Effect of a Single Oral Dose of 2,3,7,8-Tetrachlorodibenzo-p-dioxin on Immune Function in Male NC/Nga Mice

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Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces immunosuppression in humans and animals. However, the effect of TCDD on Th2-type immune responses such as allergic reactions has been unclear. Using NC/Nga mice that developed atopic dermatitis-like skin lesions with marked elevation in plasma of total IgE when bred under conventional conditions, we investigated the effects of a single oral dose of TCDD on immune responses. NC/Nga mice received a single oral dose (0 or 20 μg/kg body weight) of TCDD. On day 7, treatment with TCDD alone decreased the cellularity of thymus. However, treatment with TCDD modified the cellularity of spleens and mesenteric lymph nodes (MLNs) but not of the thymus on day 28. When NC/Nga mice received ip immunization with OVA and alum on the same day as the TCDD treatment (0, 5, or 20 μg/kg body weight), TCDD markedly suppressed the concentrations of Th2-type cytokines (e.g., IL-4 and IL-5) in culture supernatants of spleen cells, whereas IFN-γ production significantly increased. TCDD exposure reduced anti-OVA and total IgE antibody titers in plasma and did not induce the development of atopic dermatitis-like lesions in the pinnae or dorsal skin of NC/Nga mice. These results suggest that in NC/Nga mice, exposure to TCDD may impair the induction of Th2-type immune responses.

Key Words: allergy; NC/Nga; TCDD; Th1/Th2; immunosuppression; cytokine.

Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces various types of immune dysfunction. House et al. (1990) reported that a single ip injection of TCDD increased susceptibility to influenza viral infection in female B6CF1 mice, but their susceptibility to Listeria monocytogenes did not vary. It also impaired resistance to infection with Trichinella spiralis in mice and rats (Luebke et al., 1994, 1995) and enhanced mortality due to influenza virus in mice (Burleson et al., 1996). Moreover, these investigators observed that exposure to TCDD had no demonstrable effect on the functions of natural killer (NK) cells or macrophages. In contrast, TCDD suppressed virus-augmented pulmonary NK activity and enhanced the susceptibility of rats to influenza virus (Yang et al., 1994). Delayed-type hypersensitivity (DTH) response to ovalbumin (OVA) and bovine serum albumin was suppressed following an injection of TCDD (Gehrs and Smialowicz, 1999; Lundberg et al., 1992). Together, these results indicate that exposure to TCDD suppresses resistance to bacterial and viral infection and DTH response, suggesting that TCDD decreases Th1-type immune responses.

Considerable advances have been made in understanding the underlying mechanisms of immunological abnormalities induced by environmental pollutants. For example, exposure to diesel exhaust particulates modulated the balance of Th1/Th2-type immune responses (Nel et al., 1998). Diesel exhaust particulates enhanced Th2-type cytokine productions such as interleukin (IL)-4 and IL-5 (Fujimaki et al., 1994; Takano et al., 1997). Karras et al. (1995) studied the ability of TCDD to mimic hallmark responses of B cells to IL-4 in vitro and showed that TCDD did not mimic the actions attributed to IL-4. Preincubation with TCDD resulted in decreased production of both IgG1 and IgE in B-cell class switching systems. In vivo studies addressing the effects of TCDD on Th2-type immune responses have not been done previously.

Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease with altered immune responses (Hanifin, 1982). The maintenance of spongiotic dermatitis is associated with elevated T lymphocyte activation, hyperstimulation of Langerhans cells, defective cell-mediated immunity, and B-cell IgE overproduction (Cooper, 1994). Recently, Grewe et al. (1998) indicated that the development of skin lesions in AD patients resulted from sequential activation of Th2- then Th1-type cells. NC/Nga mice bred under conventional conditions develop AD-like lesions, which correlate in severity with the increase in the plasma IgE level (Matsuda et al., 1997; Matsu- moto et al., 1999). In NC/Nga mice, some kinds of environmental factors might trigger AD-like signs and symptoms (Suto et al., 1999). Th2-type chemokines such as thymus- and activation-regulated chemokines and macrophage-derived chemokines participate in the pathogenesis of these lesions in this
strain (Vestergaard et al., 1999). These results suggest that NC/Nga mice are an extremely useful model for investigating the causal relationship between environmental and genetic factors in the induction of AD.

