Published in final edited form as:

Semin Immunopathol. 2013 November ; 35(6): . doi:10.1007/s00281-013-0391-7.

ROLE OF THE ARYL HYDROCARBON RECEPTOR (AHR) IN LUNG INFLAMMATION¹

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Abstract

Millions of individuals worldwide are afflicted with acute and chronic respiratory diseases, causing temporary and permanent disabilities and even death. Oftentimes, these diseases occur as a result of altered immune responses. The aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, acts as a regulator of mucosal barrier function and may influence immune responsiveness in the lungs through changes in gene expression, cell-cell adhesion, mucin production, and cytokine expression. This review updates the basic immunobiology of the AhR signaling pathway with regards to inflammatory lung diseases such as asthma, chronic obstructive pulmonary disorder, and silicosis following data in rodent models and humans. Finally, we address the therapeutic potential of targeting the in regulating inflammation during acute and chronic respiratory diseases.

Keywords

Lung; inflammation; aryl hydrocarbon receptor; chronic obstructive pulmonary disorder; asthma; silicosis

INTRODUCTION

Pulmonary immunity is a multifaceted process, involving tolerance to self-antigens and innocuous environmental contaminants, while at the same time preserving the capability to appropriately respond to invading pathogens. Injury or damage to the lungs disrupts immune homeostasis by provoking an initial inflammatory response followed by a compensatory anti-inflammatory response—represented by the innate and adaptive arms of the immune system, respectively. Innate defense mechanisms incorporate the epithelial barrier, mucociliary escalator, humoral factors (e.g., antimicrobial peptides, complement proteins, and surfactant proteins) and innate cells such as macrophages, dendritic cells, monocytes, neutrophils, natural killer cells, basophils, eosinophils, innate lymphoid cells, and mast cells. In contrast, adaptive immune responses include T lymphocytes (CD8⁺, Th1, Th2, Th17, Th22, and Treg), B lymphocytes, antibody mediated responses, and granuloma formation. Due to the complexity of respiratory immune responses, the exact contributions of the innate and adaptive arms to specific respiratory diseases remain a subject of debate. It appears possible that the innate and adaptive immune systems may function independently of each

¹This article is published as part of the special issue on “Roles of aryl hydrocarbon receptor in controlling immunity”.

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The authors have no conflicts of interest.

other in some lung diseases and in synergy in others, such that disease heterogeneity in patients may, in part, be explained by the relative contributions of the two systems.

Acute inflammation is a key component of the innate host defense mechanism, yet when unchecked may result in tissue destruction and disease. In contrast, anti-inflammatory cascades suppress the intensity of the innate response, but when unrestrained, contribute to abnormal wound repair and development of fibrotic disease. Therefore, the balance between pro- and anti-inflammatory cells and their associated soluble mediators directly influences disease outcome. Many respiratory diseases are characterized by the abnormal accumulation and persistence of innate and/or adaptive immune cells in the alveoli and parenchyma, suggesting that a failure of normal homeostatic control occurs and contributes to initiation and progression of disease. Consequently, understanding those cellular and molecular events that maintain equilibrium between innate and adaptive immunity will result in more effective diagnosis and treatment of inflammatory and fibrotic lung diseases.

The aryl hydrocarbon receptor (AhR) is highly conserved evolutionarily and is expressed in the majority of immune cell types and human tissues, including the lungs—although at differing levels (1–7). The AhR is a ligand-dependent basic helix-loop-helix transcription factor that has dual roles in xenobiotic metabolism and as a participant in normal homeostasis, organogenesis, and immune activation (1, 8–11). The prototypic AhR ligand is the toxicant, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin), although numerous naturally occurring exogenous and endogenous AhR ligands have been identified (10). These low molecular weight, structurally diverse chemicals, include indoles, tetrapyroles, arachidonic acid metabolites, and tryptophan metabolites such as FICZ (6-formylindolo[3,2-b]carbazole), ITE (2-(1*H*-Indol-3-ylcarbonyl)-4-thiazol carboxylic acid methyl ester), indirubin, I₃C (Indole-3-carbinole), and BaP (benzo(a)pyrene) (6, 12–15). Of these, ITE was isolated from the porcine lung (16) and found to suppress B cell differentiation into Ig-secreting plasma cells (17). Although the ability of the AhR to bind a variety of ligands is of great interest, it remains to be seen which of these ligands are actually relevant in a physiological sense.

