Review

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Autoimmunity and the environment

The aryl hydrocarbon receptor in immunity

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Low-molecular-weight chemicals or xenobiotics might contribute to the increasing prevalence of allergies and autoimmunity. Certain chemicals can alter immune responses via their action on the cytosolic transcription factor aryl hydrocarbon receptor (AhR). AhR recognizes numerous small xenobiotic and natural molecules, such as dioxin and the tryptophan photoproduct 6-formylindolo[3,2-*b*]carbazole. Although AhR is best known for mediating dioxin toxicity, knockout studies have indicated that AhR also plays a role in normal physiology, including certain immune responses. In particular, Th17 cells and dendritic cells express high levels of AhR. We review here current evidence for the physiological role of AhR in the immune system, focussing in particular on T-cell biology.

Introduction

The incidence of autoimmune and allergic diseases in the developed world has been increasing over the last few decades and a growing body of evidence indicates that chemicals of low molecular weight (<1000 Da) are an important contributor to this phenomenon. Polycyclic aromatic compounds, which are formed during the combustion of organic materials and are therefore found in cigarette smoke, wood smoke and automobile exhaust gases, are increasingly linked to immune-related diseases. Such low-molecular-weight chemicals are also ubiquitous as food components, in life-style products (e.g. cosmetics) and as environmental pollutants. They can form protein adducts and haptenize antigens, expose cryptic antigens and act as endocrine disruptors. The immune system in turn responds to neo-antigens generated by small chemicals. Small chemicals can also take part in normal immunological differentiation and signaling pathways, with glucocorticoids a well-known example. Immunotoxicology occurs via adverse interference by small chemical xenobiotics with the immune system, such as allergic reactions to urushiol (the active ingredient of poison ivy), drug-induced autoimmunity, or immunosuppression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (referred to as dioxin hereafter). Moreover, immunopharmacologists are interested in small chemicals in their search for immunomodulating compounds that could be used to mimic or specifically block immune functions.

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The transcription factor aryl hydrocarbon receptor (AhR) is a cytosolic sensor of small synthetic compounds (called xenobiotics by toxicologists) and natural chemicals, which act as its ligands (also called agonists). Ligand binding induces a conformational change in AhR, thereby exposing a nuclear translocation site. Ligands can be of diverse chemical structure and need to meet only minimal requirements for size and planar shape. AhR is chaperoned by heat shock protein 90, p23 and AhR-interacting protein in the cytosol. Both allele and species differences account for the range of AhR ligand affinities, which can differ by orders of magnitudes (Box 1). AhR was first discovered as a mediator of dioxin toxicity, including immunotoxicity. Persistent triggering of AhR results in other pathological effects in both animals and humans [1,2].

AhR is highly conserved in evolution and is present in many cell types, albeit at differing abundance [3–5]. The selective forces that led to the high degree of conservation of the AhR amino acid sequence are unknown and its physiological function(s) are still being elucidated. At present, it is clear that AhR has a dual role as an activator of metabolism of small molecules and as a player in many cell functions, including the immune system. As discussed below, AhR might link adaptive immune responses to environmental factors. AhR null mutant mice, mice with a constitutively active AhR and several other mutants of the AhR signaling pathway have been generated and used to analyze the physiological function of AhR, including its role in the developing immune system [6]. In parallel, endogenous and/or exogenous natural AhR ligands – long enigmatic – have been discovered, providing important insights into the physiological functions of AhR. In this review we consider AhR signaling, the endogenous low-molecular-weight chemicals that trigger it and its effects on natural immunological function(s). In particular we focus on the role of AhR in T-cell biology and its suggested link to autoimmune diseases.

Brief overview of the biochemistry of AhR signaling

AhR is a member of the bHLH-PAS protein family found in organisms as diverse as *Caenorhabditis elegans* (nematode), *Drosophila melanogaster* (insect) and mammals [3]. bHLH-PAS proteins are biological sensors for a variety of stimuli, controlling neurogenesis, vascularization, circadian rhythms, metabolism and stress responses to hypoxia, among others.

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Box 1.