The incidence of allergic diseases such as asthma and AD has been increasing, particularly in developed countries (Björkstén, 1999; Burr et al., 1989; von Mutius, 2000). Environmental factors may contribute to the observed increase in allergic inflammation. We hypothesize that environmental chemicals such as TCDD and related compounds affect the balance of Th1/Th2-type immune responses directly or indirectly, resulting in increased allergic inflammation. Exposure to TCDD may induce IgE overproduction and the signs of AD-like skin lesions, or may exacerbate AD-like signs.

In this study, we administered a single dose of TCDD to NC/Nga mice with or without immunization with OVA as a dietary allergen and investigated the possible involvement of TCDD in the induction of AD-like signs. In particular, as our initial studies we have focused on features of Th2-type immune reactions.

MATERIALS AND METHODS

Animals. Male NC/Nga mice (5 weeks old) were purchased from CRJ (Tokyo, Japan) and allowed to acclimatize for 1 week before their use. Food and water were given ad libitum. The study was approved by the ethics committee for experimental animals at NIES.

Exposure to TCDD. To investigate the effect of TCDD on lymphoid organs, each mouse received a single po dose (0 or 20 μg/kg body weight) of TCDD in corn oil. On day 7 and 28, the mice were sacrificed under ether anesthesia, and thymus and spleen were collected. To clarify the effect of TCDD on Th1- and Th2-type immune reactions, each mouse received ip immunization with OVA and alum on the same day as the TCDD treatment (0, 5, or 20 μg/kg body weight) and were boosted 21 days later. On day 28 after the TCDD dose, the mice were sacrificed under ether anesthesia, and samples of the thymus, spleen, mesenteric lymph nodes (MLNs), pinnae, skin (from the animal’s back), and blood were collected.

Cell preparation and flow cytometry. Cell suspensions of each tissue were generated by forcing the samples through stainless-steel mesh into RPMI-1640 medium (pH 7.2–7.3; Dainippon Pharmaceutical Co., Osaka, Japan) containing 10% heat-inactivated fetal calf serum, 2 mM L-glutamine (Gibco BRL, Life Technologies, Grand Island, NY), 0.1 mM nonessential amino acids (Gibco BRL), 5 mM HEPES (Nacalai Tesque, Kyoto, Japan), penicillin (100 units/ml; ICN Biomedicals, Aurora, OH), and streptomycin (100 μg/ml; ICN Biomedicals). These suspensions were centrifuged, and the cells were counted and stained. The immunophenotype of the cells from each group of mice was analyzed by using a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, Mansfield, MA). The mAbs used were purchased from Pharmingen (San Diego, CA) and included phycoerythrin (PE)-labeled rat IgG anti-CD4 (clone GK1.5), fluorescein isothiocyanate (FITC)-labeled rat IgG anti-CD8a (clone 53-6.7), PE-labeled hamster IgG anti-CD3 (clone 145-2C11), FITC-labeled rat IgG anti-CD45R/B220 (clone RA3-6B2), and corresponding isotype-matched controls.

Assay for cell proliferation and cytokine levels. Cell proliferation was measured by using a colorimetric assay with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Chemicon International, Temecula, CA). Spleen cells (2.5 × 10⁶ cells/well) from each mouse were cultured with OVA in 200 μl of the supplemented RPMI-1640 medium in 96-well flat-bottomed microplates (Nunc Ltd., Roskilde, Denmark). After 72 h, the optical density (OD) at 570 and 630 nm was measured by using a microplate reader (Model 550, Bio-Rad Laboratories, Hercules, CA).

For measuring cytokine levels, spleen cells and pooled MLN cells from each mouse were plated at 2 × 10⁶ cells/well in 96-well flat-bottomed microplates and were cultured in the presence of 20 μg OVA in 200 μl of the supplemented RPMI-1640 medium. After 48 h, the culture supernatants were collected and stored at −70°C. Levels of IL-2, IL-4, IL-5, IL-12, and IFN-γ in the culture supernatants were measured by using commercially available mouse cytokine ELISA kits (Endogen, Woburn, MA, and Amersham International, Little Chalfont, Buckinghamshire, England). The absorbance at 450 and 550 nm was measured in duplicate with a microplate reader (Model 550; Bio-Rad Laboratories). The average absorbance of the standards was plotted against their cytokine concentration in each sample was determined by interpolation from the standard curve.