AhR ligands enter the cell in a poorly characterized manner and, after binding to the AhR, induce the activation of various signaling cascades (6–10, 14, 18). In the cytosol, the AhR is chaperoned by heat shock protein 90, p23, and AhR-interacting protein (AIP) (8–10, 19). After ligand binding, the AhR dissociates from Hsp90 and p23 (9, 14, 19–21), translocates from the cytosol to the nucleus, heterodimerizes with the AhR nuclear translocator, and binds to an enhancer sequence, referred to as dioxin response elements (DREs), within an array of target genes encoding for phase I and phase II metabolizing enzymes (e.g. CYP1A1), as well as the AhR repressor protein (AhRR). The AhRR competes with AhR for binding to ARNT, and this AhRR/ARNT heterodimer binds to DRE leading to repression of transcription. Finally, after ligand-binding nuclear translocation-transcriptional activation, AhR is rapidly degraded (6–10, 14, 18, 20–24). Many genes that participate in immune responses have DRE sequences in their promoters and are responsive to AhR ligands (25, 26). Activation of the AhR by TCDD results in a range of endpoints, including profound immunosuppression, intense alterations in thymocyte lineage, shifts in immune-cell subset frequencies, and aberrant cytokine secretion (3, 27, 28).

Over the last decade, the AhR has emerged as a critical regulator of the immune system (Figure 1). The majority of immune cells constitutively or inducibly express the AhR (15, 25). It is expressed in cells involved in innate immune responses, and in cells crucial for adaptive immunity. Antigen-presenting cells constitutively express the AhR and AhR activation by TCDD, as well as dietary ligands, suppressed immune and inflammatory responses in DCs (10, 23, 29–31). Mast cells also constitutively express AhR, and activation

with the high affinity ligand, FICZ, influences both Fc RI-mediated early degranulation response (exacerbation) and Fc RI-independent IL-6 and IL-17 cytokine production (inhibition) (32, 33). Similarly, the AhR is necessary for the development and function of ROR γ t^+ innate lymphoid cells, such that adult AhR-deficient mice have reduced numbers of innate lymphoid cells and are more susceptible to gastrointestinal infections (34). Moreover, the AhR markedly participates in Th cell differentiation, particularly with regards to FoxP3⁺ Tregs and IL-10 producing Tr1 cells (10, 11, 35), and Th17 and Th22 cells (10, 23). Deficiency in AhR or treatment with AhR antagonists impairs but does not ablate Th17 differentiation (36, 37). Depending on the AhR ligands utilized, the context of the extracellular and intracellular environments, and the model of pathology considered, AhR activation might either worsen or ameliorate disease (10, 38). Therefore, AhR biology is context and disease dependent, and future studies must differentiate between the dose- and cell-specific potential triggered by toxic ligands and the physiological effects triggered by endogenous ligands.

The recent identification of the AhR as a master regulator of mucosal barrier function, and the significance of AhR-mediated responses following activation by endogenous and exogenous ligands suggests that the respiratory system is sensitive to alterations in AhR expression or function (7, 39). Using both gene deficient mice and administration of AhR agonists and antagonists, investigators have demonstrated that the AhR modulates the immune response in various respiratory diseases, and shown that the lung is sensitive to AhR ligands (6, 7, 39–47). Therefore, we have organized this review relative to the relationship between a select group of inflammatory lung diseases (asthma, chronic obstructive pulmonary disorder, and silicosis) and the AhR signaling pathway. Moreover, we present current information on AhR ligands (e.g. dietary substances, tryptophan photoproducts, and environmental pollutants) relative to their potential use both as tools for understanding the role of the AhR in inflammatory lung diseases and as potential therapeutics for the treatment of various inflammatory lung diseases.

ASTHMA

Allergic asthma is a heterogeneous and multi-factorial disease that has been increasing in prevalence and severity worldwide. Current estimates indicate that ~8.2% of the U.S. population has asthma (48), with an annual health care cost of ~\$56.0 billion dollars (49). Asthma disproportionately affects minority populations, as well as those persons with lower household income, overweight and obese individuals, and those with increased exposure to environmental triggers (50, 51). Triggers range from viral infections to allergies, to irritating gases and particles in the air. Nevertheless, the combination of genetic and environmental factors that contribute to increased asthma prevalence, and the etiology of asthma are not precisely understood.

