Suppression of Allergic Immune Responses to House Dust Mite (HDM) 
in Rats Exposed to 2,3,7,8-TCDD

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Exposure to various xenobiotics, including oxidant gases, diesel exhaust, and certain pesticides, has been reported to exacerbate pulmonary allergic hypersensitivity responses. Increased lymphocyte proliferative responses to parasite antigens or increased antibody responses to sheep erythrocyte have also been reported in rats exposed to TCDD before infection or immunization. As a result, these studies were conducted to test the hypothesis that TCDD exposure exacerbates the allergic response to house dust mite antigen. Brown Norway rats were injected, ip, with 0, 1, 10, or 30 μg TCDD/kg 7 days before intratracheal (it) sensitization to semipurified house dust mite allergen (HDM). Fourteen days later, rats were challenged with HDM and immediate bronchospasm was measured. At this time point, plus 2 and 7 days later, inflammatory cells in bronchoalveolar lavage fluid (BALF), HDM-specific IgE levels in serum, and HDM-driven cell proliferation in bronchial lymph nodes and spleen were evaluated. TCDD exposure decreased both immediate bronchoconstriction and specific IgE synthesis after the HDM challenge; 7 days later, HDM-specific IgE responses remained suppressed. Total serum IgE levels were similar in all groups. HDM challenge alone significantly increased cellular and biochemical indicators of lung injury, both of which were suppressed by TCDD exposure. The proliferative response of lymph node cells, but not of spleen cells, to HDM was also suppressed at the highest TCDD dose, although the splenic response to Concanavalin A was elevated. It appears that early events in the response to HDM are affected by TCDD exposure, since message for IL5 was dramatically reduced 2 days after sensitization, but not after challenge. We therefore conclude that TCDD exposure suppressed, rather than enhanced the development of allergic immune responses and the expression of immune-mediated lung disease.

Key Words: dioxin; dust mite; allergy; immunosuppression; IgE; lung; rats.

Epidemiologic studies suggest that the rate and severity of allergic airway disease is increasing worldwide (CDC, 1998), and it has been hypothesized that exposure to certain environmental chemicals may be a predisposing factor (Institute of Medicine, 1993). For example, a positive correlation was found between the incidence of asthma and the use of carbanate pesticides by farmers (Senthilselvan et al., 1992), and several case studies have described associations between occupational or domestic use of insecticides and fungicides and the development of asthma (Bryant, 1985; Royce et al., 1993; Shelton et al., 1992). Studies in laboratory animal models of pulmonary hypersensitivity have likewise shown that exposure to a variety of chemicals increases allergic-type responses to antigens. For example, pulmonary exposure to NO2 or residual oil fly ash increases the pulmonary immune response to house dust mite allergen (Gilmour et al., 1996; Lambert et al., 1999, 2000), as does the pesticide carbaryl (Dong et al., 1998). In vitro experiments have also shown that the aromatic hydrocarbon component of diesel exhaust and 2,3,7,8-tetrachlordibenzo-p-dioxin (TCDD) increases human B cell-IgE production (Takenaka et al., 1995).

In contrast, the antibody response to sheep erythrocytes is markedly suppressed by TCDD in mice, but is increased in rats exposed to TCDD one week before immunization (Smialowicz et al., 1996). Likewise, TCDD exposure prior to infection suppressed the mouse splenic lymphoproliferative response to parasite antigens (Luebke et al., 1994) but enhanced the response in rats (Luebke et al., 1995). Because dioxin exposure increases rat antigen-specific responses under certain conditions, exposure may also favor the development of an allergic response, or increase the severity of an allergic response to certain environmental antigens. These studies were conducted to test the hypothesis that TCDD exposure exacerbates the allergic-type immune responses to house dust mite antigen in rats.