Measuring total and anti-OVA IgE titers in plasma. Plasma total and anti-OVA IgE titers were measured by ELISA (Takano et al., 1997). Briefly, 96-well microimmunoplates (Nunc) were treated overnight with antime IgE monoclonal antibody, blocked with Block Ace (Dainippon Pharmaceutical Co.), and incubated for 1 h at 37°C with 100 μl aliquots of the diluted samples or standards (mouse IgE; Seikagaku Kogyo Co., Ltd. Tokyo and anti-OVA mouse IgE; a gift from Dr. H. Nagai). After 6 washes, 100 μl bionylinated antime IgE (Pharmingen; diluted 1/1000) or bionylinated OVA was added to each well for 30 min at 37°C. After washing, each well was incubated with 100 μl streptavidin-horseradish peroxidase conjugate (Bibco BRL) for 1 h at room temperature. After an additional wash, the substrate solution was added and developed. The OD at 490 and 595 nm was determined by using a microplate reader (Bio-Rad Laboratories).

Measuring anti-OVA IgG1 and IgG2a titers in plasma. Plasma titers of antigen-specific IgG1 and IgG2a antibodies were measured by ELISA. Briefly, for titration of OVA-specific IgG1 or IgG2a, the microplates were treated overnight with OVA, blocked with Block Ace, and incubated for 1 h at 37°C with plasma samples (diluted 1:100). After 6 washes, horseradish peroxidase-labeled antime IgG1 or IgG2a antibodies (Southern Biotechnology, Birmingham, AL) were added, and the plates were incubated for 1 h. After an additional wash, the substrate solution containing o-phenylenediamine and H₂O₂ in substrate buffer (40.3 mM citric acid and 126.3 mM NaH₂PO₄) was added and developed. The OD at 490 and 595 nm was determined by using a microplate reader (Bio-Rad Laboratories).

Histological analysis. Pinnae and skin samples were fixed with 10% buffered formalin and embedded in paraffin. Each deparaffinized section was stained with hematoxylin and eosin (HE) and analyzed by using light microscope. Mast cells were stained with toluidine blue (1% aqueous solution).

Statistical analysis. All data are presented as the mean ± SE, which is indicated by bars in the figures. Statistical analysis was performed with the StatMate II Statistical analysis system for Microsoft Excel version 5.0 (Nankodo Inc., Tokyo, Japan). Data were analyzed by one-way analysis of variance with post hoc analysis using Dunnett’s multiple comparison test. A p-value less than 0.05 was considered to be significant.

RESULTS

Changes in Wet Weight and Number of Cells from the Thymus and Spleen of TCDD-Exposed Male NC/Nga Mice without Immunization

To investigate the effect of TCDD on thymus and spleen in NC/Nga mice, NC/Nga mice received a single oral dose of 20 μg/kg TCDD or vehicle (control) without immunization. TCDD administration resulted in a decrease in the number of thymocytes and in the wet weight of spleen (Table 1). The number of splenocytes in TCDD-exposed mice did not differ.
EFFECT OF TCDD ON IMMUNE FUNCTION

TABLE 1
Effects of TCDD on Thymus and Spleen in NC/Nga Mice

<table>
<thead>
<tr>
<th>TCDD (µg/kg)</th>
<th>Thymus wt (mg)</th>
<th>Thy/bw</th>
<th>No. thymocytes (×10³)</th>
<th>Spleen wt (mg)</th>
<th>Sp/bw</th>
<th>No. splenocytes (×10³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>40 ± 4</td>
<td>1.77 ± 0.20</td>
<td>7.72 ± 0.67</td>
<td>69 ± 3</td>
<td>3.05 ± 0.18</td>
<td>1.35 ± 0.12</td>
</tr>
<tr>
<td>20</td>
<td>30 ± 4</td>
<td>1.33 ± 0.16</td>
<td>4.70 ± 0.70 *</td>
<td>58 ± 2 *</td>
<td>2.57 ± 0.10</td>
<td>1.10 ± 0.14</td>
</tr>
<tr>
<td>Day 28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>37 ± 2</td>
<td>1.35 ± 0.08</td>
<td>4.72 ± 0.67</td>
<td>63 ± 3</td>
<td>2.28 ± 0.07</td>
<td>0.97 ± 0.06</td>
</tr>
<tr>
<td>20</td>
<td>37 ± 2</td>
<td>1.38 ± 0.07</td>
<td>3.44 ± 0.70</td>
<td>67 ± 2</td>
<td>2.52 ± 0.06 *</td>
<td>1.17 ± 0.70</td>
</tr>
</tbody>
</table>

