available at www.sciencedirect.com

ScienceDirect

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

Review

The immune phenotype of AhR null mouse mutants: Not a simple mirror of xenobiotic receptor over-activation

Charlotte Esser *

Institut für Umweltmedizinische Forschung, Division of Molecular Immunology, Auf̈m Hennekamp 50, 40225 Düsseldorf, Germany

ARTICLE INFO

Article history:
Received 28 August 2008
Accepted 3 October 2008

Keywords:
Arylhydrocarbon receptor
Knock-out mice
Immunity
IL-17
Cytokine balance

ABSTRACT

Intrinsic and induced cell differentiation and the cellular response to endogenous and exogenous signals are hallmarks of the immune system. Specific and common signalling cascades ensure a highly flexible and adapted response. Increasing evidence suggests that gene modulation by the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, is an important part of these processes. For decades the AhR has been studied mainly for its toxic effects after artificial activation by man-made chemical pollutants such as dioxins. These studies gave important, albeit to some extent skewed, evidence for a mechanistic link between the AhR and the immune system. AhR null mutants and other mutants of the AhR signalling pathway have been generated and used to analyse the physiological function of the AhR, including for the developing and antigen-responding immune system. In this review I look at the natural immunological function(s) of the AhR.

Contents

* Tel.: +49 211 3389 253 fax: +49 211 3190910.
E-mail address: chesser@uni-duesseldorf.de.

Abbreviations: AhR, aryl hydrocarbon receptor; ARNT, AhR nuclear translocator; DC, dendritic cells; DN, double negative; DRE, dioxin responsive element; OVA, ovalbumin; ConA, concanavalin A; PAH, polycyclic aromatic hydrocarbon; TCDD, 2,3,7,8-tetrachloro-dibenzo-p-dioxin; XRE, xenobiotic response element.

0006-2952/$ – see front matter
doi:10.1016/j.bcp.2008.10.002

1. Introduction

The aryl hydrocarbon receptor (AhR) signalling pathway is evolutionarily conserved, and can act independently or in concert with other signalling pathways. Similar to steroid hormones, the AhR molecule is a ligand-activated gene transcription factor. Residing in the cytosol chaperoned by hsp90, AIP, and p23, the AhR dissociates these proteins upon ligand binding to translocate into the nucleus. In the nucleus, the AhR dimerizes with aryl hydrocarbon nuclear translocator (ARNT), and eventually binds to small conserved promoter elements called xenobiotic response elements (XREs) for transcriptional regulation in cooperation with co-factors. The AhR is then exported to the cytosol and degraded [1].

Numerous genes contain XREs in combination with other responsive elements in promoter specific patterns, thus the ligand-bound AhR regulates a plethora of genes in a cell-, tissue- and condition-specific fashion [2,3].

Biased by its discovery as a regulator of xenobiotic metabolizing enzymes in vertebrates more than 30 years ago [4], the AhR has long been studied for its pathological activity in response to man-made environmental pollutants. In particular halogenated polycyclic aromatic hydrocarbons (PAH), such as dioxins attracted attention and raised concern. The AhR mediates toxicity, mainly through alterations of gene expression as outlined above. The toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a prototypic ligand of the AhR, and other PAHs are far-reaching and include alterations in lipid metabolism, skin physiology, tumour promotion, and embryonic development. Last, but not least, the immune system is a very sensitive target of AhR-mediated toxicity, responding at particularly low concentrations of chemical exposure [5].

As the AhR is evolutionary old, with members of the family already present in fungi, insects, or nematodes, and expressed constitutively, but tissue-specifically, a physiological role beyond responding to man-made chemicals is commonly postulated (reviewed in [6]).

1.1. *Brief excursion into the physiological roles of the AhR*

Studies with (i) persistent activation of the AhR by e.g. TCDD, (ii) with AhR null mice, hypomorphs or natural low-affinity mutants, and later (iii) with strains with cell-specific conditional AhR deletions, confirmed multiple physiological roles of the AhR (reviewed in [6]). In brief, the AhR is a regulator of cell proliferation, e.g. via induction of Cdk2, or by physical interaction with the retinoblastoma protein. It cooperates and cross-talks with other signalling pathways, shown for instance for the estrogen pathway, NFκB, or cAMP [7–9]. The AhR induces oxidative stress, and may play a role for cell migration and adhesion [6,9–11]. AhR activity is highly cell-specific and controlled at multiple levels. Receptor affinity, expression level, signalling crosstalk, feed-back inhibition by the AhR-repressor, competition for the dimerization partner ARNT, and/or competition for transcription co-factors participate in the outcome of AhR-activation [1]. The AhR is quite promiscuous and accepts chemically very different ligands [12]. The ligand determines to a considerable extent the outcome of AhR-activation, albeit only one binding site exists [13]. Subtle changes in protein conformation, or quick degradability may be reasons [14]. Last but not least, some ligands are persistent, while others have a high metabolic turnover rate. This can result in different outcomes of AhR-activation [15,16]; in addition to the anthropogenic chemicals such as PAHs, numerous natural ligands (i.e. not anthropogenic) and endogenous ligands (made by the organism itself) have been identified and continue to be found. Interestingly, UVB radiation present in sunlight turns tryptophan into 6-formyl-[3,2b] indolo-carbazole (FICZ), a high-affinity ligand [17,18]. Other endogenous ligands are heme metabolites, indigo derivatives, and leukotrienes [12,19–22]. Over-activation of the AhR by various ligands, and the ensuing consequences for the immune system are the topic of a review by Nancy Kerkvliet in this issue. My review focuses on AhR-deficiency, and the outcome for the immune system, in particular, I will discuss and compare the results derived from genetically engineered null mutant mice.

2. Murine mutants of the AhR signalling pathway

In the 90s, three groups generated AhR-deficient mouse mutants independently, by either deleting exon1 or exon2. In the null mutant made in the laboratory of Frank Gonzalez, exon1 is replaced from the translational start site onwards with a neomycin gene [23]. A Japanese group around Yoshiaki Fujii-Kuriyama replaced part of exon1 with the bacterial β-galactosidase gene joined to a nuclear localization signal, allowing to screen for AhR expression [24]. In the null mutant made in the laboratory of Christopher Bradfield, exon2 is deleted [25]. Exon2 encodes the basic-helix-loop-helix domain required for dimerization with the AhR partner protein ARNT. Moreover, the frame shift generated by the neomycin gene insertion into exon2 resulted in a stop codon in exon3. Still, theoretically a small piece of RNA might be made from exon1 spliced to exon3, potentially coding for a small truncated 23 amino acid peptide. All mice were made with 129 ES cells, and thus originally had a mixed C57BL/6 × 129 background. They were later backcrossed onto one genetic background, mostly C57BL/6. In the following I will refer to these three null mutants as $AhR^{\Delta1/\Delta1G}$ (Gonzalez), $AhR^{\Delta1/\Delta1F}$ (Fujii-Kuriyama), or $AhR^{\Delta2/\Delta2}$ (Bradfield). The prefix B6.129 or B6 will indicate whether the mice were still on a mixed genetic background when used for experiments, or had been backcrossed at least nine generations to the C57BL/6 strain and were thus congenic.

Table 1 – General phenotype of three AhR null mutant mouse strains[a].