Biochemistry of AhR signaling

AhR is a transcription factor that resides in the cytosol and is a member of the evolutionarily ancient bHLH-PAS protein family. AhR undergoes conformational change on binding of small ligands (also called agonists), which exposes a nuclear translocation site. AhR translocates to the nucleus and binds to its dimerization partner ARNT (another bHLH-PAS protein) and the AhR-ARNT complex initiates transcription of genes with promoters containing a dioxin-responsive element (DRE) consensus sequence. Ligands only need to meet minimal requirements for size and planar shape to fit into the AhR binding pocket. Consequently, a broad range of low-molecular-weight chemicals can activate AhR, albeit at different affinities ranging between 10^{-12} and 10^{-3} M. Many ligands have two carbon ring systems, such as tryptophan derivatives, flavonoids and biphenyls. The AhR system is genetically polymorphic and different alleles influence responsiveness to AhR ligands. Inbred mouse strains are classified into high- and low-responder strains on the basis of their ability to induce AhR-dependent cytochrome P450 1A1 expression. For instance, the C57BL/6 strain carries the AhR^b high-responder allele, whereas DBA/2, NOD, and 129 mice have the low-responder AhR^d allele. Experiments in cell lines have demonstrated that human AhR has at least 10-fold lower dioxin sensitivity than the known murine strains.

During canonical signaling, cytosolic AhR binds to a suitable small chemical (the ligand), which facilitates AhR translocation to the nucleus and eventually results in *de novo* transcription of target genes. The promoters of AhR target genes have the responsive element 5′-TNGCGTG-3′, termed DRE or XRE for dioxin or xenobiotic responsive elements. The genes for xenobiotic-metabolizing enzymes (e.g. cytochrome P450) are well-known targets of AhR and are often called AhR battery genes. Hundreds of other genes also have DREs [7]. Elucidation of the biochemistry of canonical AhR signaling revealed several parameters that can fine-tune AhR activity. These include ligand characteristics, adapter molecules and transcriptional co-activators or co-repressors that regulate the extraordinary cell-specific activity of AhR [8,9].

In recent years a alternative pathway of AhR signaling has also been demonstrated. For instance, AhR can bind to retinoblastoma protein, estrogen receptor (ER), the transcription factor E2F1 and to the NFκB pathway subunits RelA and RelB [10–13]. Evidence of AhR cross-talk with other signaling pathways, such as via kinases (src, JNK, p38, MAPK) or competition for transcription cofactors, has also been reported. AhR can act as a ubiquitin ligase, targeting the ER for proteasomal degradation [8,14,15]. In these signaling pathways, AhR and the other proteins sometimes mutually repress each other's function. Indeed, bioinformatics analysis points to the existence of complex signal cross-talk between AhR and further transcription factors or transcription co-activators [8,14,16]. The downstream targets of these pathways differ; for example, IL-2 can be induced via canonical signaling [17] and IL-8 via RelB [18], but the full biological complexity has yet to be elucidated.

Exogenous and endogenous ligands critically shape AhR function

Dioxin is widely used as a surrogate ligand for AhR but its use is problematic in revealing the true function of AhR because it is not quickly metabolized in the body (its half-life is ~2 weeks in mice and several years in adult humans [19,20]). Indeed, a proper understanding of AhR biology must differentiate between effects triggered by toxic ligands such as dioxin and physiological effects triggered by endogenous ligands. Simple extrapolation of data obtained for one ligand to all others would not correctly reflect what is currently known about AhR biology. At the very least, the dose-dependent and cell-specific potential of toxic ligands to kill cells must be considered.

Although it is possible that AhR might function in the absence of a ligand (i.e. spontaneously), overwhelming evidence suggests that ligands are required [21]. A number of low-molecular-weight chemicals qualify as endogenous or physiological AhR ligands (which is not necessarily the same), that is, they have binding dissociation constants (K_d) and effective concentrations at the level expected for a physiologically relevant receptor ligand [22,23]. Physical fluid shear stress (which causes oxidation of low-density lipoproteins), the second messengers cAMP and Ca^{2+}, serum and growth medium components all activate AhR responses [14,24,25]. This list of ligands is impressive and striking in its diversity; however, it remains to be determined which of these are actually relevant in a physiological or immunotoxic sense. AhR has not yet been crystallized, so information on ligand-dependent structural changes is currently lacking. Ligand-protected protease digestion studies indicated that only one binding pocket for ligands exists [26]. Because different ligands result in different outcomes [23], it is generally assumed that the AhR signaling pathway uses ligands in an adaptive fashion, depending on the tissue and situation.

