Food allergy is an increasing health problem in Western countries. Previously, it has been shown that the intensity of food allergic reactions can be regulated by regulatory T (Treg) cells. In addition, it has been shown that activation of the aryl hydrocarbon receptor (AhR) regulates T-cell responses by induction of Treg cells. Therefore, we hypothesized that activation of the AhR pathway can suppress development of food allergic responses through the induction of Treg cells. This was investigated by using a mouse model for peanut allergy. C3H/HeOuJ mice (AhRb-2) were sensitized to peanut by administering peanut extract (PE) by gavage in the presence of cholera toxin and were treated with the prototypical AhR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (0.6, 1.7, 5, and 15 µg/kg body weight) on days 3 and 11 orally. The functional role of CD4+CD25+Foxp3+ Treg cells was investigated by depleting these cells with anti-CD25 mAb during sensitization to PE. TCDD treatment dose dependently increased by AhR activation in both spleen and mesenteric lymph nodes. Depletion of CD4+CD25+Foxp3+ Treg cells dose dependently increased by AhR activation in both spleen and mesenteric lymph nodes. Depletion of CD4+CD25+Foxp3+ Treg cells markedly reversed the suppressive effect of TCDD on PE-specific antibody levels and PE-induced IL-5, IL-10, and IL-13 cytokine production. Present data demonstrate for the first time that activation of the AhR by TCDD suppressed the development of Th2-mediated food allergic responses. A functional shift within the CD4+ cell population toward CD4+CD25+Foxp3+ Treg cells appeared to underlie this effect. This suggests that the AhR pathway might provide potential therapeutic targets to treat food allergic diseases.

Key Words: aryl hydrocarbon receptor; peanut allergy; regulatory T cells; TCDD.

In Western countries, food allergy affects about 5% of young children and 3–4% of adults (Sicherer and Sampson, 2010). Most of these people only have mild allergic reactions after exposure to a food allergen, but in severe cases, anaphylaxis can be induced (Lee and Burks, 2009; Shaker and Woodmansee, 2009; Sicherer and Sampson, 2010). The major allergens causing food allergy are peanut, tree nuts, shellfish, fish, wheat, milk, egg, and soy (Sicherer and Sampson, 2010). These allergens can enter the body via the gastrointestinal mucosal immune system. At this location, immune cells must distinguish harmless food antigens and commensal bacteria from pathogens. Disruption of this delicate balance can result in the breakdown of oral tolerance, resulting in the development of food allergy (Shaker and Woodmansee, 2009; Sicherer and Sampson, 2009; Van Wijk et al., 2007).

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor regulating the expression of a wide range of genes via the transcription of dioxin-responsive elements (DREs) (Denison and Nagy, 2003). Cells participating in innate and adaptive immune responses express the AhR and many genes for cytokines, transcription factors, and mediators contain DRE sequences in their promoter regions (Kerkvliet, 2009; Sun et al., 2004). Recently, it has been shown that activation of the AhR by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), VAG539, or 2-(1’-indole-3’-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) results in the induction of regulatory T (Treg) cells in experimental autoimmune encephalomyelitis (EAE), experimental autoimmune uveoretinitis (EAU), and graft versus host disease (GvHD) (Funatake et al., 2005; Hauben et al., 2008; Marshall et al., 2008; Quintana et al., 2008, 2010; Zhang et al., 2010). In contrast, activation of the AhR by the tryptophan photoproduce 6-formylindolo[3,2-b]carbazole has been shown to induce Th17 cells (Quintana et al., 2008; Veldhoen et al., 2008). So, the AhR can regulate both Treg and Th17 differentiation, dependent on the ligand.

