AhR ligands:

Therapy or risk factor in food allergy

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Abstract

Food allergy is the result of an aberrant immune response towards harmless food antigens. Tolerance induction is an important process in the prevention of food allergy. Oral tolerance is the result of the interaction between DCs and T cells and the cytokine milieu. In food allergy no oral tolerance is established, but the immune response is skewed towards a Th2 response. Although cytokines play an important role in T cell differentiation, recent data suggest a role for the aryl hydrocarbon receptor (AhR) in T cell differentiation. AhR ligands can influence the differentiation and expansion of T cell subsets. Especially, regulatory T cells (Treg) and Th17 cells can be induced by AhR ligands.

Induction of regulatory T cells that suppress Th2 cells would be a promising therapeutic option in food allergy. Additionally, suppression of antigen presentation and cytokine production by antigen presenting cells would be an interesting drug target. When AhR ligands indeed can regulate T cell differentiation, the AhR could be an interesting target for the treatment of several immune-mediated diseases.

We review here current evidence for AhR ligands which influence immune responses. Based on the existing data of these specific AhR ligands, we discuss whether patients with food allergy would benefit from oral AhR ligands.

1. Introduction

1.1 Induction of food allergy

Food allergy or hypersensitivity consists of abnormal immune-mediated reactions towards ingested food. Food allergy can be divided into two categories. IgE mediated and non-IgE mediated reactions.

Non-IgE mediated food allergy is characterized by the production of pro-inflammatory cytokines by antigen-specific T cells. This reaction has a delayed onset and will occur hours after ingestion of the food protein. An example of non-IgE mediated food allergy is celiac disease. [1]

IgE mediated food allergy is characterized by the presence antigen-specific IgE antibodies. When food is ingested, food proteins will come in contact with the immune system present in the gut, the gut-associated lymphoid tissue (GALT). The GALT consists of the lamina propria (LP), the Peyer's patches (PP) and the mesenteric lymph node (MLN). These primary immune structures are thought to play an essential role in the regulation of immune responses in the gut. [2]

Antigen presenting cells (APCs), like dendritic cells (DCs), will process the food protein and present protein fragments on MHC-II molecules. Presented peptides on MHC-II molecules are recognized by the T cell receptor (TCR). In the presence of IL-4, produced by DCs, naïve CD4+ T cells will differentiate into Th2 cells. These effector T cells produce IL-4, IL-5 and IL-13 thereby supporting B cells to produce antigen-specific IgE antibodies. Those antibodies can bind to the FcεRI on mast cells and basophils and bind circulating antibodies. Re-stimulation with the food antigen results in cross linking of the IgE antibodies leading to degranulation of the mast cells. Histamines and cytokines are released leading to inflammatory symptoms. (Figure 1) This can happen within hours after ingestion of the food or even after several minutes. [2]

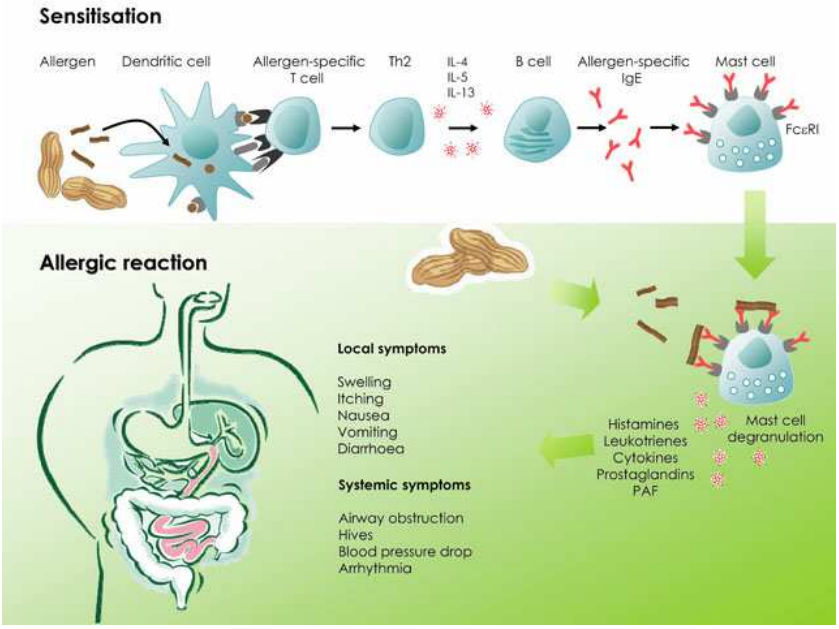

Figure 1: Induction of food allergy

Food allergy is the result of an aberrant immune response towards harmless food antigens. The immune response is skewed towards a Th2 response, associated with the cytokines IL-4, IL-5 and IL-13. This T cell response leads to the induction of IgE production towards allergens by B cells. IgE in turn binds to the FcεRI receptor on mast cells. When the allergen is re-encountered, IgE can be cross-linked, leading to degranulation of mast cells. Release of pro-inflammatory mediators by the mast cells leads to the typical allergic symptoms, such as itching or sneezing. [3]

Normally, ingestion of food proteins does not trigger an allergic response, but oral tolerance is induced towards these proteins. Oral tolerance can be induced via T cell depletion or anergy. Also active immunosuppression can occur, by the induction of regulatory T cells. How the immune system decides whether to induce tolerance towards food proteins or present the antigen by APCs to induce Th2 cells supporting B cells to produce antigen-specific IgE antibodies, is not known. It could be that dendritic cells (DCs) play a key role in the balance between tolerance and allergic sensitization. T cell activation by DCs is not only dependent on the interaction between MHC molecules and the TCR. Co-stimulatory molecules on DCs and cytokines produced by DCs will determine whether T cells are activated or tolerance is induced. DC activation by pathogen-associated molecular patterns (PAMP) could enhance the risk of inducing allergic sensitization in stead of tolerance induction. Changing DC function can influence the outcome of immune responses. Although DCs themselves are essential in T cell activation, these cells are influenced by environmental factors. Thus the whole environmental milieu will decide the outcome of immune responses. [2]

1.2 T cell differentiation in general and in relation to food allergy

Differentiation of T cells is a direct response to the cytokine milieu and antigen presentation. Dendritic cells (DCs) in the intestine and associated lymphoid tissue play a key role in the intestinal immune response. These cells regulate protective immunity and tolerance. After sampling antigens derived from the intestinal lumen, DCs migrate to the mesenteric lymph node (MLN) or Peyer´s Patches (PP). Here DCs can come in contact with T and B cells. By interacting with T cells, an inflammatory response is induced or a non-inflammatory response state is maintained. CD103+ DCs from the MLN are a specific subset of DCs, capable of inducing the generation of FOXP3+ Treg cells from naïve T cells by TGFβ. Production of retinoic acid by these CD103+ DCs is an important mechanism that contributes to the induction of FOXP3+ Treg cells and the establishment of oral tolerance. (Figure 3) [4]

Several lineage decision transcription factors play a role in the differentiation of T cells. These transcription factors are upregulated in response to specific cytokines. (As described in detail in Figure 2)

Th1 cells are helper T cells characterized by the expression of the transcription factor T-bet. In the presence of the cytokine IL-12 and IFNγ naïve CD4+ T cells differentiate into Th1 cells. Th1 cells also produce IFNγ, thereby promoting there own differentiation. Th1 cells are involved in delayed type hypersensitivity. They activate macrophages enabling them to kill intracellular pathogens. They also assist effector CD8+ T cells to differentiate into CTL effector cells. And Th1 cells support B cells to make the switch from IgM to IgG2 antibodies. [5, 6]

