin were purchased from Sigma-Aldrich Japan K. K. (Tokyo, Japan). Dimethyltin dichloride and trioctyltin chloride were obtained from Merck Japan (Tokyo, Japan) and Fluka (Buchs, Switzerland), respectively. Daidzein, daidzin, genistein, genistin, biochanin A, glycitein, glycitin, formononetin, equol, quercetin, and the various hydroxyflavones used were purchased from Funakoshi Co., Ltd. (Tokyo, Japan). The CytoTox96 non-radioactive cytotoxicity assay kit was purchased from Promega Corp., (Madison, WI, U.S.A.).

All of the reagents were of the best grade commercially available.

Culture of Mouse Spleen Cells —— The spleens of 6 week old female CBA/J mice (Oriental Yeast Co., Ltd., Japan) were resected and spleen cells suspension were prepared by the flushing method for culturing. The spleen cells were washed with RPMI1640 (Medium ICN, Aurora, OH, U.S.A.) medium containing 10% fetal bovine serum (HyClone) and then 5×10^6 cells/ml were cultured with the same medium on 48 well culture plates precoated with anti-mouse CD3 antibodies [Anti-mouse ε-T3 complex (CD3ε) monoclonal antibody-ascites, Hornby] in an atmosphere of 5% CO_2-95% air. Each chemical at the concentrations tested was added to the culture plate at the same time as the cells. All chemicals were dissolved in ethanol. The final concentration of alcohol in the assay medium was maintained so as not to exceed 0.1%(v/v). It was confirmed that this concentration of alcohol did not affect IFN-$\gamma$and IL-4 cytokine secretion levels from the cells. After incubation for 48 hr, conditioned medium was obtained by centrifugation and the cytokine concentrations were determined by enzyme-linked immunosorbent assay (ELISA) as described below. The cytotoxicity of each chemical was tested using a CytoTox96 non-radioactive cytotoxicity assay kit that determines lactate dehydrogenase (LDH) activity.

Determination of Secreted Cytokines —— The concentrations of the cytokines IL-4 and IFN-γ in the conditioned medium were determined by ELISA. Purified anti-mouse IL-4 antibody (PharMingen, U.S.A., Clone: 11B11, rat IgG_1) and

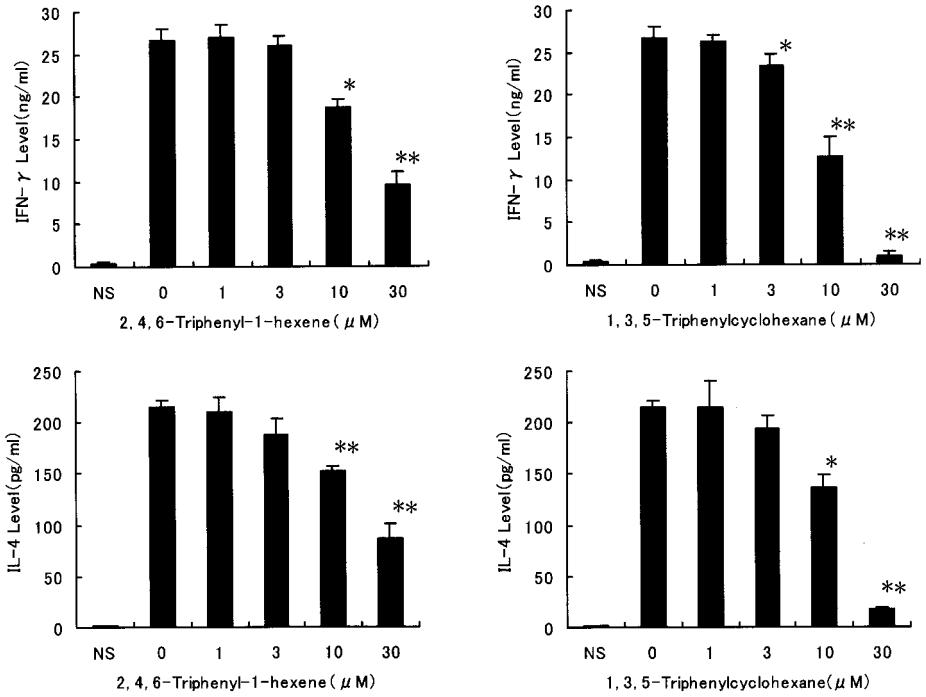

Fig. 1. Effect of Exposure to the Styrene Trimers 2,4,6-Triphenyl-1-Hexene and 1,3,5-Triphenylcyclohexane on the Secretion of Helper T Cell Subtype Specific Cytokines from anti CD3-Stimulated Mouse Spleen Cells