In food allergy, Treg cells have been shown to regulate allergic sensitization and the intensity of the food allergic response. Individuals with mutations in the FOXP3 gene suffer from the immune dysregulation polyendocrinopathy enteropathy X-linked syndrome, which can be accompanied by severe food allergy (Torgerson and Ochs, 2007). Furthermore,
depletion of CD4+CD25+ Treg cells during sensitization to peanut increases allergen-specific antibody levels and mast cell degranulation after oral challenge with peanut in mice (Van Wijk et al., 2007). In addition, it has been shown that whey-specific CD25+ Treg cells induced by dietary intervention with prebiotics are involved in the suppression of cow milk allergy in mice (Schouten et al., 2010). Previously, it has been shown that activation of the AhR impairs Th2-type immune responses by suppressing antigen-specific antibody levels and Th2-related cytokines (Fujimaki et al., 2002; Inouye et al., 2005; Ito et al., 2002; Kato et al., 2003; Luebke et al., 2001; Negishi et al., 2005; Nohara et al., 2002). In addition, one epidemiological study found a negative correlation between serum IgE and dioxin-like compounds in humans (Van Den Heuvel et al., 2002). However, no role for Treg cells was described or suggested in any of these studies. Because Treg cells play an important role in food allergic diseases and activation of the AhR is associated with the induction of Treg cells, we hypothesized that activation of the AhR can suppress development of food allergic responses through the induction of Treg cells. This was investigated by using a mouse model for peanut allergy. The prototypical AhR ligand TCDD was used to study the role of the AhR because it is the most potent AhR ligand known (thereby reducing high-dose off-target effects), it is hardly metabolized (the effects observed are not confounded by ligand metabolism) and relatively slowly excreted (half life time in mice 10–12 days) (Denison and Nagy, 2003; Kerkvliet, 2009; Van Den Berg et al., 1994).

First, the effect of different doses TCDD on peanut allergic sensitization was investigated. Next, the role of CD4+CD25+Foxp3+ Treg cells in TCDD-mediated suppression of the peanut allergic response was studied by depleting these cells with anti-CD25 mAb. Collectively, our data demonstrate that activation of the AhR by TCDD suppresses the development of food allergic responses and that a functional shift within the CD4+ cell population toward CD4+CD25+Foxp3+ Treg cells may underlie this suppression.

MATERIALS AND METHODS

Mice and reagents. C3H/HeOuJ mice (4- to 5-week-old; AhR+/−), purchased from Charles River (France), were maintained under controlled conditions (relative humidity of 50–55%, 12 h light/dark cycle, and temperature of 23 ± 2°C) in filter-topped macrolon cages with wood chip bedding. Food pellets and drinking water were available ad libitum. Prior to the start of the experiments, mice were acclimatized. All experiments were approved by the animal experiments committee of the Faculty of Veterinary Medicine, Utrecht University.

2,3,7,8-TCDD (Cambridge Isotope Lab) was dissolved in anisole (Sigma-Aldrich, The Netherlands) at 20.5 µg/ml and diluted in corn oil (Sigma-Aldrich) to the final exposure concentration (0.07% vol/vol anisole). Anisole diluted in corn oil was used as vehicle control (0.07% vol/vol). Peanut extract (PE) (30 mg/ml) was prepared from peanuts from the Golden Peanut Plant (provided by Intersnack Nederland BV, The Netherlands) as described previously (Van Wijk et al., 2005). Peanut extracts were prepared according to standard procedures and checked for protein content by BCA analysis (Pierce, Rockford, IL). Cholera toxin (CT) was purchased from List Biological Laboratories, Inc. (Campbell, CA).

Experimental design. In setup 1 (Fig. 1, setup 1), C3H/HeOuJ mice (n = 6–8 per group) were sensitized to PE by oral exposure to PE (6 mg PE, 200 µl per mouse) with CT (15 µg per mouse) on three consecutive days (days 0, 1, and 2) followed by weekly dosing (days 7, 14, 21, and 28). On days 3 and 11, mice were exposed orally to different doses of TCDD (0.6, 1.7, 5, or 15 µg/kg body weight [BW]). The role of CD4+CD25+Foxp3+ Treg cells was investigated by treating mice with anti-CD25 mAb to deplete Treg cells during sensitization with PE (Fig. 1, setup 2) (Setiady et al., 2010; Tenorio et al., 2010; Van Wijk et al., 2007). Anti-CD25 mAb treatment depletes mostly CD4+CD25+Foxp3+ cells. C3H/HeOuJ mice (n = 6–8 per group) were treated ip with purified rat anti-CD25 (IL-2Rz) mAb (clone PC61, provided by Bioceros B.V.) (200 µg per mouse) on days 4, 0, 7, 11, 14, and 18. In setup 2, TCDD (15 µg/kg BW) was...
administered orally only on day 3 to prevent interference in the Treg cell depletion by anti-CD25 mAb.