Th2 cells are classical T helper cells characterized by the expression of the transcription factor GATA-3. Th2 cells are induced by the cytokine IL-4. Th2 cells produce IL-4, IL-5 and IL-13 and help B cells to produce high affinity class-switched antibodies. Th2 cells are key immune cells in mediating allergic diseases by the production of IgE antibodies against food antigens. Re-stimulation with the specific food antigen will result in an inappropriate inflammatory immune response. [5, 6]

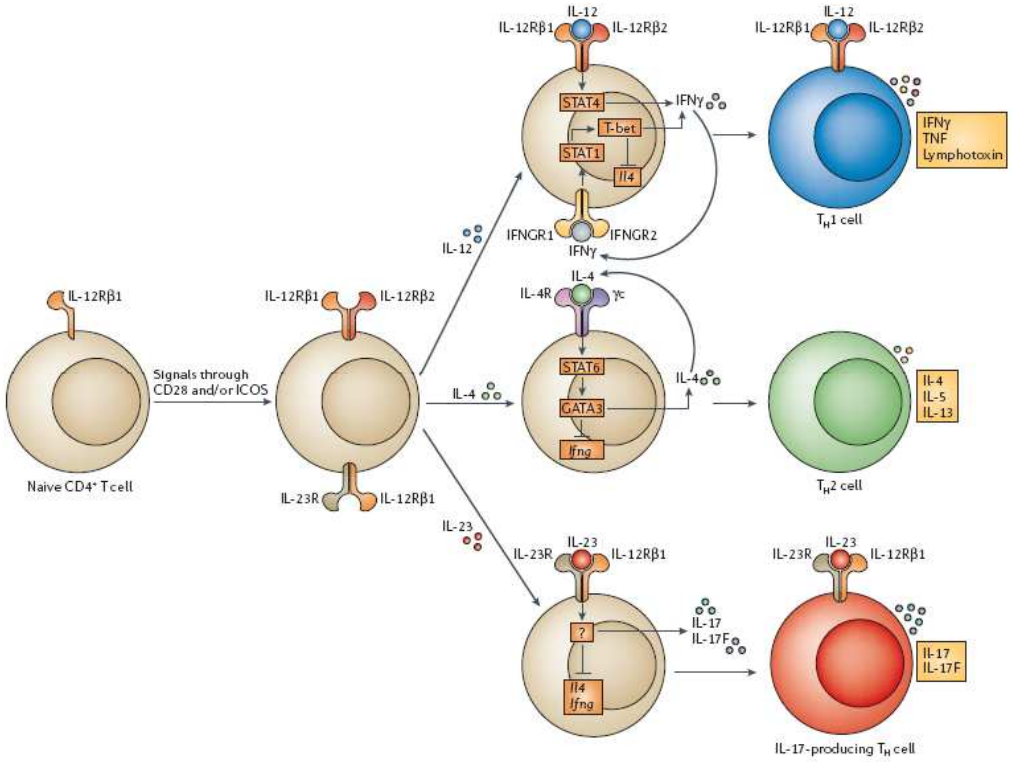

Figure 2: Differentiation of helper T cells, Th1 and Th2, and effector Th17 cells
The cytokine environment determines the terminal lineage commitment and cytokine expression profiles. IL-12, through the action of signal transducer and activator of transcription 4 (STAT4), potentiates the production of interferon-γ (IFNγ), which, through STAT1, increases the expression of the transcription factor T-bet. T-bet, in turn, increases IFNγ and IL-12Rβ2 expression, inhibits IL-4 expression, and maintains Th1-cell-lineage commitment. By contrast, IL-4, through STAT6, increases expression of GATA-binding protein 3 (GATA3). GATA3 potentiates IL-4 expression, inhibits IFNγ expression and determines Th2-cell-lineage differentiation. IL-23 functions through still poorly understood transcriptional mechanisms, it increases the differentiation of CD4+ T-cells to Th17 effector cells that produce IL-17 (and IL-22), and express high levels of IL-23R. [6]

Th17 cells are characterized by the expression of the transcription factor RORγt. These cells are generated in response to TGFβ together with IL-6. (Figure 3) Th17 cells produce IL-17 and IL-22. IL-23 is required for the production of these cytokines. Th17 cell produced cytokines will result in inflammation, the production of antimicrobial peptides and the recruitment of neutrophils. Pro-inflammatory Th17 cells are linked to autoimmune diseases. [5] If Th17 cells play a role in food allergy remains to be elucidated. It is suggested that the presence of IL17 and neutrophils indicate a role for Th17 cells in the development of non-IgE mediated food allergy. [7] But this subject needs to be further investigated.
Other IL-17 producing T cells are γδ T cells. These cells do not represent adaptive immunity, but are an innate source of IL-17. γδ T cells secrete IL-17 and IL-22 in response to IL-1 and IL-23 in the absence of TCR engagement. They also express RORγt in response to IL-1 and IL-23. [8] DCs produce IL-1 and IL-23 in response to TLR agonists and thereby induce IL-17 production from T cells. CCR6+ γδ T cells express TLR1, TLR2 and dectin-1 and can produce IL-17 upon direct contact with microbes without cytokines produced by DC or TCR engagement. However, IL-1 and IL-23 significantly enhance the production of IL-17 by γδ T cells. IL-17 production by γδ T cells leads to neutrophil recruitment. [9] γδ T cells are involved in the development of EAE and are major source of IL-17 during the development of EAE.

This early induction of IL-17 promotes IL-17 production by Th17 cells. [8]
γδ T cells can also play a role in food allergy. The precise role of γδ T cells in food allergy is unknown. But children with untreated food allergy have relatively higher levels of γδ T cells in the duodenum compared with treated or healthy controls. [10] If these higher levels of γδ T cells are a consequence of the inflammatory environment in food allergy or a risk factor for the development of food allergy is not known. However, another study showed that induction of food allergy results in a decrease in the percentage of γδ T cells in intestinal tissues and Peyer's patches in mice. In addition, blocking the γδ T cell receptor results in an enhanced food allergy response. Higher levels of IgE and Th2 cytokines in splenocytes are detected. [11] This study suggests that γδ T cells could play a regulatory role in food allergy, in contrast with the pro-inflammatory character of γδ T cells.
Treg cells are suppressive T cells that express high levels of CD25, cytotoxic T lymphocyte antigen 4 (CTLA-4) and glucocorticoid-induced tumor necrosis factor receptor (GITR). FOXP3 is the lineage decision transcription factor. Natural occurring Treg (nTreg) cells are derived from the thymus during T cell development. Inducible Treg cells (iTreg) differentiate in peripheral tissue from naïve CD4+ T cells. [2, 12]
Homeostatic DCs produce retinoic acid (RA). Together with TGFβ from the environment, RA induces the differentiation of homeostatic FOXP3+ Treg cells. (Figure 3) FOXP3+ Treg cells produce IL-10 and TGFβ and can suppress Th17 cells thereby downregulating inflammatory immune responses. Also Th1 and Th2 cells are suppressed by Treg cells, probably via the dominant role of FOXP3, which downregulates T-bet, GATA-3 and RORγt. Treg cells restrain excessive effector T cell responses to prevent damage to host tissue. Treg cells are also involved in the induction of tolerance and could thereby play an important role in food allergy. [5, 13, 14]

