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DEVELOPMENTAL EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN ALTERS POSTNATAL T CELL PHENOTYPES, T CELL FUNCTION AND EXACERBATES AUTOIMMUNE LUPUS IN 24-WEEK-OLD SNF₁ MICE

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Abstract

Background—Untreated, more than 95% of female SWR × NZB: F₁ (SNF₁) mice spontaneously develop a fatal lupus-like glomerulonephritis by 8 months-of-age, while disease onset in males is much slower.

Methods—Timed-pregnant SNF₁ mice (10/treatment) were exposed to TCDD on gestational day (GD) 12 by oral maternal gavage with 0, 40 or 80 µg/kg TCDD.

Results—Offspring of the TCDD-exposed dams showed numerous alterations in T lineage cells at 24 weeks-of-age. Females but not males showed decreased CD4⁺8⁺ and increased CD4⁺8[−] thymocytes. Females also showed increased autoreactive CD4⁺Vβ17^{a+} axillary and inguinal lymph node T cells. Con-A stimulated splenocytes from prenatal TCDD-treated mice produced decreased IL-17 in the females while males showed increased IL-2 and IFN-γ, and diminished IL-4. Mitogen-stimulated pan-lymphoproliferative responses were significantly increased across sex by TCDD. Anti-IgG and anti-C3 immune complex deposition in kidneys was present in the males after TCDD, and visibly worsened in females.

Conclusions—Developmental TCDD exposure can permanently alter T lymphopoiesis in autoimmune-prone SNF₁ mice. The alteration profile is beyond the classic immune suppression response, to also include exacerbation and induction of a lupus-like autoimmune disease.

Keywords

TCDD; prenatal exposure; T cells; autoimmunity; SNF₁ mouse

Introduction

Exposure of rodents to different immunotoxic agents during the perinatal establishment of the immune system causes persistent immune function deficits in the offspring (Dietert and Piepenbrink, 2006; Holladay and Smialowicz, 2000). For instance, pregnant rodent exposure to low levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) impaired cytotoxic T lymphocyte activity of offspring until 10 weeks of age (Holladay and others, 1991) and permanently depressed delayed-type hypersensitivity responses (Gehrs and Smialowicz, 1999). These effects of TCDD on the immune system are primarily mediated through the aromatic hydrocarbon receptor (AhR) (Hogaboam and others, 2008; Hundeiker and others, 1999) and are greatly influenced by the affinity of the AhR (high vs low) relative to the dose of TCDD encountered. For example, SNF₁ mice express a low affinity AhR and require an approximately 8-fold increase in TCDD dose to equal the high-affinity C57BL/6 response (Mustafa and others, 2008).

Besides causing immune suppression, several reports suggest TCDD may increase the risk of autoimmunity. Mice treated *in vivo* with monoclonal antibodies to MHC class I and class II molecules displayed inhibited thymocyte differentiation, similar to that occurring spontaneously in murine models of autoimmune disease (Kakkanaiah and others, 1990; Kruisbeek and others, 1985) or after TCDD (Blaylock and others, 1992). These thymic MHC class I and class II antigens are required for normal thymocyte differentiation as well as for deletion of autoreactive cells (Blaylock and others, 1992). DeWaal et al. (1992) observed reduced thymic epithelial MHC class II antigen expression in TCDD-treated mice, while Dong et al. (1997) found that TCDD down-regulated an MHC class I gene (*Q1^b*) in a mouse hepatoma cell line. The MHC *Q1^b* cDNA encodes for the $\alpha 3$ domain and transmembrane domain of the *Q1^b* class I protein, implying that the MHC gene product could interact with $\beta 2$ -microglobulin, and as such function in antigen presentation. In another study, TCDD was shown to increase the expression of MHC class II molecules on splenic Dendritic cells (DC) in the absence of antigen suggesting that TCDD modulates the processing and presentation of antigen in thymic DC, thus altering the CD4 thymocyte selection process (Vorderstrasse and others, 2003). These effects on MHC class I and II molecules by TCDD raised questions regarding the ability of TCDD to impair autoreactive thymocyte deletion (Holladay, 1999). In support of the possibility TCDD may impair T cell selection, Silverstone et al. (1994) observed T cells expressing elevated levels of CD4⁺ V β 17^a and V β 3⁺ TCR in the livers of TCDD-treated mice. Such TCR variable β (V β) chains are usually deleted in the thymus by reaction with self-MHC and minor lymphocyte stimulatory antigens (Hanawa and others, 1993; Okuyama and others, 1992) and have been associated with autoimmunity in experimental mouse models (Rocha and others, 1992).