Note. Each mouse received a single oral dose (0 or 20 µg/kg body weight) of TCDD in corn oil. On day 7 and 28, the mice were sacrificed under ether anesthesia, and thymus and spleen were collected. Thy/bw, thymus weight/body weight. Sp/bw, spleen weight/body weight. Values are mean ± SE (n = 5). *p < 0.05 vs. 0 (vehicle).

significantly. We used flow cytometry to analyze the percentage of various B- and T-cell populations in TCDD-exposed NC/Nga mice. Exposure to 20 µg/kg TCDD significantly decreased the number of CD4⁺CD8⁻ T cells in the thymus (Table 2). TCDD administration significantly decreased the number of CD3⁺ T cells (2.05 ± 0.30 × 10³) in the spleen compared with that of control mice (3.43 ± 0.29 × 10³; p < 0.05). The number of CD45R/B220⁺ B cells in the spleen from TCDD-exposed mice did not differ (data not shown).

On day 28 the wet-weight to body-weight ratio of spleen in TCDD-exposed NC/Nga mice significantly increased (Table 1). There were no significant changes in the cellularity of the thymus in TCDD-exposed NC/Nga mice compared to vehicle-exposed mice. The number of CD3⁺ T cells in the spleen from TCDD-exposed mice did not differ (data not shown). However, TCDD administration significantly increased the number of CD45R/B220⁺ B cells (8.67 ± 0.12 × 10³) in the spleen compared with that of vehicle-exposed mice (6.53 ± 0.10 × 10³; p < 0.05). Thus, exposure of NC/Nga mice to TCDD affects the T-cell subpopulations in NC/Nga mice on day 7, but not on day 28.

TABLE 2
Effects of TCDD on Thymocyte Subpopulations

<table>
<thead>
<tr>
<th>TCDD (µg/kg)</th>
<th>CD4-CD8⁻</th>
<th>CD4⁺CD8⁻</th>
<th>CD4⁺CD8⁺</th>
<th>CD4⁺CD8⁻</th>
<th>CD4⁺CD8⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.34 ± 0.04</td>
<td>6.34 ± 0.60</td>
<td>0.86 ± 0.05</td>
<td>0.19 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.39 ± 0.06</td>
<td>3.46 ± 0.58*</td>
<td>0.64 ± 0.08</td>
<td>0.21 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>Day 28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.38 ± 0.04</td>
<td>3.62 ± 0.04</td>
<td>0.56 ± 0.04</td>
<td>0.16 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.40 ± 0.06</td>
<td>2.46 ± 0.06</td>
<td>0.41 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

Note. Each mouse received a single oral dose (0 or 20 µg/kg body weight) of TCDD in corn oil. On day 7 and 28, the mice were sacrificed under ether anesthesia, and thymus was collected. Cell suspensions were centrifuged, and the cells were counted and stained. The immunophenotype of the cells from each group of mice was analyzed by using a FACSCalibur flow cytometry. Values are mean ± SE (×10³); n = 5. *p < 0.05 vs. 0 (vehicle).

Changes in Wet Weight and Number of Cells from the Thymus, Spleen, and MLN of TCDD-Exposed Male NC/Nga Mice Immunized with OVA

In OVA-immunized NC/Nga mice, exposure to various concentrations of TCDD did not affect the weight and cellularity of the thymus on day 28 (data not shown). Although the wet weights of the spleen and MLN did not vary from those in control mice (data not shown), exposure to 20 µg/kg TCDD significantly increased the numbers of CD45R/B220⁺ B cells in the spleen (Fig. 1a) and MLN (Fig. 1b). In the MLN of 20 µg/kg TCDD-exposed NC/Nga mice, the number of CD4⁺ T cells significantly decreased. In comparison, 5 µg/kg TCDD had no effect on the cellularity of the spleen and MLN.

OVA-Induced Proliferative Response in Spleen Cells

We used an MTT assay to measure the antigen-stimulated cell proliferation of spleen cells after a 72-h incubation with OVA. On day 28 TCDD significantly suppressed the cell proliferative response to OVA in spleen cells from OVA-immunized NC/Nga mice in a dose-dependent manner (Fig. 2). The proliferative response did not differ between TCDD-exposed and control mice in the absence of OVA.