In both setups, mice were challenged intragastrically with PE (12 mg per mouse) on day 30, and blood samples were taken after 30 min. On day 31, mice were sacrificed by cervical dislocation and blood, thymus (only in setup 2), spleen, mesenteric lymph nodes (MLN), and liver were isolated.

In setup 3, the early effects of TCDD and anti-CD25 mAb treatment on CD4+CD25+Foxp3+ Treg cells during sensitization to peanut were investigated. C3H/HeOuJ mice (n = 4 per group) were treated ip with purified rat anti-CD25 (IL-2Rα) mAb (clone PC61, 200 μg per mouse, provided by Bioceros B.V.) or control antibody (purified rat-GL113 mAb, 200 μg per mouse, provided by Bioceros B.V.) on days 4 and 0, and TCDD (15 μg/kg BW) was administered orally on day 3. On day 5, mice were sacrificed by cervical dissection and spleen and MLNs were isolated.

Preparation of liver microsomes. Excised livers were homogenized in Tris/HCL (50mM, 1.15% KCl, pH 7.4). The microsomal fraction was obtained from the homogenate by successive centrifugation for 25 min at 900 × g and 85 min at 100,000 × g with a Beckman Coulter Optima L-90 K centrifuge. The microsomal fraction was resuspended in a sucrose solution (0.25M). Protein concentration of the microsomes was determined by the method of Lowry using bovine serum albumin (BSA) as protein standard (Lowry et al., 1951).

Ethyoxresorufin-O-deethylase activity. Ethoxyresorufin-O-deethylase (EROD) activities in liver microsomes were determined in 10 μl sample containing 10–40 μg protein with 90 μl 50mM Tris buffer (pH 7.4) containing 5mM MgCl2, 20μM dicumarol, 2μM 7-ethoxyresorufin, and 1.5mM nicotinamide adenine dinucleotide phosphate (reduced form). A standard curve using resorufin was generated to quantify the EROD activity. Fluorescence was measured at 37°C at an excitation wavelength of 530 nm and an emission wavelength of 590 nm, every 80 s for 16 min in a Fluostar plate reader (BMG Labtechnologies GmbH, Germany). EROD activity was calculated as picomoles resorufin per minute per milligram protein.

Measurement of PE-specific IgE, IgG1 and IgG2a antibody levels in serum and mMCP-1 in plasma. PE-specific IgE, IgG1, and IgG2a antibody levels in serum were detected as previously described and are depicted in serum and mMCP-1 in plasma.

Splenic cell culture and analysis of cytokine production. After red blood cell lysis, single-cell spleen suspensions (2.5 × 10^6 cells/ml) were cultured in 200 μl complete RPMI 1640 (10% Fetal Calf Serum) in the presence of medium or PE (100 μg/ml) for 96 h at 37°C and 5% CO2. Levels of IL-5, IL-10, IL-13, IL-17a, and IFN-γ in collected supernatant were determined by commercial available sandwich ELISA (eBioscience, Austria) according to the manufacturer’s instructions. Levels of IL-4 were below detection limit.

Fluorescence-activated cell sorting analysis. Single-cell suspensions of spleen (after red blood cell lysis) and MLN (1 × 10^6 cells/ml) were stained with anti-CD4-PerCP (clone RM 4-5, BD Biosciences) and anti-CD25-PE (clone PC61, eBioscience) (setup 2) or anti-CD4-PerCP (clone RM 4-5, BD Biosciences) and anti-CD25-FITC (clone 3C7, BD Biosciences) (setup 3) in Fluorescence-activated cell sorting (FACS) buffer (PBS containing 0.25% BSA, 0.05% NaN3, 0.5mM EDTA) for 30 min at 4°C. Subsequently, cells were washed with FACS buffer and stained intracellularly for Foxp3 (Foxp3-APC, eBioscience) according to the manufacturer’s instructions. Analysis was performed on a FACScan with standard FACSflow using CellQuest software (BD Biosciences).

