Immunobiology 218 (2013) 1055–1062

Contents lists available at SciVerse ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Aryl hydrocarbon receptor activation affects the dendritic cell phenotype and function during allergic sensitization

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ARTICLE INFO

Article history:
Received 8 June 2012
Received in revised form
13 December 2012
Accepted 23 January 2013
Available online 1 February 2013

Keywords:
Aryl hydrocarbon receptor
CD103
Dendritic cells
Mesenteric lymph nodes
Regulatory T cells
Spleen

ABSTRACT

Aryl hydrocarbon receptor (AhR) activation by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) suppresses peanut sensitization by affecting T cell subsets. However, effects of AhR activation on dendritic cells (DC) in an allergic setting were not investigated yet. Therefore, we analysed the effects of AhR activation on DC phenotype *in vivo*, as well as their *ex vivo* potency to stimulate allergen-specific splenic T cells and to induce $CD4^+CD25^+Foxp3^+$ regulatory (T_{reg}) cells.

C3H/HeOuJ mice were treated with TCDD by gavage and subsequently sensitized to peanut extract (PE). After eight days, mice were sacrificed and DC in spleen and mesenteric lymph nodes (MLN) were characterized or cocultured with PE-specific $CD4^+$ T cells.

AhR activation almost doubled the absolute number of $CD11c^+CD103^+$ DC, while not affecting $CD11b^+$ DC, the absolute number of DC, the expression of the activation makers MHCII, CD86, CD80, CD40, CD54 and CD8α on $CD11c^+$ and the activation status of $CD11c^+CD103^+$ DC in the spleen. In the MLN, TCDD decreased the absolute number of DC and $CD103^+$ DC, while not affecting $CD11b^+$ DC and the expression of activation markers on DC. PE-pulsed splenic DC from TCDD-treated mice suppressed IL-5, IL-13 and IFN-γ production by PE-specific T cells, but did not induce $CD4^+CD25^+Foxp3^+$ T_{reg} cells. This suppression of cytokine production was not mediated by the TCDD-induced increase in $CD103^+$ DC in the spleen. Combined, these results indicate that AhR activation suppresses the initiation of food allergic responses by affecting DC and their interaction with effector T cells.

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Introduction

Normally, exposure to food antigens results in the induction of tolerance. However, in some individuals exposure will result in allergic sensitization (Shaker and Woodmansee 2009; Sicherer and Sampson 2009; Van Wijk et al. 2007). With a prevalence of about 5% among children and 3–4% among adults food allergy is a significant public health problem (Burks et al. 2012; Sicherer and Sampson 2010). Dendritic cells (DC) play an important role in the induction of tolerance or allergic sensitization to food allergens. These cells sample antigen from the gut lumen or underlying tissue, migrate to lymphoid organs and present there antigen to naïve T cells. This will result either in tolerance by induction of regulatory T (T_{reg}) cells expressing Foxp3 or in allergic sensitization by induction of T helper 2 (Th2) cells (Ruiter and Shreffler 2012).

Different subsets of DC have been described and each subset has a specific role in the immune system (Shortman and Liu 2002). The balance between tolerogenic $CD103^+$ DC and inflammatory $CD11b^+$ DC is important in food allergic sensitization, because oral sensitization to peanut is accompanied by an increase of inflammatory $CD11b^+$ DC and a decrease of $CD103^+$ DC in the intestine (Smit et al. 2011). Mucosal $CD103^+$ DC are important for the induction of oral tolerance, since they have been shown to induce $Foxp3^+$ T_{reg} cells in a TGF-β and retinoic acid dependent manner (Coombes and Powrie 2008; Sun et al. 2007).

