Environmental epigenetics of asthma: An update

Shuk-Mei Ho, PhD *Cincinnati, Ohio*

Asthma, a chronic inflammatory disorder of the airway, is influenced by interplay between genetic and environmental factors now known to be mediated by epigenetics. Aberrant DNA methylation, altered histone modifications, specific microRNA expression, and other chromatin alterations orchestrate a complex early-life reprogramming of immune T-cell response, dendritic cell function, macrophage activation, and a breach of airway epithelial barrier that dictates asthma risk and severity in later life. Adult-onset asthma is under analogous regulation. The sharp increase in asthma prevalence over the past 2 or 3 decades and the large variations among populations of similar racial/ethnic background but different environmental exposures favors a strong contribution of environmental factors. This review addresses the fundamental question of whether environmental influences on asthma risk, severity, and steroid resistance are partly due to differential epigenetic modulations. Current knowledge on the epigenetic effects of tobacco smoke, microbial allergens, oxidants, airborne particulate matter, diesel exhaust particles, polycyclic aromatic hydrocarbons, dietary methyl donors and other nutritional factors, and dust mites is discussed. Exciting findings have been generated by rapid technological advances and well-designed experimental and population studies. The discovery and validation of epigenetic biomarkers linked to exposure, asthma, or both might lead to better epigenotyping of risk, prognosis, treatment prediction, and development of novel therapies.
(J Allergy Clin Immunol 2010;126:453-65.)

Key words: *Pulmonary disorder, traffic-related pollutants, polycyclic aromatic hydrocarbons, microbial and viral infection, lipopolysaccharide, endotoxin, oxidant early-life programming, nutrition, maternal exposure, T_H cells, dendritic cells, macrophages, lung epithelial cells, phenotype plasticity, developmental basis of disease, gene-environment interaction, DNA methylation, histone modification, microRNA, chromatin remodeling, allergen, inflammatory response*

Abbreviations used

ACSL3:	Acyl-CoA synthetase long-chain family member 3
AXL:	AXL receptor tyrosine kinase
BaP:	Benzo[a]pyrene
DC:	Dendritic cell
DEP:	Diesel exhaust particle
DNMT:	DNA methyltransferase
ETS:	Environmental tobacco smoke
Foxp3:	Forkhead box protein 3
HAT:	Histone acetyltransferase
HDAC:	Histone deacetylase
IL:	Interleukin
MAOB:	Monoamine oxidase type B
miRNA:	MicroRNA
NF-κB:	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1
PAH:	Polycyclic aromatic hydrocarbon
PM:	Particulate matter
PTPRO:	Protein tyrosine phosphatase, receptor type, O
TLR:	Toll-like receptor
TSA:	Trichostatin A

Asthma is still poorly understood. It is not one disease but many, with some known but many unidentifiable causes underlying its development and manifestation. As such, it is referred to as a complex disease for which a subject's risk is believed to be determined by a complicated interplay of one's genetics and environmental exposures. The genetic[1] or environmental[2] explanations of asthma have been discussed and debated for many years. Our recent understanding of epigenetics as a mechanism mediating gene-environment interaction offers new opportunities to advance novel concepts and re-examine established ones about this disease.[3,4] In this review I will first discuss some key features of asthma, the basic principles of epigenetic regulation, and theories of phenotype/developmental plasticity before summarizing recent advances in environmental epigenetics that influence asthma pathogenesis. I will address future challenges and opportunities for the field, focusing on those that might help prevent asthma.

ASTHMA: MAIN FEATURES AND DISEASE EFFECT

Asthma, a chronic inflammatory disorder of the airway, is characterized by recurring episodes of airflow obstruction, wheezing, coughing, and shortness of breath.[5] However, its symptoms are highly variable, and the causes of asthma and their interactions remain largely uncertain.[6] Asthma can cause intermittent episodes or follow a more chronic course, can occur with or without atopy, usually has its onset in childhood but sometimes is not recognized until adulthood, and can be corticosteroid sensitive or resistant. The heterogeneity of asthma suggests it is influenced by a multitude of factors, including genetics, family history, age, sex, socioeconomic status, race and/or ethnicity, and a host of recognized environmental factors.

The prevalence of childhood and adult-onset asthma has increased dramatically during the last 2 to 3 decades in both

From the Department of Environmental Health, Center for Environmental Genetics, University of Cincinnati College of Medicine.

Supported by National Institutes of Health grants P30ES006096, R01ES015584, RC2ES018758, RC2ES018789, and P50ES015905.

Disclosure of potential conflict of interest: S.-M. Ho has declared that she has no conflict of interest.

Received for publication July 13, 2010; revised July 27, 2010; accepted for publication July 29, 2010.

Reprint requests: Shuk-Mei Ho, PhD, 3223 Eden Ave, Kettering Complex Suite 130, Cincinnati, OH 45267. E-mail: shuk-mei.ho@uc.edu.

0091-6749/$36.00

© 2010 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2010.07.030

developed and developing countries,[7] although there are signs of a possible leveling off of its prevalence.[8] Worldwide prevalence estimates are between 100 and 150 million persons.[9] This disorder is clearly more prevalent in more developed countries, such as the United States.[10]

Asthma has become a major health and economic burden for our nation, disproportionately affecting minorities in inner-city communities and creating concerns about major health disparities.[11]

IMMUNE CELL DYSFUNCTION AND AIRWAY HYPERSENSITIZATION

Although the cause of asthma is multifactorial, the role of specific T cells and their cytokines in the pathogenesis of allergic asthma is now well recognized.[12] The infiltration and accumulation of polarized $CD4^+$ T helper (T_H)2 cells, degranulated mast cells, and eosinophils in the bronchial mucosa are the pathological features of allergic asthma. Allergic asthma starts with an influx of naive $CD4^+$ T cells and eosinophils into the bronchial mucosa. The priming of the naive $CD4^+$ T cells to differentiate into proinflammatory T_H2 cells instead of the infection-fighting T_H1 cells in the T-cell repertoire by allergen-activated dendritic cells (DCs) is an important proposed mechanism.[13] The progressive increase in the commitment of $CD4^+$ T cells toward a T_H2 phenotype is accompanied by an upregulation of the T_H2 inflammatory cytokines, such as IL-4, IL-5, IL-9, and IL-13, and an increased expression of the transcriptional factor GATA-3.[12] In parallel, the T_H2 cells shut off the expression of interferon-γ (IFN-γ) and other T_H1 cytokines, such as IL-2. The recent discovery of T_H17 in the mediation of corticosteroid-resistant asthma sheds new light on neutrophilic asthma.[14] In short, a skewed programming of $CD4^+$ T cells toward a T_H2 or T_H17 phenotype is a primary cause of asthma and other immunodysfunctions of the airway.

As a counterbalance, naive $CD4^+$ T cells can differentiate into forkhead box protein 3 (Foxp3)–positive regulatory T (Treg) cells on transforming growth factor, β (TGF-β) stimulation. This cell type confers immune tolerance, prevents autoimmunity, and dampens allergic responses. It suppresses a T_H2 response but can promote a T_H17 response. Thus induction of Treg cell differentiation can ameliorate asthma through the suppression of a T_H2 response, but this strategy might be limited by the potential activation of a T_H17 response.[15]

In addition to the T-cell dysfunction, the interaction between epithelial cells and DCs in the airways plays a crucial role in determining the ability of inhaled allergens to initiate and maintain allergic T_H2 cell–mediated responses. On challenge with an allergen, airway epithelial cells release chemokines and cytokines to attract and activate the DCs, which migrate and settle in the basolateral space of the airway epithelium. The DCs send processes into the airway lumen and sample for allergens. Activated DCs then migrate to regional lymph nodes to interact with regulatory cells and ultimately to stimulate T_H2 cell production by naive T cells. The DCs function as key antigen-presenting cells that translate the signal from allergens on the airway surface to T cells.[16,17]

Finally, an often forgotten cell type involved in asthma is the alveolar macrophage. These cells are the predominant immune effector cells residing in the airways. They play the dual role of activating inflammatory responses sufficient to eliminate pathogens/allergens and suppressing the responses to allow for tissue repair and remodeling after inflammatory insults to the airway.[18,19] In their asthma exacerbation role they can be activated by allergens to release inflammatory mediators and cytokines that amplify the inflammatory response.[19] In the suppressive role they can ingest apoptotic inflammatory or structural cells to reduce inflammation or release cytokines and nitric oxide to promote T_H1 development.[19]

GENETICS AND ASTHMA GENES

Asthma risk is influenced by genetics. Having a parent with asthma doubles a child's risk of asthma, and having 2 affected parents increases the risk 4-fold.[20] The greater concordance of asthma among monozygotic twins compared with dizygotic twins further supports this genetic influence.[21] Findings from multiple studies of the genetic association of asthma identified 43 replicated asthma genes.[22] The most frequently replicated of these genes are tumor necrosis factor-α (*TNFA*); *IL4*; membrane-spanning 4-domains, subfamily A, member 2 (Fc fragment of IgE, high affinity I, receptor for beta polypeptide) *(FCERB)*; ADAM metallopeptidase domain 33 *(ADAM33)*; and glutathione-S-transferase pi 1 *(GSTP1)*. Other genes identified are dipeptidyl-peptidase 10 *(DPP10)*, neuropeptide S receptor 1 *(GPR154)*, and PHD finger protein 11 *(PHF11)* by means of linkage and fine mapping[22] and ORM1-like 3 *(ORMD3)*, *IL1RL1*, and phosphodiesterase 4D *(PDE4D)* by means of genome-wide association studies.[23] Most of these genes are associated with inflammation or a shift of the immune system toward a T_H2 response, whereas others are surrogate biomarkers of inflammation. None alone is sufficient to predict or explain asthma, and there is a high degree of heterogeneity in the association of these genotypes among affected subjects or populations.[22] These findings suggest that asthma genes interact in a complex manner to regulate the risk and severity of the disorder and that genetics alone is insufficient to fully explain intersubject or interpopulation variations of the disease.[1] The missing explanation could reside in gene-environment interactions,[24] which are now believed to be mediated by epigenetic mechanisms.[3-5,25]

KEY ENVIRONMENTAL FACTORS ASSOCIATED WITH ASTHMA

The rapid increase in the prevalence of asthma throughout the world over only the past few decades,[7] the huge variations observed among populations with a similar racial/ethnic background but different environmental exposures,[26] and the marked increase in the frequency of occupational asthma[2] all point to the dominance of environmental factors in asthma's etiology. Additionally, several emerging hypotheses, such as the early-life origin of asthma,[4,27] the hygiene hypothesis,[28,29] and the artificial habitat hypothesis,[30] all require explanations involving environmental contributions to asthma's etiology.