Statistical analysis. Results are presented as the mean ± standard error (SE) of four to eight mice per group. All data were logaritmically transformed (except percentages of FACS data) to achieve normal distribution and were analyzed by one-way ANOVA followed by a Bonferroni post hoc test. A value of p < 0.05 was considered as statistically significant. All statistical analyses were performed using Graphpad Prism software.

RESULTS

Effect of TCDD Treatment on Body, Spleen, and Thymus Weight and EROD Activity in Liver Microsomes

To evaluate possible overt toxic effects of TCDD, body and spleen weights were examined in experimental setup 1 on day 31 (Fig. 1). Compared with PE-sensitized vehicle control mice, TCDD treatment slightly decreased BW at doses of 5 and 15 μg/kg BW (21.31 ± 0.38 vs. 19.26 ± 0.37 [p < 0.05] and 19.49 ± 0.38 [p < 0.05], respectively). Relative weights of spleen or thymus (only measured in setup 2) were not affected by TCDD on day 31 (data not shown). The effect of TCDD on transcriptional activation of the AhR in vivo was investigated by measuring hepatic EROD activity (day 31, setup 1). No EROD activity was observed in liver microsomes from PE-sensitized mice. EROD activity increased dose dependently in TCDD-treated mice reaching maximal activity at 5 and 15 μg/kg BW (Fig. 2).

Activation of the AhR Affects Peanut-Specific Antibody Responses, Mast Cell Degranulation, and Cytokine Responses

We first investigated whether activation of the AhR inhibited the development of peanut allergy by administering increasing doses of TCDD (0.6, 1.7, 5, or 15 μg/kg BW) during sensitization (Fig. 1, setup 1). PE-sensitized mice had increased levels of PE-specific IgE, IgG1, and IgG2a (Figs. 3a–c), mast cell degranulation (measured by mMCP-1) (Fig. 3d), and PE-induced Th2-type T-cell responses (ex vivo release of IL-5, IL-10, and IL-13) (Figs. 3e–f) compared with nonsensitized vehicle control mice. Treatment with TCDD during PE-sensitization dose dependently suppressed PE-specific IgE (5 and 15 μg/kg BW), IgG1 (15 μg/kg BW), and IgG2a (15 μg/kg BW) (Figs. 3a–c). Mast cell degranulation (mMCP-1) was significantly suppressed
at 5 and 15 μg/kg BW TCDD (Fig. 3d). Furthermore, isolated spleen cells that were exposed to TCDD in vivo significantly suppressed PE-induced IL-5 (at 1, 7.5, and 15 μg/kg BW), IL-10, IL-13, and IL-17a (all at 15 μg/kg BW) (Figs. 3e–h). Particularly, at lower doses of TCDD (0.6, 1.7, and 5 μg/kg BW), a significant increase of PE-induced IFN-γ was observed compared with PE-sensitized mice (Fig. 3i). Together, these data show that AhR activation by TCDD suppresses PE-specific antibody levels, mast cell degranulation, and PE-induced cytokine responses differently, dependent on the dose of TCDD.

Activation of the AhR by TCDD Increases the Percentage of CD4+CD25+Foxp3+ Treg Cells in MLN and Spleen

