

Update on epigenetics in allergic disease

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Chronic inflammatory diseases, including allergies and asthma, are the result of complex gene-environment interactions. One of the most challenging questions in this regard relates to the biochemical mechanism of how exogenous environmental trigger factors modulate and modify gene expression, subsequently leading to the development of chronic inflammatory conditions. Epigenetics comprises the umbrella of biochemical reactions and mechanisms, such as DNA methylation and chromatin modifications on histones and other structures. Recently, several lifestyle and environmental factors have been investigated in terms of such biochemical interactions with the gene expression-regulating machinery: allergens; microbes and microbial compounds; dietary factors, including vitamin B12, folic acid, and fish oil; obesity; and stress. This article aims to update recent developments in this context with an emphasis on allergy and asthma research. (J Allergy Clin Immunol 2015;135:15-24.)

Key words: Epigenetics, allergy, asthma, DNA methylation, histone modifications

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Chronic inflammatory diseases, including allergies and asthma, are the result of complex interactions between genetic predisposition and environmental factors. Based on the individual genetic makeup, life-long exposures to various environmental, nutritional, and lifestyle factors contribute to either disease development or protection. Many of these external events directly or indirectly affect immune regulation. From a mechanistic point of view, one of the challenging questions is how such environmental components mechanistically affect immune functions.

In this regard the concept of **epigenetic regulation** has gained great interest in recent years.¹⁻³ Based on the given DNA sequence, epigenetic modifications comprise biochemical reactions that modulate and modify the accessibility of the gene transcription machinery. One such mechanism is CpG methylation

Abbreviations used

ADCYAP1R1: Membrane receptor for pituitary adenylate cyclase-activating peptide
AluYb8: DNA repetitive short interspersed nucleotide element
DNMT: DNA methyltransferase
GPR15: G protein-coupled receptor 15
GSTM-1: Glutathione-S-transferase M1
HAT: Histone acetyltransferase
HDAC: Histone deacetylase
HDM: House dust mite
FOXP3: Forkhead box protein P3
OVA: Ovalbumin
Th-POK: Th inducing POZ-Kruppel factor
Treg: Regulatory T
ZFP57: Zinc finger protein 57

directly occurring on the DNA sequence. Other mechanisms operate on the **chromatin** structure, such as biochemically modified **histone** protein tails that then subsequently allow or deny access of **transcription factors** to **gene promoter regions**.

Such epigenetic modifications play an important role in cell differentiation but also in cell activation. Although each person's cells express the identical DNA sequence (with the exception of cancer cells and some others), the epigenetic makeup differs dramatically from tissue to tissue and cell type to cell type and also in a longitudinal fashion over time. Therefore it is of great importance to delineate the biochemical processes through which external environmental exposures and "hits" have a direct effect on the epigenetic program and therefore the development of tissue and organ functions to understand the development of chronic inflammatory diseases. In this regard prenatal and postnatal life events seem to play a pivotal role in which the susceptibility of environmental hits has an especially strong effect on immune development and functions.⁴

In this article we will focus on the following environmental factors and components, which have recently gained great interest in terms of epigenetic modifications in the field of allergy and asthma: allergen exposure; bacterial microbes and microbial components; dietary factors, such as folic acid, vitamin B12, and fish oil; obesity; and stress.

SOME PRINCIPLES OF EPIGENETIC MODIFICATION

There are many examples showing that epigenetic modifications are neither permanent nor transient. Some of them are implemented into the epigenome for a short time to open or close the chromatin or to change the methylation status of a certain gene.⁵ Furthermore, epigenetic modifications are in most cases reversible. With the appropriate enzymatic machinery, the whole

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Terms in boldface and italics are defined in the glossary on page 16.

epigenome can be modified and changed.⁶ In this review we will focus on some principles of DNA methylation and histone modifications and update the knowledge about the different environmental factors and their epigenetic effects in allergic disease. There are many different epigenetic modifications affecting the status of the transcription of genes.

DNA methylation

DNA methylation is a biochemical process involving the addition of a methyl group to the DNA nucleotides cytosine or adenine. It is considered one of the epigenetic processes that lead mainly to **gene silencing** and subsequently to inhibition of the gene transcription.⁷ In general, DNA methylation occurs on the different CpGs clustered as islands on the majority of the genes. There are several million potentially methylated CpG islands throughout the genome of a single cell. They are clustered through the gene body and play a critical role within the promoter regions. Once these islands are methylated, gene transcription might not occur.⁸ On the other hand, once these CpG islands are unmethylated, an active promoter allows interaction with the various transcription factors controlling gene activation.⁹

In T cells DNA methylation plays an important role in the development, activation, and maintenance of T-cell effector function. For example, demethylation of the forkhead box protein P3 (*FOXP3*) region might favor the development of regulatory T (Treg) cells.¹⁰ During the development of T cells toward different types of T_H or effector cells, they undergo extensive epigenetic editing. The **zinc finger protein** T_H inducing POZ-Kruppel Factor (Th-POK) regulates the development of CD4⁺ T cells and inhibits the development of CD8⁺ cells through different DNA methylation of CD8-associated genes.¹¹ Furthermore, displacement of the polycomb-group protein through signal transducer and activator of transcription 6 causes long-lasting maintenance of the transacting T cell-specific transcription factor (GATA3) transcription factor and, subsequently, maintenance of T_H2 cells.¹²⁻¹⁴