Each column represents the mean ± S.E. ($n = 3$). Asterisks denote a significant difference from the control value; $*p < 0.05$ and $**p < 0.01$. NS means no stimulation with anti-mouse CD3 antibodies (NS was used in subsequent figure).

purified anti-mouse IFN-γ antibody (PharMingen, Clone: R4-6A2, rat IgG_1) were used as the immobilized antibodies. Biotinylated anti-mouse IL-4 antibody (PharMingenlone: BVD6-24G2, rat IgG_1) and biotinylated anti-mouse IFN-γ antibody (PharMingenlone: XMG1.2, rat IgG_1) were used as the antibodies for detection. Recombinant mouse IL-4 (PharMingen Baculovirus-infected T. ni cells) and IFN-γ(PharMingen Baculovirus-infected T. ni cells) were used as the standard cytokines.

Statistical Analysis —— Statistical analysis of the data was performed using Student's *t*-test. The point of minimal statistical significance was set at $p < 0.05$.

RESULTS

Effects of Various Chemicals, Including Endocrine Disruptors and Their Analogs, on the Secretion of Th1 and Th2 Specific Cytokines

The results for a typical experiment involving the exposure of mouse spleen cells to a range of concentrations (1–30 μM) of 2,4,6-triphenyl-1-hexene and 1,3,5-triphenylcyclohexan and their effects on the secretion of the Th1 specific cytokine IFN-γ and the Th2 specific cytokine IL-4 are shown in Fig. 1. The results show that the secretion of both IFN-γ and IL-4 was significantly inhibited in a dose–dependent manner. Cytotoxicity associated with these chemicals, which was estimated by the leakage of LDH activity, was not observed compared with the vehicle control for this concentration range. The effects of other styrene dimers and trimers are shown in Table 1 as the 50% inhibitory concentration.

The effects of the alkylphenols 4*n*NP, 4*n*OP, and 4-*t*-pentylphenol are shown in Fig. 2. 4*n*NP and 4*n*OP significantly inhibited the secretion of both IFN-γ and IL-4 in a dose–dependent manner. 4-*t*-Pentylphenol significantly inhibited the secretion of IFN-γ but did not inhibit the secretion of IL-4. In fact, it significantly enhanced IL-4 secretion at 30 μM. The effects of other alkylphenols and bisphenol A are shown in Table 1. Cytotoxicity associated with the alkylphenols tested was not observed at any of the concentrations examined.

The effects of the phthalate ester DEHP are shown in Fig. 3. DEHP dose–dependently inhibited the secretion of both IFN-γ and IL-4. The effects of the adipate ester DEHA are also shown in Fig. 3, however, it did not exhibit any inhibitory effect at the concentrations tested. The effects of other phthalate esters are shown in Table 1. Cytotoxicity was

Table 1. Effects of Exposure to the Various Chemicals, Including Endocrine Disruptors and Analogs, Used in the Present Study which were Evaluated Based on the IC_{50} for the Production of the Spleen Cell Specific Cytokines IFN-γ and IL-4

Chemicals	IFN-γ secretion IC_{50} (μM)	IL-4 secretion IC_{50} (μM)
Styrene dimers and trimers		
2,4-Diphenyl-1-butene	ND	ND
trans-1,2-Diphenylcyclobutane	24.5	ND
cis-1,2-Diphenylcyclobutane	ND	ND
1,3-Diphenylpropane	ND	ND
2,4,6-Triphenyl-1-hexene	16.3	21.0
1a-Phenyl-4a-(1-phenylethyl)-1,2,3,4-tetra-hydronapthalene	9.4	11.4
1,3,5-Triphenylcyclohexane	8.8	14.2
Alkyl phenols		
2-*t*-Butylphenol	ND	ND
3-*t*-Butylphenol	ND	ND
4-*t*-Butylphenol	ND	ND
4-*n*-Butylphenol	ND	ND
4-*t*-Pentylphenol	ND	ND[a)]
4-*n*-Pentylphenol	ND	ND
4-*n*-Hexylphenol	28.0	20.5
4-*n*-Heptylphenol	19.0	ND
4-*t*-Octylphenol (4*t*OP)	11.5	15.0
4-*n*-Octylphenol (4*n*OP)	13.6	11.7
4-*n*-Nonylphenol (4*n*NP)	11.8	9.6
4-*br*-Nonylphenol (4*br*NP)	9.2	20.8
4-Dodecylphenol	6.9	15.8
Bisphenol A	ND	ND
Phthalate Esters		
Phthalic Acid	ND	ND
Diethyl Phthalate	ND	ND
Dipropyl Phthalate	22.5	ND
Di-*n*-butyl Phthalate (DBP)	15.4	15.1
Di-*n*-pentyl Phthalate	13.8	18.2
Di-*n*-hexyl Phthalate	12.8	10.0
Butyl Benzyl Phthalate	18.9	20.0
Di(2-ethylhexyl) Phthalate (DEHP)	12.7	12.8
Dicyclohexyl Phthalate (DCHP)	6.6	9.0
Diethylhexyl Adipate (DEHA)	ND	ND