MATERIALS AND METHODS

Animals. Female Brown Norway rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) were housed under standard conditions and used at approximately 10 weeks of age, after a 2-week acclimatization period. Serological
FIG. 1. Experiment time line (days). In the first series of experiments, rats were exposed to vehicle or TCDD once, by ip injection. Seven and 9 days (days –14 and –12) later, all animals were sensitized to HDM by it instillation. Two weeks after the first immunization (day 0), rats were challenged with HDM; subgroups of 5 rats from each treatment group were sampled on days 0, 2 (peak of the inflammatory response), and 7 (resolution of inflammation and peak of the antibody response) (Series 1, solid triangles). In Series 2 experiments (striped triangles), groups of 5 animals each were treated as described above and sacrificed 2 days (day –10) after the second sensitization.

testing of sentinel and a subgroup of experimental animals produced no evidence of infection. The Institutional Animal Care and Use Committee approved all procedures before studies were begun.

TCDD exposure. TCDD (purity >98% by gas chromatography-mass spectroscopy analysis, Radian Corp., Austin, TX) was dissolved in acetone and added to corn oil (Sigma Chemical Co., St. Louis, MO). The acetone was removed under vacuum and the stock solution of TCDD was diluted with corn oil to prepare dosing solutions containing 0.2, 2.0, or 6.0 µg TCDD/mL. Animals were dosed by intraperitoneal injection of 5 µl of dosing solution/g of body weight, providing doses of 0 (corn oil), 1, 10, or 30 µg TCDD/kg of body weight. One injection was given 7 days before the first sensitization (on day –21; see Fig. 1).

Experimental design. Exposure, sensitization, and assay days are summarized in Figure 1. Experiments were first done to determine the whole animal effects of TCDD exposure on the expression of dust-mite allergy (“Series 1” in Fig. 1). Subgroups of 5 rats from each exposure group were killed at 0, 2, or 7 days after the HDM challenge (see below). These days were chosen as corresponding to baseline (sensitized, but not challenged) on day 0, the anticipated peak of inflammation on day 2, and resolution of inflammation, but corresponding to baseline (sensitized, but not challenged) on day 0, the anticipated peak of inflammation on day 2, and resolution of inflammation, but the peak of antibody titers and lymphocyte responses to HDM, on day 7. The same endpoints were measured on each day. These included analysis of bronchoalveolar lavage fluid (BALF) for total and differential cell counts, lactate dehydrogenase (LDH) activity, and total protein concentration. Serum immunoglobulin levels were evaluated by ELISA and semiquantitative RT-PCR was used to evaluate cytokine messenger RNA (mRNA) levels of total IgE, HDM-specific IgE, and IgG antibody levels were evaluated by ELISA, as previously described (Lambert et al., 2000).

BALF analysis. Viable cells were counted on a hemocytometer in the presence of trypan blue dye. Differential cell counts were performed on slides prepared from BALF by centrifugation (Cytospin Model II, Shandon, Pittsburgh, PA) of 50,000 cells onto glass slides, followed by staining with Diff Quik (American Scientific, Inc., Sewickley, PA). Cells were removed from the remaining BALF by centrifugation and the supernatant was assayed for protein using CoomassiePlus reagent (Pierce, Rockford, IL), and LDH (kit 228, Sigma, St Louis, MO) on a Cobas FARA instrument (Hoffman-La Roche, Branchburg, NJ).

Lymphocyte proliferation assay. Lymph-node cell suspensions were prepared using ground-glass homogenizers (Gilmour et al., 1996) and spleen-cell suspensions prepared as described by Luebke et al. (1992). The proliferation assay was performed as previously described (Gilmour et al., 1996). Lymphocyte responsiveness was expressed as net CPM; i.e., the CPM of incorporated tritiated thymidine (3H-TdR) by cells incubated with HDM, minus the CPM of cells incubated with media alone.