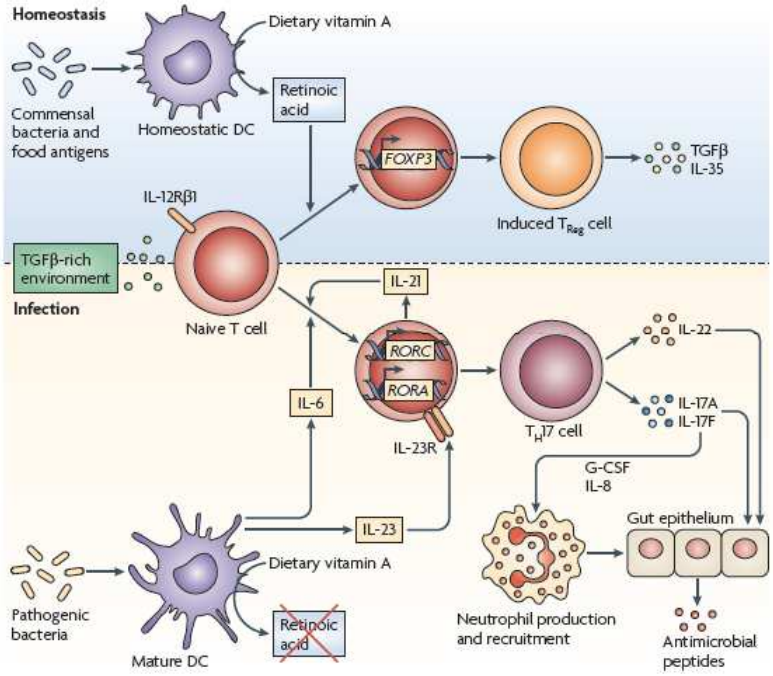

Figure 3: Differentiation of Th17 and Treg cells
During homeostasis, DCs loaded with antigens from commensal bacteria or food produce retinoic acid derived from dietary vitamin A, which favours the upregulation of forkhead box P3 (*FOXP3*) expression and the differentiation of induced Treg cells. Under conditions of microbial breach of the intestinal epithelial cell barrier, pro-inflammatory stimuli activate DCs, which mature and produce IL-6 and stop retinoic acid production, thereby inducing TH17 cell differentiation. [5]

An additional subset of regulatory T cells are the Tr1 cells. These cells do not express FOXP3, but c-Maf. Tr1 cells produce the immunosuppressive cytokine IL-10. The suppressive effect of Tr1 cells is not only mediated via IL-10 but also via granzyme B. [15] IL-27 is a growth and differentiation factor for Tr1 cells and inhibits Th17 and FOXP3+ Treg cell differentiation. IL-27 also induces production of IL-10 and IL-21, which in turn is a growth factor for Tr1 cells. [16]
The last subset of T cells are follicular helper cells. These cells reside in the B cell follicles and are essential for generating high affinity class switched antibodies and memory B cells. They continuous express CXCR5 and can secrete Th1, Th2 and Th17 cytokines. If these cells represent a real T cell subset or just a chameleon state of other subsets is not clear. [13]

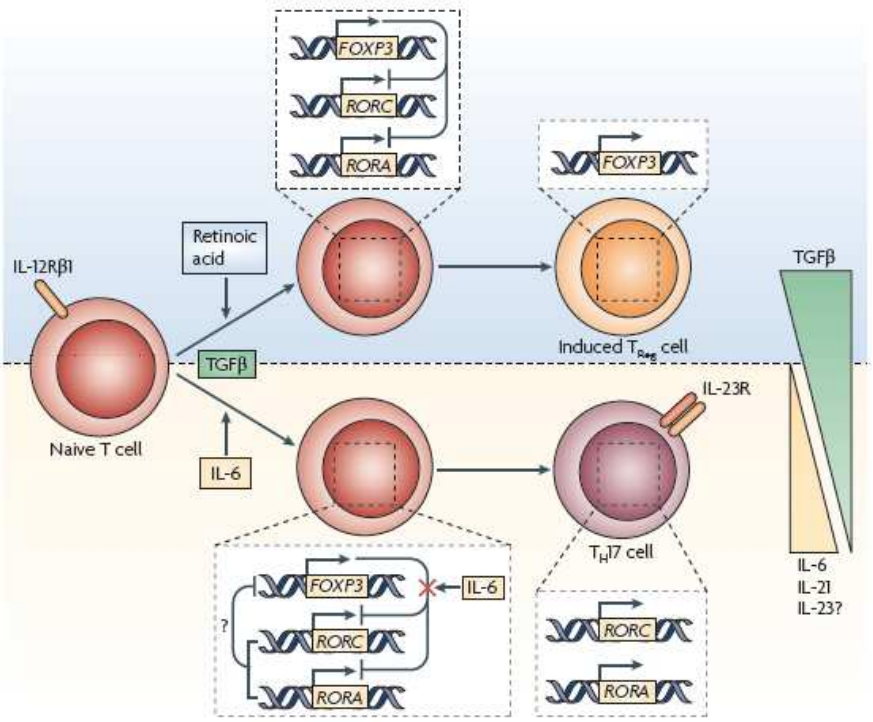

Figure 4: T cell plasticity; interplay between Th17 cells and Treg cells
Th17 and Treg cells differentiation depends on the dominance of interleukin-6 (IL-6) or retinoic acid, respectively. In the presence of retinoic acid and absence of IL-6, FOXP3 can bind and inhibit the activity of ROR transcription factors, thereby promoting Treg cell differentiation. IL-6 and other Th17 cell-inducing cytokines (such as IL-21 and IL-23) promote Th17 cell differentiation at least in part by antagonizing the FOXP3-mediated inhibition of ROR family transcription factors. [5]

Differentiated T cells are not always stable. Under changing cytokine milieu, differentiated T cells can switch to another subset. Most prominent are the Th17 cells and the FOXP3+ Treg. (Figure 4) These cells share the need for TGFβ and are less stable than Th1 and Th2 cells. The transcription factors RORγt and FOXP3 are both upregulated in naïve CD4+ T cells in response to TGFβ. FOXP3 can bind to RORγt and thereby inhibits RORγt. So FOXP3 is dominant. Under homeostatic conditions RA is present and stimulation with TGFβ will result in Treg cell differentiation. Under inflammatory conditions, the cytokine IL-6 is produced by DCs (see Figure 3). The inhibition of FOXP3 on RORγt is counteracted by IL-6 and IL-6 promotes strong RORγt transcription and silencing of FOXP3. This leads to Th17 cell differentiation. [5, 13]

Also other subsets of T cell can switch under a changing cytokine milieu. (Figure 5) When a viral infection is present, high levels of IFN and IL12 are produced. Under these circumstances Th2 and Th17 can switch to a Th1 phenotype. Also Treg cells can switch to a Th1 phenotype under certain circumstances. Although Treg cells show high level of plasticity, conversion of any effector or helper T cell subset to a Treg cell phenotype is not possible. [5, 13]

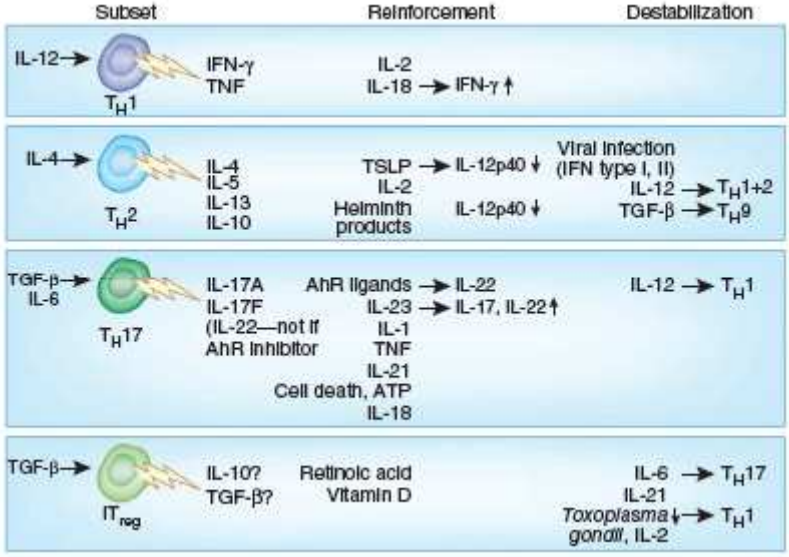

Figure 5: Overview of CD4+ T cell subsets
Reinforcement and destabilization of CD4+ T cell subsets. These four CD4+ subsets are induced under certain conditions but also can be reinforced or destabilized by other conditions, as discussed in the text. [13]

