Epigenetics mediate environment : gene effects on occupational sensitization

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Purpose of review
Epigenetics is the study of stable modifications of fixed genomes that direct which genes are expressed and which are silenced. Epigenetic changes are modulated by environmental exposures, making epigenetics the interface between genes and environment. This has particular relevance in understanding the effect of occupational exposures on the expression of allergic disease. The goal of this review is to describe how epigenetic changes affect transcription potential, and to examine more closely the effect of specific environmental and occupational exposures on epigenetic variations that alter allergy gene transcripts and the inflammatory milieu.

Recent findings
Gene transcription is activated when specific CpG sites are demethylated and histones are acetylated, and, conversely, silenced when sites are methylated and histones deacetylated. The development of Th1 and Th2 phenotypes, and expression of Treg cells, are now known to be modulated by epigenetic mechanisms. Workplace exposures such as tobacco smoke, particulates, diesel exhaust, polyaromatic hydrocarbons, ozone, and endotoxin, among others, suppress Treg development, and enhance expression of inflammatory cytokines and allergic phenotypes by epigenetic means.

Summary
Epigenetic manipulation to open and close transcription sites provides flexibility of gene expression in response to changing environmental cues. It may also be the window whereby allergic disease in the workplace can be reduced by targeted environmental interventions.

Keywords
allergy, environment, epigenetics, T cells, workplace

INTRODUCTION

Epigenetics is the heritable modification of DNA packaging that determines which genes can be read and transcribed, and which are mothballed. Although heritable from parent to child, and potentially stable between cell cycles, epigenetic regulation of DNA transcription can also be modified by a number of external factors to allow flexible responses to a changing environment. It is wherein the rubber hits the road between environmental exposures and appropriate genome driven responses. Given the complexity and sophistication of this process, ubiquitous among mammals, epigenetic regulation of gene expression can generally be assumed to benefit the host. It is a highly developed mechanism to adapt to changing environmental conditions. There are, however, settings wherein epigenetic modification of genome transcription is maladaptive.

A number of different disease processes, among them cancer, atherosclerosis, mental retardation syndromes, autoimmune, and allergic processes are in part controlled by epigenetic processes. The focus of this review is the epigenetic regulation of asthma and allergy in the workplace. Although there are no specific studies, as yet, in occupational settings, there are obvious connections between exposures applicable to the workplace that could potentiate the development of asthma and allergies. This article will review the established epigenetic mechanisms affecting gene transcription, and

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KEY POINTS

- Gene expression is regulated by epigenetic modifications that determine which loci are transcribed and which are silenced.
- These epigenetic modifications are influenced by environmental exposures such as air pollution, tobacco smoke, and other sources of oxidant stress, along with the microbial environment, pesticides, and toxins.
- The development of T helper cell phenotypes and T regulatory cells is known to be under epigenetic control.
- The occupational environment includes many oxidant stressors, microbial exposures, and toxic chemicals that affect epigenetic marks, and thus may affect the expression of an allergic or tolerant phenotype.
- Better understanding of the interface between occupational exposures and their epigenetic effects on allergic gene expression may enable us to modify the working environment and prevent occupational allergic diseases.

describe what is known regarding the effect of such mechanisms on T-cell development and differentiation. Finally, we will examine the outcome of exposures found in the workplace and known to influence epigenetic changes and gene expression relevant to occupational allergic disease.

EPIGENETIC MECHANISMS

Several different processes affect DNA packaging that determines what portions of the genome are available for transcription (reviewed in [1▪▪]). Methylation of DNA sequences, modification of histones, and chromatin remodeling are coordinated processes, and the specific enzymes needed for each process often cluster in the same complex. DNA methylation, specifically of CpG dinucleotides, leads to gene silencing [2]. The mechanisms are thought to include steric inhibition of transcriptional activator binding (e.g. Sp1 and Myc) or by binding methyl CpG binding domain proteins (e.g. MBD proteins 1–4, MeCP2, UHRF, Kaiso or ZBTs) that may recruit histone modifiers, chromatin remodeling complexes, or other proteins that create a closed chromatin configuration. In general, CpG sequences are relatively rare, comprise only 1–3% of total DNA, and most of these dinucleotides are methylated. In contrast, CpG rich clusters of 1–4 kb, termed CpG islands, tend to cluster at the 5′ untranslated regions (UTRs) of promoter regions and the first exons of many genes, and these are mostly unmethylated [3▪▪]. Hence CpG islands tend to correspond to open chromatin structures and active transcription. The earliest studies on the effect of methylation to suppress gene expression were on the X chromosome Barr Bodies – the duplicate X in women that is preferentially silenced [4]. DNA methylation is accomplished by several subtypes of the DNA methyl transferases (DNMT). DNMT1 is considered the maintenance methyltransferase because this isoform acts to maintain methylation states during mitosis and in the daughter cells. DNMT3A and DNMT3B initiate de-novo methylation [5], although the triggers for this activity are only partly identified. Age, sex, genetic polymorphisms, and environmental exposures are some factors associated with altered methylation [6]. DNMT2 is another identified isoform, although its specific function remains unclear.