IC_{50} could not be determined at 30 μM (ND and ND[a)]), 10 μM (ND[b)]), 1.0 μM (ND[c)]) and 0.3 μM (ND[d)]). *a*): IL-4 secretion was significantly enhanced at 30 μM. *c*): IL-4 secretion was extremely enhanced at 1.0 μM, significantly. *d*): IL-4 secretion was extremely enhanced at 0.3 μM, significantly.

not observed in any of the experiments at the range of concentrations examined (up to 30 μM).

The effects of exposure of the mouse spleen cells to daidzein, genistein, and genistin, which have been classified as phytoestrogens, are presented in Fig. 4. Daidzein and genistein at the higher concentrations (10 and/or 30 μM) dose–dependently inhibited the secretion of both IFN-γ and IL-4, with genistein being comparatively more potent at inhibiting the secretion of both cytokines than daidzein. Genistein and its glycoside genistin had nearly equipotent effects on the secretion of both cytokines. The effects of other flavonoids are presented in Table 1. Cytotoxicity was not observed in any experiment at the range of concentrations tested (up to 30 μM).

The effects of exposure of the mouse spleen cells to various organotins were examined, and those of butyltin and phenyltin compounds are shown in

Table 1. Continued

Chemicals	IFN-γ secretion IC_{50} (μM)	IL-4 secretion IC_{50} (μM)
Flavonoids		
Daidzin	ND	ND
Daidzein	ND	ND
Equal	14.6	14.6
Genistin	8.5	17.7
Genistein	11.0	17.5
Glycitin	ND	ND
Glycitein	ND	ND
Biochanin A	13.8	29.0
Formononetin	30.0	ND
4′-Hydroxyflavone	13.5	ND
5-Hydroxyflavone	18.0	25.4
6-Methoxyflavone	15.7	27.0
6-Hydroxyflavone	23.6	ND
7-Hydroxyflavone	20.5	ND
Quercetin	4.8	8.8
Organotins		
Methyltin trichloride	ND[b)]	ND[b)]
Dimethyltin dichloride	2.6	10.0
Trimethyltin chloride	0.42	0.255
Butyltin trichloride	6.6	2.2
Dibutyltin dichloride (DBT)	0.096	0.132
Tributyltin chloride (TBT)	0.074	0.096
Tetrabutyltin	2.8	ND[b)]
bis(Tributyltin) oxide (TBTO)	0.014	0.033
Dioctyltin dichloride	1.98	0.86
Trioctyltin chloride	3.5	4.6
Phenyltin trichloride	0.39	ND[c)]
Diphenyltin dichloride	0.25	ND[d)]
Triphenyltin chloride (TPT)	0.10	0.059
Tetraphenyltin	ND[c)]	ND[c)]

Fig. 5. Inhibition of secretion of the Th1 specific cytokine IFN-γ and the Th2 specific cytokine IL-4 were observed at extremely low concentrations of TBTO, TBT, and DBT compared to the other organotin compounds. Since cytotoxicity was not observed at concentrations below 0.3 μM, it is clear that extremely low concentrations of these three compounds inhibit the secretion of both cytokines. Tetrabutyltin and butyltin trichloride inhibited the secretion of both cytokines at relatively higher concentrations (1–10 μM). Neither of these butyltins exhibited cytotoxicity at this concentration range. The effects of phenyltin compounds on the secretion of IFN-γ and IL-4 are shown in Fig. 5. As was the case for some of the butyltin compounds, triphenyltin chloride, diphenyltin dichloride, and phenyltin trichloride inhibited the secretion of IFN-γ at extremely low levels at which cytotoxicity was not observed. However, diphenyltin dichloride and phenyltin trichloride enhanced the secretion of IL-4 at 0.3 μM and 1.0 μM, respectively, concentrations at which cytotoxicity was not observed. Tetraphenyltin did not affect IL-4 production at concentrations from 0.03 to 1.0 μM. The effects of other organotin compounds are shown in Table 1.