1.3 Role for the AhR in T cell differentiation

TGFβ is one of the most important factors for T cell differentiation. Besides TGFβ, the aryl hydrocarbon receptor (AhR) also appeared to be a prominent factor in T cel differentiation. In 2008, two articles about the AhR were published in Nature. In these articles an important role for the AhR in T cell differentiation was shown. The AhR is a ligand-activated transcription factor which is also upregulated in response to TGFβ. Quintana *et al* observed that the AhR ligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) enhances the induction of FOXP3 Treg cells by TGFβ in mice. In AhR deficient mice, less FOXP3 Treg cells are induced compared to wild type mice. Stimulation with another AhR ligand, 6-formylindolo[3,2-b]carbazole (FICZ), results in induction of Th17 cells. So AhR ligands can regulate Treg and Th17 cell differentiation. [17]
Veldhoen *et al* also observed that stimulation with the AhR ligand FICZ increases the expansion of Th17 cells by IL-6 and TGFβ in mice. In AhR deficient mice Th17 cell differentiation is significantly reduced compared to wild type mice. [18]
The AhR can also interact with Stat1 (and Stat5), which are negative regulators of Th17 cell differentiation. The AhR can downregulate these molecules to regulate Th17 cell development. [19, 20]
These results show an important role for AhR signaling in T cell differentiation. Although cytokines are the major players in inducing T cell differentiation, AhR ligands can influence the differentiation and expansion of T cell subsets. Stimulation with AhR ligands induces a different kind of T cell differentiation. Dependent on what AhR ligand is used, T cell differentiation can be manipulated.

It is probably the nature of the AhR ligand which decides the outcome of the differentiation. If AhR ligands indeed can regulate T cell differentiation, the AhR could be an interesting target for treatment of several immune-mediated diseases.

1.4 Objectives

Induction of regulatory T cells that suppress Th2 cells would be a promising therapeutic option in food allergy. Next to the effects on T cells, it is suggested that AhR ligation influences DC function. As antigen presentation and cytokine production by APCs (like DCs) is needed to induce food allergic responses, the effects of AhR ligands on DCs can also contribute to the therapeutic effects of these compounds. We review here current evidence for AhR ligands influencing immune responses. Based on the existing data of these specific AhR ligands, we discuss whether patients with food allergy would benefit from oral AhR ligands.

2. Aryl hydrocarbon receptor

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor, present within the cytosol. The protein exists of 848 amino acids, of which 10 are cleaved from the N-terminus. The receptor is a member of the bHLH-PAS protein family. The N-terminal basic helix-loop-helix domain is responsible for DNA binding, but also supports dimerization. The C-terminal is highly variable and contains a transcription activation domain. The two PAS (Per-Arnt-Sim) repeats in the middle contain the ligand binding region, they bind to DNA and mediate the dimerization with the AhR nuclear translocator (ARNT). (Figure 6)

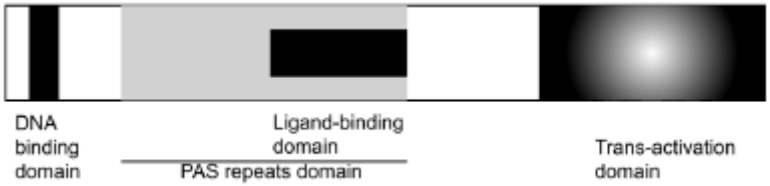

Figure 6: Cartoon of the primary structure of the aryl hydrocarbon receptor
The basic helix–loop–helix DNA-binding structure is shown as a black bar, and the two Per–Arnt–Sim (PAS) domain repeats are shown as a light gray box, enclosing a black box identifying the minimal residues necessary for ligand binding. The C-terminal transactivation domain is shown as a black- and gray-shaded region. [21]

The AhR is chaperoned in the cytoplasm by Hsp90, p23 and AIP (AhR interacting protein/ARA9/XAP2). These molecules are necessary because the AhR protein requires a complex folding pathway. When a ligand binds to the AhR, it induces a conformational change resulting in exposure of a nuclear translocation site. Hereafter the AhR translocates to the nucleus and forms a dimer with the ARNT. This complex can bind to promoters containing a dioxin responsive element (DRE) or xenobiotic responsive element (XRE) consensus sequence, 5'-GCGTG-3', and induce transcription. (Figure 7) The best known genes that are under transcriptional regulation of the AhR are the cytochrome p450 genes (CYP) resulting in the upregulation of metabolizing enzymes. [21, 22, 23]
For most AhR ligands this means that by interacting with the AhR, they induce their own metabolizing enzymes. [24] Due to this quick metabolism of AhR ligands, most ligands do not occupy the AhR for a long time. Transcriptional activity does not only depend on the AhR, but also on the presence of co-repressors or co-activators, adaptor molecules and the AhR ligand characteristics. AhR transcriptional activity results also in the upregulation of the AhR repressor protein (AhRR). (Figure 7) This protein downregulates AhR signaling by acting as a transcriptional repressor. It can also bind to the ARNT and thereby suppressing AhR signaling. Ligand bound AhR is also rapidly degraded by the proteasome after entering the nucleus. [21, 23]

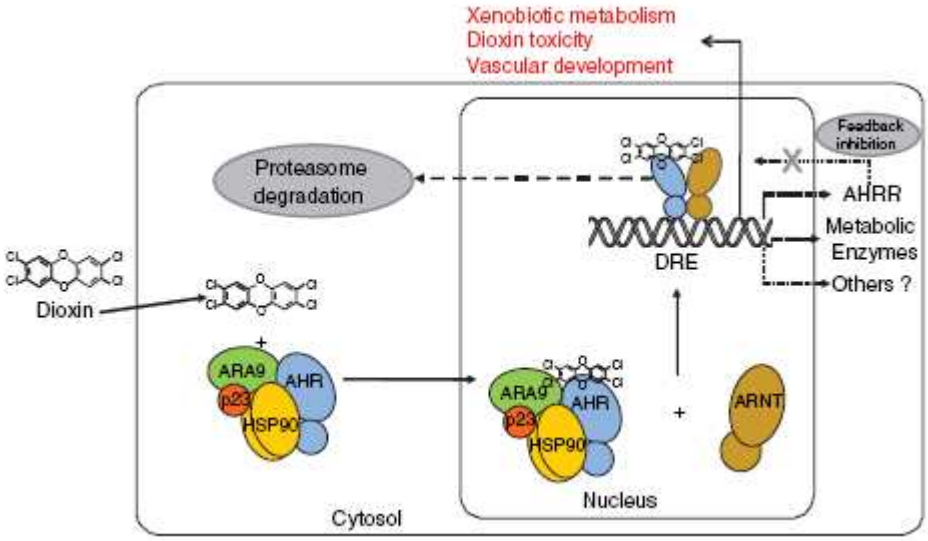

Figure 7: Schematic overview of AhR signaling
Hydrophobic ligands enter the cell via diffusion through the cell membrane and bind to the AhR in the cytosol. Ligand binding causes conformational changes leading to the exposure of a nuclear translocation site. The AhR complex translocates to the nucleus and binds its heterodimeric partner, the aryl hydrocarbon receptor nuclear translocator (ARNT). This complex can bind promoters containing dioxin response elements (DREs), upstream of target genes. Signalling through the AhR is down-regulated by the proteasome and via a feed-back loop involving the aryl hydrocarbon receptor repressor (AHRR). Expression of this AHRR protein is up-regulated by AhR signalling. [23]