Living in a developed country is a strong risk factor for asthma.[10] This increased risk might, in part, be related to potent indoor and outdoor allergens and irritants present in such an environment. Outdoor allergens and air pollutants that have been shown to trigger or exacerbate asthma include microbial and viral pathogens, airborne particulates, ozone, diesel exhaust particles (DEPs), pollens, outdoor molds (eg, *Alternaria alternata*), environmental tobacco smoke (ETS), cold air, and humidity.[2,31] Equally important are a host of indoor allergens that have been demonstrated to induce airway inflammation, such as those derived from dust mites, cockroaches, mice, and pets; particles generated from indoor combustion of tobacco, wood, and other plant

fuels; and biological agents (eg, indoor endotoxin), products from gram-positive bacteria, and 1,3-β-glucans from molds.[2] Other environmental factors affecting asthma include pharmaceuticals (eg, paracetamol[32]) and a variety of nutrients and dietary agents (eg, omega-6 polyunsaturated fatty acids, saturated fat, vitamins C and D, β-carotene, magnesium, selenium, sodium, and components in a Mediterranean diet[33]). Worthy of note is that many of these indoor and outdoor asthma inducers/triggers also have demonstrable reprogramming effects on the immature airway during early life, leading to altered asthma risk in later life (see the next section for further details).

Moreover, occupational asthma, which accounts for 5% to 15% of cases of asthma in adult workers, has more than 250 suspected causative agents,[2] including isocyanates, flour, grain dust, airborne particles, colophony, latex, animal dander, aldehydes, and wood dust.[2,34,35] The severity of such occupational asthma is usually dependent on the concentration of the allergen and the duration of exposure. However, because many workers tend to change their jobs once they have the disease, occupational asthma is underdiagnosed in the general population. Unfortunately, for many the symptoms can persist for years after the exposure is removed, thus significantly affecting the health and socioeconomics of our work force.

EARLY-LIFE ORIGIN OF ASTHMA: WINDOWS OF PROGRAMMING

Most cases of asthma are now considered to originate in early life and therefore belong to a long list of complex diseases that are "programmable" by specific early-life environmental exposures.[36] The prenatal period (during growth of the airways and development of the immune system) is a critical window of programming. In this regard maternal exposure to ETS, traffic-related pollutants, viral infection, dust mites, and certain nutritional factors during pregnancy have been shown to increase the risk of asthma in offspring.[33,37-40]

The second critical window is during early childhood, especially during the first year of life (during the expansion of alveoli and rebalancing of the immune responses). Thus severe lower respiratory tract viral infections; exposure to airborne environmental irritants (ETS and DEPs), dust mite allergens, and therapeutics (paracetamol); and deficiency in some nutritional elements, such as vitamin D,[33,38,40-45] during infancy or early childhood have been shown to increase childhood asthma risk. In contrast, exposure to dog or cat allergens is associated with protection from later childhood wheeze in some,[46] but not all,[47] cohort studies. Additionally, exclusive breast-feeding for longer than 4 months[48,49] and intake of probiotics that promote beneficial intestinal microbiota[50,51] have modest protective effects against wheeze and asthma. Synergistic effects among allergens/irritants have been observed. For example, exposure during infancy to indoor combustion-related pollutants has been reported to sensitize children to dust mite–induced asthma in later childhood.[52] This type of interaction is worthy of further investigation because most exposures comprise a mixture of allergens or inducers.

Similarly, adult-onset asthma is under early-life influences.[53-55] Respiratory tract infections during infancy are associated with a greater incidence of chronic obstructive lung disease.[56,57] Prenatal active or passive exposure to tobacco smoke[58] and traffic-related exposure to polycyclic aromatic hydrocarbons (PAHs)[59] are associated with low birth weight and very preterm birth, 2 conditions that have positive correlations with adult lung deficiencies.[56,60]

Thus it has become clear that most cases of asthma of both childhood and adult onset originate in early life. What remains elusive is how exposure in early life can permanently change one's susceptibility to asthma throughout life. One proposed mechanism is Barker's hypothesis of developmental plasticity,[36,61] which contends that during early life, in response to an environmental disruption (eg, infection, hyponutrition, and ETS) most bodily organs, through the use of developmental plasticity, can establish an altered phenotype that is expected to better suit the needs of later life. Such responses are longer-term adjustments made by an organ that is based on present guesses about probable future needs. These adjusted phenotypes are usually beneficial to the health of the subject. However, exceptions arise when early guesses do not match later-life demands. A high degree of mismatch between the "adaptive trait" established in early life and demands in later life might increase the risk of disease. In the case of asthma, it has been proposed that exposures to pathogens, metabolic changes, and other environmental factors during prenatal or postnatal life trigger the early airways to undergo a different course of development, resulting in a phenotype of increased sensitivity to allergens or irritants, hyperresponsiveness, and a skewed T_H2 response.[4] These alterations in airway and T_H cell phenotype create a lasting vulnerability to asthma in later life.

The mechanisms underlying environmental reprogramming of the early airway and T-cell phenotype remain unclear. However, a growing body of literature now suggests that the link resides in epigenetics, which is responsible for partitioning and remodeling of the genome into active and inactive domains and creating long-lasting changes in transcriptional programs of the airway and T_H cells that favor asthma pathogenesis.[62-67]

MECHANISMS OF EPIGENETIC REPROGRAMMING

Epigenetics is the study of mitotically heritable changes in phenotype (alterations in gene expression) that occur without direct alterations of the DNA sequence.[68,69] These epigenetic changes include methylation of DNA by the covalent addition of a methyl group to a cytosine residue in a CpG site[70]; posttranslational modification of the amino acid tails of histones by means of acetylation, phosphorylation, methylation, sumoylation, or ubiquitylation[71]; and aberrant expression of microRNAs (miRNAs), each of which is capable of posttranscriptionally regulating the expression of a cohort of cognate target genes.[72] Collectively, these 3 major epigenetic mechanisms affect interactions of DNA with transcriptional factors, transcript stability, DNA folding, nucleosome positioning, chromatin compaction, and higher-order nuclear organization in a manner that determines whether a gene or a set of genes is silenced or activated and when and where a gene will be expressed. They therefore play crucial roles in determining the transcriptional programs of differentiating or differentiated tissues. Before I discuss examples of early-life reprogramming of the airways and related immune responses by environmental agents through epigenetic mechanisms,[4,5,25,73] I will briefly outline how these mechanisms can alter gene expression and hence the phenotype of cells and organs on a long-term basis.

CpG dinucleotides are underrepresented in the mammalian genome (1% to 2%) but tend to cluster as CpG islands in gene promoter regions. Hypermethylation of promoter CpG islands is

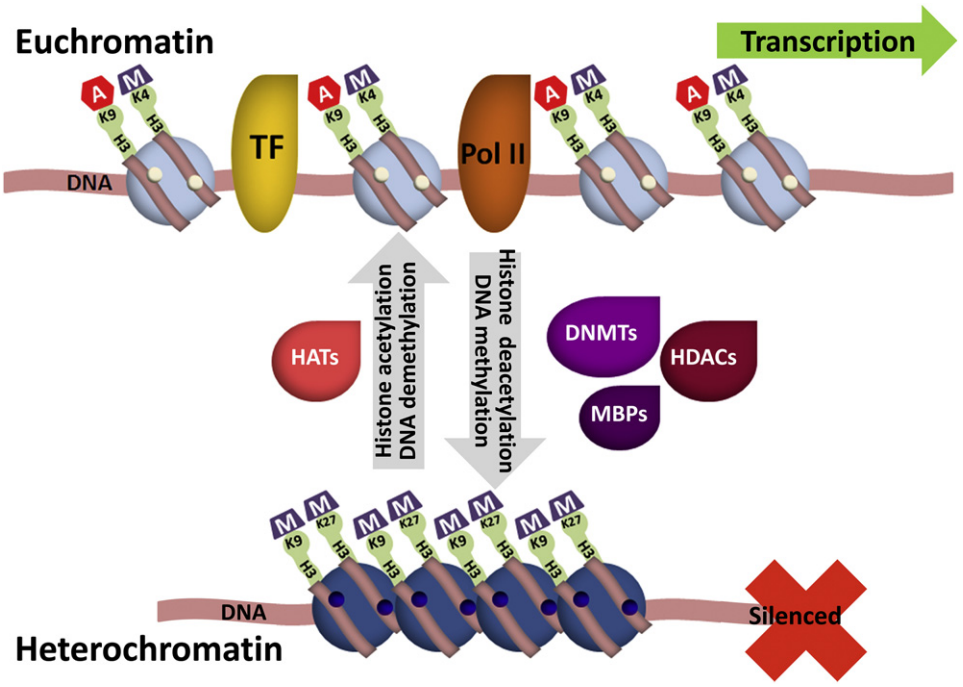

FIG 1. DNA methylation and histone modification collaborate in regulating gene expression. DNA methylation refers to the covalent addition of a methyl group to a cytosine *(C)* residue in a CpG dinucleotide (*solid circles*, methylated cytosine; *open circles*, unmethylated cytosine). The carboxyl ends of histones have specific amino acids that are sensitive to posttranslational modifications. These 2 major epigenetic mechanisms collaborate to package genes in euchromatin (active chromatin) or heterochromatin (silenced chromatin), a packaging that determines whether a gene or a set of genes is silenced or activated. CpG sites are underrepresented in the mammalian genome but tend to cluster as CpG islands (CGIs) in gene promoter regions. Hypermethylation of promoter CGIs is associated with transcriptional silencing *(red X)* because of loss of affinity for transcriptional factors *(TF)* and accessibility by the transcriptional machinery (represented by *Pol II* in this figure). The heterochromatin has increased affinity for methylated DNA-binding proteins *(MBPs)*, which further recruit histone deacetylases (HDACs), DNA methyltransferases (DNMTs), and other corepressors. Methylated promoters are associated with unique repressive histone markers, which classically include trimethylation of histone 3 *(H3)*, lysine *(K)* 9, and H3-K27. Unmethylated promoters are associated with gene activation *(green arrow)*. They have reduced affinity for MBPs, increased affinity for histone acetyltransferases *(HATs)*, and histone marks associated with active chromatin, including acetylated H3-K9 and trimethylated H3-K4. Histone modifications are believed to mediate more rapid responses to environmental influences, whereas DNA methylation provides gene silencing over a longer time frame. *A*, Acetylation; *M*, methylation; *Pol II*, RNA Polymerase II.

commonly associated with transcriptional silencing, possibly because the methylated promoter has reduced affinity for transcriptional factors[74] and increased affinity for methylated DNA-binding proteins (eg, methyl CpG binding protein 2 [MeCP2], methyl-CpG binding domain protein [MBD] 1, MBD2, MBD3, and MBD4), which further recruit histone deacetyltransferases and other corepressors. Methylated promoters also are associated with unique repressive histone markers,[75] which classically include trimethylation of histone 3, lysine 9, and histone 3–lysine 27. Conversely, unmethylated promoters are associated with gene activation, reduced affinity for methylated DNA-binding proteins, and histone marks associated with active chromatin, including acetylated histone 3–lysine 9 and trimethylated histone 3–lysine 4. Histone modifications are believed to mediate more rapid responses to environmental influences,[4,76,77] whereas DNA methylation mediates gene silencing over a longer time frame. Thus the 2 mechanisms work closely in gatekeeping the active and inactive states of a gene or parts of the genome (Fig 1).

