SHORT COMMUNICATION

Comparison of the T Cell-Independent Antibody Response of Mice and Rats Exposed to 2,3,7,8-Tetrachlorodibenzo-p-dioxin

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2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is an environmental contaminant that produces adverse effects on the immune system of experimental animals. In this study, the effect that TCDD has on the antibody plaque-forming cell (PFC) response to the T cell-independent (TI) antigen trinitrophenyl-lipopolysaccharide (TNP-LPS) was compared in adult female B6C3F1 mice and F344 rats. Mice or rats were given a single intraperitoneal injection of TCDD at doses ranging from 1 to 30 μg/kg, 7 days prior to immunization with TNP-LPS by intravenous injection. Three days later body, spleen, thymus, and liver weights were measured and the PFC response to TNP-LPS was determined. Thymus weights were decreased at 10 and 30 μg TCDD/kg, whereas spleen weights were decreased and liver weights increased in mice dosed at 3, 10, and 30 μg/kg. Mice dosed at 10 and 30 μg TCDD/kg had suppressed PFC responses and serum hemagglutination titers. In rats, thymus weights were decreased and liver weights increased at 3, 10, and 30 μg TCDD/kg; however, the PFC response and serum hemagglutination titers to TNP-LPS were suppressed only at 30 μg/kg TCDD. TCDD did not affect splenic lymphocyte subsets evaluated by flow cytometry. These results indicate that TCDD suppresses the TI antibody response to TNP-LPS in both B6C3F1 mice and F344 rats, with mice more sensitive to suppression by TCDD than rats. © 1996 Society of Toxicology

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) exposure of experimental animals results in a variety of alterations in the integrity of the immune system. The consequences of TCDD exposure include reductions in lymphoid tissue mass and cellularity, alterations in innate and specific immunity, and increased susceptibility to challenge with a variety of infectious agents (Holsapple, 1995).

While several different experimental animal species have been employed in the evaluation and characterization of TCDD-induced immunotoxicity, few studies have attempted to compare simultaneously the effects of TCDD on the immune system of two or more species. An early study by Vos et al. (1973) compared the effects of TCDD on immune function in mice, rats, and guinea pigs; however, different parameters were examined and different antigens were employed to elicit immune responses in these various species.

More recently, a direct comparison of the effect that TCDD has on the antibody plaque-forming cell (PFC) response to the T cell-dependent antigen SRBC in mice and rats was reported by Smialowicz et al. (1994). In this study, it was found that the PFC response to SRBC was dramatically different between B6C3F1 mice and F344 rats exposed to TCDD. TCDD caused a dose-related suppression of the PFC response in mice, whereas it failed to suppress and in fact enhanced the PFC response to SRBC in F344 rats. Despite this differential effect of TCDD on the antibody response to SRBC by B6C3F1 mice and F344 rats, both rodent species were equally sensitive to TCDD-mediated hepatic CYP1A1 and CYP1A2 induction (Smialowicz et al. 1994).

The present study was undertaken to determine if a similar dichotomy occurs in trinitrophenyl-lipopolysaccharide (TNP-LPS)-immunized and TCDD-exposed B6C3F1 mice and F344 rats. TNP-LPS, a T cell-independent (TI) type 1 antigen, was chosen for this study because of the minimal influence that T cells and T-cell cytokines as well as accessory cells play in the antibody response to this antigen (Elbridge et al., 1985). It has been demonstrated that purified splenic mouse and rat B cells are triggered by type 1 antigens such as TNP-LPS to undergo proliferation, differentiation, and immunoglobulin production. These B-cell responses can be augmented by the presence of T cells or T-cell factors. In contrast, both T cells and adherent accessory cells are required for B-cell responses to type 2 antigens, such as TNP-Ficoll, and to the T cell-dependent antigen SRBC (Bradley-Mullen, 1982; Eldridge et al., 1985).

The results presented here indicate that the antibody response to TNP-LPS, unlike the response to SRBC, was suppressed in both mice and rats following TCDD exposure.
However, similar to the effect of TCDD on the antibody response to SRBC, mice were more sensitive than rats to TCDD-induced suppression of the TNP-LPS response.

**MATERIALS AND METHODS**

**Animals.** Female B6C3F1 mice and Fischer 344 [CDF(F344)CrlBr] rats (Charles River Laboratory, Raleigh, NC) were 8 to 10 weeks old at the time of dosing. All procedures that involved animals were reviewed and approved by the Institutional Animal Care and Use Committee prior to initiation of experiments.