The incidence of nephritis is low in autoimmune NZB mice, but when this strain is crossed with normal SWR mice, almost 100% of the female SNF₁ hybrids develop a fatal glomerulonephritis. The progression of autoimmune disease in these lupus-like SNF₁ mice, as in the human version of the disease, is critically dependent on accelerated antibody production subsequent to inappropriate activity of CD4⁺ T helper cells. This autoimmune nephritis is thus characterized by IgG deposition in kidney glomeruli (Mohan and others, 1993).

Silverstone et al. (1998) exposed SNF₁ mice to TCDD during mid-gestation, and reported an early onset of glomerulonephritis not normally observed in the males at 24 weeks-of-age. Mechanisms underlying this early induction of autoimmunity in the male SNF₁ mice by TCDD are not known, and are important for understanding environmental influences on disease initiation and expression. In the current study, we hypothesized that prenatal exposure to TCDD in SNF₁ mice may produce permanent defects in T cell development,

maturation and function that will correspond to acceleration or exacerbation of autoimmune lupus. Detecting such changes is necessary to guide later studies aimed at determining how TCDD may act as potential risk factor in the development of autoimmune diseases.

Methods and Materials

Mice and TCDD exposure

SWR × NZB (SNF₁) mice were obtained by breeding male NZB mice with female SWR/J mice, both from Jackson Laboratories (Bar Harbor, ME). Mice (4–5 weeks-of-age) were acclimated to the animal care facility for at least 2 weeks prior to breeding. Briefly, 60 SWR females were bred to 30 NZB males overnight in cages containing one NZB male per two SWR females. Plug positive mice, evaluated the next morning, were designated gestation day (GD) 0. Pregnant SWR mice were orally gavaged on GD 12 with 0, 40 or 80 µg/kg TCDD dissolved in corn oil (N= 10 pregnant mice/treatment). The SNF₁ offspring were weaned at 20–21 days and separated by treatment, allowed to mature to 24 weeks-of-age, and evaluated for changes in immune status. All animals were fed a commercial pelleted diet, provided water *ad libitum*, and housed under controlled conditions of temperature (22 °C), humidity (40–60%), and lighting (12:12 light:dark cycle). Animal maintenance, care and use were approved prior to initiation of experiments and at all times were in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines at Virginia Tech.

Body weights and tissue collection

Mice at 24 weeks-of-age were euthanized by cervical dislocation and weighed. Left untreated, females are in early stages of lupus nephritis and males are free of clinical signs (Eastcott and others, 1983). The thymus, spleen, and axillary and inguinal lymph nodes were immediately collected post-euthanasia under aseptic conditions, using dissection scissors and curved forceps. The spleen and thymus were weighed and then all tissues were placed individually into pre-labeled sterile Petri dishes (Corning, Corning, NY), containing 8 mL of RPMI-1640 culture medium (Mediatech, Herndon, VA). Dishes were placed on ice until tissue dissociation.

Cell dissociation and enrichment

Each organ was gently dissociated over a stainless steel sieve screen (Sigma, St. Louis, MO) using curved forceps. Cells were then pipetted through the sieve screen following dissociation to remove debris. Cells were washed in incomplete RPMI-1640 for 10 min, 240 × g, and 23 °C. The supernatant was discarded and, with the exception of spleen, the cell pellet was resuspended in 8 mL of RPMI-1640. Spleen cells were resuspended in 1 mL incomplete RPMI-1640. To each tube, 2 mL of 0.83% ammonium chloride lysis buffer (ACK, pH 7.29) were added, to lyse red blood cells, and tubes were incubated for 5 min at 23 °C. After lysis incubation, the cells were resuspended in 5 mL of incomplete RPMI-1640 and washed twice (7 min, 290 × g and 7 °C). The splenic leukocyte-rich cells were then resuspended in 5 mL complete RPMI-1640 media containing 10% heat-inactivated FBS (Atlanta Biologicals, Atlanta, GA), 2 mM L-glutamine (ICN, Costa Mesa, CA), 50 IU/mL penicillin (ICN), and 50 mg/mL of streptomycin (ICN), and maintained at 7–10 °C.