A tryptophan photoproduct is a high-affinity AhR ligand

Two tryptophan-derived molecules of an indolo[3,2-*b*]carbazole type were identified in 1987 [27]. They were assumed to be endogenous AhR ligands because they could bind to the receptor with the highest affinity of any known compound, including dioxin. These two compounds were detected in experiments designed to produce oxidation products of adenine for testing as possible AhR ligands. An unfiltered high-pressure mercury UV lamp was used in the oxidation of adenine and tryptophan was added as a photo-sensitizing molecule. This led to the unexpected observation that dilute aqueous solutions containing UV-exposed tryptophan could compete efficiently with ^{3}H-labeled dioxin for AhR binding. Moreover, it was later shown that AhR-mediated expression of cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) was amplified in human, mouse and rat cells exposed to visible or UV light in the presence of tryptophan [28,29]. Intensive studies to characterize the chemically active tryptophan photoproducts identified the compounds 6-formylindolo[3,2-*b*]carbazole (FICZ) and 6,12-diformylindolo[3,2-*b*]carbazole (dFICZ) and a procedure to synthesize these has now been described [30]. To date, FICZ has been detected in aged batches of tryptophan, in light-exposed solutions of tryptophan, in cell culture media and in living cells [31,32]. Recently, sulfate conjugates of the compound FICZ were found to be present in human urine [33]. FICZ-induced autocatabolic breakdown via the cytochrome P450

enzymes CYP1A1, 1A2 and 1B1 is highly efficient and seems to be limited only by the rate of diffusion of the compound [33,34]. The high specificities and catalytic efficiencies observed, which lead to low intracellular steady-state levels of FICZ, support the argument that this molecule is an endogenous AhR ligand of high importance. Dioxin and FICZ are both lipophilic molecules that are similar in size and planarity. They bind to AhR with very high affinity and are efficient inducers of the AhR gene battery, including xenobiotic-metabolizing enzymes (XMEs). The different kinetic patterns of AhR responses are the most important differences between the two compounds because FICZ, in contrast to dioxin, is metabolized by XMEs. This results in efficient autoregulatory feedback and transient activation of AhR.

The formation of a chemical messenger molecule with high AhR binding affinity thus explains cutaneous and extracutaneous induction of CYP1A1 enzyme activity after dermal exposure to UV light [32,35]. It remains to be shown whether UV light mediates enhanced metabolism of polycyclic aromatic hydrocarbons and other environmental pollutants to which humans are exposed; if so, this could play a role in rapid elimination of topical carcinogenic substances [36]. Equally speculative, UV-generated FICZ might also provide an explanation for the photosensitivity observed in lupus erythematosus [37]. In the epidermis, keratinocytes and Langerhans cells (LCs) express AhR and are therefore targets of light-generated FICZ. Surprisingly, in dioxin-treated LCs, canonical AhR signaling is repressed and LCs do not express any of the metabolic enzymes normally induced by AhR activation. However, the biological significance of this is not clear.

AhR and the immune system

Immunotoxicological evidence

In vitro and *in vivo* immunotoxicological studies with dioxin in relation to persistently activated AhR have revealed drastic changes in thymocyte lineage decisions, shifts in immune-cell subset frequencies, aberrant cytokine secretion and many other effects on immune functions (Figure 1). Among the most sensitive outcomes of dioxin exposure in animals is strong systemic immunosuppression of the humoral, cellular and innate immune responses. The cause–effect relationships are largely unclear and the fact that only a few immune cell subsets (see below) actually express AhR (although many seem to be affected by dioxin) suggests that the answers will be very complex. For more details on immunotoxicology, readers are referred to recent reviews [2,38].

Exposure routes and ligands

Environmental exposure to AhR ligands is typically via the barrier organs of the gut, lung and skin, which are also highly active immune sites in their own right. The oral route accounts not only for 90% of human dioxin exposure [39], but also for many other AhR ligands, including normal dietary components such as flavonoids and indoles present in fruit and vegetables. Examples of quantitatively relevant dietary components are quercetin in apples, resveratrol in red wine and indole-3-carbinol in many cruciferous plants [39] (Table 1). Apart from UV-generated FICZ, the skin comes into contact with AhR ligands present in cosmetics and plants, as well as those generated by the common skin-residing yeast *Malassezia furfur*. Dietary ligands are often found in blood and although

