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Polycyclic aromatic hydrocarbons, tobacco smoke, and epigenetic remodeling in asthma

E. C. Klingbeil,
Division of Allergy and Immunology, Stanford University School of Medicine, 300 Pasteur Drive, Rm. S-303, Stanford, CA 94305-5107, USA

K. M. Hew,
Division of Allergy and Immunology, Stanford University School of Medicine, 300 Pasteur Drive, Rm. S-303, Stanford, CA 94305-5107, USA

U. C. Nygaard, and
Division of Allergy and Immunology, Stanford University School of Medicine, 300 Pasteur Drive, Rm. S-303, Stanford, CA 94305-5107, USA; Division of Environmental Medicine, Norwegian Institute of Public Health, Oslo, Norway

K. C. Nadeau
Division of Allergy and Immunology, Stanford University School of Medicine, 300 Pasteur Drive, Rm. S-303, Stanford, CA 94305-5107, USA, knadeau@stanford.edu

Abstract

Environmental determinants including aerosolized pollutants such as polycyclic aromatic hydrocarbons (PAHs) and tobacco smoke have been associated with exacerbation and increased incidence of asthma. The influence of aerosolized pollutants on the development of immune dysfunction in asthmatics has been suggested to be mediated through epigenetic remodeling. Genome accessibility and transcription are regulated primarily through DNA methylation, histone modification, and microRNA transcript silencing. Epigenetic remodeling has been shown in studies to be associated with Th2 polarization and associated cytokine and chemokine regulation in the development of asthma. This review will present evidence for the contribution of the aerosolized pollutants PAH and environmental tobacco smoke to epigenetic remodeling in asthma.

Keywords

Asthma; Polycyclic aromatic hydrocarbons; Diesel exhaust particles; Environmental tobacco smoke; Epigenetic remodeling

Introduction

Asthma is the result of immune dysfunction that causes inflammation in the bronchial mucosa [1]. Specific immune cells are linked to asthma pathogenesis. In particular, immune

ekling@stanford.edu.

cell profiling in allergic asthmatics has indicated a polarization toward CD4+ Th2 cells [1]. Also, degranulated mast cells and eosinophils have been found to have similar linkages. Furthermore, asthma is associated with impaired function of regulatory T cells (Tregs), cells that maintain tolerance in healthy individuals by exerting suppressive effects on a variety of immune cells involved in allergic disease progression, including activated T cells, eosinophils, basophils, antigen-presenting cells, and mast cells [1]. The cytokine polarization of particular cell lines can depend on genetic background, but is stimulated by environmental determinants. Studies have indicated that outdoor and indoor allergens, environmental pollutants, diet, and stress contribute to priming an individual to be vulnerable to asthma [1]. In addition, studies show that genetic variants can affect the level of impact from exposure [2]. Also, those individuals living close to busy roads may be more susceptible to developing asthma [3]. A study conducted in New York City children living at four-way intersections showed a significant increase in wheezing. Those populations in the study that lived juxtaposed to highways showed high levels of IgE, which has been associated with asthma [4]. This review will focus primarily on the contribution of the aerosolized pollutants polycyclic aromatic hydrocarbons (PAHs) and environmental tobacco smoke (ETS) to epigenetic remodeling in asthma.

Epigenetic remodeling

Maternal and neonatal exposure to aerosolized pollutants has been associated with increased development of asthma. The prenatal and neonatal period is critical to the development of the immune system and tissue growth in the airway. Aerosolized pollutants can influence development and future phenotype of those systems [1]. The influence of aerosolized pollutants on the development of immune dysfunction in asthmatics has been suggested to be mediated through epigenetic remodeling, a mechanism compatible with the suggested early life programming of this disease. Gene expression modification through epigenetic mechanisms has three primary targets: CpG methylation; amino acid tail modification on histones; and aberrant microRNAs expression (Fig. 1) [1]. Histone modification via methylation occurs post-translationally while miRNAs can control expression of other genes post-transcriptionally [1]. Changes to these targets can influence DNA folding, DNA–transcription factor interaction, transcript stability, and other methods of gene silencing (heterochromatin) or activation (euchromatin) [1].

CD4+ T cells in atopic asthmatics are primed to differentiate into Th2 cells, characterized by secretion of the cytokines IL-4, IL-5, IL-9, and IL-13, but no IFN-gamma. In particular, DNA methylation has been associated with changes in IL-4 and IFN-gamma transcription [1]. In agreement, it has been shown in mouse models that increases in IgE levels are associated with hypomethylation at IL-4 promoter CpG sites and hypermethylation of IL-4 and IFN-gamma promoter CpG sites [5]. Histone acetylation was associated with IL-4, IL-13, IL-5, IFN-gamma, CXCL10, and FOXP3+ transcription patterns [1]. Lastly, miRNA-mediated silencing has been found to repress transcripts associated with HLA-G, IL-13, IL-12p35, TGF-beta, and the POU domain [1]. These studies indicate an important role of epigenetic remodeling on the immune system.