DNA methylation requires the activity of DNA methyltransferases (DNMTs). DNMT1 facilitates the replication of the DNA methylation pattern between cell generations (maintenance methylation), and DNMT3a and DNMT3b mediate *de novo* methylation of DNA.[78,79] The mechanism of DNA demethylation is less clear. Loss of binding to methylated DNA-binding proteins might allow the promoter to enter a transcriptional state. However, the association of methylated DNA with MBD2 or MBD4 has been proposed to induce active DNA demethylation, a hypothesis currently under active debate.[80,81]

Histone modifications (marks) are believed to change gene expression by remodeling the chromatin of the promoter, the coding region of target genes, or both. They serve to recruit specific chromatin modeling enzymes (DNMTs and demethylases) and methylated DNA-binding proteins and shift the position of the nucleosomes,[82,83] thus maintaining either an active or an inactive transcriptional environment. They are known to transduce extracellular signals (eg, insulin-like growth factor 1[84]) to activate genomic events. Histone modifications work conjointly with DNA methylation to achieve short- and long-term changes in transcriptional programs through transient or permanent reorganization of the chromatin architecture.[85] Histones are modified

by specific enzymes that include histone acetyltransferases (HAT), histone deacetylases (HDAC), and histone methyltransferases.[86] Their antagonists hold great promise as epigenetic pharmaceuticals.

miRNAs function as posttranscriptional regulators of cognate target gene expression.[72] They are a class of small noncoding RNAs produced from either their own genes or introns/exons of other genes. They bind to target mRNAs with complete or incomplete complementarities, degrade/modify target mRNAs, or both and modulate protein translation.[87] It is now known that one miRNA can target hundreds of mRNAs and that one mRNA can be regulated by different miRNAs. Thus although the field is still in an early stage of development, it has great potential to reveal a new level of epigenetic regulation.

EPIGENETICS REGULATES THE IMMUNE RESPONSES ASSOCIATED WITH ASTHMA

Epigenetics is now recognized as a key mechanism underlying the establishment and maintenance of the T_H2 bias in asthmatic patients.[5,88] Exposure to allergens induces an immune response that triggers the differentiation of a naive T_H cell into a T_H2 cell, expressing the cytokines IL-4, IL-5, and IL-13, which are responsible for the allergic response.[89] Loss of DNA methylation and increased association with activating histone marks conjointly establish and maintain a euchromatin configuration at the T_H2 locus of T_H2 cells, allowing recruitment of the transcriptional machinery to this region for a rapid and coordinated expression of the T_H2 cytokines. The early response is marked by rapid increases in *IL4* expression because the GATA-3 transcriptional factor binding sites within the first intron of the gene loses CpG methylation and the *IL4* locus gains histone 3–lysine 9 acetylation and trimethylation of histone 3–lysine 4.[90-93] With lineage commitment, additional demethylation occurs in the 5′ end of the gene, which is essential for sustaining a high level of IL-4 expression.[91] In parallel, the expression of IFN-γ in T_H2 cells is silenced by repressive histone marks[92] and increased promoter CpG methylation.[94,95] In contrast, T_H1 differentiation is associated with methylation of the 3′ end of the *IL4* locus.[91] Furthermore, T_H2 polarization is associated with loss of IFN-γ expression, which is thought to be mediated by methylation of specific CpGs in its promoter region.[94,95] Specifically, methylation of CpG^{-53}, an activator protein 1–binding site in the proximal promoter of *IFNG*, results in inhibition of cAMP-responsive element binding protein 1 (CREB) and activating transcription factor 2 (ATF2)/c-Jun binding to this *cis*-regulatory element and sustained gene silencing.[95,96] Hence mounting evidence suggests that the development of a polarized T_H2 phenotype is a result of major chromatin remodeling brought about by multiple, coregulatory epigenetic changes on genes regulating T_H differentiation.

Moreover, the T_H1/T_H2 ratio is exquisitely sensitive to histone acetylation/deacetylation regulation.[73] In this regard inhibition of endogenous HDAC activity with trichostatin A (TSA) can shift recall responses toward a more T_H2-like phenotype by changing the T_H1/T_H2 ratios 3- to 8-fold and increasing T_H2-associated (IL-13, 139%; IL-5, 168%) and reducing T_H1-associated (IFN-γ, 76%; CXCL10, 47%) recall responses.[97] Of significance to glucocorticoid-resistant asthma, upregulation of class II HDACs restores steroid responsiveness in the airways.[98] Treatment with inhibitors for both class I and II HDACs, but not those only effective for class I enzymes, induces $Foxp3^+$ production and boosts the suppressive function of $Foxp3^+$ Treg cells on T_H2-mediated allergic response.[99] In addition to T_H2 polarization, a recent study has shown that human Treg cells can differentiate into T_H17 cells through epigenetic plasticity that can be modulated by histone/protein deacetylase activity.[100] It has been noted that neutrophilic asthma might involve T_H17 polarization.[101] Taken together, these findings have significant clinical ramifications because new antiasthma strategies seeking to target specific HATs/HDACs might have great utility in the future management of asthma.[102]

Finally, emerging evidence suggests that miRNAs are involved in the pathogenesis of immunologic diseases, including asthma.[103] A single nucleotide polymorphism at the 3′ untranslated region of *HLA-G*, an asthma-susceptibility gene,[104] was shown to be a putative target site for 3 miRNAs: miR-148a, miR-148b, and miR-152.[105] A recent study demonstrated that the inflammatory airway of a lung-specific *IL13* transgenic mouse overexpressed miR-21 and underexpressed miR-1.[106] It also revealed that *IL-12p35*, a predicted target of miR-21 and a cytokine germane to T_H cell polarization, was significantly downregulated in the murine inflamed airway. In human bronchial epithelial cells *mi*R-146a expression was found to be upregulated in response to TGF-β1 plus cytomix (a mixture of IL-1β, IFN-γ, and TNF-α)–induced apoptosis and, it was also found that a mimic for this miR can upregulate Bcl-XL and signal transducer and activator of transcription 3 (an acute-phase response factor) phosphorylation, improve human bronchial epithelial cell survival, and contribute to tissue repair and remodeling.[107] Furthermore, selective knockdown of miR-126 expression was shown to suppress the asthmatic phenotype, resulting in diminished T_H2 response, inflammation, airways hyperresponsiveness, eosinophil recruitment, and mucus hypersecretion.[108] At the molecular level, downregulation of miR-126 inhibited T_H2 polarization by increasing the expression of POU domain class 2–associating factor 1, which activates the transcription factor PU.1, leading to loss of GATA-3 expression. These new findings support the notion that miRNA-based oligonucleotide therapies will be an emerging class of antiasthma regimens.

In aggregate, multiple epigenetic mechanisms regulate a handful of asthma-related genes known to initiate and maintain the asthma phenotype and its symptoms. Table I[73,90-92,94-96,97,99,105-108] summarizes these genes and their relevance to the disorder.

ENVIRONMENTAL FACTORS EXERT EPIGENETIC INFLUENCES ON ASTHMA

Recent findings regarding the regulation of multiple aspects of asthma pathogenesis by epigenetics raise the fundamental question about whether environmental influences on asthma risk or its manifestations are mediated through similar epigenetic changes found to contribute to this disorder. Current knowledge of the effects of environmental agents found to be epigenetically active and to contribute to the pathogenesis of asthma is summarized below and in Figure 2 and Table II.[108-135]

Tobacco smoke

Exposure to tobacco smoke represents a major risk factor for the development of asthma.[136,137] Enhanced sensitization to allergens has been observed in human subjects and laboratory animals exposed to tobacco smoke. Early-life exposures clearly increase asthma risk in later life.[138] The epigenetic action of tobacco

TABLE I. Asthma-related genes known to be regulated by epigenetic mechanisms

Gene	Mechanism of epigenetic regulation	Relevance to asthma	References
IL4	Demethylation of an intronic sequence that binds GATA-3	Increases IL-4 secretion in T_H lymphocytes	90
IL4	Increase in H3-K9 acetylation and H3-K4 trimethylation	Increases lineage commitment of precursor T_H cells to T_H2 cells	92
IL4	Extensive demethylation of the 5′ flanking region of the *IL4* promoter	Sustains high levels of IL-4 secretion from T_H2 cells	91
IL4	Methylation of the 3′ end of the *IL4* locus	Promotes differentiation of precursor T_H cells into T_H1 cells	91
IFNG	Methylation of an activator protein 1–binding site in the proximal promoter resulting in reduced CREB and ATF2/c-Jun binding to this site	Associated with the loss of gene expression and the establishment of a T_H2 polarization phenotype	94-96
IL13, IL5	Increased histone acetylation	Increases T_H2-associated cytokine expression	73
IFNG, CXCL10	Increased histone acetylation	Inhibits T_H1-associated recall responses and expression of these cytokines	97
FOXP3+	Class II HDAC inhibitors	Increases $Foxp3^+$ expression and enhances the suppressive function of $Foxp3^+$ Treg cells on T_H2 response	99
HLA-G	miR-148a, miR-148b, and miR-152	Targets a single nucleotide polymorphism at the 3′ untranslated region of the gene	105
IL13	miR-21, miR-1	Overexpressed in *IL13* transgenic mice	106
IL-12p35	miR-21	Downregulates gene expression in murine inflamed airway	106
TGFB	miR-146a	Might mediate TGF-β plus cytomix–induced apoptosis	107
POU domain class 2 associating factor 1	miR-126	Increases expression of the transcription factor	108

ATF2, Activating transcription factor 2; *CREB*, cAMP-responsive element binding protein 1.

smoke can be direct or indirect through the induction of oxidative stress.

One epigenetic action of tobacco smoke is mediated through the disruption of HAT/HDAC homeostasis in immune cells of the airways. A recent study comparing biopsy specimens and bronchoalveolar lavage alveolar macrophages from healthy nonsmoking subjects and age-matched healthy tobacco smokers found that tobacco smoke suppressed HDAC2 expression and overall HDAC activity and enhanced expression of inflammatory mediators, such as GM-CSF, IL-8, and IL-1β–induced TNF-α.[109] Importantly, tobacco smoke markedly attenuated dexamethasone inhibition of cytokine release in these cells and hence might cause steroid resistance. Treatment of the macrophages with the HDAC inhibitor TSA reversed the proinflammatory changes and glucocorticoid responsiveness in the macrophages, supporting the possible usefulness of this class of drug as an adjuvant for asthma treatment. Because the treatment of a macrophage cell line with hydrogen peroxide mimicked the effects of tobacco smoke on HDAC activity and glucocorticoid responsiveness, it has been suggested that part of the action of tobacco smoke can be mediated through the induction of oxidative stress. Because macrophages function to fine tune allergen-induced airway inflammation (see above), an epigenetic disruption of their function likely contributes to asthma and other airway diseases.

In addition to modulating HAT/HDAC activities, tobacco smoke can exert epigenetic action through alteration of DNA methylation status in gene promoters or regulatory sequences.

Multiple studies have shown that tobacco smoke induces promoter hypermethylation of p16 (cyclin-dependent kinase inhibitor 2A [melanoma, p16, inhibits CDK4; INK4a]), a purported tumor suppressor involved in cell-cycle regulation in non–small cell lung cancer cells.[110-112]

Other lung cancer–related genes whose methylation status can be affected by smoking include cytochrome P450, family 1, subfamily A, polypeptide 1 *(CYP1A1)*[113]; Ras association (RalGDS/AF-6) domain family member 1 *(RASSF1A)*[114]; and fragile histidine triad gene *(FHIT)*.[115] However, it remains to be determined whether these epigenetic changes are the result of exposure to tobacco smoke or are pathological changes associated with carcinogenesis.