As described above, the best known target of AhR signaling is the induction of cytochrome p450 enzymes. The presence of the AhR and signaling by endogenous ligands is also involved in the physiologic development of mice. Defects in AhR null mice are of vascular nature. AhR null mice show unclosed ductus venosus (DV). The DV is a fetal portocaval shunt of the developing liver, which normally closes immediately after birth. When the DV fails to close, this causes aberrant hepatovascular blood flow and altered disposition of small molecules requiring hepatic clearance. The AhR plays a critical role in the regulation of normal vascular and hematopoietic development. [25]

3. AhR binding and activating ligands: Effect of various AhR ligands on immune response

AhR ligands can be divided in three groups, endogenous ligands, exogenous ligands and dietary ligands. AhR ligands are known to activate the AhR receptor and induce transcriptional activity. In most studies it is investigated whether AhR ligands could induce CYP1A1 mRNA. (Table 1) Only a few AhR ligands are known to play a role the immune system. How those AhR ligands affect immune cells is described below. [14, 25-27]

Endogenous ligands	**Effect on immune response**	**AhR mediated**	**Reference**
indigo	unknown		25
indirubin	Treg cell differentiation	unknown	36
lipoxin A4	unknown		14, 25, 27
bilirubin	Treg cell differentiation, suppression APCs	unknown	14, 28, 29, 30
kynurenine	Treg cell differentiation	yes	31-35, 40
FICZ	Th17 cell differentiation	yes	18, 25
ITE	reduction of EAE severity	unknown	17, 25
Exogenous ligands	**Effect on immune response**	**AhR mediated**	**Reference**
halogenated dioxins: TCCD	Treg cell differentiation	yes	15, 17, 18, 37-39, 41
polycyclic aromatic hydrocarbon: benzo(a)pyrene, 3-methylcholanthrene	suppression of DC and macrophage function	yes	42, 43
benzimidazoles: omeprazol	unknown		14
anti-allergenic drugs: M50345, M50367, VAF347	suppression of Th2 cell differentiation	yes	37
Dietary ligands	**Effect on immune response**	**AhR mediated**	**Reference**
flavonoids: apigenin, quercetin, naringenin	suppression of DC and macrophage function	unknown	26, 27, 45, 47-49
Indole-3-carbinol derivatives (I3C)	unknown		25, 27

Table 1: Overview of well-known AhR activating ligands

3.1 Endogenous ligands affecting immune responses:

The endogenous AhR ligand bilirubin is a heme degradation product. Heme oxygenase-1 (HO-1) degrades heme to generate biliverdin, carbon monoxide and ferrous iron. Then biliverdin reductase converts biliverdin into bilirubin. Bilirubin is a very potent antioxidant and in high levels present in human serum. [28, 29]

Heme degradation products are tested as immunosuppressive agents in the treatment of islet allograft rejection. Transplantation of islets is an important approach in the treatment of diabetes type 1. But the immunosuppressive agents generally used have very toxic side effects. [28, 30] Administration of HO-1, carbon monoxide or bilirubin to donor or recipient mice leads to prolonged allograft survival and antigen-specific tolerance without any detectable toxicity. Administration of bilirubin reduces the expression of TNFα, caspase-12 and iNOS. Hereby, inflammation and apoptosis of the transplanted islets is reduced. In addition, FOXP3+ Treg cells are induced and these regulatory T cells surround the transplanted islets. These Treg cells also induce

tolerance towards a second transplanted skin graft. Adoptive transfer experiments indicate that the FOXP3+ Tregs are induced by *de novo* generation of Tregs. Depletion of FOXP3+ Treg cells abrogates the prolonged allograft survival induced by bilirubin. This suggests an important role for inducible Treg cells in islet allograft survival. [28, 30] When HO-1, carbon monoxide and bilirubin are combined as immunosuppressive treatment in islet allograft transplantation the effects are comparable or even more pronounced. [30]
In these transplantation studies the effects of bilirubin are not tested in AhR deficient mice. So whether the induction of FOXP3+ Treg cells in this setting is AhR dependent remains to be elucidated. But because bilirubin is a potent AhR activator and AhR activation can play a role in T cell differentiation, it could be that bilirubin acts via AhR to induce regulatory T cells.
The effect of bilirubin is furthermore tested in an autoimmune setting. Experimental autoimmune encephalomyelitis (EAE) in mice is a model for multiple sclerosis in humans. The observation that exogenous bilirubin ameliorates EAE in mice suggests that bilirubin also has immunosuppressive activities in autoimmune diseases. Depletion of endogenous bilirubin or HO-1 dramatically exacerbates the course of EAE, this further supports the immunosuppressive character of bilirubin. [29]
To investigate the mechanism by which bilirubin induces immunosuppression, CD4+ T cells were stimulated with proteolipid protein peptide (PLP) in the presence of APCs. By adding bilirubin to this culture, production of the Th1 cytokines IL-2 and IFNγ and the Th2 cytokines IL-10 and IL-4 is suppressed. Bilirubin also reduces the expression of co-stimulatory molecule CD28 on T cells and inhibits the activity of CD80 and CD86 in macrophages and dendritic cells. This suggests that bilirubin inhibits antigen-specific T cell responses by inducing anergy in reactive T cells. Not only co-stimulatory molecules are affected by bilirubin, NFκB could not translocate to nucleus in T cells and expression of MHCII molecules on macrophages and dendritic cells is also inhibited by bilirubin, probably by inhibiting STAT1 phosphorylation. These results suggest that bilirubin not only acts on T cells but also on APCs like macrophages and dendritic cells. Whether the described effects are mediated via AhR remains to be elucidated. Whether bilirubin is always present in the body at significant levels to play a role in the process of autoimmunity or graft rejection is not known. Because depletion of endogenous bilirubin exacerbates EAE, it is suggests that the presence of endogenous bilirubin could play an immunosuppressive role by preventing severe signs of EAE or delaying the onset of EAE. It is clear that supplementation with bilirubin or HO-1 diminishes immune related diseases via suppression of T cells and APCs. [29]

Another endogenous AhR ligand which can affect immune cells is kynurenine. Kynurenine is product of tryptophan catabolism. Indoleamine 2,3-dioxygenase (IDO) is an enzyme that converts tryptophan into kynurenine. Kynurenine itself is a potent activator of the AhR. The carboxylic acid moiety is responsible for maximal transcriptional activity. [31]
T cell differentiation depends on the interaction of T cells with dendritic cells (DCs). Especially CD103+ DCs can drive specific T cell differentiation. Such DCs are present in high numbers in the gut-associated lymphoid tissue (GALT). DC expansion at mucosal sites is correlated with induction of oral tolerance. IDO is highly expressed in CD103+ DCs and plays a role in the induction of FOXP3+ Treg cells. When IDO is depleted, the ability of CD103+ DCs to induce FOXP3+ Treg cells is suppressed, resulting in less antigen-specific Treg cell expansion and reduction of oral tolerance.