Many different physiological mechanisms contribute to the inflammation, obstruction, and airway remodeling underlying asthmatic patients' wheezing, cough, and dyspnea. In addition, clinical symptoms may differ vastly between patients, suggesting that clinicians are dealing with overlapping conditions of a syndrome, rather than a single disease. The immune response to allergen exposure in the asthmatic airway is complex and involves many innate and adaptive immune cell types (including but not limited to epithelial cells, innate lymphoid cells, mast cells, eosinophils, dendritic cells, and T lymphocytes) and their soluble mediators. There is a need to identify and understand those mechanisms that underlie the asthma syndrome, which in turn will enable us to develop biomarkers and novel therapies to facilitate disease prevention and treatment on an individual level.

Decades of research suggest that murine models of allergic airway inflammation are a useful tool for investigating the pathogenesis of asthma. The majority of which involve

intraperitoneal (i.p.) injections of protein allergen, frequently ovalbumin (OVA), emulsified in aluminum hydroxide (alum) during the sensitization phase followed by direct allergen delivery to the airways in the challenge phase to induce a profoundly biased Th2-type response within the airways. The disease phenotype is characterized by features of airway hyper-reactivity; high lung eosinophil counts; elevated levels of IL-4, IL-5, and IL-13 within the bronchoalveolar lavage fluid; and serum IgE production. Under these conditions, many researchers have shown a profound role for IL-4, IL-5, and IL-13 in allergic airway disease (52). However, with the realization that simple blockade of these molecules in patients was ineffective beyond those with eosinophilic disease, there has been an emergence of interest in the role of other T cell phenotypes, cytokines, and innate cells in disease pathogenesis (53).

Recently, the IL-1 family cytokines (e.g. IL-1, IL-18 and IL-33), epithelial-derived thymic stromal lymphopoietin (TSLP), and IL-25 have received attention with regard to their role in allergic airway inflammation. These cytokines are upstream of IL-4 and IL-13 and may have greater therapeutic potential, since they play an important “gate keeper” role in mucosal homeostasis. In recent years, IL-9, IL-17, and IL-22 have been put forward as important mediators of asthma pathogenesis, since IL-9-, IL-17-, and IL-22-producing CD4⁺ T cell subsets (designated Th9, Th17, and Th22) have been implicated in asthma. Currently, various therapeutic strategies are under investigation to target Th9, Th17, and Th22 cells, which include blocking their differentiation and amplification, inhibiting or neutralizing the cytokines, and suppressing the transcription factors specific for Th differentiation.

Several studies suggest that the AhR signaling pathway may represent one such alternative target and AhR ligands may prove beneficial in asthma. The AhR ligands TCDD, curcumin, and quercetin all suppress allergic airway inflammation in rodents (54–56). Perinatal exposure to TCDD reduced allergic sensitization in an IL-4 dependent manner (57). TCDD exposure resulted in an immediate and sustained reduction in bronchoconstriction, IL-5, eosinophilia, and specific IgE synthesis after house dust mite challenge in rats (54). Similarly, TCDD suppressed the generation of OVA-specific Abs in plasma, and IL-4 and IL-5 levels were reduced upon ex vivo re-stimulation of CD4⁺ T cell isolated from mice exposed to TCDD (55, 58, 59). Quercetin (a plant-derived flavonoid with AhR agonistic activity) reduced IL-4 production and expression of GATA3, a Th2 transcription factor, in lung tissues of ovalbumin (OVA)-sensitized and -challenged mice while increasing IFN- γ production and expression of T-bet, a Th1 transcription factor (60). Curcumin (an AhR ligand rich in Indian spice) inhibited recruitment of eosinophils into the lung airways, airway hyper-responsiveness, and expression of IL-4 (56). FICZ (a tryptophan photoproduct) reduced pulmonary eosinophilia, OVA-specific IgE, and levels of IL-4 and IL-5 in mice sensitized and challenged with OVA (47). Lastly, recent studies in our laboratory showed that I3C (a dietary compound produced through the breakdown of cruciferous vegetables) attenuated OVA-induced allergic asthma in mice. In these studies, mice were sensitized with alum-precipitated OVA (i.p.) on day -7 and challenged with OVA intranasally (day 0). On days -2, 0, and +2, mice were exposed to water (vehicle) or 100 mg/kg I3C via oral gavage. At day +4, mice were sacrificed to collect whole lung lavage and lung digests. As anticipated, total cell numbers recovered from the whole lung lavage were increased in OVA-sensitized mice compared with un-sensitized control mice, an effect that was reduced by I3C (data not shown). As revealed in Figure 2, I3C was very effective at preventing pulmonary eosinophilia, greatly reducing the percent and absolute number of infiltrating eosinophils relative to OVA-sensitized mice. Because cytokines including IL-4/IL-13 and IL-5 are closely associated with the pathogenesis of asthma and eosinophil recruitment, we next examined expression of a panel of cytokine and chemokines in the lavage fluid using murine Multiplex assays (IL-1, IL-4, IL-5, IL-6, IL-12p40, IL-13, RANTES, KC, MCP-1, GM-CSF, IFN- γ and TNF- α). Of these, IL-4, IL-5, and IL-13 were