A more direct piece of evidence linking tobacco smoke and DNA methylation can be found in a recent study that reported hypomethylation of the monoamine oxidase type B *(MAOB)* promoter in PBMCs of smokers (former and current) compared with nonsmokers.[116] Moreover, the degree of methylation of the *MAOB* promoter was inversely correlated with platelet expression of MAO-B protein. Of significance to our understanding of the long-term effect of epigenetic changes, hypomethylation of the *MAOB* promoter persisted long after (>10 years) the subjects in this study had stopped smoking.

DNA methylation might also be an epigenetic mechanism that can explain the lifelong effect of exposure to tobacco smoke *in utero* on asthma risk.[139,140] Breton et al[117] recently examined DNA methylation status in buccal cells from a cohort of children born to mothers who did or did not smoke during pregnancy. Children exposed to maternal smoking had lower methylation of the *AluYb8* repeat element, indicating global DNA hypomethylation. In addition, they also identified, using a CpG loci screen, differential methylation of 8 genes between those children exposed and not exposed *in utero* and validated the hypermethylation of 2 genes, AXL receptor tyrosine kinase *(AXL)* and protein tyrosine phosphatase, receptor type, O *(PTPRO)*, in the exposed children. *AXL* is a receptor tyrosine kinase that promotes antiapoptosis, mitogenesis, invasion, and cell survival,[141] whereas *PTPRO* is a protein tyrosine phosphatase receptor involved in differentiation and axonogenesis of central and peripheral nervous system neurons during gestation.[142] At this point, it is unclear how these genes function to alter asthma risk.

However, of special interest to the concept of gene-environment interaction, differences in smoking-related effects on long

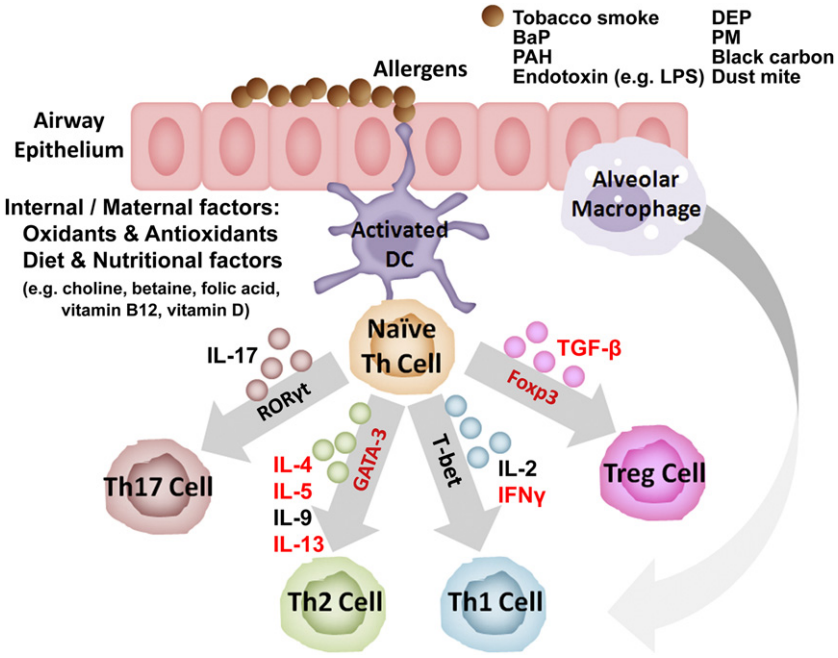

FIG 2. Environmental factor–induced immune cell regulation of allergic airway responses. Inhaled allergens derived from environmental factors, such as tobacco smoke, PAHs, endotoxin, DEPs, PM, and dust mites, in the immature or leaky airways are sampled by DCs. The allergen-activated DCs serve to prime the naive $CD4^+$ T cells to differentiate into proinflammatory T_H2 cells instead of the infection-fighting T_H1 cells in the T-cell repertoire. The progressive increase in the commitment of $CD4^+$ T cells toward a T_H2 phenotype is driven by T_H2 cytokines, such as IL-4, IL-5, IL-9, and IL-13, and heightened expression of GATA-3. In parallel, the T_H2 cells shut off the expression of IFN-γ and other T_H1 cytokines, such as IL-2. In patients with neutrophilic corticosteroid-resistant asthma, T_H17 differentiation is increased. TGF-β–driven naive $CD4^+$ T cells differentiating into $Foxp3^+$ Treg cells confer immune tolerance and dampen allergic responses. Alveolar macrophages play a dual role in pathogen/allergen elimination and suppression of the responses for airway repair and remodeling. Allergen-triggered oxidative stress, dietary methyl donors, and nutritional factors, such as vitamin D, modulate these immune/airway reprogramming events. Cytokines and transcriptional factors colored red are known to be modulated by epigenetic events. Retinoic acid receptor–related orphan receptor γt *(RORγt)*, GATA-3, T-box transcription factor *(T-bet)*, and Foxp3 are transcriptional factors promoting the differentiation of the respective T cells.

interspersed repetitive element-1 (*LINE1*) methylation were observed only in children with the common glutathione-S-transferase mu 1 *(GSTM1)* null genotype, thus suggesting that variations in genotype involved in the metabolism of tobacco smoke can interact with epigenetics to alter asthma risks in children born to mothers who smoked during pregnancy.

PAHs

PAHs are one of the most widespread classes of pollutants of the environment and in food.[143] They are present in crude oil, coal, and tar deposits and are derived from incomplete combustion of fossil fuel, oil, garbage, and cigarettes. They are major components of airborne particulate matter (PM) of urban aerosols and are widely present in food products, including grains, vegetables, oils, and fats. PAHs are emitted into the air during the production of coke and aluminum. Cooked meats are contaminated when they are charcoal grilled, roasted, or smoked. Among the PAHs, benzopyrene (BaP) is often used as a prototype PAH for many experimental studies.

The association of asthma with particulate air pollutants, DEPs, the World Trade Center disaster, maternal smoking, and exposure to ETS, coke manufacturing, and firefighting is well documented[144-150] and might well be related to the PAH component of these environmental toxicants/pollutants. However, the evidence that indicates that PAHs are a major contributing factor of asthma is just emerging. This scarcity of information is due in part to the lack of mechanistic studies and accurate biomarkers of exposure.

Using a restriction enzyme–based microarray approach, Sadikovic and Rodenhiser[118] reported that BaP induced hypomethylation of a number of genomic repeats and sequence-specific hypomethylation and hypermethylation changes in 4 breast cancer cell lines. The investigators were able to correlate some of these changes to cell growth and the p53 status of the cell lines. Unfortunately, they subsequently discovered that this array approach was compromised by the ability of BaP to form adducts at CpG dinucleotides, thus inhibiting restriction enzyme activities and PCR amplification.[151] They then turned to investigating the effect of BaP on H3K9 acetylation at a genome-wide level in the MCF-7 breast cancer cell line and found that BaP induces hypoacetylation and hyperacetylation in genes belonging to networks regulating gene expression, DNA replication and repair, and carcinogenesis.[119] Within these networks are genes involved in the organization and remodeling of chromatin, including *MTA3, HDAC1, ATRX, MBD2,* and *MBD3*. These findings are in agreement with previous studies reporting that BaP can decrease global DNA methylation,[120] inhibit DNMTs *in vitro*,[121] and

TABLE II. Environmental factors known to lead to epigenetic changes that influence the asthma phenotype

Environmental factors	Epigenetic effects	Relevance to asthma	References
Tobacco smoke	Suppresses HDAC2 expression and overall HDAC activity in macrophages	Enhances the expression of inflammatory mediators (GM-CSF, IL-8, IL-1β, and TNF-α)	109
Tobacco smoke	Induces hypermethylation of the promoter of *p16; CYP1A1*, *RASSF1A*, and *FHIT* in lung cancer cells	Relevance in asthma unknown	110-115
Tobacco smoke	Induces *MAOB* promoter hypomethylation in PBMCs	Might serve as a biomarker of smoking-induced asthma	116
Maternal tobacco smoke	Induces global DNA hypomethylation (*AluYb8* but not *LINE1*) and *AXL* and *PTPRO* promoter hypermethylation in children	Might serve as biomarkers of *in utero* exposure	117
BaP	Induces hypomethylation of a number of genomic repeats and sequence-specific hypomethylation and hypermethylation changes in breast cancer cells	Relevance to asthma unclear	118
BaP	Induces H3K9 acetylation at the genome level, leading to hypoacetylation and hyperacetylation in genes belonging to networks regulating gene expression, DNA replication and repair, and carcinogenesis (including *ATRX*, *MBD2*, *MBD3*, *HDAC1*, and *MTA3*)	Relevance to asthma not known	119
BaP	Decreases global DNA methylation, inhibits DNMTs *in vitro*, and interferes with recruitment of methylation machinery	Might affect expression of asthma-related genes	120-123
Maternal PAH exposure from traffic pollution	Increased maternal exposure associated with increased hypermethylation of the *ACSL3* promoter in umbilical cord blood DNA of offspring	Hypermethylation of *ACSL3* promoter in umbilical cord blood associated with increased asthma risk in childhood	124
Oxidants	Posttranslationally modifies the HDACs and creates HAT/HDAC stoichiometry imbalance	Contribute to the enhancement of IL-1β–stimulated inflammatory cytokine production (eg, IL-8, IL-6, CXCL1, CXCL2, and CXCL3) in the inflamed airways	125,126
LPS	Might be an miRNA-146a target	Contributes to LPS priming	127
LPS	Drives TLR signaling through Akt1-regulated expression of let-7e and miR-155	Contributes to macrophage hypersensitivity and endotoxin tolerance	128
Inhaled DEPs	Induce hypermethylation at specific CpGs of the *IFNG* promoter and hypomethylation at the *IL4* promoter in splenic $CD4^+$ cells	Hypersensitize mice to intranasal *Aspergillus fumigatus* exposure	129
PM-10	Increases HAT activity and acetylated histone 4; remodels the *IL8* promoter; action mediated through the induction of oxidative stress	Increases IL-8 expression and release from human alveolar basal epithelial cells	130
Exposure of elderly to ambient black carbon but not PM2.5 for 4 to 7 d	Induces hypomethylation of *LINE1*	Might exacerbate asthma in this population	131
Methyl donors and coenzymes	Affects DNMT activities and prevents aberrant global hypomethylation of the genome	Deficiencies in methyl donors predisposes to complex diseases, including asthma	132,133
Maternal diet rich in methyl donors	Favors lymphocyte maturation into a T_H2 phenotype	Increases the risk of allergic airway disease in offspring	134
Maternal folic acid supplementation	Increases the risk of wheeze and lower respiratory tract infections in progeny up to 18 mo of age	Explains developmental reprogramming of asthma risk	135
Dust mite antigens	Induce expression of miRNA-126 and activates TLR4	Increase inflammation, a T_H2 response, airway hyperresponsiveness through suppression of GATA-3	108

CYP1A1, Cytochrome P450, family 1, subfamily A, polypeptide 1; *FHIT*, fragile histidine triad gene; *H*, histone; *K*, lysine; *RASSF1A*, Ras association (RalGDS/AF-6) domain family member 1.

interfere with recruitment of the methylation machinery.[122,123] Although these studies have firmly established an epigenetic effect for BaP, their direct relevance to asthma remains debatable.