Cytokine Levels in the Culture Supernatants of Spleen and MLN Cells

TCDD reduced the number of CD4⁺ T cells in MLN and inhibited the proliferative response to OVA in spleen cells. To investigate the effect of TCDD on the modulation of cytokine profiles, we cultured spleen cells from TCDD-exposed, OVA-immunized mice with OVA for 48 h. Compared with those of vehicle-exposed, OVA-immunized mice, concentrations of the Th2-type cytokines IL-4 and IL-5 in the culture supernatants from TCDD-exposed mice were suppressed markedly (Figs. 3a and 3c). In particular, IL-5 production showed a dose-dependent decrease (Fig. 3c). In contrast, the amount of IFN-γ, a Th1-type cytokine, significantly increased in mice exposed to 5 or 20 µg/kg TCDD (Fig. 3b), and a 20 µg/kg dose of TCDD suppressed the production of IL-2 (Fig. 3d). Levels of IL-12 in
the culture supernatants from spleen cells of TCDD-exposed, OVA-immunized and vehicle-exposed, OVA-immunized mice were below the limit of detection.

To assess the effect of 20 μg/kg TCDD on cytokine production by MLN cells, we cultured pooled MLN cells with or without OVA. Although the level of IL-4 in the culture supernatants of vehicle-exposed, OVA-immunized mice was 5 pg/ml (mean of 3 mice), we detected no IL-4 in the cultures from TCDD-exposed, OVA-immunized mice. The production of IFN-γ was 1.6-fold higher in the culture supernatants of MLN cells from TCDD-exposed, OVA-immunized mice than that from vehicle-exposed, OVA-immunized mice (Fig. 4).

Antibody Production in Plasma of TCDD-Exposed Mice

To examine systemic changes in the effect of TCDD, we measured the total IgE concentrations in the plasma of TCDD-exposed NC/Nga mice without immunization. Exposure to 20 μg/kg TCDD significantly decreased total IgE levels (24 ± 3 ng/ml; n = 5) in the plasma of NC/Nga mice compared with that of vehicle-exposed mice (37 ± 5 ng/ml; n = 5; p < 0.05). In OVA-immunized mice, quantities of total and OVA-specific IgE were markedly decreased by exposure to 20 μg/kg TCDD (Figs. 5a and 5b), but not by exposure to 5 μg/kg TCDD. Although plasma levels of anti-OVA IgG1, a Th2-type antibody isotype, were decreased significantly in mice that received 20 μg/kg TCDD (Fig. 6a), TCDD’s effect was greater on titers of anti-OVA IgG2a, a Th1-type antibody isotype (Fig. 6b). The concentration of anti-OVA IgG2a was dose-dependently and significantly decreased in the TCDD-exposed, OVA-immunized mice.

Histology of Pinnae and Dorsal Skin of TCDD-Exposed NC/Nga Mice

In all groups of OVA-immunized and nonimmunized NC/Nga mice, we failed to find histologic evidence of the induction of AD-like lesions in the pinnae and dorsal skin samples of TCDD-exposed NC/Nga mice. There was no striking difference in thickening of the epidermis and the infiltration of inflammatory cells in the dermis of pinnae between vehicle-exposed and TCDD-exposed NC/Nga mice (Figs. 7a and 7b). Exposure to TCDD did not affect the number of mast cells in the pinnae (Figs. 7c and 7d). These findings suggest that exposure to TCDD did not induce AD-like skin lesions in NC/Nga mice.
We investigated whether exposure to TCDD enhances Th2-type immune responses and induces AD-like skin lesions, and failed to observe such wounds in nonimmunized and OVA-immunized NC/Nga mice treated with TCDD. There was no striking difference in thickening of the epidermis and the infiltration of inflammatory cells in the dermis of pinnae between vehicle-exposed and TCDD-exposed NC/Nga mice. Exposure to TCDD did not affect the number of mast cells in the pinnae and dorsal skin samples of TCDD-exposed NC/Nga mice. Under the conditions we used in our experiments, total IgE levels in plasma of TCDD-exposed mice were lower than those of vehicle-exposed NC/Nga mice.

Our results indicate that TCDD markedly suppresses not only IgE and IgG1 (Th2-type antibody isotypes) concentrations but also IgG2a, (Th1-type antibody isotype) level in OVA-immunized NC/Nga mice. Our observation of suppression of Th1-type antibody isotypes is consistent with previously reported results showing that TCDD suppressed IgG2a antibody produced in response to P815 tumor allografts (Kerkvliet et al., 1996). In a DO11.10 adoptive transfer system in mice, exposure to TCDD markedly suppressed anti-OVA IgG2a antibody but not anti-OVA IgG1 antibody (Shepherd et al., 2000). There are a few reports of the effect of TCDD on Th2-type immune responses. Recently, Luebke et al. (2001) observed that TCDD exposure decreased house dust mite allergen-specific IgE synthesis in Brown Norway rats. Total IgE levels were not affected by TCDD exposure. Taken together, these results suggest that exposure to TCDD suppresses Th2 as well as Th1-type immune responses. TCDD seems incapable of aggravating allergic diseases that are predominantly induced by the production of Th2-type antibody.