Cell enumeration

Cells were enumerated and size-analyzed using a Beckman Multisizer 3® Cell Coulter (Beckman Coulter, Fullerton, CA) according to the manufacturer's protocol. Briefly, a 10 µL aliquot of enriched cell suspension was transferred to a plastic counting-chamber containing 10 mL of PBS (Mediatech). The plastic chamber was capped, mixed by repeated gentle

inversion, and counted. The cells were enumerated and adjusted to 5.0×10^6 cells/mL in complete RPMI-1640 media.

Flow cytometric evaluation of cell-surface markers

Cell suspensions ($5 \times 10^5/100 \mu\text{L}$) from the thymus, spleen, and lymph nodes were dispensed into individual wells of a 96-well round-bottom tissue culture plate (Corning). Monoclonal antibodies (mAbs) with phycoerythrin (PE) fluorescent labels were used according to manufacturer's (BD Pharmingen; San Diego, CA) recommendation at a concentration of $0.2 \mu\text{g}/\mu\text{L}$; mAbs with fluorescein isothiocyanate (FITC) fluorescent labels were similarly used at the recommended concentration of $0.5 \mu\text{g}/\mu\text{L}$. Cells were stained as previously described (Mustafa et al., 2008). Briefly, lymphocyte aliquots (5×10^5 cells/ $100 \mu\text{L}$) from thymus, spleen, and lymph nodes were incubated with the following primary mAbs: PE-anti CD4, FITC-anti CD8, FITC-anti CD25, FITC-anti V β 3 TCR (KJ25), or FITC-anti V β 17^a TCR (KJ23) (BD Pharmingen). For double staining protocols, mAbs with different fluorescent labels were simultaneously added to the sample. Following staining, cells were washed and evaluated on a Coulter Epics XL flow cytometer (Beckman Coulter). From each sample, 10000 events were collected and analyzed using the FlowJo software (Tree Star, San Carlos, CA). Dead cells, clumps, and debris were excluded electronically by gating on forward scatter (FSC) versus side scatter (SSC).

Immunohistochemistry of the kidneys: C3 and IgG deposition

Kidneys were collected at the time of euthanasia and sectioned for immunohistochemistry. The immunohistochemistry section was embedded in OCT media (Miles, Elkhart, IN) and frozen at -80°C for cryosections. Frozen kidneys were cut into $5 \mu\text{m}$ sections and stained with FITC conjugated antibodies. Briefly, tissue sections were thawed at room temperature and dried for 30 min. Slides were fixed in acetone for 10 min and then washed with PBS thrice for 3 min/wash. Goat anti-mouse IgG diluted 1:100 (MP Biomedicals, Santa Ana, CA) or goat anti-mouse C3 diluted 1:100 (MP Biomedicals) were incubated with tissues sections in a humid chamber for 60 min at 23°C . The sections were then rinsed thrice for 5 min/wash with PBS. The slides were mounted using VectashieldTM mounting media (Vector Labs, Burlingame, CA) and then examined using an Olympus BX-60 fluorescence microscope (Center Valley, PA). The severity of glomerulonephritis and immune complex deposition was scored using a range from 0 to 3+, where 0 corresponded to a non-autoimmune healthy mouse and 3+ to the maximal alteration observed in the study. All slides were scored in a blinded manner independently by an experienced investigator (co-author RG). Scores were averaged for the final tissue score.