In a recent study[124] we identified, using an unbiased screening method, a novel epigenetic marker for PAH-associated asthma. Hypermethylation of the acyl-CoA synthetase long-chain family member 3 *(ACSL3)* promoter in umbilical cord white blood cells of children born to mothers with variable but well-documented levels of PAH exposure was highly correlated with increased maternal exposure and risk of asthma symptoms before age 5 years. *ACSL3* belongs to the acyl-CoA synthetase long-chain (ACSL) family of genes that encode key enzymes in fatty acid metabolism.[152] It is expressed in lung and thymus tissue.[153,154] Thus hypermethylation of this gene in T_H cells or lung tissues is expected to diminish fatty acid use and β-oxidation energy production and possibly influence the phospholipid composition of the

membranes. Interestingly, *ACSL3* is located in 2q36.1, which has recently been shown to be associated with regions of the asthma-susceptibility loci in specific populations.[155,156]

Finally, 2 CpG-rich regions in the promoter of INF-γ were found to undergo hypermethylation when human airway smooth muscle cells or lung cancer cells were exposed to BaP. *INFG* promoter in umbilical cord white blood cell DNA was found to associate positively with maternal PAH exposure and increased risk of childhood asthma (Ho, unpublished data). Because silencing of *INFG* is directly linked to the development of T_H2 polarization, these findings should provide a new angle to the investigation of the environmental genetics of asthma.

Microbial infection, inflammation, and oxidative stress

Both epidemiological and experimental studies have shown that microbial exposure in early life can protect against asthma but that exposure in later life predisposes to the disorder.[157-160] These contradicting outcomes could be explained by multiple mechanisms, including developmental plasticity altered during early life by epigenetic events. The first of such mechanisms might be related to the well-documented fact that infections promote the generation of oxidants[161,162] and proinflammatory mediators[163] in the airways. These intermediates in turn can exert epigenetic modifications on transcriptional programs of cytokines. In this regard damages by oxidants are known to trigger methylation. The formation of hydroxymethylcytosine as a result of oxidative stress or the generation of halogenated cytosines as a result of the release of hypochlorous acid from neutrophils or of hypobromous acid from eosinophils can lead to methylation.[164] Thus an increase in oxidants could promote cytosine methylation-mediated gene silencing that might have long-lasting effects.

Oxidants and proinflammatory mediators also regulate histone acetylation/deacetylation balance in the airways.[125,126] H_2O_2 can alter the histone acetylation and deacetylation balance through posttranslational modification of HDACs. An imbalance in HAT/HDAC stoichiometry contributes to the enhancement of IL-1β–stimulated inflammatory cytokine production (IL-8, IL-6, CXCL1, CXCL2, and CXCL3) in the inflamed airways. Modifications in the histone marks associated with gene loci of these cytokines can produce long-lasting epigenetic effects in their transcriptional programs.

A second explanation might be related to the biphasic nature of the response of the innate immune system to endotoxin released from bacterial cells. Prior exposure of innate immune cells like monocytes/macrophages to small amounts of endotoxin causes them to become refractory to subsequent challenges by endotoxin, a phenomenon known as endotoxin tolerance. This might explain why endotoxin exposure is associated with protection from asthma in some studies[39,125,165] but with the development or exacerbation of asthma in others.[166] An important mechanism underlying this endotoxin tolerance is epigenetic reprogramming of IL-1β–mediated TNF-α release in these immune cells. Exposure to endotoxin or LPS induces chromatin remodeling of the proinflammatory gene *IL1B* promoter nucleosome and epigenetic gene silencing of *TNFA* that involves aberrant retention of the heterochromatin-binding protein 1α, altered histone modifications, and loss of nuclear factor κ light polypeptide gene enhancer in B-cells 1 (NF-κB) RelA/p65 binding to its promoter.[167-169] A recent study further reported upregulation of miRNA-146a as a plausible mechanism of LPS priming.[127] Another study demonstrated that Akt1-regulated expression of let-7e and miR-155 might be responsible for tuning the LPS-driven Toll-like receptor (TLR) signaling in macrophage sensitivity and tolerance to endotoxin.[128]

In summary, the relationship between microbial exposure and asthma is complex; the intricate interplays among infection, inflammation, oxidative stress, and endotoxin tolerance likely involve multiple levels of epigenetic regulation.

PM, DEPs, and other outdoor pollutants

Epidemiological studies have shown that PM, DEPs, and other outdoor airborne pollutants are associated with adverse respiratory health effects, including asthma.[2,170,171] Several of these have been shown to exert their actions through epigenetics.

DEPs are one of the major components of PM. In a murine asthma model a 3-week exposure to inhaled DEPs was found to hypersensitized mice to intranasal exposure to *Aspergillus fumigatus*. The combined treatment increased IgE production and induced hypermethylation at CpG(−45), CpG(−53), and CpG(−205) sites of the *IFNG* promoter and hypomethylation at CpG(−408) of the *IL4* promoter in DNA from splenic $CD4^+$ cells.[129]

DEPs or PM can also exert their action in the airways through the induction of oxidative stress.[172] Treatment of A549 cells (adenocarcinomic human alveolar basal epithelial cells) with either PM-10 or H_2O_2 increased IL-8 expression and release, which was augmented by cotreatment with TSA, an HDAC inhibitor, but blocked by cotreatment with an antioxidant. Both PM-10 and H_2O_2 treatment increased HAT activity and the level of acetylated histone 4 and remodeled the *IL8* promoter region. These data suggest that the action of PM-10 is mediated by oxidative stress, which in turn triggers histone acetylation–induced remodeling of the chromatin associated with cytokine release in the lungs.[130]

Baccarelli et al[131] found that increased exposure of elderly participants (718) to ambient particulate pollutants for a short duration (4 hours to 7 days) was associated with DNA hypomethylation of *LINE1*, but not *Alu*, repetitive elements in their blood DNA samples. Interestingly, black carbon, but not PM2.5, showed this association. These findings lay the groundwork for future investigation of whether these global methylation changes or alterations in specific genes are linked to exposure-related health outcomes.

Diet and nutritional factors

In mammalian cells, during mitosis, the maintenance of the fidelity of the methylation pattern in the newly synthesized DNA strand is dependent on the availability of diet-derived methyl donors and cofactors required for the synthesis of S-adenosylmethionine. The concentration of S-adenosylmethionine affects DNMT activities and prevents aberrant global hypomethylation of the genome, which could be a cause of congenital diseases and aging.[173] In agouti mice a deficiency in methyl donors or their coenzymes, such as choline, betaine, folic acid, and vitamin B12, *in utero* predisposed the offspring to many complex diseases.[132,133] However, the evidence demonstrating that nutritional factors can directly influence epigenetic programming of T cells and airway tissues is still limited.

A recent report found that exposure of pregnant mice to a diet rich in methyl donors favored lymphocyte maturation into a T_H2 phenotype and increased the risk of allergic airway disease in the offspring.[134] The maternal diet induced methylation changes in 82-gene loci in the offspring. Among these genes, Runt-related transcription factor 3 (*Runx3*), a gene known to suppress allergic airway disease, was found to be hypermethylated, along with concordant transcriptional silencing of *Runx3* in progeny. These findings demonstrate that dietary factors can modify asthma risk through epigenetic mechanisms during a susceptible period of developmental reprogramming.[3,4] They are in agreement with findings from a large-scale cohort study, the Norwegian Mother and Child Cohort Study (>32,000 children), which showed that maternal folic acid supplementation increased the risk of wheeze and lower respiratory tract infections in progeny up to 18 months of age.[135] In aggregate, these findings call into question the safety of supplementing maternal diets with methyl donors or their coenzymes.

A growing body of evidence now suggests a protective effect of vitamin D against asthma,[174-176] but little is known about whether its action is mediated through epigenetics. This line of investigation should prove promising in the future because a combination of vitamin D with an epigenetic therapy might be highly effective.

Dust mites and other indoor allergens

An emerging concept for a mechanism potentially causing asthma is that the innate immune system inappropriately senses allergens as foreign and dangerous and responds with a programmed adaptive T_H2 immune response. TLRs differentially sense microbial and viral bioproducts and act as sentinels for the activation of innate host defense pathways. LPS, a cell-wall component of gram-negative bacteria, activates cells through TLR4 and the common TLR adaptor protein myeloid differentiation primary response gene 88, resulting in activation of transcription and proinflammatory pathways. LPS is also a prominent constituent of asthma-inducing house dust mite allergens and can instruct the immune response to inhaled antigen to generate T_H2 responses.

TLRs act as sentinels for activating innate host defense in response to inhaled antigens and play a pivotal role in programming a T_H2 immune response. Exposure to house dust mite antigens activates TLR4 and increases the expression of a unique set of miRNAs that includes miRNA-16, miRNA-21, and miRNA-126.[108] Selective blockade of miRNA-126 leads to amelioration of asthma symptoms and a diminished T_H2 response, inflammation, and airway hyperresponsiveness through an miR-126–mediated suppression of GATA-3 expression. These data open the door for future asthma therapies based on miRNAs or their antagomirs.

The major indoor allergens include arthropod allergens, animal dander mammalian allergens (from pets or pests), and fungal allergens. Nevertheless, no information is available on their epigenetic action in the airways or asthma-related immune systems. Future research on how indoor allergens program airways and the immune system through epigenetics is of critical importance because modern living involves spending nearly 90% of time indoors.

WHAT ARE THE GAPS IN THE DATA?

First, can we identify unique and specific epigenetic marks that are linked to each allergen or environmental inducers of asthma? Can these epigenetic changes be developed into exposure biomarkers or disease predictors? Can epigenetic biomarkers with high sensitivities and specificities for an environmental factor be used for formulating regulatory policies? How much overlap do environmental epigenetic biomarkers have among different classes of asthma inducers or triggers? Can environmental genetics contribute to our fundamental understanding of asthma's etiology?

Second, when are the critical developmental periods of airway and immune cell programming by environmental factors for childhood and adult asthma? How long will the epigenetic memories last, and are they reversible by later-life events, including removal of the environmental inducers, the use of epigenetic disruptors (eg, dietary methyl donors), and epigenetic therapeutics, including HDAC inhibitors and miRNA antagomirs?

Third, once an environmental inducer is removed, will its presumed long-lasting epigenetic action gradually disappear? Can this reversal be accelerated through the adoption of lifestyle changes, treatment with targeted therapies, or both? In this regard the permanency of early-life programming and the effectiveness of later-life modifiers need to be understood.

Fourth, how can environmental epigenetics explain cosensitization between 2 or more classes of allergens? Can it explain remission, tolerance, and treatment resistance? More importantly, can it be used to predict individual or population-based variability to susceptibility or treatment? In this regard the identification of susceptible subjects or populations by means of epigenotyping will provide new measures for disease surveillance, prevention, and management. Furthermore, identification of the environmental culprit for a subject's asthma could lead to personalized management of the disease. If this can be extended to exposed populations, such as schoolchildren, the elimination of the irritant or allergen in their environment will have significant public health ramifications.