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor which is expressed by cells of the innate and adaptive immune system and is best known for its role in mediating toxicity of xenobiotics, in particular of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Nowadays, it is evident that the AhR to plays an important role in the immune system (Denison and Nagy 2003; Kerkvliet 2009; Sun et al. 2004). Previously, we have shown that activation of the AhR by TCDD suppresses sensitization to peanut by decreasing precursor and effector T cells

Abbreviations: AhR, aryl hydrocarbon receptor; DC, dendritic cells; FACS, fluorescence activated cell sorting; GvHD, graft *versus* host disease; ITE, 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester; MLN, mesenteric lymph nodes; PBS, phosphate buffered saline; PE, peanut extract; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; Th2, T helper 2; T_{reg}, regulatory T.

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http://dx.doi.org/10.1016/j.imbio.2013.01.004

and by inducing a functional shift towards $CD4^+CD25^+Foxp3^+$ T_{reg} cells (Schulz et al. 2011, 2012). Interestingly, activation of the AhR has been associated with the induction of tolerogenic DC and $CD4^+CD25^+Foxp3^+$ T_{reg} cells. For example, in a mouse model for graft *versus* host disease (GvHD) transfer of splenic $CD11c^+$ cells from mice treated with the AhR ligand VAG539 increased the percentage of $CD4^+CD25^+Foxp3^+$ T_{reg} cells and promoted graft acceptance (Hauben et al. 2008). Moreover, splenic DC isolated from mice treated with the AhR ligand 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) expressed more CD103. In addition, these DC increased the percentage of $Foxp3^+$ T_{reg} cells *in vitro* dependent on retinoic acid (Quintana et al. 2010). However, it was also reported that TCDD treatment reduces the number of splenic DC, increases the expression of CD86, CD40 and CD54 on splenic DC and enhances their ability to provide activation signals to T cells, resulting in dysregulation of immune responses (Vorderstrasse and Kerkvliet 2001; Vorderstrasse et al. 2003). Altogether, these findings show that the role of DC in AhR-mediated suppression of immune responses is yet not clear and may depend on many factors, including the disease model and the AhR ligand used.

Because DC play an important role in allergic sensitization and AhR activation has been shown to affect functions of DC, we investigated the effect of AhR activation on DC in a mouse model for peanut allergy. For this purpose, we studied the effect of TCDD on DC phenotype and populations *in vivo* during sensitization, their interaction with peanut-specific $CD4^+$ T cells *ex vivo* and their capacity to induce $CD4^+CD25^+Foxp3^+$ T_{reg} cells *ex vivo*.

Materials and methods

Mice and reagents

Female C3H/HeOuJ mice (4–5 week old) purchased from Charles River (France) were maintained under controlled conditions (relative humidity of 50–55%, 12 h light/dark cycle, temperature of $23 \pm 2\,^{\circ}C$) 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 Utrecht University.

2,3,7,8-TCDD (Cambridge Isotope Lab, USA) was dissolved in anisole (Sigma Aldrich, The Netherlands) at 20.5 μg/ml and diluted in corn oil (Sigma–Aldrich, The Netherlands) to the final exposure concentration (0.07%, v/v anisole). Anisole diluted in corn oil was used as vehicle control (0.07%, v/v).

Peanut extract (PE) (30 mg/ml) was prepared from peanuts (Intersnack Nederland BV, The Netherlands) as described previously (Van Wijk et al. 2005) and checked for protein content by BCA analysis (Pierce, IL). Cholera toxin (CT) was purchased from List Biological Laboratories, Inc. (CA).

Experimental design

For phenotypic analysis of DC after TCDD treatment, mice ($n = 3$ per group) were treated with vehicle or TCDD (15 μg/kg BW) on day 0 by gavage. The dose of TCDD used was based on previous research (Funatake et al. 2005; Schulz et al. 2011). On day 8 mice were sacrificed by cervical dislocation and spleens and mesenteric lymph nodes (MLN) were isolated.

To detect possible effects of TCDD treatment on DC subsets in the spleen and MLN, mice ($n = 4$ per group) were treated with phosphate buffered saline (PBS) or sensitized to PE (6 mg PE, 200 μl/mouse) mixed with CT (15 μg/mouse) on three consecutive days (day 3, 4, 5) by gavage. TCDD (15 μg/kg BW) was administrated on day 0 by gavage. On day 8, mice were sacrificed by cervical dislocation and spleens and MLNs were isolated.