The major regulatory enzymes of DNA methylation are DNA methyltransferases (DNMTs). There are different DNMTs that play unique roles in the DNA methylation process.¹⁵ DNMT1 is the major DNMT and is important for maintenance of the DNA methylation status of a gene. Most of the genes are silenced in their normal state. DNMT1 retains gene silencing in its usually normal state (Table I).¹⁶ DNMT3a and DNMT3b are the main enzymes for *de novo* methylation and mediate methylation-independent gene repression. DNMT3a can colocalize with heterochromatin protein and methyl-CpG-binding protein (Table I).¹⁷ They also interact with DNMT1, which might be a cooperative event during DNA methylation.¹⁸⁻²²

Histone modifications

Histones are highly alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes. DNA is usually wrapped around 2 copies of the core histones H2A, H2B, H3, and H4.²³ The main mechanism of regulating chromatin is through posttranslational modifications of various protein tails of these histones.²⁴ From a biochemical point of view, the major histone modifications are acetylation, methylation, phosphorylation, ubiquitination, and sumoylation. The effects of such modifications range from gene activation²⁵ to gene silencing²⁶ and can have some DNA repair functions as well.²⁷

Histone acetylation of different lysine residues represents one of the best studied examples in this regard, resulting in activation of transcription (Fig 1).²⁵ This activation is catalyzed by histone acetyltransferases (HATs). HATs transfer the acetyl group from the acetyl-CoA cofactor to the N ϵ nitrogen of a lysine side chain within histones.²⁸

There are 2 main families for HATs. Type A HATs are located in the nucleus and involved in the regulation of gene expression through acetylation of nucleosomal histones. Gcn5, p300/CpG-binding protein, and TAFII250 are some examples of type A HATs that cooperate with activators to enhance transcription.

GLOSSARY

ARACHIDONIC ACID: A polyunsaturated omega-6 fatty acid that is the counterpart to the saturated arachidonic acid found in peanut oil.

CHROMATIN: A complex of DNA, proteins, and RNA that tightly compact DNA to fit into the cell.

CPG SITES: Regions of DNA where 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.

EPIGENETIC REGULATION: Modifications of gene expression that are not caused by changes in the DNA sequence but by histone modification, DNA methylation, and other mechanisms.

GENE PROMOTER REGIONS: Specific regions of DNA that initiate the transcription of a particular gene.

GENE SILENCING: Epigenetic regulation that prevents the expression of a gene.

HISTONE: The protein component of chromatin responsible for compacting DNA.

LONG-CHAIN POLYUNSATURATED FATTY ACIDS: Lipids that are composed of omega-3 and omega-6 fatty acids, which are thought to be instrumental in the prevention of neurodegeneration and inflammation.

METHYLATION-SENSITIVE HIGH-RESOLUTION MELTING (MS-HRM): PCR products subjected to thermal denaturation show different melting profiles. MS-HRM compares melting profiles of PCR products from unknown samples with profiles specific for PCR products derived from methylated and unmethylated control DNAs.

MHC II: Presents processed antigen that is derived from extracellular proteins to T-cell receptors.

PHORBOL 12-MYRISTATE 13-ACETATE/IONOMYCIN: Can be used to stimulate T-cell activation, proliferation, and cytokine production.

T-CELL RECEPTOR: A molecule expressed on the surface of T cells that recognizes antigens bound to MHCs.

TRANSCRIPTION FACTORS: Proteins that bind to specific DNA sequences, which control the rate of transcription of genetic information from DNA to mRNA.

V(D)J RECOMBINATION: The somatic assembly of component gene segments that encode antigen recognition sites of receptors expressed on B and T lymphocytes.

ZINC FINGER PROTEINS: A variety of protein structures that use zinc to stabilize DNA folding.

TABLE I. Different DNMTs and their function and role in patients with allergy and asthma

DNMT	Function	Role in allergy and asthma
<i>DNMT1</i>	Maintenance of DNA methylation status	• Loss of <i>DNMT1</i> changes expression of IL-2 and IFN-γ. It causes silencing of the T_H2 gene in CD8 T cells and <i>vice versa</i> and plays a role in the development of different T-cell subsets.^{14,19} • Downregulation of <i>DNMT1</i> increased ear thickness, typical of allergic skin inflammation.^{15,20} • Increased expression of <i>DNMT1</i> on initiation of monocyte differentiation to iDCs, followed by significant downregulation of <i>DNMT1</i> on maturation.^{16,21}
<i>DNMT2</i> (TRDMT)	RNA cytosine methyltransferase	Not known
<i>DNMT3a</i>	<i>De novo</i> methylation of DNA	• Limits expression of IL-13 in T_H2 cells and allergic airway inflammation.^{17,22} • Increased expression of <i>DNMT3A</i> on initiation of monocyte differentiation to iDCs.^{16,21}
<i>DNMT3b</i>	<i>De novo</i> methylation of DNA	• Upregulation of <i>DNMT3B</i> during maturation of iDCs to mDCs.^{16,21}

iDCs, Immature dendritic cells; mDCs, myeloid dendritic cells.