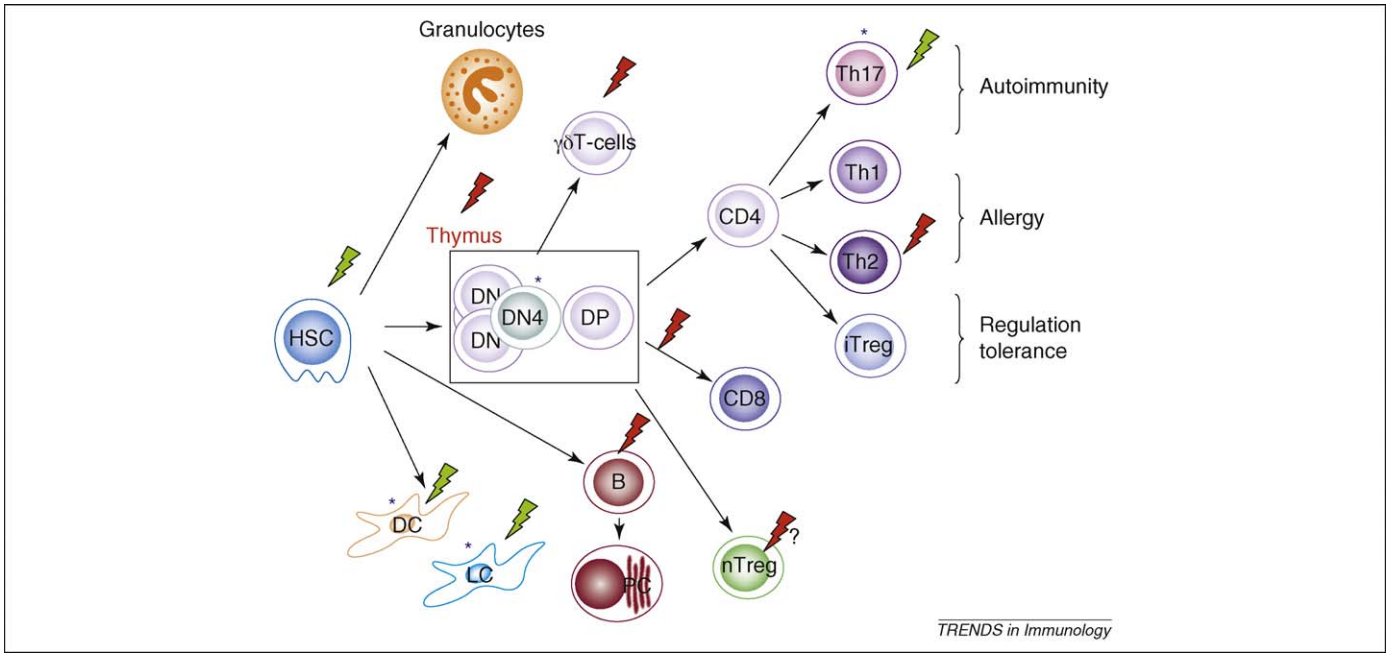

Figure 1. Schematic compilation of current evidence of the influence of AhR on immune function and differentiation. The major lymphoid and myeloid differentiation lines are shown. The red flashes indicate cells where AhR overactivation by *in vivo* or *ex vivo* exposure to dioxin has been reported to result in immunotoxic effects (e.g. biased differentiation, lack of cytokine secretion, or altered function). It is important to note that many of these effects are presumably indirect effects because some of the cells affected express little or no AhR. Blue asterisks indicate cells for which AhR expression has been experimentally confirmed by either RNA measurements or Western blotting. Green flashes indicate evidence of a direct physiological role for AhR (e.g. necessary for cell-specific expression of characteristic genes, involved in maturation or differentiation). DC, dendritic cells; DN, double negative thymocyte; DP, double positive thymocyte; HSC, hematopoietic stem cell; LC, Langerhans cell; nTreg, natural T regulatory cell; PC, plasma cell; iTreg, induced T regulatory cell.

Table 1. Some clinically or quantitatively relevant ligands of AhR[a].