Fifth, can epigenetic marks in surrogate tissues, such as buccal cells, cord blood, amniocentesis fluid, and skin cells, be used to predict the pathophysiological changes in the target tissues, such as the airway and the immune cells? This question is critically important for advancing epidemiologic studies in large cohorts, especially those studying childhood asthma.

I thank Nancy K. Voynow for her professional editorial assistance; Ethan Chung, Yuk Yin Cheung, PhD, and Xiang Zhang, PhD, for their invaluable assistance in preparing the manuscript; and Randall Goldblum, MD, PhD, and Terumi Midoro-Horiuti, PhD, for helpful critiques of the manuscript.

REFERENCES

1. Vercelli D. Advances in asthma and allergy genetics in 2007. J Allergy Clin Immunol 2008;122:267-71.
2. Peden D, Reed CE. Environmental and occupational allergies. J Allergy Clin Immunol 2010;125(suppl 2):S150-60.
3. Vercelli D. Genetics, epigenetics, and the environment: switching, buffering, releasing. J Allergy Clin Immunol 2004;113:381-6.
4. Miller RL, Ho SM. Environmental epigenetics and asthma: current concepts and call for studies. Am J Respir Crit Care Med 2008;177:567-73.
5. Kumar RK, Hitchins MP, Foster PS. Epigenetic changes in childhood asthma. Dis Model Mech 2009;2:549-53.
6. Lemanske RF Jr, Busse WW. Asthma: clinical expression and molecular mechanisms. J Allergy Clin Immunol 2010;125(suppl 2):S95-102.
7. Eder W, Ege MJ, von Mutius E. The asthma epidemic. N Engl J Med 2006;355:2226-35.
8. Moorman JE, Rudd RA, Johnson CA, King M, Minor P, Bailey C, et al. National surveillance for asthma—United States, 1980-2004. MMWR Surveill Summ 2007;56:1-54.

9. World Health Organization. Bronchial asthma. 2000. WHO fact sheet no. 206. Available at: https://apps.who.int/inf-fs/en/fact206.html. Accessed July 27, 2010.
10. Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). Eur Respir J 1998;12: 315-35.
11. Busse WW, Mitchell H. Addressing issues of asthma in inner-city children. J Allergy Clin Immunol 2007;119:43-9.
12. Ray A, Khare A, Krishnamoorthy N, Qi Z, Ray P. Regulatory T cells in many flavors control asthma. Mucosal Immunol 2010;3:216-29.
13. Hammad H, Lambrecht BN. Recent progress in the biology of airway dendritic cells and implications for understanding the regulation of asthmatic inflammation. J Allergy Clin Immunol 2006;118:331-6.
14. Barnes PJ. New molecular targets for the treatment of neutrophilic diseases. J Allergy Clin Immunol 2007;119:1055-62.
15. Nouri-Aria KT. Foxp3 expressing regulatory T-cells in allergic disease. Adv Exp Med Biol 2009;665:180-94.
16. Kuipers H, Lambrecht BN. The interplay of dendritic cells, Th2 cells and regulatory T cells in asthma. Curr Opin Immunol 2004;16:702-8.
17. Hammad H, Lambrecht BN. Dendritic cells and epithelial cells: linking innate and adaptive immunity in asthma. Nat Rev Immunol 2008;8:193-204.
18. Takizawa H, Beckmann JD, Shoji S, Claassen LR, Ertl RF, Linder J, et al. Pulmonary macrophages can stimulate cell growth of bovine bronchial epithelial cells. Am J Respir Cell Mol Biol 1990;2:245-55.
19. Peters-Golden M. The alveolar macrophage: the forgotten cell in asthma. Am J Respir Cell Mol Biol 2004;31:3-7.
20. Gilliland FD, Berhane K, Rappaport EB, Thomas DC, Avol E, Gauderman WJ, et al. The effects of ambient air pollution on school absenteeism due to respiratory illnesses. Epidemiology 2001;12:43-54.
21. Los H, Postmus PE, Boomsma DI. Asthma genetics and intermediate phenotypes: a review from twin studies. Twin Res 2001;4:81-93.
22. Weiss ST, Raby BA, Rogers A. Asthma genetics and genomics 2009. Curr Opin Genet Dev 2009;19:279-82.
23. Boezen HM. Genome-wide association studies: what do they teach us about asthma and chronic obstructive pulmonary disease? Proc Am Thorac Soc 2009; 6:701-3.
24. London SJ, Romieu I. Gene by environment interaction in asthma. Annu Rev Public Health 2009;30:55-80.
25. Adcock IM, Tsaprouni L, Bhavsar P, Ito K. Epigenetic regulation of airway inflammation. Curr Opin Immunol 2007;19:694-700.
26. Wong GW, Chow CM. Childhood asthma epidemiology: insights from comparative studies of rural and urban populations. Pediatr Pulmonol 2008;43:107-16.
27. Sly PD, Boner AL, Bjorksten B, Bush A, Custovic A, Eigenmann PA, et al. Early identification of atopy in the prediction of persistent asthma in children. Lancet 2008;372:1100-6.
28. Strachan DP. Family size, infection and atopy: the first decade of the "hygiene hypothesis." Thorax 2000;55(suppl 1):S2-10.
29. Martinez FD. The coming-of-age of the hygiene hypothesis. Respir Res 2001;2: 129-32.
30. Maziak W. The asthma epidemic and our artificial habitats. BMC Pulm Med 2005;5:5.
31. von Mutius E. Gene-environment interactions in asthma. J Allergy Clin Immunol 2009;123:3-11.
32. Nuttall SL, Langford NJ, Kendall MJ. Frequent paracetamol use linked to asthma. Lancet 2000;355:1648-9.
33. Kim JH, Ellwood PE, Asher MI. Diet and asthma: looking back, moving forward. Respir Res 2009;10:49.
34. Beach J, Russell K, Blitz S, Hooton N, Spooner C, Lemiere C, et al. A systematic review of the diagnosis of occupational asthma. Chest 2007;131:569-78.
35. Dykewicz MS. Occupational asthma: current concepts in pathogenesis, diagnosis, and management. J Allergy Clin Immunol 2009;123:519-28.
36. Barker DJ. A new model for the origins of chronic disease. Med Health Care Philos 2001;4:31-5.
37. Patel MM, Miller RL. Air pollution and childhood asthma: recent advances and future directions. Curr Opin Pediatr 2009;21:235-42.
38. Dietert RR, Zelikoff JT. Early-life environment, developmental immunotoxicology, and the risk of pediatric allergic disease including asthma. Birth Defects Res B Dev Reprod Toxicol 2008;83:547-60.
39. Finch CE, Crimmins EM. Inflammatory exposure and historical changes in human life-spans. Science 2004;305:1736-9.
40. Sporik R, Holgate ST, Platts-Mills TA, Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. N Engl J Med 1990;323:502-7.
41. Litonjua AA, Weiss ST. Is vitamin D deficiency to blame for the asthma epidemic? J Allergy Clin Immunol 2007;120:1031-5.
42. Kuehni CE, Spycher BD, Silverman M. Causal links between RSV infection and asthma: no clear answers to an old question. Am J Respir Crit Care Med 2009; 179:1079-80.
43. Wu P, Dupont WD, Griffin MR, Carroll KN, Mitchel EF, Gebretsadik T, et al. Evidence of a causal role of winter virus infection during infancy in early childhood asthma. Am J Respir Crit Care Med 2008;178:1123-9.
44. Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson F, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. Am J Respir Crit Care Med 2005;171:137-41.
45. Carroll KN, Wu P, Gebretsadik T, Griffin MR, Dupont WD, Mitchel EF, et al. The severity-dependent relationship of infant bronchiolitis on the risk and morbidity of early childhood asthma. J Allergy Clin Immunol 2009;123:1055-61.
46. Remes ST, Castro-Rodriguez JA, Holberg CJ, Martinez FD, Wright AL. Dog exposure in infancy decreases the subsequent risk of frequent wheeze but not of atopy. J Allergy Clin Immunol 2001;108:509-15.
47. Lau S, Illi S, Platts-Mills TA, Riposo D, Nickel R, Gruber C, et al. Longitudinal study on the relationship between cat allergen and endotoxin exposure, sensitization, cat-specific IgG and development of asthma in childhood—report of the German Multicentre Allergy Study (MAS 90). Allergy 2005;60:766-73.
48. Kull I, Almqvist C, Lilja G, Pershagen G, Wickman M. Breast-feeding reduces the risk of asthma during the first 4 years of life. J Allergy Clin Immunol 2004;114:755-60.
49. Elliott L, Henderson J, Northstone K, Chiu GY, Dunson D, London SJ. Prospective study of breast-feeding in relation to wheeze, atopy, and bronchial hyperresponsiveness in the Avon Longitudinal Study of Parents and Children (ALSPAC). J Allergy Clin Immunol 2008;122:49-54.
50. Bjorksten B. Effects of intestinal microflora and the environment on the development of asthma and allergy. Springer Semin Immunopathol 2004;25:257-70.
51. Kalliomaki M, Salminen S, Arvilommi H, Kero P, Koskinen P, Isolauri E. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. Lancet 2001;357:1076-9.
52. Ponsonby AL, Dwyer T, Kemp A, Couper D, Cochrane J, Carmichael A. A prospective study of the association between home gas appliance use during infancy and subsequent dust mite sensitization and lung function in childhood. Clin Exp Allergy 2001;31:1544-52.
53. Stern DA, Morgan WJ, Halonen M, Wright AL, Martinez FD. Wheezing and bronchial hyper-responsiveness in early childhood as predictors of newly diagnosed asthma in early adulthood: a longitudinal birth-cohort study. Lancet 2008;372:1058-64.
54. Orfei L, Strachan DP, Rudnicka AR, Wadsworth ME. Early influences on adult lung function in two national British cohorts. Arch Dis Child 2008;93:570-4.
55. Lawlor DA, Ebrahim S, Davey SG. Association of birth weight with adult lung function: findings from the British Women's Heart and Health Study and a meta-analysis. Thorax 2005;60:851-8.
56. Barker DJ, Godfrey KM, Fall C, Osmond C, Winter PD, Shaheen SO. Relation of birth weight and childhood respiratory infection to adult lung function and death from chronic obstructive airways disease. BMJ 1991;303:671-5.
57. Edwards CA, Osman LM, Godden DJ, Campbell DM, Douglas JG. Relationship between birth weight and adult lung function: controlling for maternal factors. Thorax 2003;58:1061-5.
58. Windham GC, Hopkins B, Fenster L, Swan SH. Prenatal active or passive tobacco smoke exposure and the risk of preterm delivery or low birth weight. Epidemiology 2000;11:427-33.
59. Perera FP, Rauh V, Tsai WY, Kinney P, Camann D, Barr D, et al. Effects of transplacental exposure to environmental pollutants on birth outcomes in a multiethnic population. Environ Health Perspect 2003;111:201-5.
60. Shaheen SO, Barker DJ, Shiell AW, Crocker FJ, Wield GA, Holgate ST. The relationship between pneumonia in early childhood and impaired lung function in late adult life. Am J Respir Crit Care Med 1994;149:616-9.
61. Bateson P, Barker D, Clutton-Brock T, Deb D, D'Udine B, Foley RA, et al. Developmental plasticity and human health. Nature 2004;430:419-21.
62. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33(suppl):245-54.
63. Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. Nat Rev Genet 2007;8:253-62.
64. Ling C, Groop L. Epigenetics: a molecular link between environmental factors and type 2 diabetes. Diabetes 2009;58:2718-25.
65. Tang WY, Ho SM. Epigenetic reprogramming and imprinting in origins of disease. Rev Endocr Metab Disord 2007;8:173-82.
66. Vaag A, Poulsen P. Twins in metabolic and diabetes research: what do they tell us? Curr Opin Clin Nutr Metab Care 2007;10:591-6.
67. Cook JD, Davis BJ, Cai SL, Barrett JC, Conti CJ, Walker CL. Interaction between genetic susceptibility and early-life environmental exposure determines tumor-suppressor-gene penetrance. Proc Natl Acad Sci U S A 2005;102:8644-9.