Cytokine evaluation. Approximately 100 mg of caudal lung lobe tissue was removed, weighed, snap-frozen on dry ice, and stored at –70°C in 1.0 ml of TRI Reagent (Sigma, St. Louis, MO); the tissue was later homogenized and total RNA was extracted according to the manufacturer’s instructions. Semi-quantitative PCR assays were performed as previously described (Dong et al., 1998; Lambert et al., 1999). Briefly, RNA concentrations were measured by a GeneQuant spectrophotometer (Pharmacia Biotech, Inc., Piscataway, NJ) set at 260 nm wavelength, and RNA purity was assessed by the ratio of absorbance at 260 and 280 nm. Complimentary DNA (cDNA) was synthesized using primers for interleukin 4 and 5 as described by Siegling et al. (1994). TNFa, IFNγ, and G3PDH cDNAs were synthesized according to the instructions described in detail (Dong et al., 1998; Lambert et al., 1998). The enhanced pause is a unit-less measure of airflow obstruction, which reflects changes in pulmonary function related to quantitative differences in time and extent between inspiration and expiration (Lambert et al., 1998). Briefly, rats were placed in a plethysmograph for 10 min to obtain a baseline, then removed, anesthetized with halothane, and challenged by intratracheal instillation of 10 µg HDM. Animals were returned to the plethysmograph and enhanced pause values were recorded at 1-min intervals for 20 min.

Sample collection. On each assay day, animals were weighed, anesthetized by intraperitoneal injection of pentobarbital, and weighed. Blood was collected by cardiac puncture. BALF was obtained from each animal as previously described (Gilmour et al., 1996). The spleen and main bronchial lymph nodes were removed aseptically and placed in culture medium.

Serum immunoglobulin levels. Serum was separated from clotted blood samples by centrifugation and aliquots of serum frozen at –80°C for later analysis. Total IgE plus HDM-specific IgG and IgE levels in thawed serum samples were measured by ELISA, as previously described (Lambert et al., 2000).

Immediate hypersensitivity. The bronchoconstrictive response to HDM challenge, as measured by enhanced pause, was evaluated on day 0 (day of challenge with HDM) in 5 rats at each dose level, using methods previously described in detail (Dong et al., 1998; Lambert et al., 1998). The enhanced pause is a unit-less measure of airflow obstruction, which reflects changes in pulmonary function related to quantitative differences in time and extent between inspiration and expiration (Lambert et al., 1998). Briefly, rats were placed in a plethysmograph for 10 min to obtain a baseline, then removed, anesthetized with halothane, and challenged by intratracheal instillation of 10 µg HDM. Animals were returned to the plethysmograph and enhanced pause values were recorded at 1-min intervals for 20 min.

Series 1 Assays

Series 2 Assays

DNA Challenge

TCDD Injection

Sensitization

-21 -19 -17 -15 -13 -11 -9 -7 -5 -3 -1 0 2 4 6 8 10 12 14 16 18 20 22
accompanying commercial primers (Clontech, Palo Alto, CA). Amplifications were performed in thermal cycler strip tubes using a 9600 GeneAmp PCR System (Perkin-Elmer Corporation, Norwalk, CT). Concentrations of specific cDNA sequences were quantified by separating 10 μl of each amplification mixture through a 2% agarose gel. Integrated optical densities (IOD) of amplification products that resolved as single bands of a predicted size were divided by the IOD of the G3PDH DNA band to normalize for any differences in the amount of input RNA.

Flow cytometry. Phenotypic analysis of bronchial lymph node cells was done 2 days after the second sensitization and 2 days after challenge, on animals in the second series of experiments. Cells were dual-labeled with monoclonal antibodies to CD5 (OX19, phycoerythrin-tagged, PharMingen, San Diego, CA) and IgG kappa light chain (OX12, FITC-tagged, Serotec, Raleigh, NC) to identify T and B cells, respectively. T cells were subtyped by staining with a cocktail of monoclonal PE-labeled anti-CD4 and FITC-labeled anti-CD8 (PharMingen). Appropriate isotype controls were included. Details of the procedure can be found in Ladics et al. (1998).