Next, we investigated whether AhR activation affected CD4+CD25+Foxp3+ Treg cells on day 31 (Fig. 1, setup 1). Treatment with TCDD during sensitization to PE increased the percentage of CD4+CD25+Foxp3+ Treg cells in MLN starting at a dose of 1.7 μg/kg BW and in spleen starting at a dose of 5 μg/kg BW (Figs. 4b and g). Within the CD4+ population, the percentage of CD25+Foxp3+ Treg cells was increased in both organs at all doses of TCDD (Figs. 4a and f). In the spleen, no effect on the absolute number of CD4+CD25+Foxp3+ Treg cells was observed (Fig. 4k). TCDD treatment decreased the percentage and number of CD4+CD25−Foxp3+ (Figs. 4h and l) and CD4+CD25+Foxp3+ (Figs. 4i and m) in the spleen at 5 and 15 μg/kg BW. In the MLN, the percentage of CD4+CD25−Foxp3+ cells was decreased at 15 μg/kg BW (Fig. 4c), whereas the percentage of CD4+CD25+Foxp3+ cells was increased at 5 and 15 μg/kg BW TCDD (Fig. 4d). The percentage of CD4− cells in the MLN and the absolute number of these cells in the spleen decreased dose-dependently by TCDD treatment (Figs. 4e and n). Together, these data show that TCDD treatment during sensitization to peanut results in a shift toward CD4+CD25+Foxp3+ Treg cells within the CD4− T-cell population.

Depletion of CD4+CD25+Foxp3+ Treg Cells Abrogates the Suppressive Effect of AhR Activation by TCDD on the Peanut Allergic Response

Then, we determined whether the increased percentages of CD4+CD25+Foxp3+ Treg cells observed after AhR activation were responsible for suppression of the peanut allergic response. For this purpose, CD4+CD25+Foxp3+ Treg cells were depleted using Treg cell-depleting anti-CD25 mAb before and during sensitization to peanut and TCDD treatment (15 μg/kg BW).

The early effects of TCDD and/or anti-CD25 treatment during sensitization to PE were investigated on day 5 (Fig. 1, setup 3). Anti-GL113 mAb was used as a control antibody. TCDD treatment of PE-sensitized mice increased the percentage of CD25+Foxp3+ Treg (Fig. 6e). Treatment of PE-sensitized mice with anti-CD25 mAb decreased the percentage of CD25+Foxp3+ Treg cells. In PE-sensitized mice that
were treated with TCDD and anti-CD25 mAb (Fig. 5d), the percentage CD25\(^+\)Foxp3\(^+\) T\(_{reg}\) cells within the CD4\(^+\) population was 4.10 versus 2.94% in PE-sensitized mice that were treated with anti-CD25 mAb only (Fig. 5b). Within the CD4\(^+\) population in the MLN, anti-CD25 mAb treatment decreased the percentage CD25\(^+\)Foxp3\(^+\) T\(_{reg}\) cells (from 5.55 to 0.97%, \(p < 0.001\)), whereas no effect of TCDD treatment on the percentage of CD25\(^+\)Foxp3\(^+\) T\(_{reg}\) cells was observed (5.55 vs. 5.9%) (data not shown). The percentage of CD25\(^+\)Foxp3\(^+\) T\(_{reg}\) cells within the CD4\(^+\) population MLN from mice treated with TCDD and anti-CD25 mAb was 0.81% (data not shown). Furthermore, TCDD treatment did not affect the percentage and number of CD4\(^+\) and CD8\(^+\) cells in the spleen and slightly increased the percentage of CD19\(^+\) cells on day 5 (Fig. 6).