	B6.129AhR$^{\Delta1/\Delta1F}$	B6.129AhR$^{\Delta1/\Delta1G}$	B6.129AhR$^{\Delta2/\Delta2}$
General pathology			
TCDD resistance	Yes	Yes	Yes
Failure to induce Cyp1a1, Cyp1a2	Yes	Yes	Yes
Postnatal lethality	Yes (50%)	Yes (40–50%)	No
Growth retardation	Yes	Yes	Yes
Fertility	Decreased	Decreased	Decreased
Liver pathology	Yes	Yes	Yes
Failure to close ductus venosus	n.d.	n.d.	Yes
Immune pathology			
Smaller PALS		No	Yes
Enlarged spleen		Yes	No
Retarded seeding of spleen	n.d.	No	Yes
Spleen, thymus subsets		Normal	Normal

n.d.= not detected.
[a] Compiled from various references. For details see the International Mouse Strain Resource http://www.informatics.jax.org/imsr/index.jsp.

These mouse strains display some physiological changes (see Table 1). As expected, all AhR null mouse strains failed to induce of xenobiotic metabolizing enzymes by TCDD exposure; TCDD lethality and TCDD-mediated toxicity were abrogated. Other phenotypes differed, as reviewed by Lahvis and Bradfield [26] for their own null mutant and the B6.129AhR$^{\Delta1/\Delta1G}$ mouse. Growth rates were lower, and fertility was decreased in all three null mutant strains, but neonatal lethality observed only in B6.129AhR$^{\Delta1/\Delta1G}$ and B6.129AhR$^{\Delta1/\Delta1F}$ mice. Liver pathology was present in all strains, but not entirely congruent and sometimes less pronounced [24,26].

The immune system of the mice differed in a number of aspects. Thus, only in the B6.129AhR$^{\Delta1/\Delta1G}$ strain a lower splenocyte count at two weeks after birth, and smaller periarterial lymphoid sheaths at four weeks after births were observed. This was not observed in the B6.129AhR$^{\Delta2/\Delta2}$ strain; in contrast, at six weeks of age the splenocyte number was higher than in control animals. Lahvis and Bradfield pointed out in their comprehensive review from 1998 that differences might have been due to the mixed genetic backgrounds of the AhR null mice (C57BL/6 × 129), as the strains had not been backcrossed to an pure genetic background strain. By now, all AhR null mutants are available on the C57BL/6 background. In general, studies done after 2000 (AhR$^{\Delta2/\Delta2}$), 2003 (AhR$^{\Delta1/\Delta1G}$), and 2004 (AhR$^{\Delta1/\Delta1F}$) used mice backcrossed onto C57BL/6. Unfortunately, the early observations on the immune differences in mixed-background null mutants have never been analyzed in direct comparison again for genetically congenic mice. Different animal husbandry, such as food or materials used for cage bedding, can affect the phenotype, at least to some extent. Table 1 compares the general phenotype of the null mutants, and Table 2 lists other currently available mouse mutants of the AhR signalling system.

3. The immune system in AhR-deficient mice

The immune system is a complex organ with highly diverse functions, short- and long-distance interactions, and memory capacities. Immune cells communicate directly with each other by cell surface structures, or over considerable distances via lymphokines and chemokines. Lymphoid organs provide relevant spatial structures for direct communication of immune cells. Immune cells follow their intrinsic programmes, and/or adapt to external cues, relayed into the cells by a number of signal transduction pathways. Continuously differentiating cells in hematopoiesis and mature cell homeostasis is a distinct feature of the immune system. All immune responses in all immune cells pass at some point through the executive steps of up- or down-regulation of genes, which are tightly controlled. Major pathways in immune cells are G-Protein coupled receptors, the MAP-kinases, NFκB, or the Janus kinase (JAK)-STAT pathways. Others are direct ligand activation of latent transcription factors (such as glucocorticoid receptors); all signalling is tightly controlled and interconnected. The AhR as an externally triggered latent transcription factor is strikingly abundant in most immune cells. Always thought noteworthy, AhR abundance equals or surpasses that of the liver in many immune cell types [3]. The AhR mediates cellular responses to small molecular weight chemicals (albeit often toxic) and emerges as a signalling pathway used in differentiation and function of immune cells [6,5,27].

3.1. *Early phenotyping indicated immune impairment but raised the issue of differences between null mutant strains*

As pointed out above, the immune phenotypes of AhR null mutants with a mixed genetic C57BL/6 × 129-background differed. Only in the B6.129AhR$^{\Delta1/\Delta1G}$ mice, the seeding of the peripheral lymphoid organs with T and B cells was retarded, e.g. the splenic periarterial lymphatic sheaths were smaller. Spleens of young B6.129AhR$^{\Delta1/\Delta1G}$ had significantly lower cell numbers, which normalized only after several weeks of age [23]. The fetal thymus of B6.129AhR$^{\Delta1/\Delta1G}$ mice contained significantly fewer thymocytes at gestation day 15. The frequency of CD4$^+$ thymocytes and thymic emigrants in fetal thymus organ cultures, prepared from gestation day 15 foetuses and cultivated for six days doubled compared to C57BL/6 thymi, indicating an effect of the AhR on normal development of thymocyte subpopulations [28]. Adult thymus cellularity and overall pattern of lymphocytes subpopulations

Table 2 – Mouse strains with mutants for AhR and ARNT genes.

Name	Description of gene-defect	Commercially available[a]	Original reference
Gene targeted mice			
B6.129-*Ahr*^{tm1Gonz} (AhR$^{\Delta1/\Delta1G}$)[b]	Targeted mutation of exon1 C57BL/6 background	No; available via repository of the Mouse Model for Human Cancer Consortium MMHCC	[23]
Ahr^{tm1Yfk} (AhR$^{\Delta1/\Delta1F}$)	Replacement of exon1 with the NLS-LacZ gene (*note*: this mouse available also on DBA/2 background)	No; available via Riken Bioresource Centre	[24]
B6.129-*Ahr*^{tm1Bra}/J (AhR $^{\Delta2/\Delta2}$)	Replacement of exon2 with the neomycin resistance gene. on C57BL/6 background	Yes	[25]
AhR^{fxneo} (hypomorph AhR)	Diminished expression of AhR[d] allele in liver, kidney, heart, lung to about 10% of normal (i.e. hypomorph expression)	No	[68]
B6.129(FVB)-*Ahr*$^{tm3.1Bra}$/J (Ahr^{fx})	exon2 of AhR flanked by loxP recombination sites	Yes	[69]
AhR^{nls}	Mutation in exon2 leading to deficiency in nuclear localization and DRE binding	No	[70]
CA-tg	Constitutively active AhR, transgenic insertion	No	[71]
B6.$^{Cg\text{-}Tg(hCD2\text{-}CA\text{-}AhR/GFP)A}$ (CA-tg-T-cell-specific)	Constitutively active AhR, active only in T cells gene activity can be followed by GFP expression	No; available via Riken Bioresource Centre	[72]
CA-tg-keratinocytes	Transgenic insertion of constitutively active AhR under the control of the human K14 promoter, to express specifically in keratinocytes	No	[73]
hAhR knock-in	Replacement of mouse AhR gene with human AhR gene by homologous recombination	No; available via Riken Bioresource Centre	[74]
B6.129-*Arnt*^{tm1Mcs}/J (ARNT^{ko})	Insertion of a PGK-neomycin resistance cassette into exon6 (coding for the bHLH domain)	Yes	[75]
ARNT-1^{ko}	Targeted mutation of exon6; embryonically lethal	No	[76]
ARNT-2^{ko}	In-frame replacement of ARNT-2 locus with NLS-LacZ sequence at exon6; protein lacks HLH domain and thus dimerization and DNA binding; homozygotes are embryonically lethal	No	[77]
ARNT-2^{ko}	Targeted deletion of bHLH region; embryonically lethal	Yes	[78]
ARNT^{flox}	Exon6 flanked by loxP recombination sites	No	[76]
AhR-repressor^{ko}	In-frame replacement of exon2 and part of Intron2 by a NLS-lacZ cassette. The protein lacks the bHLH domain necessary for DNA binding and dimerization	No	[79]