Lymphocyte proliferation assay

Splenocytes were plated into each well (5×10^5 cells/ $100 \mu\text{L}$ per well) of a 96-well round-bottom tissue-culture plate (Corning Cell WellsTM, Corning). Cells were exposed to mitogens as follows: $100 \mu\text{L}$ of: Concanavalin A (Con A, $10 \mu\text{g}/\text{mL}$, Sigma); or phorbol myristate acetate (PMA, $10 \text{ ng}/\text{mL}$, Sigma) plus ionomycin ($0.5 \mu\text{g}/\text{mL}$, Sigma) in complete media. Non-stimulated control cultures contained $100 \mu\text{L}$ of complete media alone. Triplicate wells were used for each stimulant. Following 48 h incubation, $20 \mu\text{L}$ of alamarBlueTM dye (Serotec, Raleigh, NC) (10% of incubation volume) were added to each well of the culture plates. At 24 and 48 h post addition, degree of absorbance was determined under dual wavelength (570 and 600 nm) using a Molecular Devices plate reader (Menlo Park, CA).

Cytokine ELISA Assays

Splenocytes were plated into each well (1 mL in complete media; 5×10^6 cells per well) of a 24-well tissue-culture plate (Corning). Cells were co-cultured with 1 mL Con A (10 $\mu\text{g/mL}$) and incubated at 37 °C, 5% CO₂ for 48 h. The plates were centrifuged (7 °C, 250 $\times$ g, 7 min), and the supernatants were transferred to sterile 12 $\times$ 75 mm cultured tubes (Fisher). Supernatants were stored at – 80 °C until use. The levels of interleukin 2 (IL-2), IL-4, IL-10, IL-12, and interferon-gamma (INF- γ) were determined using ELISA kits (Ready-to-use; ebioscience, San Diego, CA) according to the manufacturer's instructions. IL-17 levels were measured using the Searchlight Mouse Cytokine Array (Pierce Biotechnology Inc. Rockford IL) according to the manufacturer's instructions and imaged using a cooled CCD camera and analyzed using the Arrayvision 8.0 software.

Statistical analysis

Data were expressed as arithmetical mean $\pm$ SEM. Analysis of variance (ANOVA) was used with Dunnett's test to establish significant differences in the same sex groups between treatment groups and control. The pregnant dam was maintained as the statistical unit in all cases such that each offspring analyzed represented a separate dam (one pup/sex/treatment). Group size was six SNF₁ offspring per sex for all experiments (N=6). Results described as different in this report indicate significantly different at $p \leq 0.05$.

Results

Body and organ weights, and organ cellularity

Body weight of the 24-week-old adult SNF₁ offspring was decreased in the males by prenatal exposure to 40 and 80 $\mu\text{g/kg}$ TCDD and 80 $\mu\text{g/kg}$ TCDD in the females. Thymic weight was decreased by 80 $\mu\text{g/kg}$ TCDD in the male offspring only, while thymic cellularity was decreased in both sexes by 80 $\mu\text{g/kg}$ prenatal TCDD. There were no significant differences in splenic weight or the spleen/body weight ratio across treatment groups. In contrast, splenic cellularity was increased, in males but not females, by 80 $\mu\text{g/kg}$ TCDD (Table 1).

Thymic T cell differentiation

Female, but not male, offspring of dams dosed with TCDD exhibited significant thymic phenotypic changes at 24 weeks-of-age. The relative expression of thymic CD4⁺CD8⁺ cells was decreased in females by the 80 $\mu\text{g/kg}$ prenatal TCDD dose. In addition, CD4⁺CD8⁺ thymocytes were significantly increased in females at this same TCDD exposure level (Table 2).

Spleen T cells

The relative percentage of spleen T cells expressing CD4/CD8 markers did not change in any treatment group, in either sex. However, the absolute number of T cells expressing CD4 was significantly increased in the 40 $\mu\text{g/kg}$ TCDD females and 80 $\mu\text{g/kg}$ TCDD males when compared to corresponding controls (Table 3).

T cell subset expression in lymph nodes

The combined axillary and inguinal lymph node CD4/CD8 T cell phenotypes were not different across treatment group or sex compared to controls. However, the 80 $\mu\text{g/kg}$ TCDD females showed significantly increased percentages of CD4⁺V β 17^{a+} TcR T cells. The T cells expressing CD4⁺CD25⁺, which include T regulatory and activated T cells, were increased in the 80 $\mu\text{g/kg}$ female and male treatment groups (Table 4).