Endogenous
FICZ, 6-formylindolo[3,2-*b*]carbazole (tryptophan photoproduct)
Bilirubin (product of heme metabolism by the liver)
Lipoxin A4 (eicosanoid with anti-inflammatory properties)
ITE [2-(1′*H*-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester] (isolated from lung tissues)
Environmental pollutants (formed during combustion of organic material)
2,3,7,8-tetrachlorodibenzo-*p*-dioxin
Benz[*a*]pyrene
Dietary
Quercetin (present in apples and onions)
Indol-3-carbinol (present in many Brassicaceae, e.g. cabbage)
Resveratrol (present in red wine)
Curcumin (a spice frequently used in Indian cuisine)
Drugs (synthetic)
M50367 {3-[2-(2-phenylethyl) benzoimidazole-4-yl]-3-hydroxypropanoic acid}
VAF347 {[4-(3-chloro-phenyl)-pyrimidin-2-yl]}

[a]For comprehensive lists and a discussion of immunological relevance see Refs [4] and [23].

not rigorously demonstrated for all ligands, it can be expected that most ligands circulate freely in the body, where they can activate AhR. Surprisingly, almost no research has been carried out on the effects of AhR ligands (exogenous or endogenous) on mucosal lymphoid tissue, especially the gut, which conceivably could be different from the effects on peripheral immune cells.

Evidence from AhR-deficient mice

A good understanding of the physiological role of AhR, in particular its role in the immune system, has still not been achieved. Information regarding its physiological role(s), however, has been provided by studies of mouse lines expressing the high- AhR^b versus low-affinity AhR^d alleles, by recombinant mouse models in which AhR has been mutated and by the use of cre-lox conditional knockout mice [6]. AhR knockout mice have lower life expectancy and fecundity and exhibit a spectrum of hepatic defects and skin abnormalities without exposure to exogenous chemicals, suggesting important roles of AhR in many physiological functions [38,40]. For instance, hyperproliferative, fibrotic and inflammatory lesions were one of the original observations made in AhR-deficient mice, in addition to abnormalities in vascular and hematopoietic development [41]. AhR-deficient mice also exhibit liver deformation characterized by a failure to close the ductus venosus (a blood vessel shunt in the liver) after birth, resulting in lifelong impairment of blood flow through the liver [42].

AhR-deficient mice do not have an overt immunological phenotype, although one of the knockouts generated had decreased lymphocyte numbers in the spleen and lymphocyte infiltration of lung, intestine and urinary tract. However, these immunological alterations were not detected in a second, more widely used AhR knockout mouse [43]. The reasons underlying these discrepancies have not yet been resolved but might be related to the general health status of different animal facilities. However, as detailed in a recent review [6], AhR acts as an important co-factor in infections. For instance, AhR-deficient mice infected with *Listeria monocytogenes*, an intracellular bacterium, were more susceptible to infection but developed enhanced resistance to re-infection [44]. Furthermore, AhR-deficient mice have an increased leukocyte count and a diminished capacity to maintain the hematopoietic stem cell compartment [40]. Regarding the skin, primary LCs isolated from mice that lack AhR poorly upregulate CD40 and CD80 after *ex vivo* maturation and lose the capacity to produce tolerogenic indoleamine-2,3-dioxygenase [45]. It is conceivable that this points to a role for AhR in LC maturation and host immune responses.

AhR and interactions with NFκB

With regard to the role of AhR signaling in immune functions, the non-canonical interactions between NFκB, Stat1, Stat5 and the AhR are especially intriguing. NFκB activators such as the inflammatory cytokines IL-1β, IL-6, TNFα and lipopolysaccharide suppress constitutive and AhR-induced CYP1A1, suggesting a link between inflammation, oxidative stress and CYP expression [46]. Conversely, AhR agonists have been shown to repress or transactivate NFκB response genes [18,46]. Further evidence of cross-talk between AhR and other signaling modules has come from studies showing that AhR can be co-immunoprecipitated with Stat1 and Stat5 [47]. Presumably, this interaction is relevant for the abrogation of Stat1 signaling and hence Th17 differentiation [47] (see below).

AhR and T cell subsets

Immune cell-specific AhR expression

Liver and lung exhibit high levels of AhR expression. However, certain hematopoietic stem cells, some dendritic cells, particular subsets of thymocytes and T-cells have similar or even higher levels of AhR expression than the liver [5,48,49]. Immune functions and immune cells can be targeted directly or indirectly by AhR activity. Unfortunately, data on AhR protein levels are not available for many immune cell subpopulations, limiting the interpretation of causative AhR-ligand effects. Recently, studies using cell sorting combined with RT-PCR and Western blotting have identified Lin-Sca^+ and Sca^- progenitor cells in bone marrow (BM), double negative ($CD4^-CD8^-$) DN4 cells in thymus, $CD4^+$ Th17 cells, BM-derived dendritic cells and LCs as subpopulations with high AhR levels [40,45,50]. A careful and wide-ranging re-analysis of highly pure immune cell subsets for AhR expression would be in helpful in untangling the cell-specific actions of AhR signaling.