**Dosing.** Dosing solutions of TCDD (>98% purity, Lot No. MLB-15091-55, as determined by gas chromatography—mass spectrometry (GC-MS, Radian Corp., Austin, TX) were prepared from a stock solution containing 1 mg/kg in 10 ml of corn oil (Sigma Chemical Co., St. Louis, MO). The stock solution was prepared by dissolving TCDD in acetone, mixing the acetone solution with corn oil, and then removing the acetone by evaporation (DeVito et al., 1993). Animals were weighed and dosed with a single intraperitoneal injection of TCDD in corn oil at doses ranging from 1 to 30 μg/kg body wt, in a volume of 10 ml/kg for mice and 5 ml/kg for rats. Control mice and rats received a single intraperitoneal injection of corn oil in volumes of 10 and 5 ml/kg, respectively.

**Immune parameters.** Seven days following TCDD dosing, mice and rats were immunized with a single intravenous (iv) injection of 0.2 ml of 125 μg/ml or 0.5 ml of 40 μg TNP-LPS/ml (List Biological Laboratory, Inc., Campbell, CA) in sterile saline, respectively. Three days following immunization the antibody response to TNP was determined using the splenic PFC assay as described (Smialowicz et al., 1991). TNP-haptenated SRBC were prepared using trinitrobenzenesulfonic acid (Sigma), as described by Rittenberg and Pratt (1969), and were used for developing PFC to TNP. Serum hemagglutination titers to TNP were also determined at the time of PFC assay (Smialowicz et al., 1992). Body, spleen, thymus, and liver weights were also determined.

Expression of splenic lymphocyte surface markers from TCDD-dosed and TNP-LPS-immunized mice and rats was measured using multparameter flow cytometry (Smialowicz et al., 1994). The monoclonal antibodies employed were fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse Lyt-2 (CD8, Becton-Dickinson, Mountainview, CA); phycoerythrin (PE)-conjugated rat anti-mouse L3T4 (CD4, Becton-Dickinson); FITC-conjugated rabbit anti-mouse IgM (Zymed Laboratories, Inc., San Francisco, CA); FITC-conjugated rabbit anti-rat W3/25 (CD4, Sera-Lab, Accurate Chemical and Scientific Corp., Westbury, NY); PE-conjugated mouse anti-rat OX8 (CD8, PharMingen, San Diego, CA); and FITC-conjugated rabbit anti-rat IgM (Zymed). All antibodies were pretitrated for optimal fluorescence prior to staining of cells from TCDD-exposed animals. Cells were analyzed using an EPICS Profile II flow cytometer (Coulter Electronics, Hialeah, FL) equipped with an argon-ion laser operated at a wavelength of 488 nm. Green fluorescence from FITC emission was measured using a 525-nm band-pass interference filter, and yellow fluorescence from PE emission was measured using a 575-nm band-pass interference filter. A 550 nm dichroic filter was used to separate the PE and FITC signals. Dead cells and debris were excluded by collecting lymphocyte population data gated on forward and 90° light scatter.

**Data analysis.** Data were analyzed by one-way analysis of variance (ANOVA), with post hoc analysis using Dunnett’s multiple comparison t test (RSI Release 4, 1988). Differences between control and treatment groups were considered statistically significant when p < 0.05.

**RESULTS**

Table 1 shows the organ weight and PFC data for mice exposed to TCDD and immunized with TNP-LPS. Spleen weights were variably reduced in mice dosed with 3 or 10 μg TCDD/kg (experiment 1) or 30 μg TCDD/kg (experiment 2), whereas liver weights were variably increased in mice dosed at 3, 10, or 30 μg TCDD/kg. Thymus weights were consistently reduced in mice exposed to 10 or 30 μg TCDD/kg (experiments 1 and 2).

Exposure of mice to 10 or 30 μg TCDD/kg consistently suppressed the PFC response expressed as either PFC/10⁶ spleen cells or PFC/spleen (Table 1). These animals also had suppressed serum hemagglutination titers to TNP-SRBC. While the number of PFC/spleen was reduced in mice exposed to 3 μg TCDD/kg in experiment 2, neither the PFC/10⁶ cells nor the serum hemagglutination titer was suppressed in these mice.

Rats exposed to TCDD at 3, 10, or 30 μg/kg had reduced thymus weights and increased liver weights; their spleen weights were not affected (Table 2). The PFC response to TNP-LPS was suppressed only in rats exposed to 30 μg TCDD/kg. These same rats also had reduced serum hemagglutination titers.