Name	Mouse strain	Commercially available	Original reference
Natural mutants			
AhR$^{b\text{-}1}$ (responsive)	C57BL/6, C58, and MA/My	Yes	[80,81]
D2.B6-Ahr$^{b\text{-}1}$/J (responsive)	Responsive AhR$^{b\text{-}1}$ allele from C57BL/6 crossed onto the DBA/2 background	Yes	
AhR$^{b\text{-}2}$ (responsive)	BALB/cBy, A, and C3H	Yes	
AhR$^{b\text{-}3}$ (responsive)	*Mus spretus*, *M. caroli*, and MOLF/Ei.	Yes	

Table 2 (*Continued*)

Name	Mouse strain	Commercially available	Original reference
AhR^{d} (unresponsive)[c]	DBA/2, AKR, and 129	Yes	
B6.D2-Ahr^{d} (unresponsive)	Non-responsive AhR^{d} allele from DBA/2 strain backcrossed onto C57BL/6 strain	Yes	[82]

[a] Available via Jackson Laboratories, http://www.jaxmice.jax.org; information on vendor given to the best of my knowledge. Riken Bioresource Center http://www.brc.riken.jp or MMHCC Repository http://mouse.ncifcrf.gov/.
[b] For reasons of clarity, in the following the two AhR$^{\Delta2/\Delta2}$ strains will be designated by the letters G, or F, or to indicate the laboratories (Frank Gonzalez or Yoshiaki Fujii-Kuriyama) where the strains were made.
[c] Affinity for ligand is 10–100 times lower than in AhR^{b} alleles. (C57BL/6 × DBA/2) F_1 mice are responsive. Nucleotide and amino acid sequence differences between Ahr$^{b\text{-}1}$ and Ahr^{d} have been determined.

(determined by surface staining and FACS analysis) were neither significantly affected in B6.129AhR$^{\Delta1/\Delta1G}$ nor in B6.129AhR$^{\Delta2/\Delta2}$ mice [29–31]. For the Δ2/Δ2 knock-out, this result was confirmed later in genetically pure B6AhR$^{\Delta2/\Delta2}$ mice [30]. Other and more detailed stainings were not done; as reviewed in detail by Nancy Kerkvliet in this issue, the development and differentiation of lymphoid and myeloid cells change considerably in ligand-induced mice. The hematopoietic compartment contains the targets for TCDD [32], but nothing is yet known about the effects of AhR-deficiency on the lymphoid precursors generated in the foetal liver, and damage to the hematopoietic cells from this tissue is conceivable.

It has been suggested that the underlying cause for retarded seeding and low lymphoid cell count in the lymph nodes and spleen might be a changed emigration from the thymus, or a failure to home efficiently to the appropriate organ, but this has not been experimentally addressed so far. T cells from B6AhR$^{\Delta2/\Delta2}$ mice seed the spleen the same as AHR$^{+/+}$ T cells after intravenous injection [33,34]. The phenotypic changes reported yet need to be confirmed and extended to clarify these issues for congenic mice.

B6.129AhR$^{\Delta1/\Delta1G}$ mice are more susceptible to infections with *Helicobacter hepaticus*, an opportunistic infection indicating immunodeficiency [29,35]. The underlying cause may be specific defects in the immune system, or other, general damages resulting from AhR-deficiency. Only one published report directly compared B6.129AhR$^{\Delta1/\Delta1G}$ and B6AhR$^{\Delta2/\Delta2}$ mice: Immune responses against two model antigens showed that both strains elicited normal and competent immune responses. First, injection of allogeneic P815 tumour cells to induce a cellular immune response gave normal CTL activity. Frequency of effector CTL cells and of alloantibodies generated were almost equal in both strains. Second, immunization with sheep red blood cells generated a strong antibody response [36]. However, only B6.129AhR$^{\Delta1/\Delta1G}$ but not B6AhR$^{\Delta2/\Delta2}$ mice showed splenomegalia and an increase in B cells frequency after P815 immunization [36]. In contrast, immunization with the protein antigen ovalbumin (OVA), inciting a humoral immune response, did not enhance spleen cell number in the B6.129AhR$^{\Delta1/\Delta1G}$ mice; OVA-specific antibody titres were normal, and immunoglobulin class switching had occurred after repeated OVA injections. This indicated an intact memory response in B6AhR$^{\Delta1/\Delta1G}$ mice [37]. However, in unimmunized mice, CD8+ T cell frequency in the spleen was higher than in wild-type mice (see below; [37]) Table 3 summarizes the various findings regarding the immune phenotype of AhR null mutants.

3.2. AhR-dependent changes in differentiation of immune cells

As outlined above, phenotypic characterization and simple immunization schemes indicated no gross impairment or functional changes of the immune system if the AhR was missing. This could reflect, for instance, that triggering the AhR is not a necessary default setting in the core immune system, with an endogenous ligand always ''on duty''. Rather, the AhR could be present to respond in special circumstances, integrating e.g. environmental signals, or fine-tuning some immune responses. It is well-established that the AhR has an obligatory role in immune dysfunction after (xenobiotic) ligand exposure. Numerous experiments with over-activation of the AhR had shown (in particular by the high-affinity, persistent ligand TCDD) that AhR action affects various immune cells and particularly influences processes of differentiation. The systemic outcome of AhR over-activation all in animals analyzed is immunosuppression [5]. Human exposure to TCDD also results in slightly altered immune functions, as shown by in vitro data (often from PBMC) and epidemiological studies. For instance, helper T cell responses were impaired even 20 years after exposure. Available data indicate that infectious disease is increased in childhood, especially when exposure takes place in the period of pregnancy and nursing. However, a correlation between exposure and reduced prevalence of allergies was found [38–40]. Human studies are hampered by many factors in particular high inter-individual variation, and lack of access to exposure data, robust biomarkers, and knowledge of AhR genotype associations [41].

Immunosuppression is an operational term for a functional immunological deficit, with multiple causes. For TCDD and other AhR ligands, the system evaded any simple solution as to the cell-type responsible. Thus, dysfunctions of dendritic cells, T cells, B cells and others have been shown to contribute to AhR-mediated immunosuppression.

Experiments to pinpoint such responsible cells further and to identify AhR-mediated dysfunctions were done, but the picture remains inconclusive. For instance, thymus involution, a hallmark of TCDD exposure, was looked at in the context of AhR-deficiency. Bone marrow cells from B6.129AhR$^{\Delta1/\Delta1G}$ and also B6AhR$^{\Delta1/\Delta1G}$ successfully reconstituted the thymus of

Table 3 – Immune competence of AhR$^{-/-}$ mouse strains[a].