Effects of Estrogen and the Estrogen Receptor Antagonist ICI 182780 on the Secretion of Th1 and Th2 Specific Cytokines

The effects of exposure of the mouse spleen cells to a typical estrogen, estradiol were examined. No effect was observed on the secretion of either IFN-γ

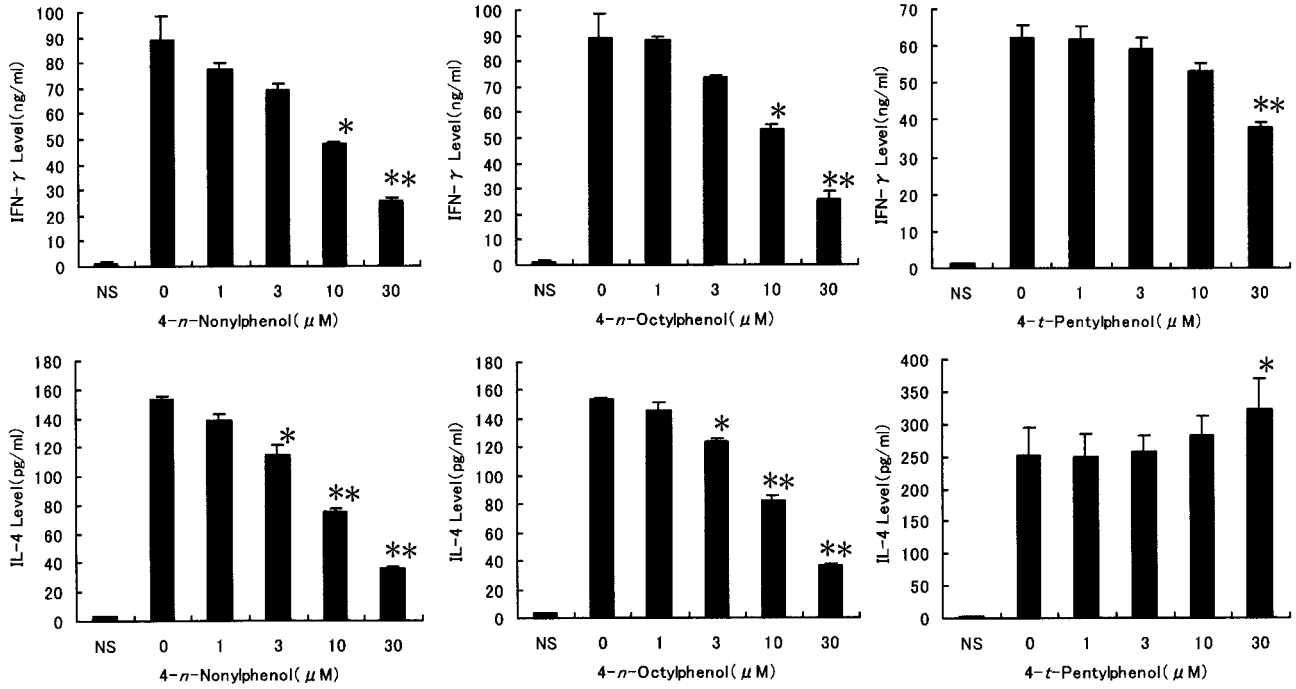

Fig. 2. Effect of Exposure to the Alkylphenol Compounds 4*n*NP, 4*n*OP, and 4-*t*-Pentylphenol on the Secretion of Helper T Cell Subtype Specific Cytokines from anti CD3-Stimulated Spleen

Each column represents the mean ± S.E. ($n = 3$). Asterisks denote a significant difference from the control value; *$p < 0.05$ and **$p < 0.01$.