The effects of TCDD and/or anti-CD25 treatment on the peanut allergic response were investigated on day 31 (Fig. 1, setup 2). PE-specific serum levels of IgE, IgG1 (Figs. 7a–b), and PE-induced T-cell responses (ex vivo release of cytokines IL-5, IL-10, IL-13, IL-17a, and IFN-\(\gamma\), Figs. 7e–i) were markedly increased by depletion of CD4\(^+\)CD25\(^+\)Foxp3\(^+\) T\(_{reg}\) cells. This confirms earlier findings that T\(_{reg}\) cells are important in regulating allergic sensitization (Van Wijk et al., 2007). This also shows that anti-CD25 treatment did probably not extensively deplete effector T or Th cells. Remarkably, the suppressive effect of TCDD on PE-specific IgE and IgG1 (Figs. 7a and b) and IL-5 (Fig. 7e) was partly reversed after depletion of CD4\(^+\)CD25\(^+\)Foxp3\(^+\) T\(_{reg}\) cells, whereas PE-specific IgG2a and PE-induced T-cell cytokine responses (IL-10, IL-13, IL-17a, and IFN-\(\gamma\)) were comparably high as in anti-CD25-treated PE-sensitized mice (Figs. 7c and f–i). It must be noted that anti-CD25 mAb treatment depletes T\(_{reg}\) cells for about 7–10 days after the last injection (day 18),
which allowed CD4⁺CD25⁺Foxp3⁺ T_reg cells to have partly recovered on day 31 (Tenorio et al., 2010; Van Wijk et al., 2007). However, supplementary data show that 11 days after the last anti-CD25 mAb treatment the effect of CD25 depletion is still apparent (Supplementary data 1). Together, these findings clearly show that TCDD increases the percentage of CD4⁺CD25⁺Foxp3⁺ T_reg cells already during the initiation of the immune response and that these cells are involved in the suppression of peanut allergic sensitization after AhR activation by TCDD.

**DISCUSSION**

T_reg cells have been shown to play an important role in allergic sensitization and the intensity of food allergic responses (Schouten et al., 2010; Torgerson and Ochs, 2007; Van Wijk et al., 2007). Recently, it has been shown that activation of the AhR results in the induction of T_reg cells (Funatake et al., 2005; Hauben et al., 2008; Marshall et al., 2008; Quintana et al., 2008; Zhang et al., 2010). Therefore, we examined whether activation of the AhR by TCDD suppresses Th2-mediated food allergic responses through the induction of T_reg cells. Data of the present study demonstrate for the first time that activation of the AhR by TCDD dose dependently suppressed various parameters of food allergic sensitization. In addition, the proportion, but not the absolute number, of CD4⁺CD25⁺Foxp3⁺ T_reg cells dose dependently increased due to TCDD treatment. Importantly, depletion of CD4⁺CD25⁺Foxp3⁺ T_reg cells markedly reversed the suppressive effect of TCDD on PE-specific antibody levels and Th2-related cytokines. This suggests that activation of the AhR by TCDD induces a moderate but functional shift in the CD4⁺ cell population toward CD25⁺Foxp3⁺ T_reg cells, resulting in suppression of peanut allergic sensitization.

Previously, it has been shown that activation of the AhR (by TCDD or M50354) impairs Th2-type immune responses (Fujimaki et al., 2002; Inouye et al., 2005; Ito et al., 2002; Kato et al., 2003; Luebke et al., 2001; Negishi et al., 2005; Nohara et al., 2002). However, no role for T_reg cells was described or suggested in these studies. Recently, it has been shown that activation of the AhR by ITE, VAG539, or TCDD (50 μg/kg BW vs. 15 μg/kw BW in our experiments) suppresses GvHD and Th1/Th17-mediated EAE and EAU through a relatively modest induction of CD4⁺Foxp3⁺ T_reg cells (Hauben et al., 2008; Quintana et al., 2008, 2010; Zhang et al., 2010). However, in these studies, the effect of AhR activation on CD4⁺Foxp3⁺ T_reg cells was only shown in percentages, whereas no data about absolute numbers of CD4⁺Foxp3⁺ T_reg cells were provided. Therefore, it is not clear whether AhR activation also increased the number of CD4⁺Foxp3⁺ T_reg cells or only induced a shift within the CD4⁺ population toward CD4⁺Foxp3⁺ cells, as seen in our experiments.