68. Goldberg AD, Allis CD, Bernstein E. Epigenetics: a landscape takes shape. Cell 2007;128:635-8.
69. Bird A. Perceptions of epigenetics. Nature 2007;447:396-8.
70. Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. Trends Biochem Sci 2006;31:89-97.
71. Kouzarides T. Chromatin modifications and their function. Cell 2007;128:693-705.
72. Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A. Identification of mammalian microRNA host genes and transcription units. Genome Res 2004;14:1902-10.
73. Bhavsar P, Ahmad T, Adcock IM. The role of histone deacetylases in asthma and allergic diseases. J Allergy Clin Immunol 2008;121:580-4.
74. Bogdanovic O, Veenstra GJ. DNA methylation and methyl-CpG binding proteins: developmental requirements and function. Chromosoma 2009;118:549-65.
75. Tiwari VK, McGarvey KM, Licchesi JD, Ohm JE, Herman JG, Schubeler D, et al. PcG proteins, DNA methylation, and gene repression by chromatin looping. PLoS Biol 2008;6:2911-27.
76. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, et al. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 2006;125:315-26.
77. Locksley RM. Nine lives: plasticity among T helper cell subsets. J Exp Med 2009;206:1643-6.
78. Hermann A, Gowher H, Jeltsch A. Biochemistry and biology of mammalian DNA methyltransferases. Cell Mol Life Sci 2004;61:2571-87.
79. Siedlecki P, Zielenkiewicz P. Mammalian DNA methyltransferases. Acta Biochim Pol 2006;53:245-56.
80. Lal G, Bromberg JS. Epigenetic mechanisms of regulation of Foxp3 expression. Blood 2009;114:3727-35.
81. Patra SK, Bettuzzi S. Epigenetic DNA-(cytosine-5-carbon) modifications: 5-aza-2'-deoxycytidine and DNA-demethylation. Biochemistry 2009;74:613-9.
82. Clapier CR, Cairns BR. The biology of chromatin remodeling complexes. Annu Rev Biochem 2009;78:273-304.
83. Cheung P, Allis CD, Sassone-Corsi P. Signaling to chromatin through histone modifications. Cell 2000;103:263-71.
84. Sun LY, D'Ercole AJ. Insulin-like growth factor-I stimulates histone H3 and H4 acetylation in the brain in vivo. Endocrinology 2006;147:5480-90.
85. Kondo Y. Epigenetic cross-talk between DNA methylation and histone modifications in human cancers. Yonsei Med J 2009;50:455-63.
86. Miremadi A, Oestergaard MZ, Pharoah PD, Caldas C. Cancer genetics of epigenetic genes. Hum Mol Genet 2007;16(Spec No 1):R28-49.
87. Cannell IG, Kong YW, Bushell M. How do microRNAs regulate gene expression? Biochem Soc Trans 2008;36:1224-31.
88. van PN, Le GG, McConnell MJ. Epigenetic regulation of Th2 cytokine expression in atopic diseases. Tissue Antigens 2008;72:91-7.
89. Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. Blood 2008;112:1557-69.
90. Tykocinski LO, Hajkova P, Chang HD, Stamm T, Sozeri O, Lohning M, et al. A critical control element for interleukin-4 memory expression in T helper lymphocytes. J Biol Chem 2005;280:28177-85.
91. Lee DU, Agarwal S, Rao A. Th2 lineage commitment and efficient IL-4 production involves extended demethylation of the IL-4 gene. Immunity 2002;16:649-60.
92. Fields PE, Kim ST, Flavell RA. Cutting edge: changes in histone acetylation at the IL-4 and IFN-gamma loci accompany Th1/Th2 differentiation. J Immunol 2002;169:647-50.
93. Wei G, Wei L, Zhu J, Zang C, Hu-Li J, Yao Z, et al. Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. Immunity 2009;30:155-67.
94. White GP, Watt PM, Holt BJ, Holt PG. Differential patterns of methylation of the IFN-gamma promoter at CpG and non-CpG sites underlie differences in IFN-gamma gene expression between human neonatal and adult CD. J Immunol 2002;168:2820-7.
95. Jones B, Chen J. Inhibition of IFN-gamma transcription by site-specific methylation during T helper cell development. EMBO J 2006;25:2443-52.
96. Young HA, Ghosh P, Ye J, Lederer J, Lichtman A, Gerard JR, et al. Differentiation of the T helper phenotypes by analysis of the methylation state of the IFN-gamma gene. J Immunol 1994;153:3603-10.
97. Su RC, Becker AB, Kozyrskyj AL, Hayglass KT. Epigenetic regulation of established human type 1 versus type 2 cytokine responses. J Allergy Clin Immunol 2008;121:57-63.
98. Ito K, Chung KF, Adcock IM. Update on glucocorticoid action and resistance. J Allergy Clin Immunol 2006;117:522-43.
99. Wang L, Tao R, Hancock WW. Using histone deacetylase inhibitors to enhance Foxp3(+) regulatory T-cell function and induce allograft tolerance. Immunol Cell Biol 2009;87:195-202.
100. Koenen HJ, Smeets RL, Vink PM, van RE, Boots AM, Joosten I. Human CD25highFoxp3pos regulatory T cells differentiate into IL-17-producing cells. Blood 2008;112:2340-52.
101. Schaub B, Liu J, Schleich I, Hoppler S, Sattler C, von Mutius E. Impairment of T helper and T regulatory cell responses at birth. Allergy 2008;63:1438-47.
102. Szyf M. Epigenetics, DNA methylation, and chromatin modifying drugs. Annu Rev Pharmacol Toxicol 2009;49:243-63.
103. Pauley KM, Chan EK. MicroRNAs and their emerging roles in immunology. Ann N Y Acad Sci 2008;1143:226-39.
104. Hunt JS, Petroff MG, McIntire RH, Ober C. HLA-G and immune tolerance in pregnancy. FASEB J 2005;19:681-93.
105. Tan Z, Randall G, Fan J, Camoretti-Mercado B, Brockman-Schneider R, Pan L, et al. Allele-specific targeting of microRNAs to HLA-G and risk of asthma. Am J Hum Genet 2007;81:829-34.
106. Ou TX, Munitz A, Rothenberg ME. MicroRNA-21 is up-regulated in allergic airway inflammation and regulates IL-12p35 expression. J Immunol 2009;182:4994-5002.
107. Liu X, Nelson A, Wang X, Kanaji N, Kim M, Sato T, et al. MicroRNA-146a modulates human bronchial epithelial cell survival in response to the cytokine-induced apoptosis. Biochem Biophys Res Commun 2009;380:177-82.
108. Mattes J, Collison A, Plank M, Phipps S, Foster PS. Antagonism of microRNA-126 suppresses the effector function of TH2 cells and the development of allergic airways disease. Proc Natl Acad Sci U S A 2009;106:18704-9.
109. Ito K, Lim S, Caramori G, Chung KF, Barnes PJ, Adcock IM. Cigarette smoking reduces histone deacetylase 2 expression, enhances cytokine expression, and inhibits glucocorticoid actions in alveolar macrophages. FASEB J 2001;15:1110-2.
110. Yanagawa N, Tamura G, Oizumi H, Takahashi N, Shimazaki Y, Motoyama T. Frequent epigenetic silencing of the p16 gene in non-small cell lung cancers of tobacco smokers. Jpn J Cancer Res 2002;93:1107-13.
111. Kim DH, Nelson HH, Wiencke JK, Zheng S, Christiani DC, Wain JC, et al. p16(INK4a) and histology-specific methylation of CpG islands by exposure to tobacco smoke in non-small cell lung cancer. Cancer Res 2001;61:3419-24.
112. Digel W, Lubbert M. DNA methylation disturbances as novel therapeutic target in lung cancer: preclinical and clinical results. Crit Rev Oncol Hematol 2005;55:1-11.
113. Anttila S, Hakkola J, Tuominen P, Elovaara E, Husgafvel-Pursiainen K, Karjalainen A, et al. Methylation of cytochrome P4501A1 promoter in the lung is associated with tobacco smoking. Cancer Res 2003;63:8623-8.
114. Kim DH, Kim JS, Ji YI, Shim YM, Kim H, Han J, et al. Hypermethylation of RASSF1A promoter is associated with the age at starting smoking and a poor prognosis in primary non-small cell lung cancer. Cancer Res 2003;63:3743-6.
115. Kim JS, Kim H, Shim YM, Han J, Park J, Kim DH. Aberrant methylation of the FHIT gene in chronic smokers with early stage squamous cell carcinoma of the lung. Carcinogenesis 2004;25:2165-71.
116. Launay JM, Del PM, Chironi G, Callebert J, Peoc'h K, Megnien JL, et al. Smoking induces long-lasting effects through a monoamine-oxidase epigenetic regulation. PLoS One 2009;4:e7959.
117. Breton CV, Byun HM, Wenten M, Pan F, Yang A, Gilliland FD. Prenatal tobacco smoke exposure affects global and gene-specific DNA methylation. Am J Respir Crit Care Med 2009;180:462-7.
118. Sadikovic B, Rodenhiser DI. Benzopyrene exposure disrupts DNA methylation and growth dynamics in breast cancer cells. Toxicol Appl Pharmacol 2006;216:458-68.
119. Sadikovic B, Andrews J, Carter D, Robinson J, Rodenhiser DI. Genome-wide H3K9 histone acetylation profiles are altered in benzopyrene-treated MCF7 breast cancer cells. J Biol Chem 2008;283:4051-60.
120. Wilson VL, Jones PA. Inhibition of DNA methylation by chemical carcinogens in vitro. Cell 1983;32:239-46.
121. Wilson VL, Jones PA. Chemical carcinogen-mediated decreases in DNA 5-methylcytosine content of BALB/3T3 cells. Carcinogenesis 1984;5:1027-31.
122. Weisenberger DJ, Romano LJ. Cytosine methylation in a CpG sequence leads to enhanced reactivity with benzo[a]pyrene diol epoxide that correlates with a conformational change. J Biol Chem 1999;274:23948-55.
123. Zhang N, Lin C, Huang X, Kolbanovskiy A, Hingerty BE, Amin S, et al. Methylation of cytosine at C5 in a CpG sequence context causes a conformational switch of a benzo[a]pyrene diol epoxide-N2-guanine adduct in DNA from a minor groove alignment to intercalation with base displacement. J Mol Biol 2005;346:951-65.
124. Perera F, Tang WY, Herbstman J, Tang D, Levin L, Miller R, et al. Relation of DNA methylation of 5'-CpG island of ACSL3 to transplacental exposure to airborne polycyclic aromatic hydrocarbons and childhood asthma. PLoS One 2009;4:e4488.
125. Rahman I. Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung diseases. J Biochem Mol Biol 2003;36:95-109.