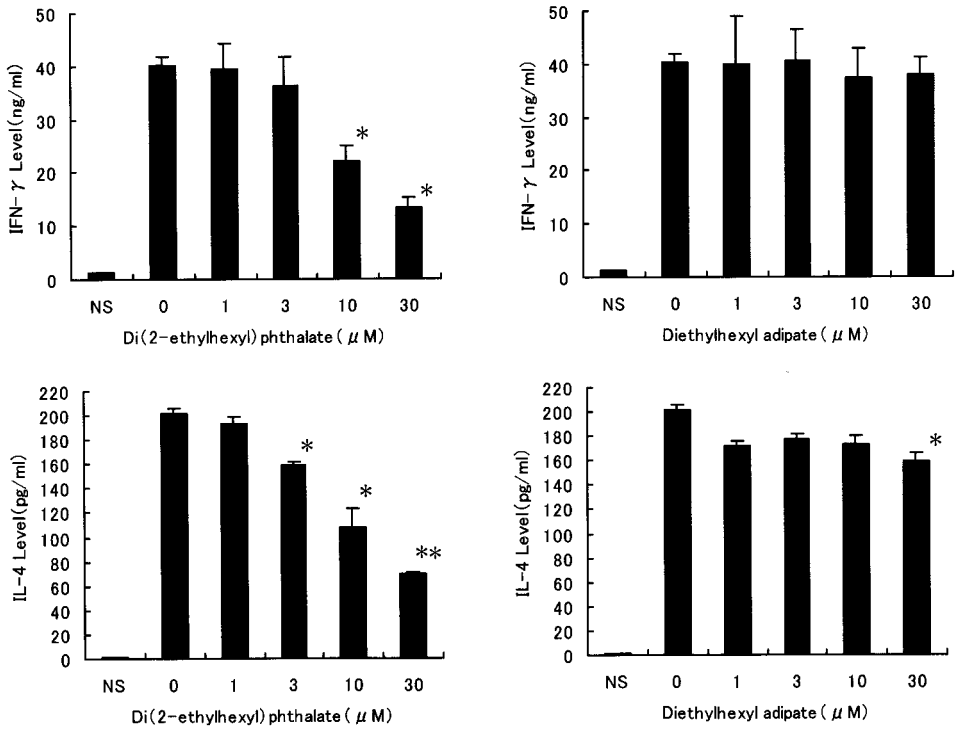

Fig. 3. Effect of Exposure to the Phthalate Ester DEHP and Adipate Ester DEHA on the Secretion of Helper T Cell Subtype Specific Cytokines from anti CD3-Stimulated Spleen Cells

Each column represents the mean ± S.E. ($n = 3$). Asterisks donate a significant difference from the control value; *$p < 0.05$ and **$p < 0.01$.