Phenotypic analysis of the lymphocyte subsets present in the spleens of mice and rats exposed to an immunosuppressive dose (i.e., 10 or 30 μg/kg, respectively) of TCDD failed to demonstrate any change relative to controls. The number of viable cells obtained from the spleens of mice exposed to 10 μg/kg TCDD was reduced (Table 3).

**DISCUSSION**

The results of this study demonstrate that TCDD exposure of mice and rats suppresses the antibody response to the TI type 1 antigen TNP-LPS. To our knowledge, this is the first study that has demonstrated that a TI antibody response can be suppressed by TCDD in the rat. The data also indicated that B6C3F1 mice were more sensitive than F344 rats to TCDD-induced suppression of this response.

The doses of TCDD that resulted in suppression of the PFC and serum antibody responses to TNP-LPS in mice also caused alterations in thymus and liver weights. On the other hand, in rats, suppression of these antibody responses to TNP-LPS occurred at a TCDD dose (i.e., 30 μg/kg) above which resulted in thymus and liver weight alterations (i.e., 3 and 10 μg/kg). This indicates that the latter manifestations of TCDD-induced toxicity in the rat are more sensitive parameters than is immunosuppression.

The fact that TCDD suppressed the antibody response to TNP-LPS in rats is in striking contrast to earlier work from this laboratory which demonstrated a marked difference between mice and rats in the effect that TCDD has on the primary antibody response to the T cell-dependent antigen SRBC (Smialowicz et al., 1994). It is well recognized that lower acute doses of TCDD are required to suppress the primary antibody response in aryl hydrocarbon (Ah)-".
TABLE 1
Effect of 2,3,7,8-Tetrachlorodibenzo-p-dioxin on the Relative Organ Weights and Antibody Plaque-Forming Cell Responses to Trinitrophenyl-lipopolysaccharide in Female B6C3F1, Mice

<table>
<thead>
<tr>
<th>TCDD (µg/kg)</th>
<th>Relative spleen weight</th>
<th>Relative thymus weight</th>
<th>Relative liver weight</th>
<th>PFC/10⁶ spleen cells</th>
<th>PFC (×10⁻⁴)/spleen</th>
<th>Titer (log₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.87 ± 0.23</td>
<td>1.25 ± 0.05</td>
<td>57.86 ± 1.27</td>
<td>1033 ± 109</td>
<td>22.91 ± 3.47</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>7.55 ± 0.22</td>
<td>1.09 ± 0.08</td>
<td>62.33 ± 2.25</td>
<td>1128 ± 123</td>
<td>22.13 ± 2.17</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>6.79 ± 0.25</td>
<td>1.03 ± 0.11</td>
<td>68.45 ± 1.62</td>
<td>868 ± 73</td>
<td>16.50 ± 1.54</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>6.43 ± 0.26</td>
<td>0.73 ± 0.03</td>
<td>73.20 ± 1.73</td>
<td>591 ± 48</td>
<td>9.72 ± 1.36</td>
<td>—</td>
</tr>
</tbody>
</table>

* Six mice per group. Results are means ± SE.
* p < 0.05.
' p < 0.01.

TABLE 2
Effect of 2,3,7,8-Tetrachlorodibenzo-p-dioxin on the Relative Organ Weights and Antibody Plaque-Forming Cell Responses to Trinitrophenyl-lipopolysaccharide in Female F344 Rats

<table>
<thead>
<tr>
<th>TCDD (µg/kg)</th>
<th>Relative spleen weight</th>
<th>Relative thymus weight</th>
<th>Relative liver weight</th>
<th>PFC/10⁶ spleen cells</th>
<th>PFC (×10⁻⁴)/spleen</th>
<th>Titer (log₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.54 ± 0.26</td>
<td>1.14 ± 0.12</td>
<td>59.14 ± 1.33</td>
<td>852 ± 57</td>
<td>20.55 ± 2.19</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>1</td>
<td>7.33 ± 0.55</td>
<td>1.16 ± 0.05</td>
<td>61.81 ± 1.23</td>
<td>807 ± 52</td>
<td>17.13 ± 1.24</td>
<td>5.0 ± 0.1</td>
</tr>
<tr>
<td>3</td>
<td>6.39 ± 0.20</td>
<td>0.94 ± 0.05</td>
<td>63.46 ± 0.88</td>
<td>741 ± 52</td>
<td>12.59 ± 1.08</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>10</td>
<td>6.52 ± 0.22</td>
<td>0.70 ± 0.07</td>
<td>65.13 ± 1.49</td>
<td>542 ± 50</td>
<td>9.14 ± 1.68</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>30</td>
<td>6.27 ± 0.28</td>
<td>0.55 ± 0.05</td>
<td>69.93 ± 2.06</td>
<td>368 ± 40</td>
<td>5.58 ± 0.83</td>
<td>2.2 ± 0.2</td>
</tr>
</tbody>
</table>

* Six rats per group. Results are means ± SE.
* p < 0.05.
' p < 0.01.
the SRBC response in both rat strains, it is not known if the observed enhanced response is unique to the F344 strain.