Statistical analysis. Data are expressed as mean ± SEM. Immediate bronchoconstriction data were analyzed by comparing all groups, using repeated-measures analysis of variance (ANOVA, SAS Institute, Cary, NC). Group differences were considered significant if the test statistical type I error was <0.05. Other endpoints were evaluated using Dunnett's t-test for comparing multiple groups to a control.

RESULTS

Post-challenge Experiments (Series 1)

Challenge of control rats with 10 μg HDM caused an immediate bronchoconstrictive response that resolved over the intervening 20 min (Fig. 2). Exposure to the lowest dose of TCDD did not alter the response to challenge; however, animals exposed to 10 μg TCDD/kg were slower to respond to challenge and returned to baseline more rapidly than controls. Exposure to 30 μg TCDD/kg prevented antigen-specific immediate airway responses.

HDM-specific IgE and IgG antibodies were markedly elevated in the serum of controls and of the lowest TCDD-exposure group at 7 days after HDM challenge but were suppressed in the 2 higher TCDD dosages (Fig. 3). Total IgE levels were not affected by TCDD exposure (data not shown).

In this model of allergic lung disease, HDM challenge causes an increase in number of BAL cells that peaks at 2 days and resolves over a 1-week period (Fig. 4). A shift from macrophage (MΦ) predominance (days 0 and 7) to approximately equal percentages of MΦ and eosinophils (day 2) was observed in controls. Exposure to TCDD antagonized the accumulation of eosinophils in BALF, significantly decreasing eosinophil percentages in groups exposed to 10 or 30 μg/kg. As a result, relatively more MΦs were present in the lungs of treated rats 2 days post-challenge. Exposure to TCDD also hindered the influx of leukocytes into the lung, completely blocking influx at 10 and 30 μg TCDD/kg on day 2 post-challenge (Fig. 4, total cell counts). One week after HDM challenge, lung eosinophilia began to resolve, although all groups of TCDD-exposed rats still had significantly fewer eosinophils present in BALF than did controls.

The concentration of protein (a marker of increased vascular permeability) and lactate dehydrogenase (LDH) activity (a
marker of cell lysis) peaked in BALF 2 days after antigen challenge (Fig. 5). TCDD exposure produced a dose-related decrease of these markers. LDH activity 2 days after challenge appeared to be more sensitive to chemical exposure, as significantly less activity was detected at even the lowest dose of TCDD.

Lymphocyte proliferative responses to HDM or the T-cell mitogen Con A were evaluated in bronchial lymph nodes and spleen cell cultures (Table 1). The response to HDM was suppressed on days 0 and 2 post-challenge by exposure to 10 or 30 μg TCDD/kg; suppression 7 days after challenge only occurred at the highest dose of TCDD. Splenocyte responses to HDM stimulation were weak in all groups on all assay days, and no significant difference between treated and control groups was observed. However, the spleen-cell response to Con A was generally elevated by exposure to the higher doses of TCDD.

No TCDD-related changes were detected in lung tissue concentrations of mRNA for IFNγ, TNFα, or IL4 on days 0, 2, or 7 after challenge (Fig. 6). Message for IL5 was detected only sporadically in treated and control animals.

Post-sensitization Experiments (Series 2)

To determine the effects of TCDD exposure on initial immune system priming, a second study was done to examine the effect of TCDD exposure on cytokine induction, lymphocyte surface markers, and the development of immunity 2 days after sensitization. These experiments utilized both sensitized and non-sensitized animals that were injected with corn oil or TCDD 11 days before sacrifice. Titers of HDM-specific IgE and IgG were below the assay limits of detection at 48 h after the second sensitization (data not shown); non-sensitized animals were not tested for HDM-specific antibodies.
The BALF concentration of leukocytes was greater in sensitized control rats than in non-sensitized animals, and exposure to TCDD decreased the sensitization-related cellular influx (Fig. 7 insert). TCDD exposure decreased the percentage of eosinophils in the BALF of both sensitized and non-sensitized rats (Fig. 7). Although the percentage of eosinophils was similar in sensitized and non-sensitized controls, the absolute number of eosinophils was increased (data not shown) by the increased cellularity of sensitized rat BALF.