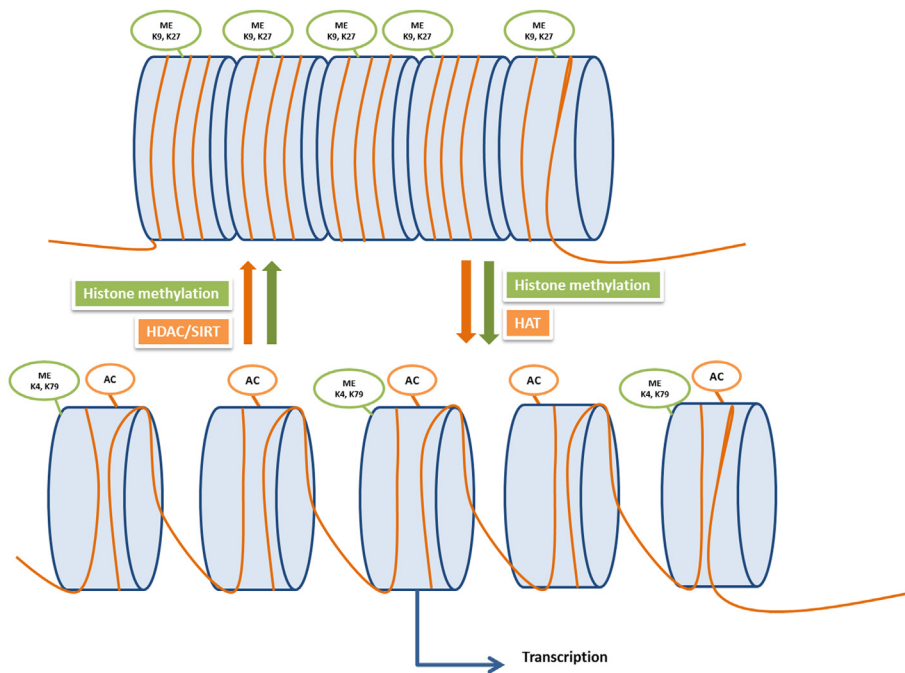


FIG 1. Histone modifications. The illustration shows both histone acetylation and histone methylation and the different modifications on lysine residues with regard to histone methylation causing either opening of the chromatin or closure. AC, Acetylation; K, lysine residue where the acetyl or methyl group is attached; ME, methylation.

Type B HATs are located in the cytoplasm and are responsible for acetylating newly synthesized histones before their assembly into nucleosomes.²⁹ On the other hand, histone methylation proved to be more complex. For example, histone methylation on the lysine residues 4 and 79 and arginine residue 17 (K4, K79, and R17) can lead to active transcription of the gene,³⁰ whereas histone methylation on other lysine residues, such as K9 and K27, renders the promoter region inactive, resulting in gene silencing (Fig 1).³¹ Furthermore, phosphorylation of serine residues at histone H3 is a highly dynamic process that creates specific combinatorial patterns, together with acetylation and methylation marks, at neighboring lysine residues that are read by specific detector proteins.^{32,33} Table II lists a number of different chromatin modifications with reference to their main effects on gene activation or silencing.

Histone modifications in T cells play an important role in the development of T cells toward different T-cell subsets, playing a

pivotal role in both physiologic and pathologic conditions. For example, gene-specific targeting of H3K9 methylation causes efficient gene silencing in many T_H1 or T_H2 effector genes.³¹ Furthermore, Th-POK, the primary CD4⁺ transcription factor, recruits different histone deacetylases (HDACs), which silence many of the CD8 genes. As a result, T cells are pushed toward development of the CD4⁺ subset.³⁴ Lymphocyte development is controlled by either repression or activation of different genes. There is some evidence that histone acetylation can directly affect different T-cell functions, as suggested by Han et al,³⁵ showing that HDAC4 and P300 build a complex with GATA3 causing deacetylation on the IL-5 promoter suppressing production of IL-5. The antigen specificity of the T cells is determined by expression of the *T-cell receptor*, which is the result of assembly of antigen receptor genes by *V(D)J recombination*. Gene expression and changes in V(D)J recombination are affected by different epigenetic modifications primarily in histones, altering chromatin

TABLE II. Examples of epigenetic modifications and consequences on gene activation status

			Gene activation	Gene silencing
DNA methylation (cytosine)				+
Modification	Histone	Residue		
	Acetylation	H2A K5	+	
		H2B K5, K12, K15, K20	+	
		H3 K4, K14, K18, K23, K27	+	
		H4 K8, K16	+	
Methylation	H3	K4, K79, R17	+	
		H3 K9, K27		+
	H4	R3	+	
		H4 K20		+
Ubiquitination	H2A	K119		+
	H2B	K120	+	

K, Lysine residues where the acetyl or methyl group can bind.

folding to render it inaccessible for nuclear factors. G9a, a H3K9 methyltransferase, promotes the development of B lymphocytes to their mature phase by H3K9 methylation.³⁶ Fig 2 shows a more detailed listing of epigenetic modifications and the responsible effector enzymatic machinery playing the most important role in these changes.