AhR and T-helper cells, regulatory T-cells and dendritic cells

Considering the wide-ranging immunosuppressive effects of dioxins, some researchers have investigated the potential of AhR ligands to manipulate immune responses. In particular, two compounds with AhR-binding capacity have been studied. VAF347, a water-soluble ligand of AhR, suppressed graft rejection in a mouse transplantation model [51]. The evidence presented so far suggests that shifts in T subset frequencies, as well as effects on dendritic cells, are relevant for these effects [52]. Another ligand, a compound named M50367, has been tested in

models of allergy [53]. The results suggest that inhibition of allergy is due to suppression of Th2 differentiation from naïve T-cells. Although the findings are intriguing, it must be pointed out that neither naïve T-cells nor Th1 or Th2 cells express AhR. Moreover, attempts to manipulate the immune system with compounds that bind to a transcription factor that is also present in many non-immune cells and for which systemic triggering can be highly toxic must be viewed with the utmost caution.

AhR and Th17 cells

A very defined role for AhR in the immune system was revealed by analysis of transcription factors expressed in differentiated CD4 effector T-cell subsets. Th17 cells, a relatively new addition to the group of effector T-cells, express high levels of AhR [50] (Box 2). Although AhR expression was also reported in regulatory T (Treg) cells [54], the levels of expression are extremely low compared with Th17 cells and so far no clear-cut mechanistic role for AhR has been reported in Treg cells. AhR activation results in expansion of Th17 cells, as well as increased cytokine production. Moreover, in a model of experimental autoimmune encephalomyelitis (EAE) AhR activation caused earlier onset of the disease and more severe pathology. Although AhR is not required for Th17 differentiation, its activation in these cells promotes further functional differentiation. This link between a transcription factor that responds to a wide range of environmental pollutants and the Th17 program opens intriguing possibilities regarding the potential of such factors to augment autoimmune conditions that are crucially dependent on Th17 cells, such as EAE (or human multiple sclerosis), rheumatoid arthritis (RA) and myocarditis. Because environmental factors clearly contribute to autoimmune disease manifestation, further studies of AhR involvement should provide important clues to an understanding of disease parameters such as relapse or induction of spinal cord inflammation in multiple sclerosis. Box 3 lists some environmental factors that could be related to AhR activation and autoimmune disease. It should be noted that Th17 cells are not only involved in certain autoimmune diseases, but also play important and beneficial roles in defense against several types of microbial pathogens and are probably involved in maintaining barrier functions of the gut.

Box 2.

Th17 cells and their development

Th17 cells are a new effector T-cell subset in the CD4 lineage. These cells differentiate in response to IL-6 produced by antigen-presenting cells such as dendritic cells and macrophages and to TGF-β, which is produced by many cells types. Antigen-presenting cells might be the most critical factor in the induction of Th17. Co-factors that enhance differentiation are IL-1β, TNFα and IL-21. IL-23 released by dendritic cells and macrophages is an essential component for the maintenance but not the induction of Th17 cells. The transcription factors RORγt and RORα define the Th17 lineage and together with STAT3 are essential for induction of IL-17 production. By contrast, AhR is not required for initial differentiation of Th17 cells but promotes their expansion and is essential for their production of IL-22. Although the discovery of Th17 cells was linked to the etiology of autoimmune diseases such as EAE and RA, their true function is most likely the recruitment and activation of neutrophils and protection against certain microbial pathogens.

Box 3.

Environmental links of AhR to autoimmune diseases

Epidemiology, anecdotal evidence and mechanistic studies suggest links between autoimmune diseases and environmental exposure to small chemicals and/or AhR ligands or xenobiotic-metabolizing enzyme activity. Such links include:

- Dioxin and rheumatoid arthritis [69]
- Smoking and rheumatoid arthritis [70]
- Smoking and psoriasis [72]
- UV light and systemic lupus erythematosus [71]
- Cytochrome P450 RNA levels and multiple sclerosis [73]