Previous studies have shown that TCDD treatment suppressed the production of Th1-type cytokines (IL-2, IFN-γ, and TNF) by spleen cells (Kerkvliet et al., 1996), and decreased levels of the T cell-derived cytokines IL-2 and IL-10 (Shepherd et al., 2000). Although exposure to TCDD increased IL-2 production by spleen cells from rats that had been injected with Staphylococal Enterotoxin B, the production of IL-1, IL-6, and TNF did not differ from those in control animals (Huang and Koller, 1998). In our study, levels of IL-4 and IL-5 (Th2-type cytokines) and of IL-2 in the culture supernatants of spleen cells from TCDD-exposed, OVA-immunized mice were decreased significantly compared with those for vehicle-exposed, OVA-immunized mice. In contrast, culture supernatants from TCDD-exposed, OVA-immunized mice showed significantly increased concentrations of IFN-γ. IL-18, in cooperation with IL-12, mediates the release of IFN-γ from Th1-type T lymphocytes, but we were unable to document any TCDD-associated changes in the concentrations of IL-12 in culture supernatants or IL-18 production in plasma (data not shown).

![FIG. 3. Cytokine levels in culture supernatants of spleen cells from TCDD-exposed, OVA-immunized NC/Nga mice. On day 28, spleen cells from TCDD-exposed, OVA-immunized and vehicle-exposed, OVA-immunized mice were cultured for 48 h, then the cytokine levels in the supernatants were determined by ELISA. (a) IL-4, (b) IFN-γ, (c) IL-5, (d) IL-2. Each value is mean ± SE (n=4–6). *p < 0.05 vs. 0 (vehicle + OVA), **p < 0.01 vs. 0 (vehicle + OVA), †p < 0.05 vs. 5 μg/kg TCDD + OVA.]

![FIG. 4. Cytokine levels in culture supernatants of MLN cells from TCDD-exposed, OVA-immunized NC/Nga mice. On day 28, pooled MLN cells from TCDD-exposed, OVA-immunized and vehicle-exposed, OVA-immunized mice were cultured for 48 h, then IFN-γ level in the culture supernatants was determined by ELISA.]

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Why the production of IFN-γ from the spleen cells of TCDD-exposed mice was enhanced remains uncertain. The cytokine profile of MLN cells from TCDD-treated animals was similar to that of spleen cells, and the enhanced production of IFN-γ may be derived from non-T lymphocytes among the spleen and MLN cells. One possibility is that exposure to TCDD may activate spleen- or MLN-associated NK cells, and that these cells produce the IFN-γ. In fact, injection of male A/J mice with TCDD induced increased activity of NK cells in the blood and spleen (Funseth and Ilbäck, 1992). However, other previous reports have demonstrated that exposure to TCDD alone has no effect on spontaneous splenic NK activity (House et al., 1990; Mantovani et al., 1980; Yang et al., 1994). Another possible mechanism for the TCDD-associated increase in IFN-γ levels in supernatants of spleen cell cultures is that TCDD stimulated B cells of the spleen and MLN to produce the cytokine. We found that the numbers of B cells in the spleens and MLNs of TCDD-exposed NC/Nga mice were significantly higher than in the vehicle-exposed mice. In fact, Yoshimoto et al. (1998) observed that cultured B cells produce IFN-γ. Further, a single oral dose of 10 μg/kg TCDD enhanced pulmonary IFN-γ levels but suppressed IFN-γ production by mediastinal lymph node cells (Warren et al., 2000). Further studies addressing the production of IFN-γ in subpopulations of splenic or lymph node cells of NC/Nga mice are needed.

The mechanisms underlying TCDD-associated immunosuppression remain unknown. In this study, the proliferative response of spleen cells to OVA was suppressed markedly in TCDD-exposed mice, whose plasma also showed significantly reduced levels of both Th1- and Th2-regulated antibody isotypes. TCDD affected differentiating but not naive resting T cells (Rhile et al., 1996), and induced increased apoptosis among activated, but not resting, T cells (Pryputniewicz et al.,...
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REFERENCES