EPIGENETIC CONTROL IN FETAL IMMUNE DEVELOPMENT

Activation of naive CD4⁺ helper T cells through the T-cell receptor and *MHC II* peptide complex induces rapid T-cell activation and differentiation. Effector helper T cells are classified according to the type and pattern of cytokines they produce. The main subsets are T_H1, T_H2, T_H9, T_H17, and Treg cells, although there are others. Both T_H1 and T_H2 cells have been extensively studied since the initial description of helper T-cell subsets,³⁷ and the epigenetic modification of T cells starts very early during the activation/differentiation program within the naive noncommitted precursors. DNA methylation is considered the main epigenetic mechanism controlling T_H1 expression. IFN- γ (*IFNG*) production is low in neonatal CD4⁺ T cells compared with those from adults, and this is closely associated with hypermethylation of the *IFNG* promoter.³⁸ Moreover, Treg cell function is also impaired in the neonatal period compared with that seen in adults.^{39,40} In the naive T-cell state histones in both the *IFNG* and *IL4* loci are hypoacetylated.⁴¹ Furthermore, DNA methylation at T_H1 cytokine genes supports the development of T_H2 cells.⁴² This is further supported by histone acetylation, which provides accessibility to both *IFNG* and *IL4* loci for both T_H1 and T_H2 cell development.⁴³ The induction of *IFNG* expression by H2.0-like homeobox protein depends on a permissive epigenetic state of the *IFNG* gene locus, the molecular context of the immature T_H cells, or both.⁴⁴ In addition to the classical T_H1 and T_H2 genes, recent evidence suggests that DNA methylation and histone acetylation also occur at the *IL10* locus during T-cell differentiation into both T_H2 and Treg cells.⁴⁵

ENVIRONMENTAL FACTORS AND EPIGENETIC MODIFICATIONS

Allergens

The effect of different allergens on epigenetic modifications is not fully known. There are few studies providing insights into the

effect of allergens on epigenetics in patients with asthma and allergy. In our laboratory we tested the hypothesis that allergen sensitization and development of the T_H2 immune response are closely linked to epigenetic programming of the previously naive T cell during development of effector status. To test this concept, BALB/C mice were sensitized to ovalbumin (OVA), and an allergic asthma protocol was applied.⁴⁶ OVA caused a significant increase in DNA methylation at the *IFNG* promoter after allergen sensitization/challenge in CD4⁺ T cells, which correlated with decreased IFN- γ cytokine expression, whereas only minor changes were observed at the cyclophilin seven suppressor 1 (*CNS1*) locus. Furthermore, the increase in DNA methylation at the *IFNG* promoter could be reversed with the DNMT inhibitor 5-aza-2'-deoxycytidine *in vitro* and *in vivo*, which prevented the development of experimental allergy.⁴⁶ This was a cell type-selective effect because only CD4⁺ T cells were affected and not CD8⁺ or natural killer T cells.

Moreover, house dust mite (HDM) can also elucidate epigenetic modifications in asthmatic patients. Pascual et al⁴⁷ investigated DNA methylation of 3 well-characterized populations of patients with HDM allergy, patients with aspirin-intolerant asthma, and control subjects. They focused on CD19⁺ B cells in these populations. The data indicate that epigenetic changes occur at different loci relevant for immune responses. One such locus is a newly identified candidate gene, cytochrome P450 26A1 (*CYP26A1*), which encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are mono-oxygenases that catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids, and other lipids. Differential methylation patterns and expression levels are found between study groups.⁴⁷ Furthermore, in an animal allergic reaction model, HDM-exposed mice showed an increase in airway hyperresponsiveness and inflammation together with structural remodeling of the airways. Methylated DNA immunoprecipitation next-generation sequencing revealed that particularly the TGF- β signaling pathway is epigenetically modulated by chronic exposure to HDM.^{14,48}

Microbes

Environmental microbes are considered to play an important role in shaping the immune response, particularly early in life. One real-life model of allergy protection is traditional farming exposure, which was proposed initially by Braun-Fahrlander et al.⁴⁹ Children growing up in a traditional farming environment had a lower risk of respiratory allergic disease later in life. There is increasing evidence that at least some of the protective effects are mediated through epigenetic modifications. The effect of maternal farming exposure was determined in 84 pregnant mothers. Cord blood was collected after delivery and stimulated with different microbial stimuli. A higher number of FOXP3⁺ cells was detected in cord blood mononuclear cells from newborns born of mothers with extensive farming exposure. Furthermore, the Treg cell function was more efficient with farming exposure. This was associated with demethylation of the FOXP3 promoter in offspring of mothers with farm milk exposure compared with control mothers.⁵⁰ In addition to that, a subsample of 46 samples from the Protection Against Allergy: Study in Rural Environment (PASTURE) study showed hypomethylation in different regions of the *ORMDL1* and signal transducer and activator of transcription 6 (*STAT6*) genes in cord blood from farm