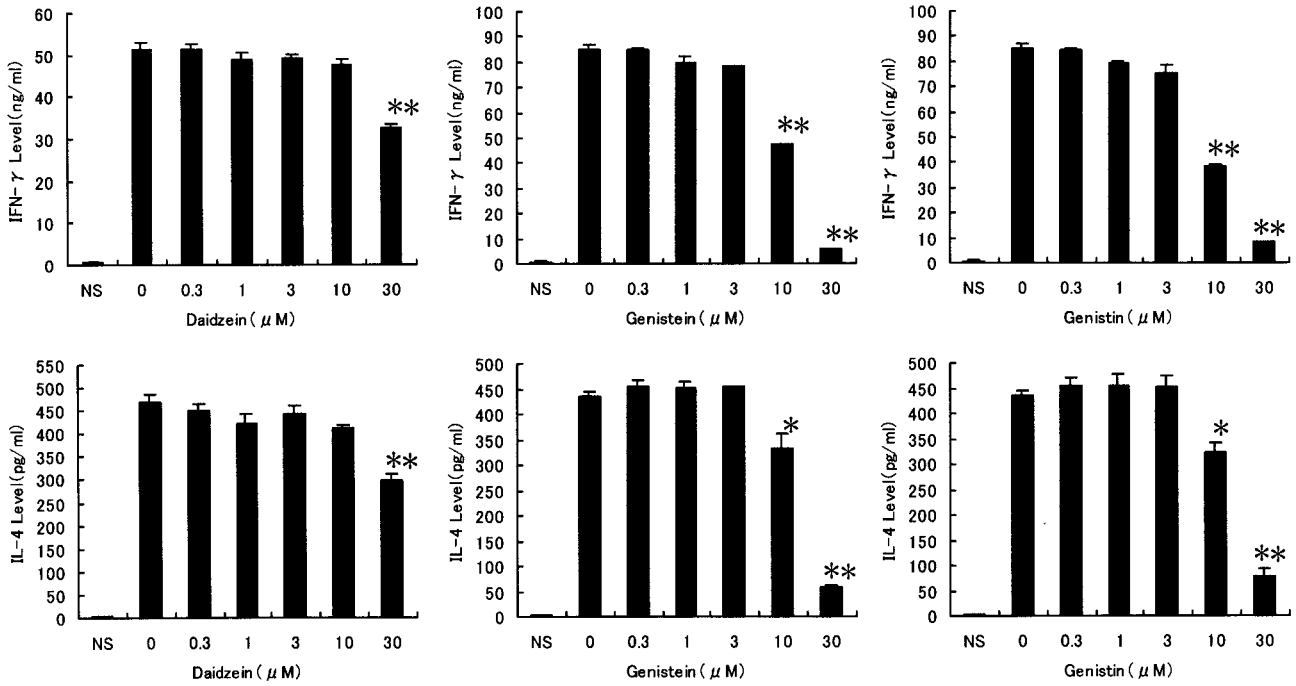

Fig. 4. Effect of Exposure to the Isoflavones Daidzein, Genistein, and Genistin on the Secretion of Helper T Cell Subtype Specific Cytokines from anti CD3-Stimulated Spleen Cells

Each column represents the mean ± S.E. ($n = 3$). Asterisks denote a significant difference from the control value; $*p < 0.05$ and $**p < 0.01$.

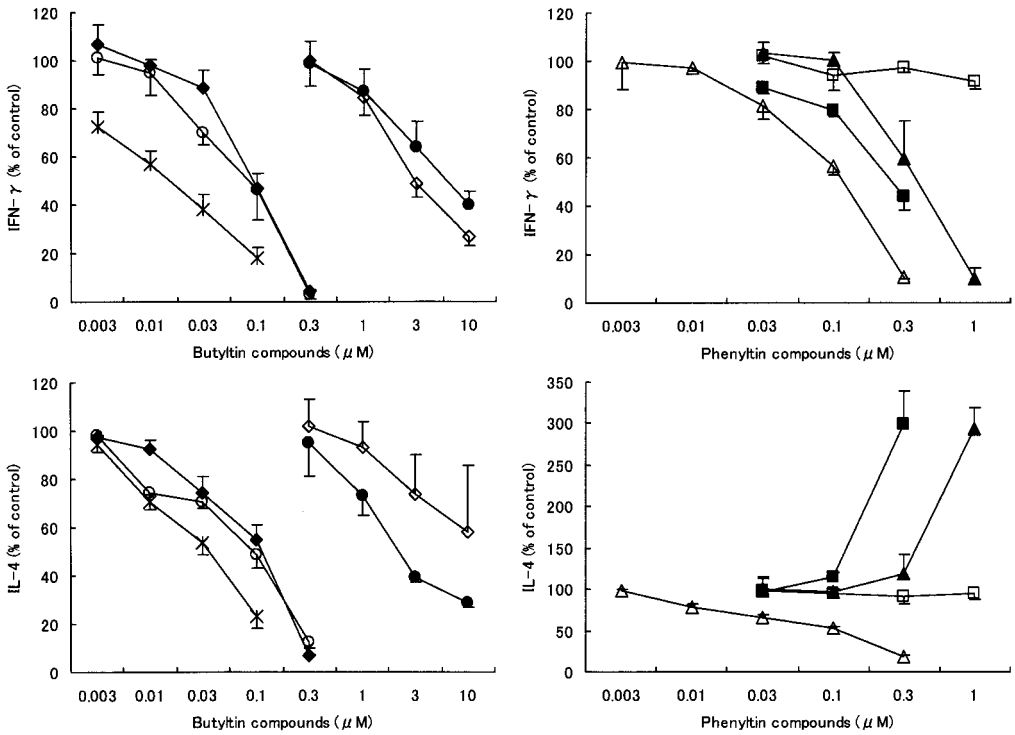

Fig. 5. Effect of Exposure to Butyltin and Phenyltin Compounds on the Secretion of Helper T Cell Subtype Specific Cytokines from anti CD3-Stimulated Spleen Cells

Each value is the mean ± S.E. ($n = 3$) of triplicate assay. ●: butyltin trichloride, ◆: DBT, ○: TBT, ◇: tetrabutyltin, ∗: TBTO, ▲: phenyltin trichloride, ■: Diphenyltin dichloride, △: TPT, □: tetraphenyltin.

or IL-4 at the concentrations of 0.003–10 μM used with our assay method (data not shown).