Genes linked to immunoregulation such as Foxp3, transforming growth factor (TGF)-β, and IL-10 contain DREs in their promotor regions (Kerkvliet, 2009; Sun et al., 2004). Therefore, it is likely that immunoregulatory cells such as Foxp3⁺ T_reg cells can be induced directly by AhR activation. In support of the importance of AhR in the actual generation of Foxp3⁺ T_reg cells, naïve T cells from AhR knockout mice showed impaired T_reg-cell development in vitro (Kimura et al., 2008). Also, mutant AhR CD4⁺Foxp3⁺ cells with reduced affinity for AhR ligands appeared less capable of differentiating into CD4⁺Foxp3⁺ both in vivo and in vitro (Quintana et al., 2010). In addition, it has recently been shown that AhR...
AhR ACTIVATION SUPPRESSES SENSITIZATION TO PEANUT

FIG. 6. TCDD treatment increased the percentage, but not the number, of CD4+CD25+Foxp3+ Treg cells and did not affect the percentage and number of CD3+CD4+ and CD3+CD8+ and the number of CD19+ cells in the spleen on day 5. Mice were injected with anti-CD25 (clone PC61) mAb or control antibody (mAb anti-GL113) prior and during sensitization to peanut and treated with 15 µg/kg BW TCDD on day 3 (setup 3). On day 5, mice were sacrificed and the percentage and number of CD4+CD25+Foxp3+ (a and c), CD3+CD4+ (b and f), CD3+CD8+ (c and g), and CD19+ (d and h) cells in the spleen were examined by FACS analysis. For detection of CD25 expression, clone 3C7 was used. Values are presented as mean ± SE (n = 4), *p < 0.05 compared with PE + CT + anti-GL113.

activation by TCDD in naïve human T cells induces Foxp3+ Treg cells in the presence of TGF-β1 (Gandhi et al., 2010). Despite the strong indications that CD4+CD25+Foxp3+ Treg cells are instrumental in TCDD-induced suppression of sensitization to peanut, it cannot be excluded that TCDD interferes with other cells containing AhR and relevant DRE-containing genes. Among these are other regulatory T cells, e.g., CD4+CD25+Foxp3+ cells (Funatake et al., 2005), which were also increased in our study by TCDD treatment in MLN and Tr1 cells-producing regulatory IL-10 (Apetoh et al., 2010; Gandhi et al., 2010). However, IL-10 can also stimulate functions of innate immunity and Th2-related immunity (differentiation of B cells), depending on the cell type producing it (Moore et al., 2001). In our experiments, in vivo TCDD treatment suppressed ex vivo PE-induced IL-10 production dose dependently along with PE-specific antibody levels. This suggests that PE-induced IL-10 is rather a Th2-related cytokine than a regulatory cytokine in the present peanut allergy model.

The observed decrease in numbers of CD4+ T cells after TCDD treatment may result from an effect of TCDD on thymocyte differentiation (Camacho et al., 2005; Kamath et al., 1998; Temchura et al., 2005) or from a direct suppressive effect of TCDD on peripheral T cells on days 4–5 (Dearstyn and Kerkvliet, 2002; Funatake et al., 2004; Shepherd et al., 2000). Importantly, in our experiments, TCDD treatment did not affect the percentage and number of splenic CD4+ and CD8+ T cells on day 5. Interestingly, the percentage, but not the number, of CD4+CD25+Foxp3+ Treg cells was already increased on this day, i.e., during initiation of sensitization. However, our data cannot exclude that TCDD treatment directly deletes or suppresses the function of peripheral (naïve) T cells and/or other cells of the immune system, resulting in suppression of the peanut allergic reaction.

Our data show an increase in IFN-γ production at lower doses (0.6, 1.7, and 5 µg/kg BW) of TCDD in ex vivo PE-restimulated splenocyte cultures. Furthermore, IL-5 was already decreased at these doses of TCDD. This agrees with previous findings that in vivo TCDD treatment increased antigen-induced IFN-γ production and suppressed antigen-induced IL-5 production by splenocytes (Fujimaki et al., 2002; Negishi et al., 2005; Nohara et al., 2002). Interestingly, a role for the AhR in skewing the Th1/Th2 cytokine balance toward Th1 has been described by others (Kato et al., 2003; Negishi et al., 2005). Unpublished results from our laboratory and published results from others have shown that IFN-γ is involved in regulating the intensity of (peanut) allergic reactions (Yoshida et al., 2002). Therefore, shifting the Th1/Th2 cytokine balance toward Th1 might be an additional mechanism through which TCDD could suppress sensitization to peanut in this study. However, TCDD-induced IFN-γ production by other cells (e.g., phagocytic cells) cannot be excluded (Neff-LaFond et al., 2007).