Additionally, more IL-17-producing and IFNγ producing T cells are generated when IDO is depleted. [32] Furthermore, when IDO is blocked with 1-methyl-DL-tryptophan (1-MT), addition of kynurenine restores the ability to induce FOXP3+ Treg cells. [33] Induction of FOXP3+ Treg cells by kynurenine is AhR dependent, as in AhR null mice kynurenine does not induce generation of FOXP3+ Treg cells from naïve CD4+ T cells. [34]
This suggests an important role of IDO enzymatic activity and the production of kynurenine in the induction of FOXP3+ Treg cells and regulation of inflammation in the gut. [32-35]

In addition, 6-formylindolo[3,2-b]carbazole (FICZ) is a potent activator of the AhR. FICZ is an UV light product of tryptophan. Ligation of the AhR by FICZ induces transcription of cytochrome P450 metabolizing enzymes. [18] FICZ increases the expression of Th17 cells and IL-22 production in mice in the presence of IL6 and TGFβ. In AhR-deficient mice, ligation with FICZ resulted in reduced numbers of Th17 cells and almost no IL-22 production, so the effects of FICZ on T cell differentiation are AhR dependent. Stimulation with FICZ also increases the severity of EAE in mice. This can be prevented by neutralizing antibodies to IL-17A. [18]
Thus the endogenous AhR ligand FICZ induces AhR dependent expansion of Th17 cells. This AhR dependent expansion of Th17 contributes to the development and severity of EAE.

Furthermore, indirubin is a potent AhR activating ligand. Treatment of mice with indirubin significantly enhances the percentage of FOXP3+ Treg cells compared to control mice. The cell number of total immune cells and CD4+ T cells are reduced by indirubin, which shows the immunosuppressive effects of bilirubin. Because the percentage of FOXP3+ Treg cells is higher in indirubin treated mice, it is suggested that FOXP3+ Treg cells are less sensitive to indirubin than helper and effector T cells. [36]

3.2 Exogenous ligands affecting immune responses:
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most investigated AhR ligand. It is one of the most potent AhR ligands known and possesses wide immunosuppressive activities. TCDD inhibits the differentiation into Th1, Th2 and Th17 cells, while inducing or preserving FOXP3+ Treg cells. In contrast to almost all AhR ligands, TCDD is not metabolized and is persistently bound to the AhR. This probably contributes to the toxicity of TCDD. TCDD has been shown to suppress allograft responses, allergic responses and autoimmune responses. How TCDD induces a shift in T cell differentiation, thereby suppressing Th1, Th2 and Th17 cell differentiation and inducing Treg cell differentiation, is not known. [37]
It is possible that TCDD induces apoptosis in effector T cell population and that Treg cells are resistant to this TCDD induced apoptosis. Hereby Treg cells are not induced by TCDD, but the proportion of Treg cells is enhanced compared to effector T cells. It could also be that expansion of Treg cells is really due to TCCD-induced *de novo* generation of Treg cells. Most studies indicate that TCDD induces FOXP3+ Treg cells. But whether there is an increase in absolute numbers of FOXP3+ Treg cells or there is a proportional increase in FOXP3+ Treg cells is not mentioned. Increase in FOXP3+ Treg cells by TCDD results in an immunosuppressive phenotype, which favors the outcome of various immune-related diseases. The immunosuppressive effects are mediated via the AhR because in AhR deficient mice these effects of TCDD were

abrogated. [15, 17, 18, 38, 39]
Additionally, it was shown that TCDD enhances IDO expression via an AhR dependent mechanism, resulting in the generation of FOXP3+ Treg cells [34, 40] When IDO is blocked with an IDO inhibitor, 1-methyl-DL-tryptophan (1-MT), induction of FOXP3+ Treg cells is abolished. [40]
Activation of the AhR by TCDD does not only induce FOXP3+ Treg cells, but also regulatory Tr1 cells. Human TCDD treated T cells produce IL-10 and induce apoptosis in effector T cells. This is AhR dependent as in AhR knock-down T cells the effect of TCDD was abrogated. The AhR interacts with c-Maf (Tr1 transcription factor) to upregulate IL-10. TCDD also induces AhR dependent upregulation of granzyme B. Granzyme B mediates the immunosuppressive effect of these TCDD treated T cells. [15]
Thus, TCDD induces both FOXP3+ Treg cells and immunosuppressive Tr1 cells via an AhR dependent mechanism, acting on DCs and directly on T cells.
A recently published paper showed that TCDD does not always induce Treg cells and tolerance. In this article, TCDD was given before oral tolerance induction by three times oral ovalbumin (OVA). Hereafter, the mice were three times challenged with OVA i.p. After the first parenteral immunization with OVA, TCDD suppresses the IgG1 antibodies against OVA. However, after the second and third immunization anti-OVA antibodies are not suppressed, but increased in TCCD treated mice compared to untreated, tolerance-induced mice. So TCDD treatment breaks tolerance after repeated OVA challenges. TCDD treated mice had more Th17 cells and IL-6 producing DCs, compared to untreated immunized mice. [41]
These results are the opposite of the findings described above. Although TCDD is known to induce FOXP3 Treg cells, apparently TCDD does not always induce regulatory T cells, but can also break oral tolerance.

Polycyclic aromatic hydrocarbons (PAH) are another group of very potent exogenous AhR ligands. They have immunosuppressive properties, mainly affecting DC or macrophage function. Most common PAHs are benzo(a)pyrene (BP), dimethylbenz(a)anthracene (DMBA) and 3-methylcholanthrene (MC).
BP influences the differentiation of monocytes into DCs. When BP is added to a blood monocyte culture with GM-CSF and IL-4, CD1a, being a marker for immature DCs, is upregulated. Additionally, the expression of the activation markers CD80 and CD40 is inhibited by BP. Similar results are obtained with DMBA and MC. Not only differentiation of monocytes into DCs is suppressed, the activation of DCs by LPS is also significantly reduced. BP downregulates the expression of CD80, CD86, CD83, CD40 and HLA-DR in LPS-stimulated DCs. Furthermore, the production of IL-12 is suppressed by BP and BP treated DCs can not induce antigen-specific T cells. [42]
PAHs like BP do not only suppress DC function, but also macrophages are affected by PAHs. BP, DMBA and MC suppress the differentiation of monocytes into macrophages, observed by the downregulation of the expression of CD80,CD86, CD64, CD11, CD29, CD49 and HLA-I molecules. In this experiment, the proliferation of antigen-specific T cells is also suppressed by PAHs. [43]
It is shown that the immunosuppressive effects of PAHs on macrophages are indeed AhR dependent. The monocytes express functional AhR and treatment with PAHs induces upregulation of CYP1A1 expression. When the experiments are repeated in the presence of the AhR antagonist α-naphtoflavone, the inhibitory effects of the PAHs are counteracted and mature macrophages are produced. When another PAH, benzo(e)pyrene (BeP), which does not interact with the AhR, is used, differentiation of

monocytes into macrophagesis not suppressed. These results show that the immunosuppressive properties of PAHs on macrophages are mediated via the AhR. [43]

The anti-allergic drug M50354 and its ethyl ester derivative M50367 are benzoimidazole derivatives. They bind to the AhR and induce CYP1A1 and CYP1B1 activity. These drugs suppress Th2 cell development, skewing T cell differentiation towards a Th1 phenotype. [44] They suppress Th2 differentiation by inhibition of GATA3. This suppression of Th2 cell differentiation is AhR dependent. [14]
The anti-allergic drug Tranilast is a derivative of the tryptophan metabolite 3-hydroxyanthranilic acid. Tranilast can bind and activate the AhR and has been shown to suppress EAE in a mechanism linked to Treg cells. [14]
These drugs can be effective in the treatment of allergies. The suppressive mechanism is of these drugs is AhR dependent. This supports the role for AhR ligands in the treatment of immune mediated diseases.