	B6.129AhR$^{\Delta1/\Delta1F}$	B6.129AhR$^{\Delta1/\Delta1G}$	B6.129AhR$^{\Delta2/\Delta2}$	Reference
(A) Mixed genetic background				
Fetal thymus cellularity, gestation day 15		Decreased		[28]
Fetal CD4+ thymocytes		Increased		[28]
Adult thymus cellularity		Normal	Normal	[26,29]
CD4/CD8 subset pattern		Normal	Normal	[26,29,30]
Helicobacter pylori infection		Frequent		[29]
Competence in humoral (anti-SRBC) and cellular immune response		Normal		[36]
Splenomegalia and increase in B cells after immunization		Yes		[26]
Expression of co-stimulatory molecules on splenic DC		Increased		[51]
	B6AhR$^{\Delta1/\Delta1F}$	**B6AhR$^{\Delta1/\Delta1G}$**	**B6AhR$^{\Delta2/\Delta2}$**	**Reference**
(B) Congenic genetic background				
Adult thymus cellularity			Decreased	[30]
Thymic subset pattern (CD4/CD8, CD69, Fas+ cells)			Normal	[30]
Competence in humoral (anti-SRBC) and cellular immune response			Normal	[36]
Contact hypersensitivity			Decreased	authoŕs unpublished observation
Capacity of bone marrow cells to reconstitute the thymus		Successful; more CD4+ cells in reconstituted thymus		[42]
Splenomegalia and increase in B cells after immunization			No	[36]
Memory response (humoral, class switching)		Intact		[37]
Output of CD8+ cells from thymus		Increased		[37]
Langerhans cells			Immature phenotype	Authoŕs unpublished observation
Gene changes in CD4+ T cells (microarray data)			Considerable up- and down-modulation	[48]
CD62L, GITR, CTLA4 expression on T-reg like cells			normal	[33]
IL-5 after ConA or OVA re-stimulation of spleen cells in vitro		Decreased		[37]
IL-5 secretion in inflamed lung (OVA-inhalation)			Increased	[47]
IL-5, IgE after DNP-ascaris immunization	Increased			[46]
IFN-γ, IL-12 secretion after ConA or OVA re-stimulation of spleen cells in vitro		Increased		[37]
IL4 secretion secretion after ConA or OVA re-stimulation of spleen cells in vitro		Normal		[37]
IFN-γ transcripts in CD4 cells (microarray result)			Up-regulated	[48]
	B6AhR$^{\Delta1/\Delta1F}$	**B6AhR$^{\Delta1/\Delta1G}$**	**B6AhR$^{\Delta2/\Delta2}$**	**Reference**
(C) Disease models				
Neutrophil frequency and infection-driven IFN-γ in influenza			Normal	[54]
Survival after *Streptococcus pneumonia* infection		Better		[55]
Susceptibility to *Listeria monocytogenes* infection			Increased, enhanced resistance to re-infection	[56]

Table 3 (*Continued*)

	B6AhR$^{\Delta1/\Delta1F}$	B6AhR$^{\Delta1/\Delta1G}$	B6AhR$^{\Delta2/\Delta2}$	Reference
Number of cytokine producing T cells in *Listeria monocytogenes* infection			Increased	[56]
Differentiation of Th17 cells/IL22cytokine production	Impaired		Impaired	[58–60]
Onset and severity of experimental encephalitis (depends on ligand)			Reduced	[58]

[a] For details see text.

irradiated mice. Yet, the results from B6.129AhR$^{\Delta1/\Delta1G}$ bone marrow chimeras indicated lower c-kit- Sca1+ thymocyte precursors frequency in the bone marrow, and lower frequency of very immature thymocytes (DN1 cells) in the thymus [32]. These experiments also confirmed that the thymocytes, but not the thymus epithelium, are the targets of adverse AhR action [32,42]. There was a tendency to a higher absolute number of CD4+ cells derived from the precursors in AhR$^{-/-}$ bone marrow reconstituted mice [42]. This is similar to our own observation in B6.129AhR$^{\Delta1/\Delta1G}$ that on gestation day 15 the frequency of CD4+ thymocytes had increased in the thymi of AhR$^{-/-}$ foetuses [28]. In contrast, spleen cells form naïve adult B6AhR$^{\Delta1/\Delta1G}$ mice had a higher frequency of CD8 T cells than wild-type mice [37]. A physiological role of the AhR might be initiation of developmental changes in the thymus and a skewed generation or emigration into the periphery. Recent evidence points to a role of the AhR in further differentiation of T cell subsets in the periphery as well, in particular regulatory T cells and Th17 cells (see below).

CD4+ T cell subsets are a primary target of the AhR, but the emerging evidence suggests that activation of the AhR affects the immune response only if it happens during the ongoing immune response. The generation of regulatory CD4 + CD25+ T cells by TCDD in a graft-versus-host model was not observed if the T cells did not express AHR. At the same time, adoptive transfer of B6AhR$^{\Delta2/\Delta2}$ cells into an allogenic recipient host did not lead to changes in the subpopulations of donor cells. It is as yet completely unknown whether such a generation of T-reg-like cells is physiological, i.e. controlled by endogenous AhR-ligands, or a strictly toxic event, only triggered by environmental cues. At least, no differences in surface molecules such as CD62L, GITR, or CTLA4 were detectable on peripheral CD4 + CD25+ cells from B6.AhR$^{\Delta2/\Delta2}$ versus wild-type mice [33].

3.3. *AhR and cytokines*

Many cytokine promoters contain one or more DREs, and it has long been known that the activated AhR controls transcription of some cytokine genes [43–45]. Cytokines were analyzed in AhR null mutant mice with a view to better define the role of the AhR in cytokine production during the immune response.

Allergic sensitization with DNP-*Ascaris* extracts led to a significantly higher IL-5 production, and increased IgE titre in B6AhR$^{\Delta1/\Delta1F}$ compared to AhR$^{+/+}$ mice [46]. The authors concluded that the AhR is involved in Th1/Th2 balance after sensitization. In a model of allergic lung inflammation, exposing B6AhR$^{\Delta2/\Delta2}$ mice to OVA via inhalation led to higher IL-5 increase in bronchoalveolar lymphocyte supernatants, compared to AhR$^{+/+}$ mice [47]. It is noteworthy that this is similar to the situation, where the AhR was over-activated by TCDD and where the immunotoxic failure only kicked in once the T cells were antigen activated [36].

However, IL-5 was not increased, but reduced, after non-allergic immunization of B6AhR$^{\Delta1/\Delta1G}$ mice by subcutaneous injection of OVA in Freunds adjuvant. T cell proliferation and IL-4 production was unaffected. In these null mutant mice, stimulation of spleen cells with either ConA or re-stimulation with OVA triggered higher IFN-γ and IL-12 protein production in spleen cell [37]. In agreement with this and extending the result to B6AhR$^{\Delta2/\Delta2}$ mice, we found increased transcription of IFN-γ, but not IL-4 in their purified, naive CD4 T cells [48]. No data exist on IFN-γ production by AhR-deficient CD8+ cells, NKT cells, or other possible sources for IFN-γ. Recently, the importance for IFN-γ in CD4+ and dendritic cell migration has been demonstrated [49,50]. Interestingly, splenic dendritic cells (DC) from B6.129AhR$^{\Delta1/\Delta1G}$ mice expressed less CD8α, but more LFA-1, a migration-related β2 integrin [51]. The molecule is necessary for the antigen-induced migration of DC to the lymph nodes [52]. The data suggests that the AhR is involved in balancing Th1 versus Th2 cytokines, e.g. by keeping IFN-γ and IL-12 at low level in normal mice, and controlling B cell proliferation upon infection [37], ultimately contributing to shaping inflammatory responses.