Because AhR and DREs are present in many different immune cells, it is difficult to pinpoint one of the regulatory players as the crucial one, but importantly, the net result appears to be a proportional shift to a more regulatory phenotype of the CD4+ T-cell compartment as a whole. Considering the importance of B cells in allergic responses, the situation is further complicated by fact that TCDD can also suppress formation of germinal centers (Inouye et al., 2003) and plasma cell differentiation (North et al., 2009). However,
TCDD exposure does not decrease B-cell activation and maturation during early peanut sensitization, suggesting that possible effects of TCDD on B cell are secondary to T-cell effects (Schulz, unpublished data).

The finding that TCDD appears to induce an immunoregulatory T-cell compartment, involving CD4⁺CD25⁺Foxp3⁺ T_reg cells as important contributors, suggests that activation of the AhR in cells upstream from T cells might play an important role herein. Importantly, activation of the AhR has been shown to induce tolerogenic dendritic cells (DCs) producing retinoic acid or indoleamine 2,3-dioxygenase, both associated with the development of T_reg cells (Nguyen et al., 2010; Quintana et al., 2010; Vogel et al., 2008). In other studies, TCDD has been shown to activate DCs in the presence and absence of an antigen (Vorderstrasse et al., 2003), to selectively decrease CD11c<sup>high</sup>CD8α⁺33D1⁺ splenic DCs specialized at activating CD4⁺ T cells (Bankoti et al., 2010) and to reduce the amount of splenic DCs (Bankoti et al., 2010; Vorderstrasse and Kerkvliet, 2001). The role of DCs in TCDD-induced immunoregulation is clearly not deciphered yet because TCDD-affected DCs may either stimulate T_reg cells or limit activation of effector T cells. Recently, we have demonstrated that DCs are crucial in the allergic response to peanut, by showing that inflammatory CD11b⁺ DCs stimulate and regulatory CD103⁺ and plasmacytoid DCs inhibit food allergic responses (Smit et al., 2011). This knowledge will be used to study the influence of various DC subsets in TCDD-mediated effects in food allergy further.

Besides TCDD, there are numerous other endogenous and exogenous ligands for the AhR described. Yet, most of these ligands are easily metabolized and therefore probably not sufficiently bioavailable to activate the AhR persistently (Denison and Nagy, 2003). The AhR is strongly expressed along the small intestine (Chmill et al., 2010) and based on present findings, it can be hypothesized that prolonged exposure to AhR ligands via the diet could influence the outcome of food allergic responses. Such dietary AhR ligands may potentially help to alleviate or even treat allergic responses, if they are relatively stable without causing the toxic side effects associated with dioxins. In addition, these AhR ligands should induce T_reg cells and not Th17 cells (Quintana et al., 2008, Veldhoen et al., 2008). Moreover, the existing cytokine milieu (especially the presence of IL-6 and/or TGF-β) appears to be very important in deciding whether AhR activation results in an inflammatory or a regulatory immune response (Apetoh et al., 2010; Gandhi et al., 2010; Veldhoen, 2010). Furthermore, it has been reported that TCDD impairs maintenance of oral tolerance against ovalbumin in a high-dose oral tolerance model (Chmill et al., 2010). Together, this shows that activation of the AhR pathway can affect immune responses differently, depending on the AhR ligand, the cytokine milieu, and the disease model used.

In summary, the present study showed that activation of the AhR pathway suppressed the development of Th2-mediated food allergic responses by inducing a functional shift within the CD4⁺ cell population toward CD4⁺CD25⁺Foxp3⁺ cells. This emphasizes the important role of the AhR in shaping the T-cell repertoire and the delicate balance between tolerance and
immunity. Importantly, findings of this study suggest that the AhR pathway might be a unique target for therapeutic manipulation of food allergic diseases. In addition, our data warrant further studies to investigate the therapeutic potential of natural or endogenous AhR ligands in suppressing food allergic responses.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

REFERENCES