3.3 Dietary ligands affecting immune responses:
Flavonoids are a major source of dietary AhR ligands. Most potent flavonoid AhR ligands are apigenin and quercetin. These flavonoids do not only interact with the AhR but also activate the estrogen receptor (ER). Flavonoids are present in many vegetables, fruits, herbs, flowers, seeds and beverages.
Apigenin is a non-toxic, non-mutagenic flavonoid present in onions, parsley and oranges. Apigenin can suppress the maturation of DCs. It inhibits the expression of CD80, CD86, MCH-I and MHC-II molecules and the production of IL-12. Both in vitro and in vivo experiments show the suppressive effect of apigenin on DC function. By suppressing DCs, T cell differentiation is hampered and antigen stimulation will not result in an appropriate immune response. [45]
Apigenin can also play a suppressive role in lupus autoimmune disease. Lupus autoimmune cells express high levels of COX-2 and c-FLIP. COX-2 is an enzyme that is responsible for the production of pro-inflammatory prostaglandins. By upregulating the expression of COX-2, lupus autoreactive T cells become resistant to anergy and apoptosis. Additionally, via expression of c-FLIP, lupus autoreactive T cells will become resistant to Fas-mediated apoptosis. C-FLIP binds to and inhibits caspase 8, hereby inhibiting apoptosis. [46]
Apigenin reduces autoimmune response by inhibiting the IFNγ and IL-17 response in splenocytes stimulated with lupus antigen. IgG class switched antibodies against several lupus antigens are suppressed. Furthermore, apigenin reduces the expression of COX-2 in CD4+ T cells, B cells, DCs and macrophages. By reduction of COX-2 expression, apigenin causes depletion of lupus cells autoreactive immune cells via the induction of apoptosis in these cells. Moreover, production of IL-6 by APCs is suppressed by apigenin, thereby inhibiting Th17 responses in lupus. Apigenin is not found to induce regulatory T cells. [47]
Whether the AhR plays a role in the suppressive effects of apigenin is not known. Because apigenin can activate several receptors, these immunosuppressive effects are probably the result of multiple signaling pathways. Reduction of COX-2 positive CD4+ T cells is probably not due to AhR signaling. On the other hand, suppression of DC function could be the result of AhR signaling, as it is observed with other AhR ligands.
Quercetin, another common flavonoid, can also suppress the DC maturation. Quercetin downregulates several cytokines and chemokines produced by LPS-activated DCs.

Expression of MHCII, CD40, CD80 and CD86 is downregulated by quercetin. Additionally DC migration and expansion of antigen-specific T cells is suppressed in the presence of quercetin. When the DC suppressive effects of quercetin are assessed in contact hypersensitivity (CHS) experiments in mice, it is observed that quercetin suppresses the CHS response. This suggests that quercetin can play a role in the treatment of delayed type hypersensitivity diseases. [48]
Another flavonoid, naringenin, can also inhibit the expression of pro-inflammatory cytokines in LPS-stimulated macrophages in vitro. Moreover, naringenin can inhibit the CHS response in mice and it decreases antigen-induced T cell proliferation by induction of apoptosis in vitro. [49]
These experiments show that flavonoids do have major immunosuppressive properties. Mainly characterized by the suppression of DC or macrophage maturation. Induction of apoptosis of antigen-specific T cells could be due to the suppression of activation markers on APCs, but maybe flavonoids can also directly induce apoptosis in activated T cells. Which signaling pathway is responsible for these immunosuppressive effects remains to be identified. Because flavonoids can both interact with the AhR and the estrogen receptor (ER), these two pathways might interact. Even if these pathways do not interact with each other, they might signal at the same time and the outcome of signaling will always be dependent on both the AhR and the ER. The resulting effects can only be linked to one receptor when the other one is not present (knock-out experiments).

The flavones α-naphthoflavone and β-naphtoflavone are other AhR ligands. α-naphthoflavone is an AhR antagonist and is used as AhR inhibitor. [43] However, β-naphthoflavone is an AhR agonist and can act like FICZ, by enhancing IL-17 and IL-22 production in CD4+ T cells under Th17-cell-inducing conditions. This effect of β-naphthoflavone is AhR dependent. [18]

Vitamin A is the source of retinoic acid (RA). It cannot be synthesized by the body, but has to be absorbed from the diet. Vitamin A is metabolized via the enzymes alcohol dehydrogenase and retinal dehydrogenases into RA. RA binds to homo- or heterodimers of retinoic acid receptors (RAR) and retinoid X receptors (RXR). These nuclear receptors function as ligand-activated transcription factors. CD103+ DCs are able to produce RA from vitamin A. RA is a regulator of TGFβ dependent immune responses. RA can inhibit the induction of Th17 cells and promote differentiation of FOXP3+ Treg cells. Hereby, RA from homeostatic DCs can support the induction of tolerance. RA can directly inhibit RORγt and has also direct positive regulatory effect on the transcription of FOXP3. This effect is reversible, in the presence of pro-inflammatory cytokines the regulatory response is converted into a pro-inflammatory immune response. STAT5 and STAT3 are important for the transcription of FOXP3 and IL-17 respectively. Cooperation between STAT5 and RARs results in STAT5-enhanced responsiveness of RARs to RA. So it could be that the RA mediated effect on Treg and Th17 cell differentiation is a consequence of the interaction of STAT and RAR transcription factors. [50]
RA itself is not an AhR ligand. But the RARs and the AhR might interact with each other. [51-53] So when RA binds to a RAR and additionally the AhR binds to the RAR, these two pathways can interact and RA can influence AhR signaling. Moreover, when TCDD binds to the AhR, it can affect RAR signaling. [51-53] Both the AhR and RARs are transcription factors, therefore, they might act as or bind to co-repressors or co-activators in response to ligands, hereby affecting the outcome of signaling. [54]

4. Microbiota affecting T cell differentiation

The commensal microbiota in the gut can play a role in T cell differentiation. Tolerance towards the intestinal microbiota is important and break of tolerance will lead to inflammatory diseases in the bowel or even systemic. The commensal bacteria *Bacteriodes fragilis* is able to protect mice from intestinal inflammation by suppressing IL-17 production. *B. fragilis* induces FOXP3+ Treg cells and these cells suppress Th17 cells and the production of IL-17 and IL-22. Polysaccharide A (PSA) from *B. fragilis* mediates the induction of Treg cells. Probably PSA acts via TLR2 on DCs, because in TLR2 deficient mice Treg cells are not induced in response to colonization with *B. fragilis.* [55]
Commensal bacteria can also play a role in the development of autoimmune diseases. In a mice EAE model, germ free (GF) mice do not develop EAE whereas mice colonized with specific pathogen free (SPF) microbiota do develop EAE. In GF mice CD4+ T cells from draining lymph nodes express lower amounts of IL-17 and IFNγ. The expression of Th17 transcription factor RORγt is also decreased. Additionally the proportion of FOXP3+ Treg cells is increased in GF mice. This suggests that colonization with commensal bacteria can indeed play a role in the development of inflammation and autoimmunity. [56]
This is confirmed by a study that shows that treatment of mice with oral antibiotics can protect against EAE. Oral antibiotics reduce the bacterial load in the gut and the composition of the microbiota. Treatment with oral antibiotics increases the proportion of FOXP3+ Treg cells in the GALT as well as in the central nervous system. Also increased expression of IL-10 and IL-13 is observed and decreased expression IL-17 and IFNγ is observed. So both in GF mice as in mice treated with oral antibiotics, inflammation in gut and CNS is reduced. Protection against EAE is induced and the proportion of FOXP3+ Treg cells is increased. [57]
Segmented Filamentous Bacteria (SFB) are commensal bacteria and potent inducers of Th17 cells. Colonization with SFB correlates with reduced colonization and growth of pathogenic bacteria. SFB induces Th17 cells in the GALT and the production of IL-17 and IL-22. SFB colonization also results in the production of serum amyloid A (SAA). When SAA is added to a co-culture of DCs with naïve CD4+ T cells, expression of IL-17, IL-22 and RORγt is induced, indicating differentiation of Th17 cells. Without DCs no production of Th17 cytokines is observed. DCs stimulated with SAA produce IL-6 and IL-23. This suggests that SFB induce Th17 differentiation via the production of SAA, which in turn acts on DCs to induce Th17 cell differentiation. [58]
This Th17 cell differentiation and the production of IL-17 and IL-22 by commensal bacteria is needed for protection against pathogenic bacteria. Th17 cells also mediate the production of anti-microbial peptides.
From *Candida albicans* it is known that it downregulates the mucosal immune response against fungi. Th17 mediated IL-17 expression is a major mucosal host defense against fungi. Live *C. albicans* can inhibit IL-17 production by altering the metabolism of tryptophan . In stead of IDO produced kynurenine, live *C. albicans* shifts the metabolism towards the production of 5-hydroxytryptophan by another tryptophan metabolizing enzyme, tryptophan hydroxylase. This is probably the result of downregulation of IFNγ expression and subsequently low levels of IDO. Thereby the tryptophan metabolism is shifted towards tryptophan hydroxylase. It is clear that *C. albicans* can downregulate the host mucosal immune response against fungi, although the exact mechanism remains to be elucidated. [59]
As mentioned before, GF mice harbor less IL-17 producing cells than SPF microbiota