In this study, the TCDD dose required to suppress the TNP-LPS response in B6C3F1 mice was several times higher than that with which suppressed the T cell-dependent response to SRBC (Smialowicz et al., 1994). A similar requirement for higher doses of TCDD to suppress the TNP-LPS response in Ah-responsive mice has also been reported by others. For example, Harper et al. (1994, 1995) observed suppression of the TNP-LPS response in C57BL/6 and B6C3F1 mice at ED50 values of 1.6 and 4.2 μg/kg TCDD, respectively. House et al. (1990) found that a single dose of 10 μg/kg TCDD did not suppress the PFC response to TNP-LPS in B6C3F1 mice, whereas the response to the TI type 2 antigen TNP-Ficoll, which requires helper T cells and accessory cells (Braley-Mullen, 1982; Eldridge et al., 1985), was suppressed at 1 and 10 μg TCDD/kg. Likewise, Kerkvliet and Brauner (1987) demonstrated differences in the sensitivity of T cell-dependent and -independent antibody responses to suppression by 2,3,7,8-tetrachlorodibenzo-p-dioxin in C57BL/6 mice with the rank order of suppression being SRBC > TNP-Ficoll > TNP-LPS. These results suggested that regulatory T cells, specifically helper T cells, are more sensitive to TCDD than are B cells (Kerkvliet et al., 1990). TCDD-induced suppression of the antibody response to TNP-LPS is also Ah receptor dependent in mice. The ED50 for suppression of the TNP-LPS response in congenic Ah-reponsive C57BL/6 mice was 7.0 μg TCDD/kg, whereas in congenic C57BL/6 Ah-responsive mice higher doses of TCDD (i.e., ED50 of 30.8 μg/kg) were required (Kerkvliet et al., 1990). F344 rats are also Ah responsive in that hepatic CYP1A1 and CYP1A2 induction occurs at comparable doses of TCDD in these rats and B6C3F1 mice (Smialowicz et al., 1994). Unlike B6C3F1 mice, however, in this study F344 rats required a TCDD dose of 30 μg/kg to suppress the TNP-LPS response.

Enhancement of the response to SRBC occurred in F344 rats exposed to TCDD that displayed decreased OX8+ (i.e., CD8+ suppressor/cytotoxic/natural killer cells in the rat) and increased IgM+ splenocytes, whereas no alterations in these cell subsets were observed in mice (Smialowicz et al., 1994). It is difficult to mechanistically reconcile this observation of enhanced antibody response to SRBC and reduced CD8+ suppressor T cells. An enhanced response would more reasonably be expected to occur in rats with alterations in CD4+ helper T cells; however, this subset was unaffected in either TCDD-exposed rats or mice (Smialowicz et al., 1994). In this study, it was of interest to determine if a similar pattern of reduced CD8+ cells occurred in rats dosed with TCDD and immunized with TNP-LPS; however, no difference was observed in splenic lymphocyte phenotypes between control and TCDD-exposed and TNP-LPS-immunized rats. Similarly, no difference was observed for mice. These data are not surprising given that regulatory T cells (i.e., helper or suppressor) play a minimal role in the antibody response to the TI antigen TNP-LPS (Braley-Mullen, 1982; Eldridge et al., 1985).

In conclusion, this study represents the first direct comparison of the effect that acute exposure to TCDD has on the antibody response to the TI antigen TNP-LPS in B6C3F1 mice and F344 rats. The dose of TCDD required to suppress this immune response in rats was higher than that in mice. Nevertheless, the sensitivities of mice and rats to TCDD-induced suppression of this response to TNP are relatively similar. This is in marked contrast to the observed lack of TCDD-induced suppression in rats versus mice immunized with the T cell-dependent antigen SRBC (Smialowicz et al., 1994). Taken together, these results indicate that the direction and magnitude of TCDD-induced alterations in primary antibody responses to T cell-dependent and -independent antigens are contingent on the rodent species employed. The mechanism(s) underlying these species differences remains to be elucidated.

**DISCLAIMER**

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