AhR-mediated induction of IL-22

In Th17 cells, AhR activation is a prerequisite for the production of the cytokine IL-22. IL-22, a member of the IL-10 family [55], is linked to pro-inflammatory processes such as dermal inflammation, psoriasis, inflammatory bowel disease and Crohn's disease. In addition, IL-22 induces the production of antimicrobial proteins that are needed for defense against pathogens in the skin and gut [56,57]. It also plays a protective role in models of hepatitis, whereby IL-22 neutralization exacerbates pathology and IL-22 delivery prevents it [58,59]. IL-22 is linked to psoriasis and is strongly upregulated in skin lesions from psoriasis patients [60], together with a host of IL-22–IL-17-dependent downstream molecules such as defensins 2 and 3, psoriasin (S100A7), MMP3 and MMP1 [57]. Liang *et al.* reported that IL-22 is induced in Th17 cells by the proinflammatory cytokine IL-23 [61], which is produced by macrophages and dendritic cells. It is clear that IL-23 plays a fundamental role in maintaining the survival and function of Th17 T-cells and recent data suggest that it is needed for terminal effector differentiation of these cells [62]. However, the role of IL-23 in IL-22 production appears to be downstream of AhR because Th17 cells from AhR-deficient mice cannot produce IL-22 despite the presence of IL-23 under inflammatory conditions such as immunization with Freund's complete adjuvant or injection with heat-killed mycobacteria [50]. Interestingly, low-responder DBA/2 mice were resistant to EAE induction [54] and highly susceptible to infection with *Candida albicans* [63], both important features of Th17 responses. Human AhR has at least 10-fold lower sensitivity to dioxin as determined by exposure of various cell lines to dioxin [64], but it remains to be seen whether AhR polymorphisms play a role in various IL-17–IL-22-dependent immune pathologies in humans.

Endogenous AhR ligands affect Th17 differentiation

Although AhR is most widely studied for its effects downstream of dioxin, its evolutionary conservation suggests there must be other, more physiological ligands that drive its functions [22]. An interesting group of such endogenous ligands are metabolites of tryptophan, notably the UV- and light-generated metabolite FICZ described above. Given that tryptophan is an essential amino acid and therefore has to be supplied either by food intake or in culture media, it is clear that investigation of the roles of