colonized mice. The presence of commensal microbiota does not only drive Th17 cell differentiation, also γδ T cells are induced by commensal microbiota. GF mice have significantly less IL-17 producing γδ T cells than mice colonized with SPF microbiota. Reconstitution of GF mice with SPF microbiota restores the percentage of IL-17 producing γδ T cells. So commensal microbiota do not only induce Th17 cells but also induce IL-17 producing γδ T cells. [60]
Whether the (commensal) microbiota can act via the AhR is very unlikely. No activation of the AhR by microbial products has been described. It is more likely that the microbiota act via APCs and influence the cytokine milieu, thereby inducing specific T cell differentiation. Although the microbiota does not interact with the AhR, they can play an important role in AhR signaling. The microbiota in the gut is a major environmental factor, which continuously can influence the cytokine milieu. Hereby signaling via the AhR and T cell differentiation will be affected by the microbiota. So the microbiota should be considered as a risk factor.

5. Discussion

Tolerance induction is an important process in the prevention of food allergy. Oral tolerance is the result of the interaction between DCs and T cells and the cytokine milieu. In food allergy no oral tolerance is established.
In T cell differentiation, the cytokine milieu is the most important factor. Specific cytokines will drive naïve CD4+ T cell differentiation. TGFβ signaling results in differentiation of FOXP3+ Treg cells. However, when TGFβ and IL-6 are present, differentiation of Th17 cells is induced. Activation of the AhR can result in the differentiation into FOXP3+ Treg, Th17 or regulatory Tr1 cells, dependent on the specific AhR ligand. It appears that signaling via TGFβ and the AhR results in the same outcome. From TGFβ it is known that it can induce upregulation of AhR and potentiate the activity of the AhR. Addition of AhR ligands enhances TGFβ induced differentiation of FOXP3+ Treg cells. It is clear that signaling via TGFβ and the AhR are alike. They can interact and potentiate each other.
Several AhR ligands have been shown to induce suppression of DC function, induction of Treg cells and reduction of antigen-specific T cells. In addition, anti-allergic drugs that act in an AhR dependent manner induce skewing of T cell differentiation from Th2 towards a Th1 phenotype. However, stimulation with FICZ results in induction of Th17 cells. In addition, one article showed break of tolerance by TCDD, in stead of the induction of Treg cells by TCDD.
The question remains whether AhR ligands are an option for treatment of food allergy? Based on the above described findings, I would suggest that indeed certain AhR ligands can be considered as treatment candidates. Although these ligands are not yet tested in the setting of food allergy, it would be very interesting to do so. Almost all described AhR ligands induced FOXP3+ Treg cells, which suppress all other effector and helper T cells, or they suppressed DCs and/or macrophage function, so no antigen-specific T cells are produced.
To investigate whether AhR ligands indeed are a potential treatment option, other factors should be taken into account.
Signaling via the AhR does not always involve only the AhR signaling pathway. The AhR can interact with other transcription factors. When the AhR is bound to another transcription factor, not only genes with the XRE consensus sequence in their promoter can be transcribed, also other genes can be transcribed. Best known is the interaction between AhR en NFκB signaling pathways. For example, signaling of the AhR via association with RelA and RelB results in transcription of NFκB responsive genes.
Signaling pathways also interact because ligands can bind more than one receptor. From several AhR ligands it is known that they not only activate the AhR, but also the estrogen receptor (ER). For example the flavonoids quercetin and naringenin can activate both the AhR and the ER. When signaling pathways interact, the outcome of the signaling can be changed. In this case it is hard to dissect whether the obtained results are the effect of AhR signaling.
AhR ligands can be an option for treatment of immune mediated diseases. In the case of food allergy no established oral tolerance is present and treatment is always introduced after food allergy is diagnosed. In this case AhR ligands can not break tolerance to the specific food allergen, but perhaps AhR ligands enhance the severity of the allergic response. Although AhR ligands are proposed to be potential immunoregulatory agents, other possible opposing effects of oral AhR ligands should be carefully investigated.
It also remains to be identified whether AhR ligands are stable in vivo in humans. When the AhR ligand is given orally, it should be investigated if the ligand will be

available in the gut to be absorbed. Furthermore the concentration of the ligand has to be high enough to activate the receptor and the metabolism of AhR ligands is important. Most ligands induce their own metabolism, by upregulation of CYP enzymes. So it is possible that they have a very short half life and are maybe cleared before they can induce a significant effect.
Environmental factors, like the commensal microbiota may interfere with the therapeutic outcome. All evidence suggests that the presence of commensal bacteria induce IL-17 producing T cells. This response is necessary to protect the body against pathogenic bacteria. The microbiota changes the cytokine milieu and thereby affects T cell differentiation. When AhR ligands are used to induce Treg cell differentiation and the microbiota induce IL-6 production in DCs, probably not Treg cells are induced but Th17 cells. This Th17 cell differentiation can then be enhanced by the AhR ligand. Hereby AhR ligands will enhance the inflammation in stead of reducing the inflammatory response. In addition it could also be a problem when Treg cells are induced by AhR ligands, but Th17 cells are needed to fight invading pathogens.
Another environmental factor is food. Ingested food can contain unknown AhR ligands. These ligands can also affect the signaling by AhR ligands and change the outcome of signaling.
Based on all decribed findings about AhR ligands and possible ‘risk’ factors, it can be concluded that AhR ligands could be a promising treatment option in regulating immune mediated diseases. One of the most likely candidates should be kynurenine. This tryptophan metabolite is an endogenous AhR ligand and because tryptophan catabolism is always present, kynurenine will always be produced by DCs. This could be an advantage, because there is a minimal risk of side effects. AhR dependent induction of FOXP3+ Treg cells via kynurenine could be an important mechanism of the body to induce tolerance towards every day food intake and protect the body from the microbiota in the gut. The question remains if exogenous kynurenine indeed can induce tolerance and immunoregulation in vivo. Furthermore it should be investigated whether kynurenine is not immediately metabolized and can be given orally. If so, kynurenine would be a very interesting candidate in the treatment of immune-related diseases.
Additionally, it should be known whether the FOXP3+ Treg cells induced by kynurenine indeed can suppress the allergy-mediating Th2 cells. Besides AhR induced immunoregulation, general knowledge is required about the capacity of induced FOXP3+ Treg cells to suppress food allergy.
To conclude, several AhR ligands are found to induce immunoregulation. Most of the described AhR ligands induce FOXP3+ Treg cells or suppress APCs and suppress antigen-specific T helper cells. Hereby these AhR ligands may provide an option to treat immune-mediated diseases, like food allergy, where immunoregulation is required.

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