Sensitization increased the concentration of LDH in control animal BALF, compared to non-sensitized controls. Exposure to TCDD prevented the sensitization-related increase in LDH release (Table 2).

In contrast to Series 1, lung cytokine mRNA analysis detected a marked increase in message for IL5 in control rats 2 days after sensitization, but not in rats exposed to TCDD prior to sensitization (Fig.8).

Neither TCDD exposure nor sensitization with HDM produced a change in the total number of T cells (CD5+), B cells (IgG κ chain-positive), or the relative distribution of CD4+ or CD8+ bronchial lymph node cells.

DISCUSSION

These studies were initiated because several publications collectively suggested that dioxin might enhance the immune response to house-dust mites in rats. Specifically, in vitro studies by Takenaka et al. (1995) demonstrated that TCDD can augment production of IgE by purified human B cells, and Smialowicz et al. (1996) demonstrated increased anti-sheep erythrocyte antibody (IgM) production in rats exposed to TCDD, in contrast to marked suppression in exposed mice. Furthermore, we (Luebke et al., 1995) reported that proliferative responses of spleen cells to parasite antigen were enhanced by TCDD exposure in infected rats but were suppressed in mice (Luebke et al., 1994). In the present study, we tested the hypothesis that TCDD exposure exacerbates the immune (i.e., allergic) response of BN rats to HDM. Our results clearly
indicate that exposure to TCDD before sensitization with HDM had either no measurable effect at lower doses or suppressed the response at the molecular (message for IL5 after sensitization), protein (specific IgE), cellular (T-cell function and pulmonary leukocytes) and tissue (bronchoconstriction) levels. TCDD exposure essentially prevented the marked increase in IL5 noted in control animals 2 days after sensitization (Fig. 8), indicating that exposure blocked early events in the induction of the Th2 response to HDM.

Suppression of HDM-specific immune responses by exposure to TCDD reduced the subsequent immune-mediated lung disease following intratracheal allergen challenge. Previous adoptive transfer studies using this model had demonstrated that serum IgE antibody conferred the ability to produce allergen-induced bronchospasm, while activated lymphocytes were responsible for inducing pulmonary inflammation, acute lung injury and airway hyper-responsiveness (Lambert et al., 1998). Thus, it was not surprising that these pathological features were reduced in animals with suppressed T-cell and antibody responses. Because total IgE in serum and splenic mitogen responses were unaffected by TCDD exposure, suppression appeared to be an antigen-specific event rather than a generalized reduction in immune competence.

Although our results did not support the hypothesis, the outcome of these studies is noteworthy in that suppression of antibody production occurred at doses of TCDD reported by others to enhance antigen-specific immune function. For example, Smialowicz et al. (1996) reported an increase in numbers of PFCs at doses of 30 μg TCDD/kg in F344 rats 7 days before immunization. Increased responses do not appear to be rat strain-dependent, because similar results were observed in other strains, including the BN, following exposure to 3 μg TCDD/kg (Ralph Smialowicz, personal communication). Also, Fan et al. (1996) observed increased SRBC-specific IgG titers, as well as increased delayed hypersensitivity responses to keyhole limpet hemocyanin in Sprague-Dawley rats at TCDD exposure levels of 20 μg/kg. Thus, this paper is the first to