3.4. *AhR in models of infection—innate immunity*

Studies in naïve mice and their adaptive immune responses indicated a role for the AhR in immune functions. Further experiments addressed infectious immunity, where a functional innate immune response is pivotal for complete resistance to a pathogen.

Especially the group of Paige Lawrence has worked with models of infection. Exposure to TCDD during influenza infection indicated that the AhR-activation diminishes the memory response, decreases survival, and increases pulmonary neutrophilia and IFN-γ secretion [53]. AhR-deficiency as such did not influence neutrophil frequency or infection-driven IFN-γ secretion in the lungs (B6AhR$^{\Delta2/\Delta2}$), which were comparable to wild-type mice [54]. However, survival rate in a lethal *Streptococcus pneumonia* infection model was slightly enhanced in B6AhR$^{\Delta1/\Delta1G}$, albeit less than after AhR over-activation with TCDD [55]. B6AhR$^{\Delta2/\Delta2}$ mice infected with *Listeria monocytogenes*, an intracellular parasite, were more susceptible to infection, but developed enhanced resistance to

re-infection [56]. Serum levels of inflammatory cytokines IL-6, IFN-γ, and TNF-α were comparable to wild-type mice, whereas, somewhat surprisingly, IL-10 and IL-12 levels increased upon infection. The latter finding may simply reflect the higher bacterial burden. Cytokine producing *Listeria*-specific T cell numbers after the infection equalled or surpassed in both AhR null mice those of wild-type mice. Moreover, macrophages retained their ability to ingest *Listeria* or inhibit parasite growth [56].

Again, this data showed that the AhR contributes to an optimal immune response, but is not a *sine qua non* condition in infection. The response suggested a constitutive role of the AhR in innate immunity, an idea congruent with the data on neutrophil activation by TCDD.

3.5. *AhR role in Th17 differentiation and T cell subsets*

As pointed out above, the lack of a grossly devastating immune phenotype in AhR null mutants was surprising. Recent research thus focuses on determining AhR-dependence of particular AhR-ligand-induced immunological phenomena, with a view (i) to understanding the contribution of environmental pollutants to immune disease, and (ii) to exploit the AhR system pharmacologically. Environmental factors, in particular low molecular weight chemicals, can trigger and exacerbate immune dysfunctions, such as allergy and autoimmunity. Xenobiotic metabolizing enzymes, most of which are under the control of the AhR, can be risk factors, as shown for psoriasis [57]. Many autoimmune diseases are due to the activity of Th17 cells, a newly discovered subset of CD4+ T cells specialized in secretion of IL-17 and IL-22. Ligand-induced activation of the AhR during Th17 cell differentiation markedly increased the level of AhR protein and the generation of Th17 cells and their IL-17/IL-22 production in B6AhR$^{\Delta 2/\Delta 2}$ and B6AhR$^{\Delta 1/\Delta 1F}$ mice [58,59]. Onset and pathology in experimental autoimmune encephalitis (EAE), a mouse model of multiple sclerosis, increased in FICZ treated C57BL/6 mice. The effect was abrogated in B6AhR$^{\Delta 2/\Delta 2}$ mice, which produced less IL-17, and no IL-22 by Th17 cells. Moreover, onset and severity of EAE was markedly reduced in these AhR null mice, both compared to the wild-type C57BL/6 and compared to the B6.129 mice exposed to AhR ligand FICZ before disease triggering [58]. Similar results were obtained by the group of Weiner, who used a natural mutant of the AhR, C57BL/6 mice congenic for the DBA/2.AhR^{d} allele (which has a 100–1000× lower AhR affinity for ligands) [60]. They also found induction of regulatory T cells by the persistent ligand TCDD, which confirmed previous results [33].

The differential action of two high-affinity ligands (FICZ versus TCDD), one of them persistent, the other metabolically degradable, is intriguing but not yet understood. In any case, it appears that environmental ligands of the AhR can shift the balance between the ability of the organism to tolerate or to fight ''self'' by generation and activation of T cell subsets. Along the same line, yet with a different perspective, this capability of subtly shifting T cell subsets by AhR is exploited pharmacologically. VAF347, a novel pharmacologic ligand of the AhR, induced T-reg cells, and promoted allograft tolerance [61]. The same compound inhibited allergic lung inflammation in an AhR dependent manner as the response was abrogated in B6AhR$^{\Delta 2/\Delta 2}$ mice [47]. Shifts of the Th1/Th2 balance towards Th1 dominance by AhR ligand M50354, associated with GATA-3 expression, renders the AhR pathway as novel pharmacological target for anti-allergic drugs [47]. Research on such ''selective AhR modulators'' is ongoing, and of high interest also in cancer research [62].

4. An extrinsic rather than an intrinsic role of the AhR?

Immune phenotypes of naïve mice and in infection models contributed to the understanding that AhR-over-activation is only one side of the coin, yet the better studied one. AhR over-activation by environmental pollutants is of concern for public health. Insights into AhR biology point to chances for pharmacological manipulation [61,63]. The role of the AhR in the ''untouched'' state appeared unexpectedly more subtle. However, does an ''untouched'' state really exist? Gene expression profiling for CD4 cells from B6AhR$^{\Delta 2/\Delta 2}$ mice showed that in comparison to wild-type mice hundreds of genes are up- or down-regulated if the AhR is absent [48]. The reasons are unclear but likely due to a networking of AhR-regulated genes with other expression pathways. Moreover, bioinformatic AhR analysis of targeted gene promoters (here: transcribed after TCDD exposure) coupled to experimental evidence revealed crosstalk of the AhR with multiple other signalling pathways, such as the NFκB, hypoxia, or the estrogenic pathway [64]. Albeit the AhR can in principle act in the absence of any endogenous ligand, it is likely that a relevant constitutive activation of the AhR signalling pathway exists, e.g., by endogenous or food-derived ligands [65,66]. The extent and control of this is completely unexplored.

A main function of the AhR is the adaptive clearance of a variety of small molecular weight compounds. During phase I of metabolic degradation chemical compounds reactive groups are added (e.g. hydroxyl groups), often rendering them prone to become haptens by covalently binding to proteins. We recently found that Langerhans cells of the skin, which have abundant AhR expression, were inert to up-regulation of xenobiotic metabolizing enzymes by TCDD. Possibly, this represents a protective adaptation of skin antigen presenting cells, minimizing the risk of allergic responses to the many small molecular weight chemicals which come in contact with the skin. However, when the AhR is absent, gene expression patterns changed in B6AhR$^{\Delta 2/\Delta 2}$ Langerhans cells (unpublished observation). The search for endogenous activators for the AhR and the many compounds detected stressed the different physiological roles of the AhR, but leaves many questions open as to the actual balance between intrinsic and extrinsic activation of the AhR [67].