Histones can be modified by a number of processes, including by acetylation, phosphorylation, methylation, ubiquitination (attachment of small ubiquitin molecules that act as a tag to ferry the protein to the proteasome for degradation), or sumoylation [the attachment of Small Ubiquitin-like Modifier (SUMO) proteins with similar effects]. Histone acetylation is thought to enhance transcription, and the best characterized enzymes for histone modification include histone acetyltransferases (HATs), histone deacetylases (HDACs), and histone methyltransferase [7]. Histone modifications alter gene expression by recruiting DNA demethylases and methylated DNA binding proteins to shift nucleosomes and open or close transcription sites. In general, histone modifications are thought to transduce more rapid responses to a changing environment, followed by alterations in DNA methylation sites that maintain gene silencing over longer time periods [8,9▪].

In addition, small noncoding miRNAs (microRNAs) may bind to target mRNAs with complementary sequences to interfere with translation, or cause sequence degradation. miRNAs are thought to result from their own genes, or to arise from introns/exons of other genes [10,11].

Together, DNA methylation, histone modification, and miRNAs represent coordinated processes that regulate gene silencing or expression by architectural remodeling of the genome.

EPIGENETIC EFFECTS ON T-CELL DIFFERENTIATION: T HELPER CELL 1, T HELPER CELL 2, AND T REGULATORY CELLS

A growing body of evidence clearly shows that the development and differentiation of T-cell helper subsets, and the expression and function of

T regulatory cells, are under the control of epigenetic mechanisms. Since the expression of inflammation, immunity, and tolerance must adapt quickly to environmental cues, this body of research is the clearest indication yet of the marvelous flexibility of the T-cell network to respond to shifting immunological needs in a changing milieu.

T helper cell 1/T helper cell 2 differentiation

Differentiation of T helper cells into a Th1 or Th2 phenotype is in part directed by differential methylation and acetylation, and subsequent expression or repression of Th specific genes [12▪▪]. The Th1/Th2 ratio is extremely sensitive to histone acetylation and deacetylation [13]. De-novo methylation of the intergenic region between interleukin 4 (IL-4) and IL-13 on chromosome 5q21 reduces expression of Th2 cytokines in the cellular milieu, and hence decreases skewing toward Th2 development [14]. Concurrently, CpG sites upstream to the transcription start site of the gamma interferon (γ-IFN) promoter are demethylated [15]. HDAT complexes are displaced whereas HAT activity increases, leading to histone acetylation and nucleosome repositioning to a more open position [16]. These events facilitate binding of T-bet, c-Jun (activating transcription factor 2 ATF2), and CREB (cAMP-responsive element binding protein) to γ-IFN promoter sites [17], turning on promoter activity that leads to γ-IFN expression.

In contrast, Th2 differentiation is characterized by increased methylation of these γ-IFN promoter sites along with repressive histone deacetylation [18]. The IL-13/IL-4 region undergoes extensive demethylation and histone modification, and becomes available to bind transcription factors GATA3 (one of a family of transcription factors able to bind the DNA sequence 'GATA') and signal transducer and activator of transcription 6 (STAT6). Because the GATA3 binding site is located within the first intron of the IL-4 sequence, and is first to lose CpG methylation, IL-4 production is a rapid, early response that sets the stage for the synthesis of IL-13 and IL-5 [14]. With commitment to the Th2 lineage, additional demethylation occurs at the 5′ end of the gene, necessary to maintain high levels of IL-4 synthesis [14].

In summary, the process of T helper lineage commitment requires coordinated repression of cytokines related to one phenotype, concomitant with the orchestrated expression of cytokines of the alternate phenotype. This is accomplished by the epigenetic modification of chromatin packaging that opens one set of gene sequences for expression while closing another.