126. Biswas S, Rahman I. Modulation of steroid activity in chronic inflammation: a novel anti-inflammatory role for curcumin. Mol Nutr Food Res 2008;52:987-94.
127. Nahid MA, Pauley KM, Satoh M, Chan EK. miR-146a is critical for endotoxin-induced tolerance: implication in innate immunity. J Biol Chem 2009;284:34590-9.
128. Androulidaki A, Iliopoulos D, Arranz A, Doxaki C, Schworer S, Zacharioudaki V, et al. The kinase Akt1 controls macrophage response to lipopolysaccharide by regulating microRNAs. Immunity 2009;31:220-31.
129. Liu J, Ballaney M, Al-alem U, Quan C, Jin X, Perera F, et al. Combined inhaled diesel exhaust particles and allergen exposure alter methylation of T helper genes and IgE production in vivo. Toxicol Sci 2008;102:76-81.
130. Gilmour PS, Rahman I, Donaldson K, MacNee W. Histone acetylation regulates epithelial IL-8 release mediated by oxidative stress from environmental particles. Am J Physiol Lung Cell Mol Physiol 2003;284:L533-40.
131. Baccarelli A, Wright RO, Bollati V, Tarantini L, Litonjua AA, Suh HH, et al. Rapid DNA methylation changes after exposure to traffic particles. Am J Respir Crit Care Med 2009;179:572-8.
132. Cooney CA, Dave AA, Wolff GL. Maternal methyl supplements in mice affect epigenetic variation and DNA methylation of offspring. J Nutr 2002; 132(suppl):2393S-400S.
133. Waterland RA, Jirtle RL. Transposable elements: targets for early nutritional effects on epigenetic gene regulation. Mol Cell Biol 2003;23:5293-300.
134. Hollingsworth JW, Maruoka S, Boon K, Garantziotis S, Li Z, Tomfohr J, et al. In utero supplementation with methyl donors enhances allergic airway disease in mice. J Clin Invest 2008;118:3462-9.
135. Haberg SE, London SJ, Stigum H, Nafstad P, Nystad W. Folic acid supplements in pregnancy and early childhood respiratory health. Arch Dis Child 2009;94:180-4.
136. Robays LJ, Maes T, Joos GF, Vermaelen KY. Between a cough and a wheeze: dendritic cells at the nexus of tobacco smoke-induced allergic airway sensitization. Mucosal Immunol 2009;2:206-19.
137. McLeish AC, Zvolensky MJ. Asthma and cigarette smoking: a review of the empirical literature. J Asthma 2010;47:345-61.
138. Doherty SP, Grabowski J, Hoffman C, Ng SP, Zelikoff JT. Early life insult from cigarette smoke may be predictive of chronic diseases later in life. Biomarkers 2009;14(suppl 1):97-101.
139. Carlsen KH, Lodrup Carlsen KC. Parental smoking and childhood asthma: clinical implications. Treat Respir Med 2005;4:337-46.
140. Li YF, Langholz B, Salam MT, Gilliland FD. Maternal and grandmaternal smoking patterns are associated with early childhood asthma. Chest 2005;127:1232-41.
141. Shankar SL, O'Guin K, Cammer M, McMorris FA, Stitt TN, Basch RS, et al. The growth arrest-specific gene product Gas6 promotes the survival of human oligodendrocytes via a phosphatidylinositol 3-kinase-dependent pathway. J Neurosci 2003;23:4208-18.
142. Stepanek L, Stoker AW, Stoeckli E, Bixby JL. Receptor tyrosine phosphatases guide vertebrate motor axons during development. J Neurosci 2005;25:3813-23.
143. Simon R, Gomez Ruiz JA, von HC, Wenzl T, Anklam E. Results of a European inter-laboratory comparison study on the determination of EU priority polycyclic aromatic hydrocarbons (PAHs) in edible vegetable oils. Anal Bioanal Chem 2008; 391:1397-408.
144. Bakirtas A. Acute effects of passive smoking on asthma in childhood. Inflamm Allergy Drug Targets 2009;8:353-8.
145. Delfino RJ. Epidemiologic evidence for asthma and exposure to air toxics: linkages between occupational, indoor, and community air pollution research. Environ Health Perspect 2002;110(suppl 4):573-89.
146. Li N, Hao M, Phalen RF, Hinds WC, Nel AE. Particulate air pollutants and asthma. A paradigm for the role of oxidative stress in PM-induced adverse health effects. Clin Immunol 2003;109:250-65.
147. Landrigan PJ, Lioy PJ, Thurston G, Berkowitz G, Chen LC, Chillrud SN, et al. Health and environmental consequences of the world trade center disaster. Environ Health Perspect 2004;112:731-9.
148. Millman A, Tang D, Perera FP. Air pollution threatens the health of children in China. Pediatrics 2008;122:620-8.
149. Lillienberg L, Zock JP, Kromhout H, Plana E, Jarvis D, Toren K, et al. A population-based study on welding exposures at work and respiratory symptoms. Ann Occup Hyg 2008;52:107-15.
150. Ribeiro M, de Paula SU, Bussacos MA, Terra-Filho M. Prevalence and risk of asthma symptoms among firefighters in Sao Paulo, Brazil: a population-based study. Am J Ind Med 2009;52:261-9.
151. Sadikovic B, Andrews J, Rodenhiser DI. DNA methylation analysis using CpG microarrays is impaired in benzopyrene exposed cells. Toxicol Appl Pharmacol 2007;225:300-9.
152. Mashek DG, Bornfeldt KE, Coleman RA, Berger J, Bernlohr DA, Black P, et al. Revised nomenclature for the mammalian long-chain acyl-CoA synthetase gene family. J Lipid Res 2004;45:1958-61.
153. Minekura H, Kang MJ, Inagaki Y, Suzuki H, Sato H, Fujino T, et al. Genomic organization and transcription units of the human acyl-CoA synthetase 3 gene. Gene 2001;278:185-92.
154. Fujino T, Kang MJ, Suzuki H, Iijima H, Yamamoto T. Molecular characterization and expression of rat acyl-CoA synthetase 3. J Biol Chem 1996;271: 16748-52.
155. Bouzigon E, Siroux V, Dizier MH, Lemainque A, Pison C, Lathrop M, et al. Scores of asthma and asthma severity reveal new regions of linkage in EGEA study families. Eur Respir J 2007;30:253-9.
156. Choudhry S, Taub M, Mei R, Rodriguez-Santana J, Rodriguez-Cintron W, Shriver MD, et al. Genome-wide screen for asthma in Puerto Ricans: evidence for association with 5q23 region. Hum Genet 2008;123:455-68.
157. Reed CE, Milton DK. Endotoxin-stimulated innate immunity: a contributing factor for asthma. J Allergy Clin Immunol 2001;108:157-66.
158. Braun-Fahrlander C. Environmental exposure to endotoxin and other microbial products and the decreased risk of childhood atopy: evaluating developments since April 2002. Curr Opin Allergy Clin Immunol 2003;3:325-9.
159. Macaubas C, DeKruyff RH, Umetsu DT. Respiratory tolerance in the protection against asthma. Curr Drug Targets Inflamm Allergy 2003;2:175-86.
160. Belderbos M, Levy O, Bont L. Neonatal innate immunity in allergy development. Curr Opin Pediatr 2009;21:762-9.
161. Giri DK, Mehta RT, Kansal RG, Aggarwal BB. Mycobacterium avium-intracellulare complex activates nuclear transcription factor-kappaB in different cell types through reactive oxygen intermediates. J Immunol 1998;161:4834-41.
162. Schweizer M, Peterhans E. Oxidative stress in cells infected with bovine viral diarrhoea virus: a crucial step in the induction of apoptosis. J Gen Virol 1999;80: 1147-55.
163. Backdahl L, Bushell A, Beck S. Inflammatory signalling as mediator of epigenetic modulation in tissue-specific chronic inflammation. Int J Biochem Cell Biol 2009;41:176-84.
164. Valinluck V, Sowers LC. Endogenous cytosine damage products alter the site selectivity of human DNA maintenance methyltransferase DNMT1. Cancer Res 2007;67:946-50.
165. Braun-Fahrlander C, Riedler J, Herz U, Eder W, Waser M, Grize L, et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. N Engl J Med 2002;347:869-77.
166. Douwes J, Pearce N, Heederik D. Does environmental endotoxin exposure prevent asthma? Thorax 2002;57:86-90.
167. Chan C, Li L, McCall CE, Yoza BK. Endotoxin tolerance disrupts chromatin remodeling and NF-kappaB transactivation at the IL-1beta promoter. J Immunol 2005;175:461-8.
168. Biswas SK, Lopez-Collazo E. Endotoxin tolerance: new mechanisms, molecules and clinical significance. Trends Immunol 2009;30:475-87.
169. Foster SL, Hargreaves DC, Medzhitov R. Gene-specific control of inflammation by TLR-induced chromatin modifications. Nature 2007;447:972-8.
170. Damato G, Cecchi L, D'Amato M, Liccardi G. Urban air pollution and climate change as environmental risk factors of respiratory allergy: an update. J Investig Allergol Clin Immunol 2010;20:95-102.
171. Gilmour MI, Jaakkola MS, London SJ, Nel AE, Rogers CA. How exposure to environmental tobacco smoke, outdoor air pollutants, and increased pollen burdens influences the incidence of asthma. Environ Health Perspect 2006;114: 627-33.
172. Ito T, Okumura H, Tsukue N, Kobayashi T, Honda K, Sekizawa K. Effect of diesel exhaust particles on mRNA expression of viral and bacterial receptors in rat lung epithelial L2 cells. Toxicol Lett 2006;165:66-70.
173. Gonzalgo ML, Jones PA. Rapid quantitation of methylation differences at specific sites using methylation-sensitive single nucleotide primer extension (Ms-SNuPE). Nucleic Acids Res 1997;25:2529-31.
174. Gorman S, McGlade JP, Lambert MJ, Strickland DH, Thomas JA, Hart PH. UV exposure and protection against allergic airways disease. Photochem Photobiol Sci 2010;9:571-7.
175. Dimeloe S, Nanzer A, Ryanna K, Hawrylowicz C. Regulatory T cells, inflammation and the allergic response-The role of glucocorticoids and Vitamin D. J Steroid Biochem Mol Biol 2010;120:86-95.
176. Brehm JM, Celedon JC, Soto-Quiros ME, Avila L, Hunninghake GM, Forno E, et al. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. Am J Respir Crit Care Med 2009;179:765-71.