significantly reduced and IFN γ , IL-12p40, and RANTES were significantly elevated (Figure 3), whereas IL-1 β , TNF α , IL-6, KC, MCP-1, and GM-CSF were unaffected compared to OVA-sensitized mice (data not shown). These data suggest that I3C induces a switch in the cytokine response away from Th2 towards a Th1 type of response. Studies are currently ongoing to determine the cellular and molecular mechanisms underlying the ability of I3C to attenuate allergic airway inflammation.

Allergic responses are characterized by the production and secretion of “Th2” cytokines (e.g. IL-4/IL-13 and IL-5), leading to eosinophil and mast cell degranulation, goblet cell hyperplasia, mucus production, increased serum IgE levels, and smooth muscle contractions. Such responses can develop following repeated low-dose exposure to normally innocuous allergens, such as bacterial products, food items, plant pollens, or fungal spores at mucosal barriers. The presumption that allergic lung responses are solely Th2 cell mediated has been contested following the discovery of type 2 GATA3⁺/ROR γ ⁺ innate lymphoid cells (ILCs). Although Th2 cells are a major source of these cytokines during allergic asthma, ILCs also contribute to disease pathology. A characteristic feature of type 2 ILCs is their expansion and abundant production of IL-5 and IL-13 in asthma, in protease allergen-induced airway inflammation, in glycolipid induced lung allergy, in a chronic rhino-sinusitis model, and in models of helminth infection (61). Production of IL-5 and IL-13 by type 2 ILCs is mediated through IL-25, IL-33, and TSLP (62–65). Although the AhR is highly expressed in ROR γ ⁺ ILCs and in type 2 ILCs (66–69), and AhR mRNA and IL-22 protein levels are significantly increased in activated PBMCs in allergic asthma sufferers, compared to controlled asthmatics and healthy controls (70), additional work is needed to elucidate the functions of the AhR in ILCs in the lungs.

Th17 and regulatory T cells have been implicated in the pathogenesis of allergic asthma, although the exact contributions to disease etiology remain unknown. Multiple studies have documented that binding of the AhR with certain ligands favors T helper cell differentiation toward regulatory T cells, and paradoxically, binding of this same receptor with different ligands enhances Th17 effector cell differentiation. This finding has been confirmed in both *in vitro* and *in vivo* models, where different ligands are able to either ameliorate or conversely aggravate inflammatory diseases (23, 38, 71–73). In addition, the AhR is central to IL-22 expression by T cells and for Th22 differentiation (74, 75). Although treatment of mice with TCDD and other AhR ligands ameliorated allergic airway disease, details about the effects on pulmonary Th cell differentiation remain largely unknown. Taken together, AhR ligands may be important modulators of immunological responses that participate in allergic reactions. However, differences in metabolism, AhR binding and subsequent gene transcription indicates the need for further studies to investigate the role of the AhR in allergic asthma.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic obstructive pulmonary disease (COPD) affects over 200 million people worldwide and is the fourth leading cause of mortality in the United States (76, 77). The main symptoms of COPD include chronic coughing, sputum production, and breathing difficulties. COPD is preventable and treatable, but the pulmonary consequences of this disease are largely irreversible. COPD has been linked to chronic exposure to lung irritants such as noxious gases or particles released from burning cigarettes, second-hand smoke, biomass burning, chemical fumes, air pollution, and dust from the workplace or the environment (78, 79). Although there is no known cure for COPD, treatments and lifestyle changes may help alleviate symptoms and slow disease progression (80).