Deposition of anti-IgG and anti-C3 immune complexes in the kidney

Since immune complex deposition in the kidney is a common signalment in lupus patients, we employed immunofluorescent staining with antibodies to IgG and C3. Sectioned kidneys from 24-week-old SNF₁ offspring stained with anti-IgG and anti-C3 antibodies showed an increasing TCDD dose dependent trend in deposition of immune complexes, which was significant in the males (Figure 1). Anti-C3 staining produced images that very similar to IgG (data not shown).

Mitogen-stimulated splenic lymphocyte proliferation

Mitogen stimulation of enriched, cultured splenic lymphocytes was employed to assess the influence of prenatal TCDD on lymphocyte functionality in the adult mouse. Prenatal TCDD had a selective effect on splenic lymphoproliferative responses of 24 week-old SNF₁ offspring. No significant effects on stimulation responses were seen using ConA in splenocytes, at 48 or 72 h. However, the mitogenic response to PMA/Ionomycin (P/I) was significantly enhanced in females at both TCDD exposures, at 48 and 72 h. Enhanced proliferation was also seen in 80 µg/kg TCDD P/I males, at 48 h (Figure 2).

T_h1/T_h2 cytokine balance

In males, prenatal exposure to TCDD caused a shift toward a T_h1 cytokine profile in Con-A-stimulation splenic lymphocytes collected from 24-week-old mice. The 80 µg/kg TCDD males showed enhanced IL-2 and IFN-γ production, and diminished IL-4 production relative to controls. In the female mice, IL-17 production followed a decreasing trend reaching significance at 80 µg/kg TCDD (Figure 3).

Discussion

T cells were initially proposed to not play a direct role in tissue damage in SNF₁ lupus-like nephritis. However, the proliferation of glomerulus-specific autoantibodies may require interaction between autoreactive T-helper (T_h) cells and autoreactive B cells (Mohan and others, 1993). An increase in the CD4 to CD8 ratio of IdLNF1-reactive T cells, suggestive of increased T_h cells, was also detected at 22–24 weeks of age in sera of SNF₁ mice and coincided with increased IdLNF1 Ig (IgG + IgM) deposition in the kidneys (O'Garra and others, 1992). Further, the presence of pathogenic autoantibody-inducing T_h cells specific for chromatin subparticles or histones has been noted in human patients with systemic lupus erythematosus as well as in SNF₁ mice (Fournel and others, 2003). Thus, available data suggest that autoimmune nephritis in SNF₁ mice might be influenced by a T cell lesion. This is supported by a study showing that postnatal day 3 thymectomy protects SNF₁ mice from glomerulonephritis (Bagavant and others, 2002).

In the present study, SNF₁ mice exposed developmentally to TCDD varied considerably, by sex and compartment, in both type and severity of immune lesions. Thymic cellularity was approximately equally diminished in the male and female offspring. Mechanisms underlying this effect are not known, but may include bone marrow progenitor T cell damage by TCDD (Fine and others, 1989) or enhancement of precursor T cell death at the thymic level (Rajagopalan and others, 1990; Shivakumar and others, 1989). Female offspring, but not males, showed a dramatic decrease in CD4⁺8⁺ cells and increase in CD4⁺8⁻ cells. These data suggest prenatal TCDD caused permanent postnatal alterations in thymic T cell maturation in female offspring, in addition to thymocyte hypocellularity.

T cell parameters in the secondary lymphoid organs were not changed by TCDD, with two exceptions. Total spleen CD4⁺ T_h cells tended toward increased numbers in 80 µg/kg TCDD females and were significantly increased, by 79%, in males at the same dose level. Lymph

node $V\beta$ TCR expression (autoreactive phenotypes) was unchanged in males, however in females $CD3^+V\beta3^+$ TCR expression tended toward increase while $CD4^+V\beta17a^+$ TCR expression increased significantly, by 115%. Thymic dendritic cells of SNF_1 mice tend to have a lower expression of MHC class II and costimulatory molecules (Michaels and others, 2005). The increase in peripheral $V\beta^+$ T_h cells concentrations, above controls, may indicate that TCDD further compromised thymic deletion of autoreactive TCR (negative selection), a possibility previously suggested based on diminished thymic MHC class II antigen expression by TCDD (Holladay, 1999). It is also possible that TCDD may alter the presentation of nucleosomal autoepitopes by the thymic DC in these mice to exacerbate the disease (Michaels and others, 2005). Further, TCDD exposure increased the thymic $CD4^+CD8^-$ (DN) population. In SNF_1 mice, DN T_h cells are known inducers of the pathogenic IgG anti-DNA antibodies (Adams and others, 1990).