### TABLE 1
Specific and Non-specific Lymphocyte Proliferative Responses to HDM in Sensitized Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Lymph node</th>
<th>Spleen</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HDM</td>
<td>HDM</td>
</tr>
<tr>
<td>Day 0 post-challenge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>27.9 ± 6.7</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td>1 μg TCDD/kg</td>
<td>19.8 ± 4.7</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>10 μg TCDD/kg</td>
<td>9.3 ± 1.0*</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>30 μg TCDD/kg</td>
<td>4.1 ± 0.8*</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>Day 2 post-challenge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>22.0 ± 3.2</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>1 μg TCDD/kg</td>
<td>13.7 ± 1.7</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>10 μg TCDD/kg</td>
<td>9.6 ± 4.6**</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>30 μg TCDD/kg</td>
<td>6.1 ± 1.1**</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Day 7 post-challenge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>33.0 ± 3.7</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>1 μg TCDD/kg</td>
<td>30.6 ± 6.8</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>10 μg TCDD/kg</td>
<td>18.7 ± 5.6</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>30 μg TCDD/kg</td>
<td>12.9 ± 3.0**</td>
<td>1.5 ± 0.2</td>
</tr>
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</table>

*Note.* Groups of 5 BN rats were injected (ip) 0, 1, 10 or 30 μg TCDD/kg, 7 days before intratracheal sensitization, with house-dust mite antigen (HDM). Groups were killed on the day of challenge plus 2 and 7 days after challenge. Single cell suspensions were cultured with HDM or Con A, cultured for 72 h, pulse-labeled with tritiated thymidine, and harvested. Values are the mean ± standard error of net CPM × 10^-3 (CPM stimulated culture–CPM non-stimulated culture).

* *p < 0.01.
** *p < 0.05.
report suppression of thymus-dependent antibody production in adult rats exposed to TCDD.

In evaluating these apparently contradictory results, it must be considered that the enhanced systemic immune responses reported in TCDD-exposed rats followed parenteral immunization, rather than local (pulmonary) sensitization. It has been shown that both primary and secondary antibody responses to sheep erythrocytes can be induced by intravenous injection or intratracheal instillation of equal numbers of red cells (Stein-Streilein et al., 1979), indicating that either route of immunization can be effective. However, the route of antigen (allergen) exposure may still be pivotal in controlling whether suppression or enhancement is observed in xenobiotic-exposed animals. For example, Dong et al. (1998) reported an enhanced pulmonary allergic response to HDM in BN rats exposed to the pesticide carbaryl prior to systemic sensitization (i.e., subcutaneous injection) but found that the response was suppressed if HDM was given by intratracheal instillation (Wumin Dong, personal communication). One possible interpretation of these results, assuming that humans share this response pattern, is that the apparent increase in human allergic asthma is not the result of systemic antigen exposure. Rather, xenobiotic-induced exacerbation of allergic lung disease may be a special situation mediated by inhaled allergen and reactive gases (e.g., NO₂; Gilmour et al., 1996) or inhaled particulates in rodents (Lambert et al., 1999, 2000) and humans (Diaz-Sanchez et al., 1997). Future experiments will address how strongly the route of immunization influences the outcome of antigen exposure following xenobiotic exposure, and how the nature of the xenobiotic influences the process.

**TABLE 2**

Concentration of Soluble Indicators of Inflammation in BALF after Sensitization and Challenge with Dust Mite Antigen

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein (μg/ml)</th>
<th>LDH (units/l)</th>
</tr>
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<tbody>
<tr>
<td>Two days after sensitization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn oil/saline</td>
<td>346.0 ± 37.6</td>
<td>66.0 ± 5.8</td>
</tr>
<tr>
<td>TCDD/saline</td>
<td>327.2 ± 31.2</td>
<td>49.4 ± 7.1</td>
</tr>
<tr>
<td>Corn oil/HDM</td>
<td>614.0 ± 125.1</td>
<td>96.2 ± 4.7*</td>
</tr>
<tr>
<td>TCDD/HDM</td>
<td>348.4 ± 89.5</td>
<td>45.6 ± 7.1</td>
</tr>
<tr>
<td>Two days after challenge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn oil/HDM</td>
<td>481.6 ± 64.1</td>
<td>67.6 ± 4.3</td>
</tr>
<tr>
<td>TCDD/HDM</td>
<td>350.3 ± 67.6</td>
<td>44.3 ± 6.5</td>
</tr>
</tbody>
</table>

* LDH, lactate dehydrogenase.

