The environment, epigenome, and asthma

Ivana V. Yang, PhD,[a,b,c] Catherine A. Lozupone, PhD,[a] and David A. Schwartz, MD[a,b,d] *Aurora and Denver, Colo*

Asthma prevalence has been on the increase, especially in North America compared with other continents. However, the prevalence of asthma differs worldwide, and in many countries the prevalence is stable or decreasing. This highlights the influence of environmental exposures, such as allergens, air pollution, and the environmental microbiome, on disease etiology and pathogenesis. The epigenome might provide the unifying mechanism that translates the influence of environmental exposures to changes in gene expression, respiratory epithelial function, and immune cell skewing that are hallmarks of asthma. In this review we will introduce the concept of the environmental epigenome in asthmatic patients, summarize previous publications of relevance to this field, and discuss future directions. (J Allergy Clin Immunol 2017;140:14-23.)

Key words: *Asthma, DNA methylation, epigenetics, nasal epithelium, microbiome, air pollution, allergens*

Abbreviations used
DMR: Differentially methylated region
GF: Germ-free
GWAS: Genome-wide association study
OVA: Ovalbumin
PAH: Polyaromatic hydrocarbon
PM: Particulate matter
Treg: Regulatory T
ZPS: Zwitterionic coat polysaccharide

Asthma remains an important public health problem in the United States and worldwide because of high morbidity and inadequate disease control. Recent data from the International Study of Asthma and Allergies in Childhood (ISAAC) demonstrate geographic differences in asthma prevalence, suggesting that the environment and ***epigenetics*** play a key role in patients with this disease.[1] Although asthma is more problematic in children, this disease also affects the health of adults. The dynamic and unique biological responses triggered by allergens and air pollutants have proved difficult to predict and prevent. This review will focus on the epigenetic mechanisms that regulate the response to environmental exposures and are critical to primary and secondary prevention of asthma.

ETIOLOGY OF ASTHMA

Although inheritance,[2-5] parent-of-origin inheritance,[6-8] the general environment,[9-17] immunization,[18] *in utero* exposures,[19-24] and T_H2 immunity[25] play important roles in the etiology of asthma, there is no unifying mechanism accounting for these etiologic events.

Asthma concordance in monozygotic twins is only approximately 50%,[26] and the heritability of this disease is 0.40 to 0.85.[27,28] Initial ***genome-wide association studies (GWASs)*** identified 3 putative susceptibility loci (5q22.1 near *TMEM232*, 11q13.5 near *C11orf30*, and the HLA region) associated with asthma risk,[3,29] but the scope of these studies was limited. Moffatt et al[30] reported that genetic variants of *ORMDL3* contribute to the risk of childhood asthma. Recently, a meta-analysis of GWASs identified 7 asthma genetic risk loci (*HLA-DQ*, *IL33*, *ORMDL3/GSDMB*, *IL1RL1/IL18R1*, *IL2RB*, *SMAD3*, and *TSLP*[31,32]) and 10 loci near *TLR6*, C11orf30, *STAT6*, *SLC25A46*, *HLA-DQB1*, *IL1RL1*, *LPP*, *MYC*, *IL2*, and *HLA-B* that influence allergic sensitization.[33] In aggregate, approximately 50 replicated genes have been identified from association studies, several genes by using linkage and fine mapping, and 1 gene by means of a GWAS. However, the effect estimates are modest (odds ratio, 0.5-1.5), and it has been estimated that these gene variants predict less than 10% of the heritability of asthma.[34]

ALLERGENS AND ASTHMA

Allergic sensitization is a critical risk factor for childhood asthma, conferring a 4- to 20-fold increase in the risk of having the disease.[10,35] Both indoor (molds, house dust mites, cockroaches, rodents, and pets) and outdoor (pollens from trees, grass, and weeds) allergens are critical environmental triggers of asthma.[36-38] Seminal studies in this field have clearly demonstrated a role of the environment in asthma development. Exposure to house dust mite, cat, and dog allergen early in life and continuing throughout childhood determined the course of chronic airway hyperresponsiveness and impairment of lung function[35]; sensitization to dog, cat, or ***Alternaria*** species was associated with increased bronchial responsiveness in children with mild-to-moderate asthma[39]; children who were both allergic to cockroach and exposed to high levels of this allergen had significantly more asthma-related hospitalizations, days of wheezing, missed school days, and nights with lost sleep[40]; and

From [a]the Department of Medicine, University of Colorado, School of Medicine, Aurora; [b]National Jewish Health, Denver; [c]the Department of Epidemiology, Colorado School of Public Health, University of Colorado, Aurora; and [d]the Department of Immunology, University of Colorado.

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Corresponding author: Ivana V. Yang, PhD, University of Colorado School of Medicine, 12700 East 19th Ave, 8611, Aurora, CO 80045. E-mail: ivana.yang@ucdenver.edu.