Altered T cell maturation and $V\beta^+$ T_h phenotypes in SNF_1 mice, suggest that prenatal TCDD might cause inappropriate T cell function. We therefore surveyed six principle cytokines mitogen-stimulated T cells of the spleen. We observed that cytokine production was permanently changed by prenatal TCDD, in a manner different based on sex. These variations in cytokine levels between the two sexes would appear to correspond to normal sex-based differences in the disease progression at 24 weeks in this murine strain. For example, IL-10 levels were significantly increased in the females compared to the males across all treatments. Currently, it is believed that elevated levels of IL-10 reflect disease activity once lupus inflammation has been initiated. At 24 weeks, SNF_1 females are already in the active stage of the disease, which is supported by the elevated levels of IL-10.

IL-10 mitigates the pro-inflammatory function of T_h17 and T_h1 thus may explain, in part, the lower levels of IL-17 and $INF\gamma$ observed in the females of this strain at 24 weeks (McGeachy and others, 2007; Yin and others, 2002). Also, $INF\gamma$ is not believed to be required for later stages of disease which would appear to correlate with the disease progression of these female mice (Nicoletti and others, 1992). Interestingly in this study, prenatal TCDD exposure further suppressed the T_h17 and T_h1 cytokine profile as indicated by the decreased levels of IL-17 and $INF\gamma$ in these females compared to controls. One possibility for the decreased IL-17 levels may be the stage of disease progression, in that the IL-17-producing T cells may have already localized within the sites of inflammation, for instance the kidney (Kang and others, 2007). Another possibility is that TCDD modulated the IL-17-producing T cells through the AhR in a ligand-specific manner (Quintana and others, 2008). Further, since the percentage of $CD4^+CD25^+$ T cells was increased in the TCDD mice, it is possible that this reflects an expansion of an induced T-reg population (Marshall and others, 2008). It is also likely that a different set of cytokines might contribute to or even orchestrate the florid immune response at advanced stages of SLE. Recent studies have focused on type I IFNs ($IFN-\alpha$ and $IFN-\beta$) as there is growing evidence to suggest these IFNs may play a critical role in the pathogenesis of lupus (Banchereau and Pascual, 2006).

In the males, however, prenatal TCDD exposure increased the level of $INF\gamma$ and IL-2, and decreased IL-4 suggesting a skewing towards T_h1 activity. At 24 weeks, SNF_1 males usually do not manifest SLE-like symptoms, however, higher levels of $INF\gamma$ would appear to correlate with the early onset of the autoimmune disease (Karpuzoglu-Sahin and others, 2001a; Karpuzoglu-Sahin and others, 2001b). For example, increased $INF\gamma$ has been associated with development of autoimmune insulinitis (Campbell and others, 1991), lupus nephritis (Haas and others, 1997), Sjogren's syndrome (Hayashi and others, 1996) and autoimmune arthritis (Billiau, 1996).

In this study, Con A stimulation was employed to specifically evaluate the function of T cells, in particular the T_H cells. Thus, the observed cytokine profile is reflective of T cell signaling. It should be noted that this cytokine profile might shift significantly if the splenocytes had been cultured with other mitogens, for instance the B cell mitogen lipopolysaccharide (LPS) or pan-lymphocyte mitogen PMA/ionomycin. Further studies are planned to evaluate in more detail the cytokine signaling under time kinetics using additional mitogens targeting the other immune cells. Beyond altered cytokine production, splenocytes from both sexes of the prenatal TCDD-exposed SNF₁ mice displayed enhanced lymphoproliferative responses to a pan-lymphocyte mitogen stimulation at 24 weeks-of-age, supporting a hyperactivity of these cells.

Immunofluorescent staining of the kidney was employed to track lupus-nephritis and to determine if developmental TCDD enhanced autoimmune disease in the present SNF₁ mice. Immune complex IgG and C3 deposition was significantly increased in the TCDD-exposed males and displayed a dose-dependent numeric increase in females. Females and males both also showed dose-related trends toward increases in autoimmune-related kidney pathology, in the form of fibrinoid necrosis, crescents and inflammatory cells (data not shown).