Both innate and adaptive immune cells and their soluble mediators contribute to the inflammatory response in COPD. Although macrophages, neutrophils and T cells were once believed to be the prime effector cells, more recent studies have focused on defining the contributions of other leukocyte populations (e.g. dendritic cells, mast cells, and innate lymphoid cells, as well as non-immune cells such as lung fibroblasts and epithelial cells) to disease development and progression. Many inflammatory mediators including reactive oxygen species (ROS), matrix metalloproteases (MMPs), elastase, and cytokines have been implicated in the pathogenesis of COPD (81). Furthermore, as with many chronic inflammatory diseases, dysfunctional immune-regulatory systems may underlie the development and progression of COPD.

Cigarette smoking is causally linked with COPD. Therefore, exposure to cigarette smoke and its many constituents are considered major risk factors. The gaseous phase of smoke from cigarettes is comprised of thousands of dangerous chemicals such as carbon monoxide, carbon dioxide, hydrogen cyanide, ammonia, nitrogen oxides, formaldehyde, benzene, arsenic, acrolein, and many other toxic compounds (82). Additionally, tar released from cigarettes and other tobacco burning products contains significant amounts of particulate matter in association with harmful chemicals including nicotine, phenol, endotoxin and polycyclic aromatic hydrocarbons such as BaP--a well-defined AhR agonist. Collectively, chemicals found in cigarette smoke are major contributors to the inflammatory response observed in smokers although the specific cellular and molecular events that underlie these effects remain to be fully elucidated.

Over the last few years, the AhR signaling pathway in the lung and many immune-mediated lung diseases including COPD has been examined. Activation of the AhR in laboratory rodents affects many aspects of immune function and regulation (25). It has repeatedly been demonstrated to generate what can appear to be opposing effects manifested as excess inflammation and immunosuppression, effects that are also observed in the lung. This is perhaps not surprising as lung tissue contains a relatively high level of AhR expression (83–85). Using the prototypical AhR agonist TCDD to treat C57Bl/6 mice, Wong and colleagues reported an increased expression of inflammatory cytokines (TNF- α , IL-1 β , MCP-1), mucin 5AC, and a number of matrix metalloproteases (MMP-12, MMP-13) in whole lung samples (83). These effects were found to be dependent on AhR activation via DRE in the promoter/enhancer regions of these genes as no TCDD-induced increases were seen in NLS mice that harbor DRE DNA-binding defects. In a separate study evaluating hyperoxic lung injury in mice, omeprazole, a proton pump inhibitor with affinity for the AhR, was shown to attenuate lung inflammation via enhanced expression of cytochrome P450 enzymes, especially CYP1A1 (86). This protective effect was AhR-dependent as similar effects were not observed in B6.AhR^{d/d} mice that express a low-affinity AhR on a C57Bl/6 background. These contrasting studies illustrate the potential differences that can occur in the lung and other organ systems following AhR activation by disparate agonists such as TCDD and omeprazole, and further emphasize the need for comprehensive evaluation of AhR binding compounds that have the potential to affect lung function.

In addition to evaluating the effects of activation of the AhR by xenobiotics, it is critical for us to also understand the potential pathophysiologic role of the AhR in the lung. In a recent study using a mouse model of inflammation-induced pulmonary fibrosis, B6.AhR^{d/d} mice developed accelerated lung fibrosis with nearly a fourfold increase in collagen deposition when compared to C57Bl/6 wild-type controls (87). The exaggerated response of the AhR mutant mice was attributed to decreased IL-22 production in the lung as protection of lung injury (i.e. lung inflammation and fibrosis) was achieved when the B6.AhR^{d/d} mice were treated with recombinant IL-22. Interestingly, although the AhR has been identified as a critical transcription factor in the development and function of IL-22 producing CD4⁺ T

cells, this study identified a rare T cell population in the lung that was responsible for the protective secretion of IL-22 in an AhR-dependent manner, at least in this murine model of hypersensitivity pneumonitis. Similar beneficial effects following AhR-induced increases in IL-22 have recently been reported for other organs including the gut and pancreas (34, 88–91). In a mouse model of cigarette smoke-induced COPD, the sensitivity of lung cells to smoke induced apoptosis was significantly enhanced in AhR knockout mice (92). Indeed, absence of AhR expression led to decreased pulmonary cell survival primarily via mitochondrial dysfunction and decreased levels of antioxidant regulating proteins (i.e. MnSOD and CuZn-SOD). These results suggest that cigarette smoke-induced loss of structural cells in the lung, caused by alterations in AhR expression, may be a significant contributor towards the development of COPD and other lung inflammatory diseases in smokers.