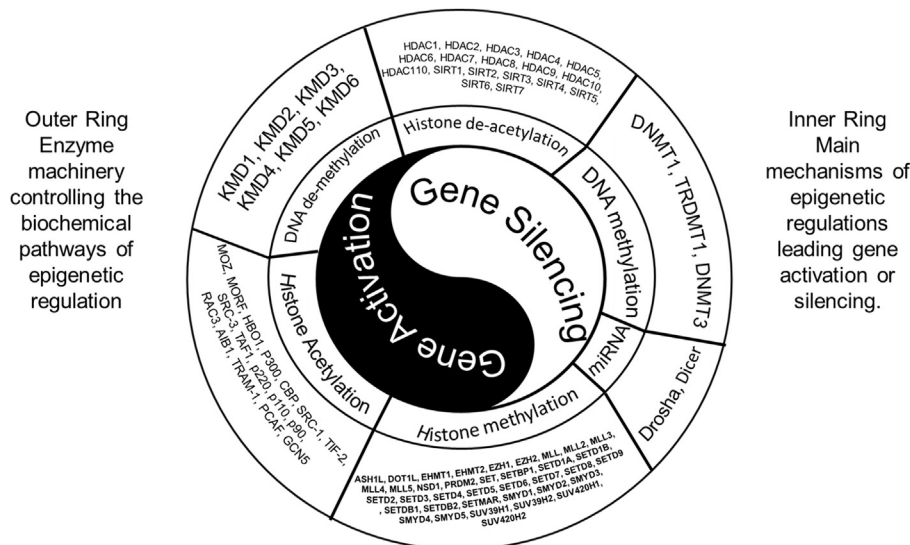


FIG 2. Yin and yang of both gene activation and gene silencing. Different epigenetic modifications and their enzymatic machinery.

TABLE III. Examples of environmental exposure on clinical phenotype mediated through epigenetic modifications: current examples

Effector	Epigenetic regulation	Clinical phenotype	Genes (cell type)	References
Allergens (OVA)	Histone deacetylation Histone acetylation	AA, COPD AA	<i>LAT</i> (CD4 ⁺) <i>PDE4E</i> (CD4 ⁺) <i>ACLS3</i> (CD4 ⁺)	48
Microbes/farm environment	DNA methylation	AA	<i>RAD50</i> (PBMC) <i>IL13</i> (PBMC) <i>IL4</i> (PBMC) <i>IFNG</i> (CD4 ⁺)	50,51
Tobacco smoke	DNA methylation Histone acetylation Histone deacetylation	COPD COPD COPD	<i>GSTM1/GSTP</i> (macrophages) <i>TNF</i> (macrophages)	61-63
Diesel exhaust/polycyclic aromatic hydrocarbons	Histone deacetylation DNA methylation	COPD, AA A	<i>FOXP3</i> (CD4 ⁺) <i>IFNG</i> (CD4 ⁺) <i>FOXP3</i> (CD4 ⁺) <i>ACSL3</i> (CD4 ⁺)	4,60,73,75
Folic acid	DNA methylation Histone Acetylation	AA AA	<i>ZFP57</i> (CD4 ⁺)	83,84
Fish oil	Histone deacetylation	Cell-culture analysis	<i>IL6</i> (macrophages) <i>TNF</i> (macrophages)	91,92
Lifestyle (obesity)	DNA methylation	AA	<i>CCL5</i> , <i>IL2RA</i> , and <i>TBX21</i> (PBMC)	100
Stress	DNA methylation	AA	<i>ADCYAP1R1</i> (PBMC)	102

A, Nonallergic asthma; AA, allergic asthma; COPD, chronic obstructive pulmonary disease; LAT, linker for activation of T cells; TBX21, T-box transcription factor.

children compared with nonfarm children. In contrast, *RAD50* and *IL13* were found to be hypermethylated (Table III).⁵¹ Further investigation in the same cohort revealed an example of gene-environment interaction. The polymorphism rs2240032 in the *RAD50* DNase I hypersensitive site region is suggestive of allele-specific transcription factor binding. This polymorphism affects methylation of the *IL13* promoter region and influences *RAD50* and *IL4* expression.⁵²