Epigenetic regulation of the forkhead box p3 gene

The forkhead box p3 (FOXP3) gene, located on the X chromosome, is the master controller of Treg cells, and it is highly conserved among a number of mammals including humans [19]. Required for Treg suppressive function, FOXP3 expression is largely controlled by epigenetic alterations in three highly conserved noncoding regions in the FOXP3 gene (reviewed in [20] and [21]). The FOXP3 upstream promoter is strongly associated with acetylated histones [22], and contains CpG motifs that are weakly methylated in naïve and activated $CD4^+$ T cells and in TGFβ induced (i)Treg cells, but are almost completely demethylated in natural (n)Treg cells [23,24]. The second highly conserved region in the FOXP3 gene is a TGFβ sensitive element that contains nuclear factor of activated T cells (NFAT) and mothers against decapentaplegic homologue 3 (SMAD) binding sites, and is associated with higher levels of acetylated histone H4. Binding of TGFβ induces the development of peripheral iTreg cells. This site may also increase the demethylation of the FOXP3 promoter, making it more available for transcription. TGFβ induced iTreg cells do not maintain constitutive FOXP3 expression if restimulated in the absence of TGFβ, suggesting that FOXP3 expression must undergo stable epigenetic modification in order to maintain a consistent repressor phenotype in these cells [25]. Lastly, the Treg-cell-specific demethylated region (TSDR), a CpG rich region of the FOXP3 locus, is always demethylated in Treg cells, but is methylated in conventional T cells [26,27]. The region is also associated with acetylated histones H3 and H4, and trimethylated lysine 4 in H3 [28]. Although the TSDR is not thought to act as a definitive on-off switch for Treg activity, it does determine FOXP3 stability [25]. Treg cells induced from conventional $CD4^+$ T cells by interaction with antigen presented by dendritic cells also display a fully demethylated TSDR [25].

Other epigenetic mechanisms negatively regulate the differentiation of natural Treg cells; among them is the SUMO E3 ligase PIAS1 (protein inhibitor of activated signal transducer and activator of transcription STAT1). PIAS1 binds to the FOXP3 promoter to recruit DNA methyltransferases that shut down FOXP3 transcription [29]. IL-6 also negatively regulates nTregs by inducing STAT3 dependent methylation of the upstream FOXP3 enhancer by DNMT1 that represses FOXP3 expression [23]. T-cell receptor (TCR) binding also controls FOXP3 expression in $CD4^+$ cells by inducing H3-K4 methylation at the FOXP3 promoter and intronic enhancer. However, continued TCR stimulation

leads to loss of these epigenetic marks and subsequent inhibition of FOXP3 expression [30].

ENVIRONMENTAL FACTORS THAT MODULATE EPIGENETIC EFFECTS ON ASTHMA AND ALLERGY

Most epigenetic modifications are dynamic, with one study [31] demonstrating a global DNA methylation change of over 20% in a subset of individuals studied for 11–16 years. Interestingly, methylation changes were found clustered in related individuals, compared with the majority who did not show significant changes, and suggesting a genetic influence. A similar study [32] of methylation patterns over 10 years found that loci in CpG islands were more likely to become methylated, whereas loci outside of such islands were more likely to demethylate, with methylation patterns stable across tissue types. Histone modifications are also dynamic, with changes over shorter time periods [33].

Epigenetic remodeling of chromatin packaging has been detected in children in prenatal, perinatal, and postnatal time periods. Maternal tobacco smoke exposure, air pollution (specifically diesel, particulate material, and PAHs), and a diet rich in folate, have all been associated with increased risk of asthma and atopy in the child (reviewed in [9▪]). Each of these exposures associates with DNA methylation and histone modification. In contrast, in-utero exposure to specific microbial exposures in farming environments [34], and with *Actinetobacter lwoffii* in a mouse model [35], associates with demethylation of the FOXP3 gene, concomitant increased FOXP3 expression, and enhanced neonatal Treg function, leading to allergy protection.

Polychlorinated biphenyl compounds (PCBs), organochlorine pesticides, dioxins, and phthalates have generally immunosuppressive effects at high doses [36]. At low doses such as those found in the ambient environment, however, allergic Th2 immune responses are supported through the estrogenic hormonal activity of these compounds, whereas Th1 immune responses are selectively inhibited [37,38], mediated by effects on global DNA methylation patterns.

Tobacco smoke has been associated with higher rates of allergic sensitization and is a major risk factor for asthma in both adults and children. Its mechanisms of action are thought to include the induction of oxidative stress that disrupts the HAT/HDAC balance in airway macrophages, and enhances the expression of inflammatory cytokines such as GM-CSF, IL-8, and IL-1β-induced TNFα [39]. Tobacco smoke reduces the inhibitory effect of steroids on cytokine release, and may be an important modulator of steroid resistance [39]. Tobacco smoke has also been found to alter DNA methylation in gene promoters and regulatory sequences, and, among other effects, induces hypermethylation and silencing of p16, a tumor suppressor [40,41]. Hypomethylation of the monoamine oxidase type B (MAO-B) promoter persists in smokers for up to 10 years after quitting [42]. Interestingly, of children exposed *in utero* to tobacco smoke, only those with the common glutathione-S-transferasemu 1 (GSTM1) null phenotype showed enduring changes in the methylation status of *LINE1*genes (long interspersed repetitive element-1), suggesting that some gene-environment effects are modulated by epigenetic changes [43].