5. Summary

Three null mutants of the AhR allowed tackling the two questions, namely (i) to what extent immunotoxic events after AhR ligand exposure to environmental chemicals are AhR dependent, and (ii) whether and how the AhR plays a role for a

functioning immune system. Some differences in immune phenotype were noted in the null mutant mice (such as splenocyte numbers at certain ages), but these differences reported early after generation of the mice can be explained by mixed genetic background, non-standardized animal husbandry, or observation parameters. Only one study directly compared immune responses between two strains, and found no differences. Unfortunately, no direct comparative data were generated for the congenic strains. Surprisingly, at first glance the immune phenotypes in AhR null mice were much less pronounced than expected from the sensitivity of mice towards extrinsic AhR over-activation with environmental pollutants. AhR-deficiency was not devastating for immune system homeostasis, organ architecture or even response to strong immunogens. However, when looking closer, the null mutants revealed a role for the AhR as an important co-factor especially in the ongoing immune response.

6. Conclusion and outlook

In conclusion, the AhR seems particularly relevant for the differentiation and balance of T cell subsets in ongoing immune responses, and for the decision of the immune system to tolerate or fight antigens. The AhR thus links the immune response to environmental factors, and may help control the risk of developing adverse immune reactions.

Further research will have to focus on the role of individual ligands in shaping these responses, elucidating the environmental risks for autoimmunity, allergy, cancer, and how the AhR pathway can be exploited pharmacologically. Eventually, also the role of the AhR in the aging immune system with its lifelong experience of environmental insults – including AhR ligands in environment and food – will be of high interest.

Acknowledgements

I thank Drs. Heike Weighardt, Bettina Jux, and Nancy Kerkvliet for critical reading of the manuscript. The work in my laboratory is supported through grants of the Bundesministerium für Umwelt, the Deutsche José Carreras Stiftung für Leukämieforschung, and the Deutsche Forschungsgemeinschaft (GRK1427).

REFERENCES

[1] Schmidt JV, Bradfield CA. Ah receptor signaling pathways. Annu Rev Cell Dev Biol 1996;12:55–89.
[2] Sun YV, Boverhof DR, Burgoon LD, Fielden MR, Zacharewski TR. Comparative analysis of dioxin response elements in human, mouse and rat genomic sequences. Nucleic Acids Res 2004;32:4512–23.
[3] Frericks M, Meissner M, Esser C. Microarray analysis of the AHR system: tissue-specific flexibility in signal and target genes. Toxicol Appl Pharmacol 2007;220:320–32.
[4] Poland A, Glover E, Kende AS. Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by hepatic cytosol. Evidence that the binding species is receptor for induction of aryl hydrocarbon hydroxylase. J Biol Chem 1976;251:4936–46.
[5] Kerkvliet NI. Recent advances in understanding the mechanisms of TCDD immunotoxicity. Int Immunopharmacol 2002;2:277–91.
[6] Barouki R, Coumoul X, Fernandez-Salguero PM. The aryl hydrocarbon receptor, more than a xenobiotic-interacting protein. FEBS Lett 2007;581:3608–15.
[7] Ohtake F, Baba A, Fujii-Kuriyama Y, Kato S. Intrinsic AhR function underlies cross-talk of dioxins with sex hormone signalings. Biochem Biophys Res Commun 2008;370:541–6.
[8] Thatcher TH, Maggirwar SB, Baglole CJ, Lakatos HF, Gasiewicz TA, Phipps RP, et al. Aryl hydrocarbon receptor-deficient mice develop heightened inflammatory responses to cigarette smoke and endotoxin associated with rapid loss of the nuclear factor-kappaB component RelB. Am J Pathol 2007;170:855–64.
[9] Oesch-Bartlomowicz B, Huelster A, Wiss O, Antoniou-Lipfert P, Dietrich C, Arand M, et al. Aryl hydrocarbon receptor activation by cAMP vs. dioxin: divergent signaling pathways. Proc Natl Acad Sci USA 2005;102:9218–23.
[10] Nebert DW, Roe AL, Dieter MZ, Solis WA, Yang Y, Dalton TP. Role of the aromatic hydrocarbon receptor and [Ah] gene battery in the oxidative stress response, cell cycle control, and apoptosis. Biochem Pharmacol 2000;59:65–85.
[11] Weiss C, Faust D, Schreck I, Ruff A, Farwerck T, Melenberg A, et al. TCDD deregulates contact inhibition in rat liver oval cells via Ah receptor, JunD and cyclin A. Oncogene 2008;27:2198–207.
[12] Denison MS, Nagy SR. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. Annu Rev Pharmacol Toxicol 2003;43:309–34.
[13] Kronenberg S, Esser C, Carlberg C. An aryl hydrocarbon receptor conformation acts as the functional core of nuclear dioxin signaling. Nucleic Acids Res 2000;28:2286–91.
[14] Backlund M, Ingelman-Sundberg M. Different structural requirements of the ligand binding domain of the aryl hydrocarbon receptor for high- and low-affinity ligand binding and receptor activation. Mol Pharmacol 2004;65:416–25.
[15] Henry EC, Bemis JC, Henry O, Kende AS, Gasiewicz TA. A potential endogenous ligand for the aryl hydrocarbon receptor has potent agonist activity in vitro and in vivo. Arch Biochem Biophys 2006;450:67–77.
[16] Zhang S, Rowlands C, Safe S. Ligand-dependent interactions of the Ah receptor with coactivators in a mammalian two-hybrid assay. Toxicol Appl Pharmacol 2008;227:196–206.
[17] Fritsche E, Schäfer C, Calles C, Bernsmann T, Bernshausen T, Wurm M, et al. Lightening up the UV response by identification of the arylhydrocarbon receptor as a cytoplasmatic target for ultraviolet B radiation. Proc Natl Acad Sci USA 2007;104:8851–6.
[18] Rannug A, Rannug U, Rosenkranz HS, Winqvist L, Westerholm R, Agurell E, et al. Certain photooxidized derivatives of tryptophan bind with very high affinity to the Ah receptor and are likely to be endogenous signal substances. J Biol Chem 1987;262:15422–7.
[19] Chiaro CR, Morales JL, Prabhu KS, Perdew GH. Leukotriene A4 metabolites are endogenous ligands for the Ah receptor. Biochemistry 2008.
[20] Savouret JF, Antenos M, Quesne M, Xu J, Milgrom E, Casper RF. 7-ketocholesterol is an endogenous modulator for the arylhydrocarbon receptor. J Biol Chem 2001;276:3054–9.
[21] Adachi J, Mori Y, Matsui S, Takigami H, Fujino J, Kitagawa H, et al. Indirubin and indigo are potent aryl hydrocarbon