In summary, male and female SNF₁ mice showed persistent changes in T cells as a consequence of GD 12 exposure to TCDD. Among these were numerous sex-specific effects, suggesting possible interactions with endogenous hormones. The TCDD-exposed mice displayed a clear enhanced autoimmune profile, including increased V β ⁺ T_H cells, increased T cell proliferative capacity, dysregulated cytokine production toward T_H1, and increased autoimmune kidney lesions. Further, it should be noted that these changes were reflective of the disease progression at 24 weeks and may change differently across sex as these animals age. Thus, these collective data show that developmental TCDD permanently alters the postnatal immune system, in a manner beyond the well-established profile of immune suppression, and that correlates with exacerbation of lupus-like autoimmune responses in SNF₁ mice.

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Figure 1.

The kidneys from 24-week-old SNF₁ mice that were prenatally exposed to 0, 40 or 80 µg/kg TCDD were collected, fixed, sectioned and stained with FITC-labeled anti-IgG and anti-C3. The above are representative images of kidneys stained with FITC-anti-IgG based on treatment and sex. The data are based on 5 mice /treatment/sex (*= $p \leq 0.05$, Dunnett's test).

Figure 2.

The lymphoproliferative responses of cultured splenocytes in 24-week-old SNF₁ mice exposed prenatally to 0, 40 or 80 µg/kg TCDD. Splenocytes were exposed to Con A or P/I for 48 h or 72 h. Values are reported as mean Δ specific absorbance $\pm$ SEM. The results are based on 5 mice/treatment/sex (* = $p \leq 0.05$, Dunnett's test).

Figure 3.

Supernatants were collected from splenocytes, of 24-week-old mice that were prenatally exposed to 0, 40 or 80 µg/kg TCDD, and cultured for 48 h with Con A (10 µg/mL). The levels of IL-2, IL-4, IL-10, IL-12, IL-17 and INF-γ were determined using commercially available murine cytokine ELISA kits. The data are presented as mean ± SEM. The results are based on 5 mice/treatment/sex (*= $p \leq 0.05$, Dunnett's test).

Table 1**Body Weight, Organ Weight & Cellularity**

		0.0 µg/kg Mean ± SEM	40.0 µg/kg Mean ± SEM	80.0 µg/kg Mean ± SEM
Body Weight Grams	<i>female</i>	26.9 ± 0.9	25.9 ± 0.3	24.1 ± 0.6 *
	<i>male</i>	42.0 ± 0.8	36.1 ± 1.3 *	33.7 ± 1.1 *
Thymic Weight Grams	<i>female</i>	0.042 ± 0.004	0.052 ± 0.006	0.038 ± 0.004
	<i>male</i>	0.042 ± 0.002	0.038 ± 0.003	0.032 ± 0.001 *
Thymic Cellularity Cells (Millions)	<i>female</i>	35.3 ± 4.6	26.0 ± 3.5	20.9 ± 2.6 *
	<i>male</i>	22.2 ± 1.4	21.2 ± 1.8	14.4 ± 1.8 *
Thymus: Body Weight Ratio	<i>female</i>	0.0015 ± 0.0001	0.0020 ± 0.0002	0.0016 ± 0.0001
	<i>male</i>	0.0010 ± 0.0001	0.0011 ± 0.0001	0.0009 ± 0.0001
Splenic Weight Grams	<i>female</i>	0.190 ± 0.047	0.217 ± 0.040	0.172 ± 0.026
	<i>male</i>	0.114 ± 0.016	0.111 ± 0.008	0.108 ± 0.009
Splenic Cellularity Cells (Millions)	<i>female</i>	106.3 ± 13.4	151.8 ± 22.3	144.5 ± 22.7
	<i>male</i>	64.4 ± 5.8	65.2 ± 4.8	88.8 ± 10.3 *
Spleen: Body Weight Ratio	<i>female</i>	0.007 ± 0.001	0.008 ± 0.002	0.007 ± 0.001
	<i>male</i>	0.003 ± 0.00	0.003 ± 0.00	0.003 ± 0.00

n = 6 mice /treatment/sex

* $p \leq 0.05$, Dunnett's test.