PAHs (polyaromatic hydrocarbons) are important components of ambient air pollution and are also widely found in food products. Benzo-a-pyrene (BaP) has been shown to form adducts at CpG dinucleotides, affects histone acetylation status, modulates global DNA methylation and inhibits DNMT function [44]. Two CpG rich regions in the IFN-γ promoter were found to be hypermethylated in human airway smooth muscle cells exposed *in vitro* to BaP, suggesting another mechanism whereby air pollutants are able to enhance the development of Th2 type allergic responses [1▪▪].

Diesel exhaust particles (DEPs), another important constituent of particulate air pollution, have been shown to increase IgE production to environmental allergens. A potential mechanism is suggested by the finding of increased methylation of several sites of the IFN-γ promoter, indicating gene silencing, along with decreased methylation of a CpG site in the IL-4 promoter that may lead to increased IL-4 expression [45]. Part of the DEP effect may be modulated by oxidative stress that affects histone acetylation and cytokine responses [46].

Endotoxin and exposure to a farming microbial environment are also known to affect the development of atopy and asthma in both children and adults. Some studies implicate epigenetic changes as the mechanism by which this is accomplished. Farm environments are shown to produce a general increase in DNA methylation at the TSDR [34]. Endotoxin has been shown to demethylate histone H3 at the IL-1β promoter [47], and silence the TNF gene by specific chromatin remodeling [48]. Endotoxin has been detected on particulates of 10 and 2.5 μm, and may modulate some of the effects of particulate air pollution on the lungs (reviewed in [49▪]). In support of this is the finding that TLR4−/−mice do not develope increased airway hyperresponsiveness (AHR) to ozone or residual oil fly ash (ROFA) as compared with wild type mice [50]. Endotoxin is also a significant

Table 1. 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)	[56]
Tobacco smoke	Induces hypermethylation of the promoter of *p16; CYP1A1*, *RASSF1A*, and *FHIT* in lung cancer cells	Relevance in asthma unknown	[40,41,57–60]
Tobacco smoke	Induces *MAOB* promoter hypomethylation in PBMCs	Might serve as a biomarker of smoking-induced asthma	[42]
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	[43]
BaP	Induces hypomethylation of a number of genomic repeats and sequence-specific hypomethylation and hypermethylation changes in breast cancer cells	Relevance to asthma unclear	[61]
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	[62]
BaP	Decreases global DNA methylation, inhibits DNMTs *in vitro*, and interferes with recruitment of methylation machinery	Might affect expression of asthma-related genes	[44,63–65]
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	[66]
Oxidants	Posttranslationally modifies the HDACs and creates HAT/HDAC stoichiometry imbalance	Contribute to the enhancement of IL-1β–stimulated inflammatory cytokine production (e.g. IL-8, IL-6, CXCL1, CXCL2, and CXCL3) in the inflamed airways	[67,68]
LPS	Might be an miRNA-146a target	Contributes to LPS priming	[69]
LPS	Drives TLR signaling through Akt1-regulated expression of let-7e and miR-155	Contributes to macrophage hypersensitivity and endotoxin tolerance	[70]
Inhaled DEPs	Induce hypermethylation at specific CpGs of the IFNG promoter and hypomethyl-ation at the IL-4 promoter in splenic $CD4^+$ cells	Hypersensitize mice to intranasal *Aspergillus fumigatus* exposure	[45]
PM-10	Increases HAT activity and acetylated histone 4; remodels the IL-8 promoter; action mediated through the induction of oxidative stress	Increases IL-8 expression and release from human alveolar basal epithelial cells	[46]
Exposure of elderly to ambient black carbon but not PM2.5 for 4–7 days	Induces hypomethylation of *LINE1*	Might exacerbate asthma in this population	[71]
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	[72,73]

Table 1 (*Continued*)

Environmental factors	Epigenetic effects	Relevance to asthma	References
Maternal diet rich in methyl donors	Favors lymphocyte maturation into a Th2 phenotype	Increases the risk of allergic airway disease in offspring	[74]
Maternal folic acid supplementation	Increases the risk of wheeze and lower respiratory tract infections in progeny up to 18 months of age	Explains developmental reprogramming of asthma risk	[75]
Dust mite antigens	Induce expression of miRNA-126 and activates TLR4	Increase inflammation, a Th2 response, airway hyperresponsiveness through suppression of GATA-3	[51]