Individual exposure to 10 μM of 2,4,6-triphenyl-1-hexene, 1,3,5-triphenyl-cyclohexan, 4*n*OP, 4*n*NP, DEHP, daidzein and genistein, and 0.1 μM of TBT and TPT is sufficient to inhibit the secretion of either cytokine from mouse spleen cells. The addition of ICI 182780 (1.0 μM), which was added to the medium 1 hr prior to the addition of sample chemical, did not restore the inhibition of secretion of either cytokine (data not shown). On the other hand, exposure to 4-*t*-pentylphenol (30 μM), diphenyltin dichloride (0.3 μM), or phenyltin trichloride (1.0 μM) inhibited the secretion of IFN-γ, but enhanced the secretion of IL-4 at the same concentrations. The addition of ICI 182780 (1.0 μM) did not restore the inhibition or enhancement in secretion of the respective cytokine (data not shown).

Risk Assessment of Various Chemicals, Including Endocrine Disruptors and Their Analogs, on the Secretion of Th1 and Th2 Specific Cytokines

Table 1 shows the risks associated with exposure to various chemicals, including endocrine disruptors and their analogs, used in the present study that were evaluated by determining the concentrations which inhibited production of the spleen cell specific cytokines IFN-γ and IL-4 by 50% (IC_{50}). The results show that organotin compounds, in particular TBTO, TBT, and DBT, inhibited the secretion of the Th1 and Th2 specific cytokines at concentrations that were much lower than those for the other compounds tested. It was also observed that DEHP, DCHP, the alkyl phenols OP (4*t*, 4*n*) and NP (4*br*, 4*n*), and other compounds inhibited the secretion of Th1 and Th2 specific cytokines at comparatively high concentrations. More noteworthy, diphenyltin dichloride and phenyltin trichloride enhanced the secretion of IL-4 at the comparatively low concentrations of 0.3 μM and 1.0 μM, respectively, although these compounds inhibited IFN-γ secretion at the same concentrations. Tetraphenyltin did not affect the secretion of either cytokine. In addition, 4-*t*-pentylphenol enhanced IL-4 secretion, although it inhibited IFN-γ secretion at a comparatively high concentration (30 μM).

DISCUSSION

In the present study, we have attempted to examine the effects of various chemicals, including typical endocrine disruptors and their analogs, on the secretion of Th1 and Th2 specific cytokines as part of an effort to elucidate their effects on the homeostasis of biological immunoreactions. Lymphocyte progenitor cells produced in bone marrow differentiate and mature in the primary immune organ, the thymus, while at the same time eliminate T-cells that react with self-antigens. The progenitor cells are then distributed to and circulate in the secondary immune organs, namely the spleen and lymph nodes. In the present research, primary cultures of spleen cells were conducted and then T-cell selective antigen stimulation was carried out by stimulation with CD3 antibodies.

It has been reported that various kinds of styrene dimers and styrene trimers are present in polystyrene products which are widely employed as food containers,[9] and these compounds have been put on the list of potential endocrine disruptors. It has been suggested that these dimers and trimers may migrate from the polystyrene packaging material into the food, particularly from the polystyrene containers used in Japanese instant noodles that contain a large fat content and use boiling water during preparation.[10,11] Phthalate esters, especially DEHP and DBP, are widely used as plasticizers in plastic products, suggesting that they may migrate from plastic food containers and packaging into the food itself. Alkylphenol compounds, which are obtained by the decomposition of plastic raw materials and industrial surfactants, have also been put on the watch list of potential endocrine disruptors. 4NP, a degradation product of the antioxidant tris(nonylphenyl) phosphite as well as surfactants such as nonyl ethoxylates, may be a residue in plastic products. In recent years, plastic film for wrapping foods, which is widely used in supermarkets and households, is an example of one of its uses. Plant-derived chemicals with estrogen-like activity are called phytoestrogens. Soybeans and soybean products contain large amounts of isoflavones, which are a type of phytoestrogen. They have been included on the list of endocrine disruptors because of their estrogenic activity. In the present research, we have studied flavonoids, plant constituents that may be ingested as foods, particularly isoflavones that are present in large quantities in soy and soybean prod-