Polycyclic aromatic hydrocarbons and tobacco smoke

PAHs are formed during partial combustion of organic compounds and compose a large portion of aerosolized diesel, wood, and industrial emissions. Within the class of PAHs, benzo[a]pyrene (BaP) is the most highly studied. PAHs have been of particular interest in their association with asthma due to patterns in epigenetic remodeling and specifically DNA methylation [6].

When PAHs are inhaled, their small molecular weight allows them to easily come into contact with and/or enter cells lining the bronchial mucosa [6]. Once inside the cytoplasm of bronchial epithelial cells, PAHs will interact with abundant aryl hydrocarbon receptors (AhRs), which dissociate from their inactive complex (HSP90, XAP2, P23) [6]. Aryl nucleotide receptor translocator (ANRT) will then dimerize with the PAH and AhR to form an active complex. This complex is then translocated within the nucleus where it can behave as a transcription factor for xenobiotic response elements (XRE) (Fig. 2) [6].

One of the XREs is found in the *CYP1A1* and *CYP1B1* gene codings for cytochrome P450 (CYP), which is transcribed during this process [7]. CYP functions normally in a two-phase process to metabolize carcinogenic agents to an inactive state. Detoxification through mono-oxygenation prepares these agents for excretion [7]. The activation of PAHs to their mutagenic state by CYP is an unintended consequence of xenobiotic metabolism. Of its forms, CYP1A1 and CYP1B1 have the highest efficacy in catalyzing PAHs to their active form. It is speculated that varying responses to PAH exposure are likely due to the genetic variance in CYP proteins [7]. For BaP, activation begins when CYP induces the formation of an epoxide at the 7, 8 position. Epoxide hydrolases reduce the epoxide to a diol [7]. CYP performs a second catalytic event this time at the 9,10 position producing a diol-epoxide, the key characteristic of an activated PAH [7].

The PAH diol-epoxide can bind covalently to nitrogenous bases to produce DNA adducts. In a study measuring CpG methylation in cord blood, a linear regression model determined that the likelihood of adduct formation was higher in methylated DNA. The changes in methylation profiles in conjunction with adduct formation were first identified using restriction enzyme-based microarrays [8]. Due to the aromatic basis of PAHs, their planarity facilitates the ease of their interaction with nucleotide bases. The cytosines in CpG regions are often targets for adduct formation [7].

PAH-associated adduct formation at the CpG region is associated with changing methylation patterns in specific immune signaling molecules. Air pollutant exposure has been associated with up-regulation of GATA-3, a Th2 transcription factor, and prototypic Th2 cytokines including IL-4, IL-5, IL-9, and IL-13 [1]. Importantly, irregular methylation patterns in Tregs and T helper 17 have also been associated with PAH exposure and asthma [1]. In particular, PAH exposure has been reported to be associated with impaired Treg function [9], as well as with altered methylation of the Foxp3 promotor region (Hew et al., in revision). In agreement, in vitro exposure of primary Tregs to the PAH phenanthrene gave reduced methylation of CpG sites within the FOXP3 locus and reduced FOXP3 expression,

leading to impaired Treg function and conversion of Treg into a CD4(+)CD25(lo) Th2 phenotype [10].

PAHs and in particular BaP have been found to be critical constituents in tobacco smoke [6]. Consequently, smoking has been shown to up-regulate *CYP* expression much like PAH [11]. Furthermore, smoking behavior introduces CYP1A1 prevalence in the airway epithelium by 100-fold [12]. Tobacco smoke has shown evidence of inducing epigenetic modification through DNA methylation. Kohli et al. [13] reported that tobacco smoke exposure is associated with hypermethylation of the promoter region for IFN-gamma in T effector cells and Foxp3 in regulatory T cells. However, it should be noted that the effects of cigarette smoke alone are not surrogate measures for the effects of PAH, and other chemicals, like nicotine, and particulate matter in cigarette smoke probably also are contributing to epigenetic changes.