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Terms in boldface and italics are defined in the glossary on page 15.

mouse sensitization/exposure was associated with acute care visits, decreased lung function, fraction of exhaled nitric oxide levels, and bronchodilator reversibility.[41] Pet allergens might also be protective in some settings, although evidence for this is mixed.[36,42]

AIR POLLUTION AND MICROBIAL FACTORS AND THE SEVERITY OF ASTHMA

Air pollutants are known to exacerbate asthma symptoms and might also play a role in initiation of this disease.[43-45] In most urban areas, and increasingly in suburban areas, components of traffic-related emissions are a major source of air pollution. However, air pollution represents a complex exposure with inorganic and organic components. Particulate matter (PM) carries both environmental pollutants, such as polycyclic aromatic hydrocarbons (PAHs) formed during incomplete combustion of fossil fuels and oil products,[46] and immune-stimulating agents, such as pollens, endotoxin, and fungal spores. PAHs are widespread environmental contaminants formed as a result of incomplete combustion of organic materials and are a particularly toxic component of air pollution.[47,48] PAHs in PM, such as phenanthrene or other components of diesel exhaust, can also directly enhance IgE production *in vitro*.[49] Endotoxin, a structural component of membranes of gram-negative bacteria, is ubiquitous in urban and suburban environments in homes/day care centers/schools[50-53] and is a component of air pollution.[54,55] In addition to endotoxin, children who grow up in rural and inner-city areas are exposed to many other aerosolized microorganisms and their toxins.[56,57] Interestingly, germ-free (GF) mice are more prone to a T_H2 phenotype and allergic airway disease, and this risk can be reduced by exposure to a diverse microbial flora.[58] Moreover, monocolonization with the common intestinal bacterium *Bacteroides fragilis* restores T_H2/T_H1 balance by using a mechanism dependent on stimulation of T_H1 $CD4^+$ T-cell proliferation by a zwitterionic (neutral molecule with positive and negative charges) coat polysaccharide (ZPS) that directly activates $CD4^+$ T cells[59,60] and can also be potent regulatory T (Treg) cell inducers.[61] ZPSs have been characterized in commensals from multiple body sites, including *Streptococcus pneumoniae* in the upper respiratory tract, and ZPSs from different organisms have similar biological properties and can display immune cross-reactivity.[59,62,63] Interestingly, the prevalence of *Staphylococcus aureus* (which produces ZPS and is present in bedding/household dust) was inversely associated with asthma.[64,65]

INTRODUCTION TO EPIGENETICS

Epigenetic mechanisms control expression levels of genes without changing DNA sequence. Hypermethylation of cytosines within CpG islands in gene ***promoters*** leads to gene silencing, and hypomethylation leads to active transcription.[66,67] More recent studies have demonstrated that methylation of less CpG-dense regions near islands ("shores")[68,69] and within gene bodies[70,71] is also important in regulation of gene expression and alternative

GLOSSARY

ALTERNARIA: Genus of ascomycete fungi that are known to be plant pathogens and common allergens in human subjects and can lead to allergic hypersensitivity and asthma.

CpG SITES: DNA regions in which a cytosine nucleotide occurs next to a guanine nucleotide separated by only 1 phosphate. CpG islands are regions with a high frequency of CpG sites. Methylation of the cytosine within a gene can turn the gene off.

DNA METHYLATION: A process by which methyl groups are added to the DNA molecule, which can change the activity of a DNA segment without changing the sequence. When located in a gene promoter, DNA methylation typically acts to repress gene transcription.

ENHANCER: A region of DNA approximately 50 to 1500 bp long that can be bound by certain proteins called activators or transcription factors to increase the likelihood of transcription of that particular gene.

EPIGENETICS: Changes in a chromosome that affect gene activity and expression. Epigenetics can describe a heritable phenotypic change that does not derive from a modification of the genome. These changes can result from external or environmental factors or be part of normal development. Common mechanisms that produce such changes are DNA methylation and histone modification, each of which changes how genes are expressed without changing the underlying DNA sequence.

GENOME-WIDE ASSOCIATION STUDY (GWAS): Study of a genome-wide set of genetic variants in different subjects to identify associations with a trait or disease. A GWAS is also referred to as a whole-genome association study (WGAS).

HISTONE ACETYLATION: Process by which the lysine residues within the N-terminal tail protruding from the histone core of the nucleosome are acetylated and deacetylated as an essential part of gene regulation. These processes are often catalyzed by the enzymes histone acetyltransferase or histone deacetylase. Acetylation removes the positive charge on histones, thereby reducing the interaction of the N-termini of histones with the negatively charged phosphate groups of DNA. The outcome is a more relaxed chromatin structure and increased gene transcription.

IFNG: Gene that encodes the cytokine protein IFN-γ, which is a cytokine secreted by cells of the innate and adaptive immune systems and is essential for immunity against viral, bacterial, and protozoal infections. IFN-γ is an important activator of macrophages and inducer of class II MHC molecule expression. IFN-γ is produced predominantly by natural killer and natural killer T cells as part of the innate immune response and by T_H1 and cytotoxic T lymphocyte effector T cells once antigen-specific immunity develops.

INVARIANT NATURAL KILLER T (iNKT) CELLS: Specialized subset of T cells that use their T-cell receptors (TCRs) to recognize self-antigens and foreign lipid antigens. iNKT cells are a subset of CD1d-dependent NKT cells that express an invariant $TCR\alpha$ chain. They are notable for their ability to respond rapidly to produce proinflammatory cytokines and engage in effector functions.

PROMOTER: Region of DNA approximately 100 to 1000 bp long located near the transcription start sites of genes that initiates transcription of that gene.

TRANSCRIPTOME: Set of all mRNA molecules that reflect the genes that are actively expressed at any given time in one cell or a population of cells. Unlike the fixed genome, the transcriptome can be altered by external environmental conditions.

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