Flow cytometry analysis

Single cell suspensions of spleen (after red blood cell lysis) and MLN (2.5×10^6 cells/ml for extracellular stainings, 1×10^7 cells/ml for intracellular stainings) were incubated for 15 min with anti-mouse CD16/32 (clone 93, eBioscience, Austria) in fluorescence activated cell sorting (FACS) buffer (PBS containing 0.25% BSA, 0.05% NaN_3, 0.5 mM EDTA) and subsequently stained extracellularly in FACS-buffer for 30 min at $4\,^{\circ}C$ using the following FACS-antibodies: CD11c-APC (clone N418, eBioscience, Austria), CD103-PE (clone 2E7, eBioscience, Austria), MHCII-FITC (clone M5/114.15.2, eBioscience, Austria), CD86-PerCP (clone GL-1, Biolegend, USA), CD80-PE (clone 16-10A1, BD Pharmingen, USA), CD40-FITC (clone 3/23 BD Pharmingen, USA), CD54-FITC (clone 3E2, BD Pharmingen, USA), CD11b-PE (clone M1/70, BD Pharmingen, USA), CD8α-PerCP (clone 53–6.7, BD Pharmingen, USA), CD4-FITC (clone L3T4, eBioscience, Austria), CD25-PE (clone PC61.5, eBioscience, Austria), CD3e-FITC (clone 145-2C11, eBioscience, Austria), CD8α-PE (clone 53–6.7, eBioscience, Austria), CD103-APC (clone 2E7, eBioscience, Austria). Next, cells were washed with FACS-buffer and fixed with 1% formaline in FACS buffer or subsequently stained intracellularly for Foxp3-APC (clone FJK-16s, eBioscience, Austria) according to the manufacturer's instructions. Appropriate isotype controls were used in conjunction with the primary antibody staining. Cells were analysed on a BD FACSCanto II using BD FACS Diva software (BD Biosciences, USA).

Coculture of splenic DC with PE-specific $CD4^+$ T cells

Spleens were isolated 8 days after treatment of mice with vehicle or TCDD (15 μg/kg BW). Pooled splenic single cell suspensions were put on a density gradient and $CD11c^+$ DC were subsequently isolated by negative selection using a Dynabeads Mouse DC Enrichment Kit according to manufacturer's instructions (Invitrogen, The Netherlands). In separate experiments, $CD103^+$ cells were depleted using negative selection by additional incubation with biotin-anti-mouse-CD103 (clone M290, ebioscience, Austria). PE-specific $CD4^+$ T cells were enriched by immunizing mice with PE/alum (100 μg PE, 4 mg aluminium hydroxide and 4 mg magnesium hydroxide per mouse [Thermoscientific, The Netherlands]). On day 28–38, mice were sacrificed and spleens were isolated. $CD4^+$ T cells were isolated from the spleen by negative selection using a Dynal Mouse CD4 Negative Isolation Kit according to manufacturer's instructions (Invitrogen, The Netherlands). Isolated splenic $CD11c^+$ DC (6.67×10^5 cells/ml) were plated in a 12-well plate and pulsed with PE (50 μg/ml) for 24 h. Next, DC were scraped, washed with PBS and plated in a 48-well plate (8×10^5 cells/ml) together with freshly isolated $CD4^+$ T cells (8×10^6 cells/ml) (1:10) as described before (Pochard et al. 2010). After 3 days, the supernatant was collected and the frequency of $CD4^+CD25^+Foxp3^+$ T_{reg} cells was determined by flow cytometry.

Analysis of cytokine production

Levels of IL-5, IL-10, IL-13 and IFN-γ in collected supernatants were determined by commercially available sandwich ELISA (eBioscience, Austria) according to the manufacturer's instructions.

Statistical analysis

Results are presented as the mean ± standard error of the mean (SEM) of 3–4 mice per group. All data were logarithmically transformed to achieve normal distribution and were analysed by a *t*-test

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