Table 2

T cell differentiation Analysis

Thymus		0.0 µg/kg Mean ± SEM	40.0 µg/kg Mean ± SEM	80.0 µg/kg Mean ± SEM
CD4+ CDS- (%)	<i>female</i>	16.1 ± 3.7	19.0 ± 3.9	9.9 ± 0.9
	<i>male</i>	20.0 ± 4.5	21.8 ± 3.3	14.1 ± 2.2
CD4+ CO8+ (%)	<i>female</i>	31.3 ± 6.2	26.0 ± 3.7	16.2 ± 2.9 *
	<i>male</i>	34.9 ± 8.8	40.8 ± 3.0	42.2 ± 6.5
CD4- CD8- (%)	<i>female</i>	49.2 ± 9.9	50.3 ± 7.6	71.9 ± 3.3 *
	<i>male</i>	43.5 ± 12.1	35.0 ± 1.8	41.8 ± 8.4
CD4- CD8+ (%)	<i>female</i>	3.4 ± 0.7	4.8 ± 1.0	1.9 ± 0.2
	<i>male</i>	1.5 ± 0.3	2.5 ± 0.4	1.8 ± 0.3

n = 6 mice /treatment/sex

*
 $p \leq 0.05$, Dunnett's test.

Table 3

Splenic T cell Subset Analysis

Spleen		0.0 µg/kg Mean ± SEM	40.0 µg/kg Mean ± SEM	80.0 µg/kg Mean ± SEM
CD4+ CD8- (%)	<i>female</i>	32.3 ± 3.2	42.6 ± 4.1	34.3 ± 4.2
	<i>male</i>	34.9 ± 3.2	38.6 ± 3.6	43.8 ± 3.0
CD4- CD8+ (%)	<i>female</i>	4.8 ± 0.4	4.9 ± 0.3	4.6 ± 0.3
	<i>male</i>	4.1 ± 0.4	3.6 ± 0.3	4.0 ± 0.5
Absolute number CD4+ CD8- Cells (Millions)	<i>female</i>	33.269 ± 2.36	56.026 ± 8.21 *	49.891 ± 10.79
	<i>male</i>	21.957 ± 1.78	24.622 ± 1.93	39.213 ± 6.02 *
Absolute number CD4- CD8+ Cells (Millions)	<i>female</i>	5.171 ± 0.88	7.453 ± 1.30	6.602 ± 1.13
	<i>male</i>	2.559 ± 0.18	2.257 ± 0.11	3.443 ± 0.47

n = 6 mice /treatment/sex

*
 $p \leq 0.05$, Dunnett's test.

Table 4

Lymph Node T cell Subset Analysis

Lymph Nodes		0.0 µg/kg Mean ± SEM	40.0 µg/kg Mean ± SEM	80.0 µg/kg Mean ± SEM
CD4+ CD8- (%)	<i>female</i>	67.6 ± 1.1	71.6 ± 0.9	63.6 ± 2.2
	<i>male</i>	79.4 ± 1.1	79.1 ± 2.5	75.7 ± 0.7
CD4- CD8+ (%)	<i>female</i>	25.1 ± 1.6	22.0 ± 0.9	22.6 ± 2.8
	<i>male</i>	15.2 ± 1.1	11.2 ± 1.8	18.7 ± 1.8
CD4+ CD25+ (%)	<i>female</i>	7.4 ± 0.9	7.4 ± 1.3	15.2 ± 2.8 *
	<i>male</i>	3.5 ± 0.1	4.4 ± 0.4	5.1 ± 0.4 *
CD4+ Vβ17 ^a + TCR (%)	<i>female</i>	3.9 ± 1.0	3.6 ± 0.5	8.4 ± 1.7 *
	<i>male</i>	3.2 ± 0.0	3.2 ± 0.0	3.1 ± 0.0
CD3+Vβ3+TCR (%)	<i>female</i>	2.4 ± 0.5	2.3 ± 0.6	4.7 ± 1.6
	<i>male</i>	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0

n = 6 mice /treatment/sex

*
 $p \leq 0.05$, Dunnett's test.