receptor ligands present in human urine. J Biol Chem 2001;276:31475–8.
[22] Phelan D, Winter GM, Rogers WJ, Lam JC, Denison MS. Activation of the Ah receptor signal transduction pathway by bilirubin and biliverdin. Arch Biochem Biophys 1998;357:155–63.
[23] Fernandez-Salguero P, Pineau T, Hilbert DM, McPhail T, Lee SS, Kimura S, et al. Immune system impairment and hepatic fibrosis in mice lacking the dioxin-binding Ah receptor. Science 1995;268:722–6.
[24] Mimura J, Yamashita K, Nakamura K, Morita M, Takagi TN, Nakao K, et al. Loss of teratogenic response to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in mice lacking the Ah (dioxin) receptor. Genes Cells 1997;2:645–54.
[25] Schmidt JV, Su GH, Reddy JK, Simon MC, Bradfield CA. Characterization of a murine Ahr null allele: involvement of the Ah receptor in hepatic growth and development. Proc Natl Acad Sci USA 1996;93:6731–6.
[26] Lahvis GP, Bradfield CA. Ahr null alleles: distinctive or different? Biochem Pharmacol 1998;56:781–7.
[27] Esser C. The arylhydrocarbon receptor: more than a tox story. Biol Chem 2006;387:1147.
[28] Hundeiker C, Pineau T, Cassar G, Betensky RA, Gleichmann E, Esser C. Thymocyte development in Ah-receptor-deficient mice is refractory to TCDD-inducible changes. Int J Immunopharmacol 1999;21:841–59.
[29] Fernandez-Salguero PM, Ward JM, Sundberg JP, Gonzalez FJ. Lesions of aryl-hydrocarbon receptor-deficient mice. Vet Pathol 1997;34:605–14.
[30] Camacho IA, Singh N, Hegde VL, Nagarkatti M, Nagarkatti PS. Treatment of mice with 2,3,7,8-tetrachlorodibenzo-p-dioxin leads to aryl hydrocarbon receptor-dependent nuclear translocation of NF-kappaB and expression of Fas ligand in thymic stromal cells and consequent apoptosis in T cells. J Immunol 2005;175:90–103.
[31] Lahvis GP, Bradfield CA. Ahr null alleles: distinctive or different? Biochem. Pharmacology 1998;56:781–7.
[32] Staples JE, Murante FG, Fiore NC, Gasiewicz TA, Silverstone AE. Thymic alterations induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin are strictly dependent on aryl hydrocarbon receptor activation in hemopoietic cells. J Immunol 1998;160:3844–54.
[33] Funatake CJ, Marshall NB, Steppan LB, Mourich DV, Kerkvliet NI. Cutting edge: activation of the aryl hydrocarbon receptor by 2,3,7,8-tetrachlorodibenzo-p-dioxin generates a population of CD4+ CD25+ cells with characteristics of regulatory T cells. J Immunol 2005;175:4184–8.
[34] Kerkvliet NI, Shepherd DM, Baecher-Steppan L. T lymphocytes are direct, aryl hydrocarbon receptor (AhR)-dependent targets of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD): AhR expression in both CD4+ and CD8+ T cells is necessary for full suppression of a cytotoxic T lymphocyte response by TCDD. Toxicol Appl Pharmacol 2002;185:146–52.
[35] Ward JM, Anver MR, Haines DC, Melhorn JM, Gorelick P, Yan L, et al. Inflammatory large bowel disease in immunodeficient mice naturally infected with Helicobacter hepaticus. Lab Anim Sci 1996;46:15–20.
[36] Vorderstrasse BA, Steppan LB, Silverstone AE, Kerkvliet NI. Aryl hydrocarbon receptor-deficient mice generate normal immune responses to model antigens and are resistant to TCDD-induced immune suppression. Toxicol Appl Pharmacol 2001;171:157–64.
[37] Rodriguez-Sosa M, Elizondo G, Lopez-Duran RM, Rivera I, Gonzalez FJ, Vega L. Over-production of IFN-gamma and IL-12 in AhR-null mice. FEBS Lett 2005;579:6403–10.
[38] Tonn T, Esser C, Schneider EM, Steinmann-Steiner-Haldenstätt W, Gleichmann E. Persistence of decreased T-helper cell function in industrial workers 20 years after exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ Health Perspect 1996;104:422–6.
[39] Weisglas-Kuperus N, Patandin S, Berbers GA, Sas TC, Mulder PG, Sauer PJ, et al. Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. Environ Health Perspect 2000;108:1203–7.
[40] Baccarelli A, Mocarelli P, Patterson Jr DG, Bonzini M, Pesatori AC, Caporaso N, et al. Immunologic effects of dioxin: new results from Seveso and comparison with other studies. Environ Health Perspect 2002;110:1169–73.
[41] Valet G, Esser C. Data sieving analysis as a novel method to asses immunotoxic exposure to dioxins retrospectively. Int Immunopharmacol 2006;6:1374–5.
[42] Laiosa MD, Wyman A, Murante FG, Fiore NC, Staples JE, Gasiewicz TA, et al. Cell proliferation arrest within intrathymic lymphocyte progenitor cells causes thymic atrophy mediated by the aryl hydrocarbon receptor. J Immunol 2003;171:4582–91.
[43] Lai ZW, Pineau T, Esser C. Identification of dioxin-responsive elements (DREs) in the 5' regions of putative dioxin-inducible genes. Chem Biol Interact 1996;100:97–112.
[44] Jeon MS, Esser C. The murine IL-2 promoter contains distal regulatory elements responsive to the Ah receptor, a member of the evolutionarily conserved bHLH-PAS transcription factor family. J Immunol 2000;165:6975–83.
[45] Clark GC, Taylor MJ, Tritscher AM, Lucier GW. Tumor necrosis factor involvement in 2,3,7,8-tetrachlorodibenzo-p-dioxin-mediated endotoxin hypersensitivity in C57BL/6J mice congenic at the Ah locus. Toxicol Appl Pharmacol 1991;111:422–31.
[46] Negishi T, Kato Y, Ooneda O, Mimura J, Takada T, Mochizuki H, et al. Effects of aryl hydrocarbon receptor signaling on the modulation of TH1/TH2 balance. J Immunol 2005;175:7348–56.
[47] Lawrence BP, Denison MS, Novak H, Vorderstrasse BA, Harrer N, Neruda W, et al. Activation of the aryl hydrocarbon receptor is essential for mediating the anti-inflammatory effects of a novel low molecular weight compound. Blood 2008;112:1158–65.
[48] Frericks M, Temchura VV, Majora M, Stutte S, Esser C. Transcriptional signatures of immune cells in aryl hydrocarbon receptor (AHR)-proficient and AHR-deficient mice. Biol Chem 2006;387:1219–26.
[49] Wu X, Hou W, Sun S, Bi E, Wang Y, Shi M, et al. Novel function of IFN-gamma: negative regulation of dendritic cell migration and T cell priming. J Immunol 2006;177:934–43.
[50] Debes GF, Dahl ME, Mahiny AJ, Bonhagen K, Campbell DJ, Siegmund K, et al. Chemotactic responses of IL-4-, IL-10-, and IFN-gamma-producing CD4+ T cells depend on tissue origin and microbial stimulus. J Immunol 2006;176:557–66.
[51] Vorderstrasse BA, Kerkvliet NI. 2,3,7,8-Tetrachlorodibenzo-p-dioxin affects the number and function of murine splenic dendritic cells and their expression of accessory molecules. Toxicol Appl Pharmacol 2001;171:117–25.
[52] Ma J, Wang JH, Guo YJ, Sy MS, Bigby M. In vivo treatment with anti-ICAM-1 and anti-LFA-1 antibodies inhibits contact sensitization-induced migration of epidermal Langerhans cells to regional lymph nodes. Cell Immunol 1994;158:389–99.
[53] Lawrence BP, Vorderstrasse BA. Activation of the aryl hydrocarbon receptor diminishes the memory response to homotypic influenza virus infection but does not impair host resistance. Toxicol Sci 2004;79:304–14.
[54] Neff-LaFord HD, Vorderstrasse BA, Lawrence BP. Fewer CTL, not enhanced NK cells, are sufficient for viral clearance from the lungs of immunocompromised mice. Cell Immunol 2003;226:54–64.