The *GSTM1* gene product, glutathione *S*-transferase M1 (GSTM1), plays an important role in the detoxification of PAH [14]. In a study by Breton et al. [15], prenatal populations with a common *GSTM1* null genotype, which is a single gene allele, were especially vulnerable to environmentally linked epigenetic modification. The *GSTM1* null genotype has been identified to show increased susceptibility to PAH in tobacco smoke and increased incidence of asthma development [14, 16]. An analysis of children with GSTM1 null genotypes showed differential LINE-1 methylation in smoking cohorts [16], LINE-1 being critical in regulating gene expression [17]. Wan et al. [18] investigated the role of CpG site-specific methylation patterns in association with cigarette exposure to find whether G-protein-coupled receptor (GPR15) plays a critical role in immune tolerance in the mucosa by regulating Treg levels [18, 19]. This suggests that tobacco smoke induces site-specific gene modification through methylation. Several studies have also been performed to identify other genes that are associated with tobacco smoke-induced methylation and immune dysfunction. For example, a cohort study by Word et al. [20] found cigarette smoke condensate exposure to result in altered methylation patterns of tumor suppressors, *ECAD* and *RASSF1A*. Furthermore, histone modification in key regions has also come to be identified as critical to epigenetic remodeling in the presence of tobacco smoke. Critical to gene expression, histone deacetylase (HDAC2), which removes acetyl groups associated with open chromatin and gene expression, has been shown to be influenced by tobacco smoke exposure. HDAC2 is involved in protection from damage as a result of oxidative stress [21]. It has been shown that cigarette-smoke-conditioned medium reduced overall HDAC2 expression in A549 epithelial cells in vitro [22]. Further studies conducted by Adenuga et al. [23] have shown that tobacco smoke exposure down-regulates HDAC2 via phosphorylation. These studies indicate that both PAH and tobacco smoke seem to induce epigenetic modifications at several levels, which are highly relevant in a disease setting like asthma.

Epigenetic mechanisms and prenatal exposures

Studies have indicated that exposure to aerosolized pollutants, particularly during early life, has caused significant impacts on immune expansion and airway development. A meta-analysis including 79 studies showed a 30–70 % increased risk of wheezing and 21–85 %

increased incidence of asthma after prenatal and postnatal tobacco smoke exposure [24]. In agreement, perinatal nicotine exposure of subjects between prenatal day 17 and postnatal day 7 showed significant decreases in airway diameter and increases in length [25]. Prenatal exposure to air pollution during the last 7 days of pregnancy showed postnatal increases in the IL-1-beta and reduced IL-10 levels in the cord blood of neonates [26]. Early disruptions in normal levels of specific immune signaling molecules may be key in improper immune development.

Several studies suggest a role for epigenetic remodeling in such effects of prenatal air pollutant exposure. In a study of prenatal exposure to tobacco smoke, epigenome-wide mapping has shown CpG hypermethylation patterns for aryl hydrocarbon receptor repressor (AHRR) and CYP1A1 [27]. Recently, Herberth et al. [28] reported associations between maternal tobacco smoke exposure and maternal and cord blood miR223. These miRNA changes were associated with reduced Treg numbers, suggesting increased allergy risk later in life [28]. Early pregnancy exposure to air pollutants like PM2.5 has been associated with hypomethylation of placental DNA [29], while more specifically maternal exposure to PAHs is associated with hypermethylation of the IFN-gamma promoter in cord blood DNA [30]. In agreement, studies have illustrated the significance of exposure to tobacco smoke and its impact on the intrauterine environment and methylation patterns.

Identifying key genetic markers for global DNA methylation may serve to predict asthma development or act as controls in studies. A study involving smoking and non-smoking pregnant women indicated an association between low birth weight and two differently methylated regions of Insulin-like growth factor 2 (IGF2) [31]. Methylation patterns in the long interspersed nucleotide element (LINE-1) and the short interspersed nucleotide element AluYb8 were found to differ between cohorts of infants exposed and non-exposed to tobacco smoke [32]. In a study conducted by Wangsri et al. [33], cigarette smoke was found to influence methylation patterns of LINE-1 sequences in cells of the oral mucosa and higher methylation of AXL. LINE-1 is a retrotransposon that is prevalent in the human genome and critical to regulating gene expression [17]. The promoter region of the LINE-1 gene sequence contains several CpG regions. LINE-1 may be important to use as a measure of global DNA methylation patterns in asthma studies [34]. Infants that were exposed to tobacco smoke were also found to have significantly lower levels of AluYb8 methylation [15]. AluYb8 insertion has been associated with oxidative stress and DNA damage [35]. Increasing levels of AluYb8 methylation were positively associated with changes in global methylation patterns as seen in polycomb group target genes [32]. Decreased methylation of AluYb8 may then be a genetic marker for global DNA methylation patterns in asthma studies where the mother smoked in pregnancy [34].

Since ruling out genetic and environmental confounders is extremely difficult in humans, there is only scarce evidence for multigenerational epigenetic effects for any condition in humans. In animal models, however, gestational exposure to nicotine has been shown to have multigenerational and even transgenerational effects on asthma [36]. These changes were accompanied by epigenetic alterations in both lung and gonad tissues, further suggesting that epigenetic remodeling plays an important role in this picture.