Exposure to farm milk was identified as an additional independent farm-related exposure and has been associated with higher *FOXP3* demethylation in PBMCs as well.⁵³ Not only were CD4⁺ T cells affected through a farm environment, but also other

cells were a target of epigenetic regulation. From the Assessment of Lifestyle and Allergic Disease During Infancy (ALADDIN) study, 94 placentas were investigated. **Methylation-sensitive high-resolution melting** analysis was performed to semiquantitatively analyze DNA methylation of the promoter region of *CD14*. *CD14* was chosen because it plays an important role in sensing microbial compounds together with the T-cell receptor. Indeed it has been shown that placentas express higher levels of *CD14* mRNA under certain conditions. Methylation-sensitive high-resolution melting revealed a close to significant correlation between methylation of the *CD14* promoter region and *CD14* mRNA expression. DNA methylation in the *CD14* promoter

was significantly less in placentas of mothers living on a farm compared with that seen in mothers not living on a farm.⁵⁴

Several environmental microbes have been closely associated with the protective farming effect on respiratory allergies. One important example represents the gram-negative bacterium *Acinetobacter lwoffii* F78. Prenatal administration of *A lwoffii* F78 prevented development of an experimental asthma phenotype in the progeny, and this effect was dependent in the offspring on *IFNG* induction. The production of *IFNG* was induced because of inhibition of H4 acetylation in the offspring at the *IFNG* promoter. Regarding T_H2 -relevant genes only at the *IL4* promoter, a decrease could be detected for H4 acetylation but not at the *IL5* promoter or the intergenic T_H2 regulatory region conserved noncoding sequence 1 (*CNS1*).⁵⁵ In addition, pharmacologic inhibition of H4 acetylation by administration of the drug garcinol in the offspring abolished the asthma-protective phenotype.⁵⁵ The data indicate that the environmental bacterium *A lwoffii* F78 promotes asthma protection from the mother to the progeny and that epigenetic modifications, particularly at cytokine genes, account for this effect in the progeny.

In the Rural Environments birth cohort study with 298 children, Treg cell numbers were significantly increased in farm-exposed children after *phorbol 12-myristate 13-acetate/ionomycin* and LPS stimulation.⁵³ LPS, also known as lipoglycans and endotoxin, is a complex molecule consisting of a lipid and a polysaccharide composed of O-antigen, with the outer core and inner core joined by a covalent bond; the molecule domain is integrated in the outer membrane of gram-negative bacteria and elicits strong immune regulatory responses. Repeated low-dose LPS exposure in mice, particularly through the mucosal route, leads to tolerance development.⁵⁶ These changes are mediated through epigenetic modifications. Furthermore, prenatal maternal LPS exposure triggered neonatal IFN- γ , but not IL-4 and IL-2, production. OVA sensitization of prenatally LPS-exposed mice was accompanied by a marked suppression of anti-OVA IgG₁ and IgE with unchanged IgG_{2a} antibody responses paralleled by a significant reduction in IL-5 and IL-13 levels after mitogenic stimulation of splenic leukocytes. These data support the concept that LPS might already operate in prenatal life to modulate the development of allergies in offspring.⁵⁷ Antibacterial genes, such as *RANTES*, lipocalin-2 (*LCN2*), and prostaglandin E synthase (*PTGES*), mediate an increase in levels of histone H4 acetylation and H3 K4 trimethylation, and both are markers of gene activation, which act to prime these genes in response to subsequent LPS stimulation, resulting in similar or even greater levels of gene expression.⁵⁸

Tobacco smoke

Prenatal and early childhood exposure to tobacco smoke represents a major risk factor for the development of childhood asthma.⁵⁹ One important mechanism through which components derived from tobacco smoke exert toxic and detrimental metabolic functions is through affecting the epigenetic programming of different cell types. In one study lung biopsy specimens and bronchoalveolar lavage fluid macrophages from 16 nonsmoking healthy subjects and 13 age-matched cigarette smokers were analyzed. Cigarette smoking was closely associated with reduction in the expression of HDAC-2 and HDAC activity in the analyzed biopsy specimens. This was paralleled by an enhanced *IL1B*-induced expression of tumor necrosis factor (*TNF*).⁶⁰

Reduced HDAC expression might account for enhanced expression of proinflammatory mediators, such as GM-CSF, IL-8, and TNF.⁶¹⁻⁶³ Furthermore, it was shown in mouse models that an increase in IgE levels was associated with hypomethylation at the *IL4* promoter in parallel to hypermethylation at the *IFNG*^{4,64} and *FOXP3*⁶⁵ promoters. In addition, *in utero* tobacco smoke exposure significantly increased placental cytochrome P1A1 (*CYP1A1*) expression in association with differential methylation at a critical xenobiotic response element in a study with a cohort of gravidae who had smoked during their pregnancy and nonsmoking control subjects.⁶⁶

The glutathione-S-transferase M1 (*GSTM1*) gene product GSTM-1 plays an important role in the detoxification of polycyclic aromatic hydrocarbon compounds produced through tobacco smoke and environmental pollution.⁶⁷ In a study in children from the Children's Health Study in relation to a positive history of prenatal environmental tobacco smoke exposure, there was no strong clustering of gene methylation patterns by prenatal smoke exposures, but the researchers found that methylation of the DNA repetitive short interspersed nucleotide element (*AluYb8*) was decreased in association with maternal smoking during pregnancy. Increasing levels of *AluYb8* methylation were positively associated with changes in global methylation patterns, as observed in polycomb group target genes.⁶⁸