COPD pathogenesis is attributable to many different cell populations that express the AhR. With regards to immune cells, T cells contribute significantly to the development of COPD. CD8⁺ and CD4⁺ T cells are found in the airways and lung parenchyma during COPD and disease severity is associated with increases in both of these populations (93). Previous studies showed increased numbers of CD8⁺ T cells in the bronchial biopsies of smokers with COPD, and studies using CD8⁻ mice have indicated a critical role for these cells in the development of emphysema following exposure to cigarette smoke (94). It is believed that release of perforin and granzymes by CD8⁺ T cells contributes to the cell death of lung cells leading to decreased barrier function. This possibility is supported by detection of increased levels of these mediators in the sputum and BALF of COPD patients. Additionally, the production of cytokines including IL-13, IL-17 and IL-18 is thought to contribute to enhanced lung inflammation in COPD via upregulation of MMP expression as well as the recruitment of innate immune cells. COPD associated CD4⁺ effector T cells are associated with increased inflammation in the lungs of cigarette smokers, and Th1, Th2, and more recently Th17 cells, have been linked to COPD pathogenesis (81). Cigarette smoke is a selective adjuvant that augments Th17, but not Th1, cell differentiation via the AhR (95). Further, IL-17RA is required for CCL2 expression, macrophage recruitment, and emphysema in response to cigarette smoke. With regards to the role of regulatory T cells (Tregs) in COPD, evidence indicates that there is an increase in Tregs in COPD patients (81). However, these cells may not be effective in regulating inflammation in the lung, a conclusion supported by a recent study demonstrating an expansion of CD4⁺CD25⁺ T cells without regulatory function in smoking and COPD (96). Likewise, decreased indoleamine 2,3-dioxygenase activity and IL-10/IL-17 ratio has been reported in patients with COPD (97). Collectively, it is likely that in COPD that the body's regulatory system is not effective in preventing the lung damage caused by cigarette smoke and other noxious gases but instead switches to a pro-inflammatory environment, at least in part via effects conferred by activation of the AhR.

Within the innate immune system, AhR activation plays a major role in regulating inflammation. Several elegant studies have demonstrated that the AhR signaling pathway is a master regulator of the inflammatory responsiveness of macrophages, cells critical for the development of COPD (98, 99). Increased numbers of macrophages in both airways and parenchyma of COPD patients have been reported, and a positive correlation exists between macrophage recruitment to the airways and disease severity (80). Recently, a study by Monick and co-workers explored the relationship between smoking and genome wide methylation and gene expression in lung alveolar macrophages (100). They found that smoking was associated with significant changes in methylation, especially at the AhR repressor (AHRR), a major regulator of the AhR. These results suggest that AhR status in smokers is dysregulated, an effect that warrants future investigation. Other innate immune cells implicated in COPD include neutrophils, mast cells, lung epithelial cells and lung

fibroblasts. Although not directly affected by AhR activation, neutrophils are recruited to the inflamed lung following exposure to cigarette smoke. Consequently, neutrophil elastase is critical for cigarette smoke-induced emphysema (82). Recent clinical data shows increased levels of mast cell-secreted tryptase and increased numbers of mast cells in the lavage and lung tissue from smokers (82, 101). Moreover, AhR-activated mast cells produce IL-17 and histological analysis of COPD patients revealed an increase in AhR/IL-17 double-positive mast cells within bronchial lamina propria (101). AhR activation in lung epithelial cells by TCDD or BAP results in alterations in immunomodulatory genes including IL-8 and several genes involved in interferon and retinoic acid signaling, and mucin 5AC, respectively (83, 102, 103). Finally, AhR regulates the inflammatory responsiveness of lung fibroblasts, attenuating tobacco smoke-induced cyclooxygenase-2 and prostaglandin production via regulation of RelB (104). Taken together, many immune and non-immune cells in the lung are significantly affected by the AhR, ultimately contributing to the development and progression of COPD.