Conclusion

As outlined in the studies presented, aerosolized pollution may promote the development of asthma, possibly through epigenetic mechanisms. Several studies show evidence that exposure to PAHs and tobacco smoke in particular can induce epigenetic modification, and such modifications have been found to be associated with asthma. Furthermore, age or developmental stage at the time of exposure is critical due to the effects of modification through DNA methylation, histone modification, and miRNA silencing. These modifications may have the greatest consequences after exposure to prenatal and infant populations. Limiting the exposure of infants and pregnant women to tobacco smoke and air pollution is important to reduce the risk of acquiring exposure-linked asthma.

Biography

E. C. Klingbeil

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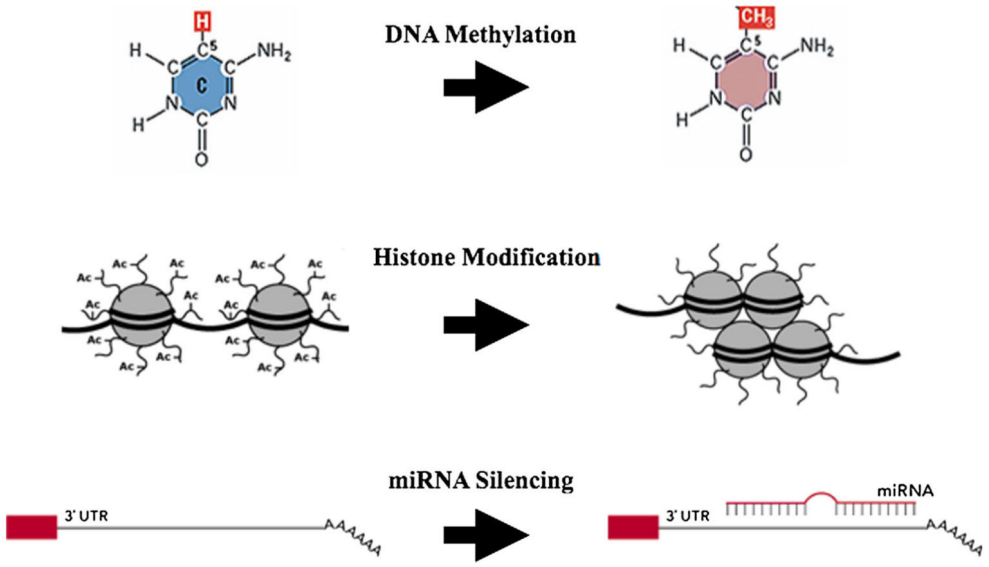

Fig. 1.

Polycyclic aromatic hydrocarbons and tobacco smoke contribute to epigenetic remodeling through DNA methylation, histone modification and microRNA silencing

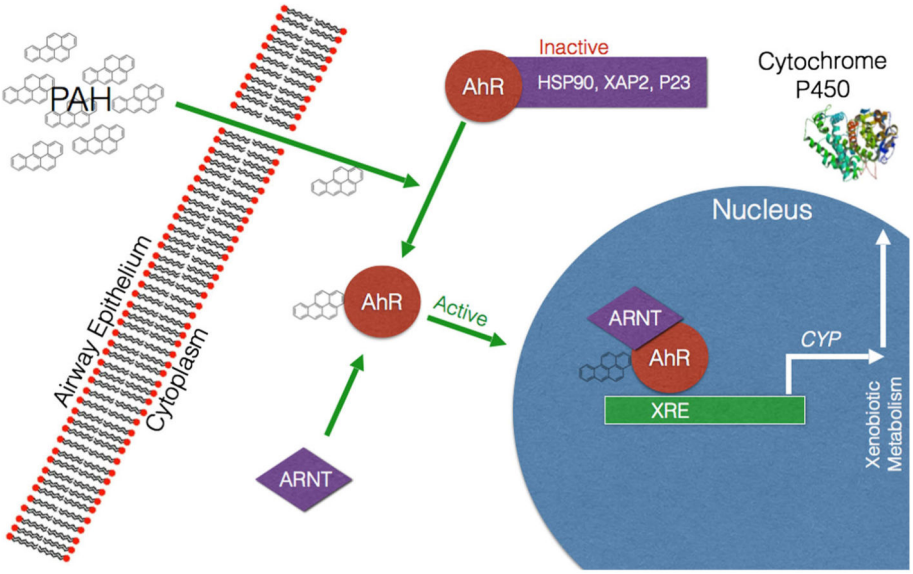

Fig. 2.

Illustration of how polycyclic aromatic hydrocarbons (PAHs) upregulate cytochrome P450 expression and activation of PAH to its mutagenic state (modified from Tsay et al. [6]).