Methylation of the DNA repetitive long interspersed nucleotide element (*LINE1*) was decreased only among children with the common *GSTM1* null genotype.⁶⁹ Moreover, decreased methylation of *AluYb8* might then be a genetic marker for global DNA methylation patterns, an indication of maternal smoking during pregnancy (Table III).⁷⁰

A genome-wide methylation analysis looking at 27,578 *CpG* sites was conducted in subjects from the International COPD Genetics Network (n = 1,085) and the Boston Early-Onset COPD study (n = 369). Fifteen sites were identified to be significantly associated with current smoking, and others were associated with cumulative smoke exposure and the time span since quitting cigarettes. Moreover, 2 loci, factor II receptor-like 3 (*F2RL3*) and G protein-coupled receptor 15 (*GPR15*), were significantly associated in all 3 analyses. *GPR15* is closely connected to development of immune tolerance in the mucosa by regulating Treg cell levels. *GPR15* is epigenetically regulated through DNA methylation and might contribute to the extended risks associated with cigarette smoking that persists after cessation.⁷¹⁻⁷³

Additional genes associated with tobacco smoke and induction of DNA methylation and immune regulation were identified in other studies. In one study exposure to cigarette smoke condensate altered the methylation patterns of the tumor suppressors *ECAD* and *RASSF1A* in patients with lung cancer.⁷⁴ Furthermore, *in vitro* studies performed with the epithelial cell line A549 showed that cigarette smoke-conditioned medium reduced overall HDAC2 expression.⁷⁵ Further studies conducted by Adenuga et al⁷⁶ have shown that tobacco smoke exposure downregulates HDAC2 through phosphorylation. These data clearly indicate that epigenetic mechanisms account for several of the detrimental effects of tobacco smoke exposure.

Diet and metabolism

There are many studies suggesting that different diets and nutrients exert their effects through epigenetic mechanisms. For

instance, a methyl donor-rich diet causes a drastic change in fur color of the agouti mice.^{77,78} Two of the most prominent methyl donors are folic acid and vitamin B12, which can affect DNA methylation status universally.⁷⁰

Folic acid. Folic acid is one of the main vitamin supplements used by many pregnant mothers, primarily to minimize the risk of neural tube defects in the offspring.^{79,80} Folate is known as a dietary factor that donates methyl groups throughout its metabolic process.⁸¹ This can lead to a change in the epigenome of the receiver. In mice maternal supplementation with folate during pregnancy resulted in hypermethylation of regulatory genes in the lung tissue, favoring the development of allergic airway disease in the offspring.⁸² Furthermore, in a human cohort using the 2 extremes of highest and lowest maternal serum folate levels measured in the last trimester of pregnancy, CD4⁺ T cells were purified from cord blood mononuclear cells, followed by genome-wide DNA methylation profiling. A comparison of these 2 extreme groups showed differential methylation at 7 regions across the genome. The largest effect was the hypomethylation of zinc finger protein 57 (*ZFP57*) transcripts.^{83,84} *ZFP57* is considered a regulator of DNA methylation during development in many cell types.⁸⁵ Furthermore, folate status had an effect not only on DNA methylation but also on histone acetylation. Levels of histone H3 and histone H4 acetylation at the *ZFP57* promoter in the high-folate group associated with high mRNA expression in CD4⁺ T cells (Table III).^{83,84} This was also associated with a higher acetylation level at the *GATA3* promoter, as shown in a recent study conducted on the same study population in our laboratory (Harb et al, submitted for publication). Also, levels of histone H3 acetylation at the *IL9* promoter were higher in the high-folate group compared with the low-folate group (Harb et al, submitted for publication). However, more mechanistic studies are needed to identify the exact mechanism of how folate as a methyl donor affects histone acetylation and how this might influence development of the allergic atopic phenotype. Thus far, there is no evidence to promote any change in the current recommendations for folate supplementation during pregnancy.

Fish oil. Fish oil is one of the main sources of omega-3 fatty acids (n-3), which are the precursors of a large number of (anti)-inflammatory mediators, including defensins, resolvins, and others.^{86,87} Consumption of fish oil capsules or even purified n-3 fatty acids is very common also during pregnancy. There have been several studies examining n-3 fatty acid consumption during pregnancy, early life, or both and the effect on the development of allergic disease. In the KOALA birth cohort study maternal blood samples (n = 1275) were collected at weeks 34 to 36 of pregnancy and analyzed for concentrations of n-6 and n-3 *long-chain polyunsaturated fatty acids*. The full spectrum of offspring's atopic manifestations (wheeze, asthma, allergic rhinoconjunctivitis, eczema, atopic dermatitis, allergic sensitization, and high total IgE levels) was assessed by using repeated parental questionnaires and measurements of total and specific IgE antibody levels until the age of 6 to 7 years. A high blood ratio of maternal n-6 versus n-3 long-chain polyunsaturated fatty acids could be associated with a lower risk of childhood eczema; however, this remained nonsignificant. A decreased risk of eczema in the first 7 months of life was associated with increasing *arachidonic acid* levels.⁸⁸ On the other hand, another birth cohort study by Willers et al⁸⁹ showed that consumption of fish or fish oil during the last month of pregnancy had no effect on allergic disease development later in life.