[55] Vorderstrasse BA, Lawrence BP. Protection against lethal challenge with Streptococcus pneumoniae is conferred by aryl hydrocarbon receptor activation but is not associated with an enhanced inflammatory response. Infect Immun 2006;74:5679–86.
[56] Shi LZ, Faith NG, Nakayama Y, Suresh M, Steinberg H, Czuprynski CJ. The aryl hydrocarbon receptor is required for optimal resistance to *Listeria monocytogenes* infection in mice. J Immunol 2007;179:6952–62.
[57] Richter-Hintz D, Thier R, Steinwachs S, Kronenberg S, Fritsche E, Sachs B, et al. Allelic variants of drug metabolizing enzymes as risk factors in psoriasis. J Invest Dermatol 2003;120:765–70.
[58] Veldhoen M, Hirota K, Westendorf AM, Buer J, Dumoutier L, Renauld JC, et al. The aryl hydrocarbon receptor links TH17-cell-mediated autoimmunity to environmental toxins. Nature 2008;453:106–9.
[59] Kimura A, Naka T, Nohara K, Fujii-Kuriyama Y, Kishimoto T. Aryl hydrocarbon receptor regulates Stat1 activation and participates in the development of Th17 cells. Proc Natl Acad Sci USA 2008.
[60] Quintana FJ, Basso AS, Iglesias AH, Korn T, Farez MF, Bettelli E, et al. Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. Nature 2008;453:65–71.
[61] Hauben E, Gregori S, Draghici E, Migliavacca B, Olivieri S, Woisetschläger M, et al. Activation of the aryl hydrocarbon receptor promotes allograft specific tolerance through direct- and DC-mediated effects on regulatory T cells. Blood 2008.
[62] Safe S, McDougal A. Mechanism of action and development of selective aryl hydrocarbon receptor modulators for treatment of hormone-dependent cancers (review). Int J Oncol 2002;20:1123–8.
[63] Selgrade MK. Immunotoxicity: the risk is real. Toxicol Sci 2007;100:328–32.
[64] Frericks M, Burgoon LD, Zacharewski TR, Esser C. Promoter analysis of TCDD-inducible genes in a thymic epithelial cell line indicates the potential for cell-specific transcription factor crosstalk in the AhR response. Toxicol Appl Pharmacol 2008.
[65] McMillan BJ, Bradfield CA. The aryl hydrocarbon receptor sans xenobiotics: endogenous function in genetic model systems. Mol Pharmacol 2007;72:487–98.
[66] Ito S, Chen C, Satoh J, Yim S, Gonzalez FJ. Dietary phytochemicals regulate whole-body CYP1A1 expression through an arylhydrocarbon receptor nuclear translocator-dependent system in gut. J Clin Invest 2007;117:1940–50.
[67] Nguyen LP, Bradfield CA. The search for endogenous activators of the aryl hydrocarbon receptor. Chem Res Toxicol 2008;21:102–16.
[68] Walisser JA, Bunger MK, Glover E, Bradfield CA. Gestational exposure of Ahr and Arnt hypomorphs to dioxin rescues vascular development. Proc Natl Acad Sci USA 2004;101:16677–82.
[69] Walisser JA, Glover E, Pande K, Liss AL, Bradfield CA. Aryl hydrocarbon receptor-dependent liver development and hepatotoxicity are mediated by different cell types. Proc Natl Acad Sci USA 2005;102:17858–63.
[70] Bunger MK, Moran SM, Glover E, Thomae TL, Lahvis GP, Lin BC, et al. Resistance to 2,3,7,8-tetrachlorodibenzo-p-dioxin toxicity and abnormal liver development in mice carrying a mutation in the nuclear localization sequence of the aryl hydrocarbon receptor. J Biol Chem 2003;278:17767–74.
[71] Andersson P, McGuire J, Rubio C, Gradin K, Whitelaw ML, Pettersson S, et al. A constitutively active dioxin/aryl hydrocarbon receptor induces stomach tumors. Proc Natl Acad Sci USA 2002;99:9990–5.
[72] Nohara K, Pan X, Tsukumo S, Hida A, Ito T, Nagai H, et al. Constitutively active aryl hydrocarbon receptor expressed specifically in T-lineage cells causes thymus involution and suppresses the immunization-induced increase in splenocytes. J Immunol 2005;174:2770–7.
[73] Tauchi M, Hida A, Negishi T, Katsuoka F, Noda S, Mimura J, et al. Constitutive expression of aryl hydrocarbon receptor in keratinocytes causes inflammatory skin lesions. Mol Cell Biol 2005;25:9360–8.
[74] Moriguchi T, Motohashi H, Hosoya T, Nakajima O, Takahashi S, Ohsako S, et al. Distinct response to dioxin in an arylhydrocarbon receptor (AHR)-humanized mouse. Proc Natl Acad Sci USA 2003;100:5652–7.
[75] Maltepe E, Schmidt JV, Baunoch D, Bradfield CA, Simon MC. Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. Nature 1997;386:403–7.
[76] Tomita S, Sinal CJ, Yim SH, Gonzalez FJ. Conditional disruption of the aryl hydrocarbon receptor nuclear translocator (Arnt) gene leads to loss of target gene induction by the aryl hydrocarbon receptor and hypoxia-inducible factor 1alpha. Mol Endocrinol 2000;14:1674–81.
[77] Hosoya T, Oda Y, Takahashi S, Morita M, Kawauchi S, Ema M, et al. Defective development of secretory neurones in the hypothalamus of Arnt2-knockout mice. Genes Cells 2001;6:361–74.
[78] Keith B, Adelman DM, Simon MC. Targeted mutation of the murine arylhydrocarbon receptor nuclear translocator 2 (Arnt2) gene reveals partial redundancy with Arnt. Proc Natl Acad Sci USA 2001;98:6692–7.
[79] Hosoya T, Harada N, Mimura J, Motohashi H, Takahashi S, Nakajima O, et al. Inducibility of cytochrome P450 1A1 and chemical carcinogenesis by benzo[a]pyrene in AhR repressor-deficient mice. Biochem Biophys Res Commun 2008;365:562–7.
[80] Thomas PE, Hutton JJ, Taylor BA. Genetic relationship between aryl hydrocarbon hydroxylase inducibility and chemical carcinogen induced skin ulceration in mice. Genetics 1973;74:655–9.
[81] Nebert DW, Goujon FM, Gielen JE. Aryl hydrocarbon hydroxylase induction by polycyclic hydrocarbons: simple autosomal dominant trait in the mouse. Nat New Biol 1972;236:107–10.
[82] Curran CP, Miller KA, Dalton TP, Vorhees CV, Miller ML, Shertzer HG, et al. Genetic differences in lethality of newborn mice treated in utero with coplanar versus non-coplanar hexabromobiphenyl. Toxicol Sci 2006;89:454–64.