CYP1A1, Cytochrome P450, family 1, subfamily A, polypeptide 1; DEP, Diesel exhaust particles; FHIT, fragile histidine triad gene; H, histone; K, lysine; LPS, lipopolysaccharide – synonym for endotoxin; RASSF1A, Ras association (RalGDS/AF-6) domain family member 1. Reproduced with permission from [1▪▪].

component of house dust mite allergens that have been shown to activate TLR4 and increase the expression of specific miRNAs including 16, 21, and 126 [51]. miRNA-126 in particular appears to enhance Th2 responses, eosinophil recruitment, and airways inflammation and hyper-responsiveness, by augmenting GATA-3 expression that promotes the secretion of IL-4, IL-5, and IL-13 [51].

Innate immune responses are affected by oxidant stress. Ozone challenge triggers increased expression of innate immune surface proteins CD11b, CD14, and TLR4, antigen presentation markers CD80, CD86, and HLA-DR, and immunoglobulin receptors CD16, CD23, FceR1 (reviewed by [49▪]). Oxidative burst and phagocytic functions are also increased, and suggest that ozone exposure may enhance the inflammatory milieu of the lung and augment the response to biologic agents.

Diet also may enhance the inflammatory milieu in which the host responds to environmental cues. A diet rich in methyl donors favors the development of a Th2 phenotype. A possible mechanism may involve the hypermethylation and silencing of Runx3 (runt-related transcription factor 3), a gene known to suppress IL-4 and activate FOXP3 [52]. Vitamin D also affects airways hyperresponsiveness [53], as reduced levels associate with higher markers of allergy and asthma severity [54]. The vitamin D receptor appears to be epigenetically controlled, and is similarly activated by DNA demethylation or histone acetylation [55].

Table 1 is a partial list of environmental exposures known to cause epigenetic changes affecting the expression of asthma and allergy genes [1▪▪,40,46,51,56–75].

POTENTIAL OCCUPATIONAL EFFECTS ON ALLERGY GENE EXPRESSION AND OCCUPATIONAL SENSITIZATION

As noted in the introduction to this review, there are no specific studies as yet of the occupational environment or related epigenetic mechanisms that may modify the development or progression of occupational allergic disease. However, what we know from studies of environmental exposures suggest similar effects are operative in the workplace. Occupational exposures may include PAHs, diesel exhaust, particulates, endotoxin, cigarette smoke, and other forms of oxidant stress, that have been shown to affect epigenetic marks and airways inflammation. The onset and progression of asthma and allergic disease specific to the workplace may be triggered by a proinflammatory milieu established through environmental effects on the epigenome. Indeed, the influence of coexposures to irritants in the context of an occupational allergen may not be solely through mucosal effects that permit access to the allergen. Another significant consequence may be via epigenetic modifications of gene expression that create an inflammatory milieu ripe for the elaboration of an allergic response. Exposure to low-dose toxins, including PCBs, organochlorine pesticides, dioxins, and phthalates, may also potentiate allergic responses to otherwise ignored workplace allergens by modifying the epigenome. The epigenetic marks of specific workplace exposures that modulate allergic responses are an area that calls for further study. Better understanding and modification of these epigenetic exposures and consequences may be more effective at reducing occupational sensitization than by a sole focus on controlling the allergen exposure itself.

CONCLUSION

Large questions remain unanswered. What is the dose of environmental co-exposures that most affects the allergic response? Is the effect to be found system wide, or is it found only in specific tissues or organ systems? This, of course, affects which tissues need be studied, and whether findings in one system, such as the blood, can be extrapolated to other systems, such as the skin or lung. Do epigenetic

mechanisms affect onset of allergic disease differently in atopic and nonatopic individuals? Do these mechanisms explain the relatively later onset of occupational allergies in the nonatopic worker? An integrated question, then, is the duration of the epigenetic effect. Does it wane over a period that can be better quantitated? Lastly, epigenetic effects provide the benefit of plasticity to immune responses. Can occupational exposures or their epigenomic effects then be deliberately modified to influence the outcome to one of tolerance rather than allergy?

In summary, epigenetics provides a measure of flexibility in regulating which genes are expressed, and which are silenced, based on environmental cues. With more knowledge, it may be the window through which we can manipulate those exposures to protect workers from occupational allergic diseases.

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Conflicts of interest

No conflict of interest is reported for this review.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

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Another provocative point of view that examines the oxidant effects of ozone and endotoxin on the airway, and how these both may affect the innate immune system and modify the epigenome.

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