ucts. Organotin compounds are added as heat degradation stabilizing agents and stabilizing agents to increase the UV resistance of products during the formation of polyvinyl chloride. They have also been used for many years around the world as antifouling agents in marine paints and fishing nets. Their use is now restricted because of concerns about their harmful effects on marine organisms and it is thought the amount being used has decreased. However, it has been reported that organotin levels in marine organisms still remain high due to biological concentration, bioaccumulation, and other phenomena, even though the concentrations polluting the marine environment are extremely low.[12–17] It is thought that humans may be exposed to the compounds *via* the food chain.

In the present study, we have examined the effects of these chemicals and these analogs on the secretion of Th1 and Th2 specific cytokines from anti CD3-stimulated mouse spleen cells. We have also studied the cytotoxicity using a CytoTox96 nonradioactive cytotoxicity assay that determines the activity of LDH that has leaked outside the cells as an index of cytotoxicity. It was found the organotin compounds TBTO, TBT, and DBT at concentrations which did not elicit any cytotoxicity inhibited the secretion of the Th1 and Th2 specific cytokines IFN-γ and IL-4 at concentrations that were much lower than those of the other organotin compounds (0.01–0.03, 0.07–0.1, and 0.096–0.132 μM for LD_{50}, respectively). Furthermore, these butyltin compounds exhibited a similar degree of inhibitory effects on IFN-γ and IL-4 secretion and did not selectively inhibit the secretion of one or the other cytokine. In addition, although the concentration of IC_{50} is higher than that of organotin compounds, 2,4,6-triphenyl-1-hexene, 1,3,5-triphenyl-cyclohexan, 4*n*OP, 4*n*NP, DEHP, dicyclohexyl phthalate, genistin, genistein and quercetin were also exhibited a similar degree of inhibitory effects on IFN-γ and IL-4 secretion and did not selectively inhibit the secretion of one or the other cytokine. It is conceivable based on the results of the present experiments that the inhibition or failure of antigen-specific immunoreactions is brought about by exposure to organotin compounds such as tributyltin and/or to other chemicals pointed out above. However, it is worth noting that diphenyltin dichloride and phenyltin trichloride enhanced the secretion of IL-4 at 0.3 μM and 1.0 μM, respectively, which are comparatively low concentrations although these compounds inhibited IFN-γ secretion at the same concentrations. Tetraphenyltin did not affect the secretion of either cytokine. In addition, 4-*t*-pentylphenol enhanced IL-4 secretion although it inhibited IFN-γ secretion at a comparatively high concentration (30 μM). Accordingly, it is thought that the failure or breakdown of the Th1-Th2 balance control mechanism, more specifically, an imbalance in the Th1-Th2 balance, in the direction of Th2, which is due to increased secretion of the Th2 specific cytokine IL-4 by exposure to these chemicals, may induce diseases which are Type I reaction allergies such as atopic dermatitis and pollinosis.

Many environmental chemicals including phthalate esters, alkylphenols, and phytoestrogens that are suspected of being endocrine-disrupting agents have a native estrogen-like structure and show weak estrogenic activity, and thus have been dubbed environmental estrogens.[18–25] Recently, the existence of the estrogen receptor α in mouse splenic T cells has been reported.[26] In this paper, it was found that some phthalate esters, alkylphenols, and flavonoids inhibit the secretion of Th1 and Th2 specific cytokines at comparatively high concentrations (4.8–30 μM for IC_{50}). Therefore, it is thought these chemicals may act *via* estrogen receptors. We examined the effects of a typical estrogen, estradiol, on the secretion of Th1 and Th2 specific cytokines, however, no effects were observed on the secretion of either cytokine. Furthermore, treatment with the estrogen receptor antagonist ICI 182780 did not restore the environmental chemical-induced inhibition or enhancement of secretion of either cytokine. These results suggest that the inhibition or enhancement of cytokine secretion by these typical environmental chemicals is not mediated through estrogen receptors. On the other hand, in this paper, CD3 antibodies were used for activation of lymphocytes from mouse spleen cells. We have to consider that a chemical which inhibit cytokine secretion affect activation process of lymphocyte by anti CD3 antibodies.

In the future, studies that examine this issue in greater detail are needed.

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