SILICOSIS

Silicosis is one of the oldest, preventable occupational lung diseases. Despite this, silicosis continues to kill thousands every year in both developing and developed countries as a result of not using proper protective measures. Silica (SiO_2) particles are ubiquitous and naturally occurring in the environment, even constituting a portion of the small particulate fraction of air pollution. Silicosis occurs as a result of exposure to respirable dust containing crystalline SiO_2 and can arise as the result of exposure through occupation (e.g. construction, mining), recreation (e.g. pottery), or environment (e.g. soil). It is irreversible and, moreover, the disease progresses even when exposure stops. Although significant efforts have been made through industrial hygiene standards to control ambient dust in the workplace, silicosis remains a prevalent health problem. Indeed, recent reports linked an epidemic of silicosis in Turkey to lack of appropriate protective measures in workers sandblasting denim jeans (105–109). As of yet, no cure exists, but strategies for palliative care may improve quality of life and slow deterioration in patient's lungs.

Studies of SiO_2 -induced alterations of the lung structure and function are important for understanding the basic mechanisms of silicosis and other interstitial lung diseases. In addition, defining the importance of AhR mediated immunity to the cellular and molecular responses to SiO_2 particles may contribute to: (a) the identification of biomarkers for monitoring exposure or lung disease activity, (2) the development of therapeutic interventions for lung disease, and (3) the development and validation of *in vitro* and *in vivo* approaches for assessing the toxicity of new materials.

Initially, pulmonary defense mechanisms control SiO_2 induced inflammation and damage; however once a threshold lung burden has been exceeded, these control mechanisms no longer prevent disease progression (110–112). SiO_2 deposition then results in cytotoxic damage, free radical production, alveolar lipoproteinosis, neutrophil recruitment, and progressive pulmonary fibrosis, as well as systemic immune deficits (113–117). SiO_2 -induced lung injury is characterized by the presence of abnormal numbers of myeloid and lymphoid derived immune cells and their associated soluble mediators (118–121). Typically, alveolar macrophages and the concomitant Nlrp3 inflammasome activation and IL-1 produced are believed to be the prime effectors responsible for initiation of disease; however, much attention has also been directed at continuing to define the contributions of other leukocyte populations such as dendritic cells, neutrophils, natural killer cells, innate lymphoid cells, and T cells, as well as non-immune cells such as lung fibroblasts and epithelial cells (119, 120, 122) to the development and progression of disease. As with many

chronic inflammatory lung diseases, it is believed that an imbalance or dysregulation in regulatory systems may either underlie the development and progression of silicosis.

In the lung, Ag presenting cells such as alveolar macrophages and dendritic cells are critical effector cells primarily mediating the binding, ingestion, and clearance of inhaled particulate matter. Their response to inhaled particulates ranges from ingestion and clearance with minimal inflammation, to apoptosis and profuse production of inflammatory cytokines and reactive oxygen intermediates. In particular, recent advances have implicated the formation of multi-protein inflammasome complexes in both inflammation and fibrosis. In particular, Nlrp3-mediated inflammasome formation is critical for caspase 1 activation and IL-1 and IL-18 processing and release in response to microbial molecules, ATP, urate crystals, silica, carbon nanotubes, and asbestos particles. Although normal activation of the Nlrp3 inflammasome contributes to host defense, excessive activation leads to auto-inflammatory diseases. Thus, it is of particular interest that macrophages derived from AhR deficient mice secrete elevated levels of IL-1, and that activation of the AhR with TCDD reduces SiO₂-induced increases in IL-1 levels *in vitro* and *in vivo*, ultimately reducing pulmonary inflammation (43). Furthermore, the AhR has been implicated in the regulation of inflammasome adaptor proteins including Pai-2 and STAT2 (123–125), such that select AhR ligands may induce signaling cascades that negatively regulate inflammasome activation and ultimately effect inflammation and fibrosis in the lungs.