**Note.** Groups of 5 BN rats were injected (ip) with corn oil or 30 μg TCDD/kg 7 days before sensitization with HDM. Groups were killed 2 days after sensitization or 2 days after challenge. Values are mean ± standard error.

FIG. 7. Relative cell distribution and total cell count in bronchoalveolar lavage fluid, 2 days after sensitization. Rats were exposed to TCDD, sensitized as described in Figure 1, and killed 2 days after the second sensitizing installation of HDM (Series 2). Cells were isolated from BALF by centrifugation, resuspended, and counted. A portion of the cell suspension was transferred to glass slides by centrifugation, fixed, and stained for differential leukocyte counts. Values are the mean ± SEM of 5 rats/group.

FIG. 8. Cytokine message in lung tissue. Rats were exposed to TCDD, sensitized as described in Figure 1, and killed 2 days after the second sensitizing installation of HDM (Series 2). Message for cytokines in caudal lung samples was evaluated by RT-PCR. Values are arbitrary densitometer units, standardized to a housekeeping gene and expressed as the mean ± SEM of 5 rats/group.
The route of antigen administration is apparently not the only factor dictating whether TCDD will cause immune system enhancement or suppression. It is also possible that the chemical or immunological properties of the administered antigen influenced the outcome of antibody synthesis. For example, Smialowicz et al. (1996) reported suppression of antibody production in rats exposed to 30 μg TCDD/kg after immunization with the type 1 T-independent antigen TNP-LPS. This finding is in accord with earlier studies suggesting that a B-cell maturation defect underlies suppression of humoral immunity by TCDD in mice (Tucker et al., 1986). It remains to be determined whether HDM antigen is processed and responded to by the rat immune system in such a way that pre-sensitization exposure to TCDD has a suppressive, rather than stimulatory effect.

Enhanced antibody responses to SRBCs in TCDD-exposed rats were accompanied by a dose-related decrease in CD4+/CD8+ lymphocytes and a dose-related increase in IgM+ lymphocytes (Smialowicz et al., 1996). We found no differences in lymph node B- and T-lymphocyte populations 2 days after the second sensitization or 2 days after challenge, suggesting that exposure-related loss of lymphocyte populations was not responsible for the observed effects. It is unlikely that the disparity between the 2 studies is simply due to kinetic differences, since the length of time between TCDD exposure and sensitization (7 days) and sensitization and assay (4 days) were similar in both studies.

When the results of these and other published experiments are considered together, it appears that the combination of xenobiotic exposure, the immunologic microenvironment where antigen is first encountered, and possibly the type of immunogen that is injected influence whether suppression or enhancement of the immune response will occur. Clearly, TCDD exposure does not have a similar effect in rats under all conditions of immunization. Further studies are required to determine how widespread these disparate effects are, both in terms of immunosuppression and development of pulmonary hypersensitivity.

It is interesting to note that Weisglas-Kuperus et al. (2000) reported an inverse relationship between the odds ratio for developing allergic disease and total maternal and toddler TCDD toxic equivalents. While the biological basis for reduced allergic responses in humans and rodents may differ, the similarity of results suggest that immunosuppression is common to both events. From the standpoint of human risk assessment, it is important to determine whether the route of immune system sensitization, coupled with previous xenobiotic exposure, affects the development of allergy and possibly asthma. This is particularly important if subsequent studies suggest that contradictory results will likely be obtained in the xenobiotic-exposed host immunized locally instead of systemically.

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