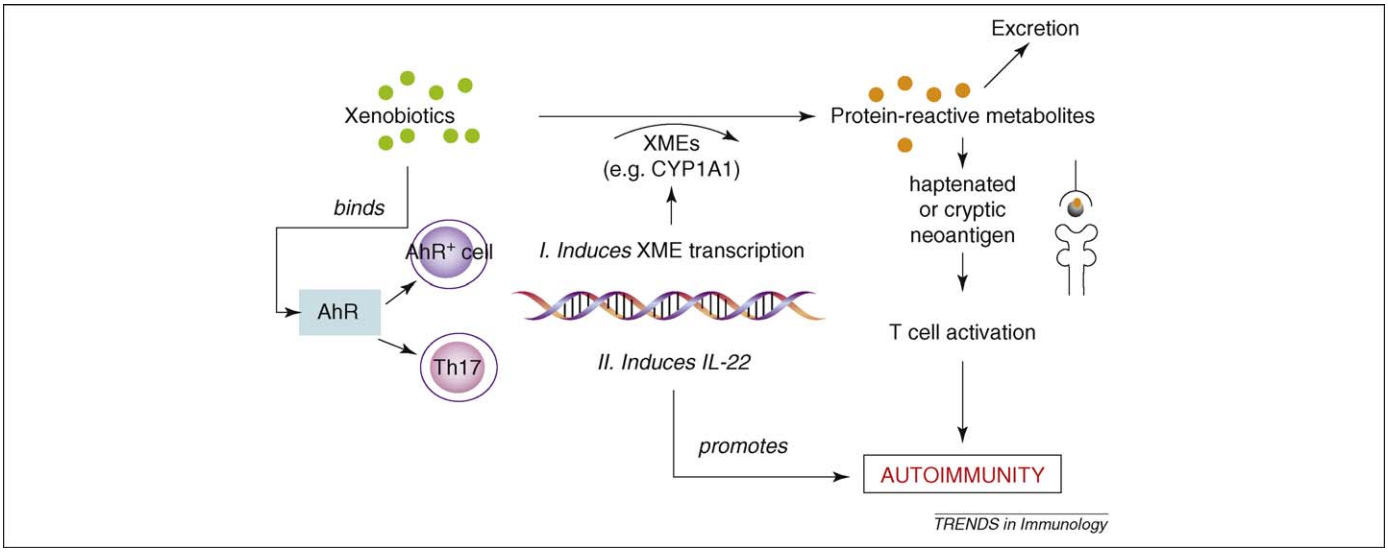

Figure 2. Two pathways by which direct gene induction via AhR could contribute to initiation and/or exacerbation of autoimmune diseases. (I) Low-molecular-weight chemicals and in particular their more reactive metabolites – formation of which is under control of AhR-inducible phase I and phase II metabolizing enzymes – (xenobiotic metabolizing enzymes, XMEs) can form protein adducts, leading to the formation of new self antigens against which no tolerance exists. For instance, the phenotype of slow acetylation by N-acetyltransferase, a phase II enzyme, is more common in patients with drug-induced lupus caused by procainamide or hydralazine [67]. Variant alleles of *CYP1A1*, which code for enzymes with higher activity, might protect against psoriasis [68]. The links between dioxin and rheumatoid arthritis (RA) and between psoriasis and smoking are strong. Smoke contains numerous strong AhR ligands, including benz[*a*]pyrene dioxin and other planar aromatic hydrocarbons [69–71]. (II) AhR can mediate induction of cytokine genes in a cell-specific manner. AhR is necessary for IL-22 secretion by Th17 cells. Where IL-22 is relevant in autoimmune disease, this might exacerbate effects. An example is the skin, where IL-22 involvement in psoriasis is known. However, it should be noted that IL-22 has other functions; in particular, it is essential in protection against infectious agents.

AhR in immune responses has to take into account background levels of endogenous ligands. This is especially true for studies involving C57BL/6 mice, which express a high-affinity version of AhR [65]. Such background activity has been demonstrated in *in vitro* studies using cell lines transfected with reporter constructs for CYP expression [66]. Similarly, we recently showed that culture medium contains AhR ligands that shape the *in vitro* differentiation of Th17 cells [48]. AhR antagonists reduce Th17 differentiation in naïve C57BL/6 CD4 T-cells to levels equivalent to those observed for CD4 T-cells from AhR-deficient mice. Interestingly, standard culture medium such as RPMI with suboptimal AhR ligand content results in poor Th17 differentiation and a failure to generate IL-22 responses from Th17 cells, highlighting the requirement for additional modulating stimuli to drive fully functional Th17 responses [17]. FICZ increases the proportion of Th17 cells and their production of IL-22 by activating the AhR [50], thus demonstrating a role for AhR in mounting an elevated response against infections. However, such functions were not evident in studies with dioxin [54].

AhR and autoimmunity: a critical perspective

Except for drug-induced autoimmune reactions, most autoimmune diseases remain idiopathic. Epidemiology, anecdotal evidence and mechanistic studies have suggested links between autoimmune diseases and environmental exposure to small chemicals and/or AhR ligands or xenobiotic-metabolizing enzyme activity. For instance, links exist between dioxin and rheumatoid arthritis and between smoking and psoriasis. Other examples are given in Box 3. Autoimmunity requires the breakdown of central and/or peripheral tolerance mechanisms. This can have a number of causes, including dysregulated T-cell functions, the cytokine milieu and aberrant antigen-presentation. Figure 2 shows how AhR can be involved in the generation of neo-antigens and the exacerbation of autoimmune diseases for which Th17 cells are the driving force of pathogenesis. Whether and which environmental AhR ligands are involved in the various autoimmune diseases are questions that remain to be answered. A link between AhR ligands and infections can also be envisioned.

Conclusions and future perspectives

AhR, a sensor of small chemical compounds, is abundant in many cells of the immune system. It is involved in the metabolism of these compounds and in regulating cell differentiation, cell cycling and important homeostatic processes. Recent evidence has shown that AhR enhances Th17 differentiation and is essential for induction of IL-22. Because Th17 cells are the driving force for some autoimmune diseases, it is possible that AhR activation exacerbates (rather than induces) Th17-mediated autoimmunity. An important consideration is the involvement of AhR ligands in mucosal immune responses that are driven by IL-22 because this cytokine seems to be critically dependent on AhR activation.

Several challenges remain. First, further epidemiological studies are needed to verify links between potential AhR ligands and autoimmune or allergic diseases in humans. Second, AhR has not been crystallized and it remains unclear what functional consequences different AhR ligands have on the immune system (dietary substances, tryptophan photoproducts, environmental pollutants). Finally, and possibly most complex, future research will need to define more precisely how and to what extent small chemicals and the AhR shape immune responses.

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