Because many Th1- and Th2-associated cytokines have been implicated in pulmonary inflammation and fibrosis, many investigators considered T cells a key participant in the initiation and progression of silicosis in rodent models. Undeniably, lymphocyte recruitment to the lungs is a prominent component of the immune response to SiO₂. However, neither NK cells nor T cells appear to play a pivotal part in the pathogenesis of fibrotic disease. In fact, lymphocytes have been implicated in limiting innate immune responses through inhibition of Nlrp3 inflammasome *in vitro* and *in vivo* (120, 126). Studies in lymphopenic mice (including *scid*, *nude*, NK-deficient, Rag1^{-/-}, or a combination (Rag1^{-/-} NK-depleted)) demonstrated that fibrosis and inflammation could occur independently of T, B, NK T, and NK cells (127, 128). Furthermore, these studies indicate that lymphocyte deficiency exacerbated IL-1 secretion and neutrophilia following SiO₂ exposure, resulting from a lack of negative controls of inflammasome activation. Although TCDD suppressed inflammation, but not fibrosis, in a model of chronic silicosis, it did not appear to significantly reduce measures of pulmonary function 12 weeks after initial exposure. In contrast, in the acute silicosis model (4 weeks after exposure), TCDD reduced lung tissue inflammatory infiltrate, as well as airway hyperreactivity (Figures 4 & 5). Due to the ability of select AhR ligands to modulate T helper differentiation and cytokine secretion, it will be of interest to further investigate the therapeutic potential of AhR-activated CD4⁺ cells in acute silicosis.

The respiratory tract contains well-defined populations of Ag presenting cells (APC) such as alveolar macrophages (AM), pulmonary macrophages (PM), and dendritic cells (DC), which are major contributors to SiO₂-induced inflammation and fibrosis (129, 130). AM and DC survey the local tissue environment, are responsible for neutralization and elimination of foreign material in the body, including non-biological particles such as SiO₂, and are capable of presenting Ag to T cells (130, 131). Traditional T cell based immune responses require APC-T cell interactions consisting of an antigenic peptide bound in a major histocompatibility (MHC) complex (132–134). Despite this, AM avidly phagocytose airborne particulates including SiO₂, secrete soluble mediators important for CD4⁺ cell differentiation and activation, and increase their Ag presenting cell activity (129, 130). Although DC alter their surface molecule expression following TCDD and I3C-induced activation (29–31), and AhR activation attenuates macrophage cytokine production in

response to SiO₂ (43), it is not known whether AhR activation may alter Ag presenting cell function and represent a control mechanism for early immune response to SiO₂.

CONCLUSION

A precise balance is necessary to maintain proper immune surveillance in the lungs, while at the same time preventing chronic inflammation, fibrosis, and autoimmunity. As we have highlighted in this review of numerous recent publications, the AhR and its ligands exhibit important immunomodulatory properties and can fine-tune the respiratory immune response. Therefore, a better understanding of the key AhR ligands and AhR signaling properties relative to the development of therapeutic and preventive approaches to inflammation and fibrosis is required. However, several points require resolution before implementation of AhR ligands in clinical practice. First, there is a need to design AhR ligands with safe pharmacological profiles. Indeed, the most studied AhR ligand, TCDD, has valuable immunomodulatory properties, but is not applicable in clinical practice because of its toxicological properties. Second, the impact of distinct AhR ligands on the immune system has to be better understood. For example, while TCDD and FICZ have opposite effects on Foxp3⁺ Tregs and Th17 cells, both promote Tr-1 cells and may induce IL-22 depending on the experimental setting. Therefore, the specificity, pharmacology and dose-effect of each AhR ligand must be prudently assessed in different inflammatory lung diseases models. Third, the effect of AhR ligands needs to be vigilantly monitored to avoid excessive responses. For example, the endogenous AhR ligand kynurenine is secreted by human tumors and promotes the generation of Tregs *in vivo* (135). Although Tregs can be beneficial in preventing autoimmune diseases, they may reduce anti-tumor immunity and prevent the development of successful immunotherapy. In conclusion, AhR ligands are promising compounds for pharmaceutical drugs. However, further studies should aim at designing a new generation of AhR ligands that could specifically target different cell types of the immune system with limited side effects. More research is required to understand the multifaceted role of the AhR in the context of inflammatory lung diseases, as well as evaluate the potential of AhR targeting for the treatment diseases in humans.

Acknowledgments

This work is supported in part by National Institutes of Health grants R01 ES013784 (D.M.S.) and R15 ES 020993A (C.A.B.). The contents of this publication are solely the responsibility of the authors and do not necessarily reflect the official views of the National Institutes of Health.

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.