Furthermore, a prospective birth cohort of 4089 new-born infants was followed for 4 years by using parental questionnaires at ages 2 months and 1, 2, and 4 years to collect information on exposure and health effects. It showed that regular fish consumption in the first year of life was associated with a reduced risk of allergic disease and sensitization to food and inhalant allergens during the first 4 years of life.⁹⁰

How does fish oil consumption alter the development of clinical phenotype? There are initial data providing mechanisms toward altered expression of the nuclear factor κ B subunit p65 through histone deacetylation in macrophages, which acts through activation of the AMPK/SIRT1 pathway *in vitro*.^{91,92} The modified expression of nuclear factor κ B affects important inflammatory regulatory pathways; however, the detailed mode of action is not fully understood, and more studies are certainly needed (Table III).

Obesity

Lifestyle changes are one of the important factors that have an effect on the epigenome. Obesity is considered one of the risk factors that predispose to different diseases, including cardiac disease, diabetes, and some forms of asthma.

In adipose tissue of diabetic twins, decreased expression levels of genes involved in oxidative phosphorylation and carbohydrate, amino acid, and lipid metabolism have been reported by several studies.⁹³⁻⁹⁵ This was accompanied by an increased expression of genes involved in inflammation and glycan degradation. Some of the most differentially expressed genes were *ELOVL6*, *GYS2*, *FADS1*, *SPP1* (OPN), *CCL18*, and *IL1RN*. Differences in methylation between monozygotic twin pairs discordant for type 2 diabetes were subsequently significant.⁹⁶

In another cross-sectional study 117 genes associated with obesity were identified by means of a genome-wide association study. Next, it was analyzed whether the methylation levels of these identified genes were also associated with obesity in 2 genome-wide methylation panels. An initial panel of 7 adolescent obese patients and 7 age-matched lean control subjects was investigated, followed by a second panel of 48 adolescent obese patients and 48 age- and sex-matched lean control subjects. The validated CpG sites were further replicated in 2 independent replication panels (46 vs 46 patients and 230 vs 413 control subjects, respectively) and in a general population of youth, including 703 healthy subjects. In particular, one CpG site in the lymphocyte antigen 86 (*LY86*) gene was identified that showed significantly higher methylation levels in the obese group.⁹⁷

With regard to asthma and allergic disease, there is evidence that obesity, a high-fat diet, or both can cause worsening of asthma in both human subjects and mice. Dietze et al⁹⁸ showed that in mice high-fat diet-induced obesity decreased the sensitization threshold in an asthma model. These findings support the concept that obesity is a risk factor for the development of allergic asthma, particularly during childhood.

In studies in 286 asthmatic patients, all subjects were evaluated by performing clinical examination, spirometry, fraction of exhaled nitric oxide measurement, and Asthma Control Test questionnaire. Ninety-six (33.6%) patients were overweight, and 45 (14.1%) patients were obese. Lung function was significantly impaired in overweight and obese asthmatic patients in comparison with normal-weight subjects. Overweight patients had double the risk and obese patients had triple the risk of having pathologic

FEV₁ lung function levels in comparison with normal-weight subjects.⁹⁹ Investigating the mechanism behind pathologic lung function, in a genome-wide DNA methylation analysis of 8 normal-weight asthmatic patients versus 8 obese asthmatic patients, Rastogi et al showed a methylation difference in several gene loci in PBMCs. PBMCs from obese asthmatic children had lower levels of promoter methylation of the *CCL5*, *IL2RA*, and T-box transcription factor (*TBX21*) genes, which are associated with T_H1 polarization. On the other hand, there was increased promoter methylation for *TGFB1*, encoding a cytokine associated with anti-inflammatory activity and Treg cell function. Furthermore, promoter methylation was also found for the gene *FCER2*, the low-affinity receptor for IgE (Table III).¹⁰⁰ These are some examples indicating a role of epigenetic alteration as a mechanism linking metabolic change (eg, obesity) and the effect on altered gene expression leading to phenotype (eg, asthma) development.

Stress

Stress represents an additional important environmental factor operating at least in part through epigenetic modifications. Psychological stress has emerged in recent years as an important additional risk factor for the pathogenesis and severity of childhood asthma.¹⁰¹ Membrane receptor for pituitary adenylate cyclase-activating peptide (*ADCYAP1R1*) is highly expressed in the hypothalamus and limbic structures, which are integral to the stress response. Recently, Chen et al¹⁰² identified a higher methylation status at the *ADCYAP1R1* gene in one of the CpG islands in the promoter. This increase was associated with asthma development, especially in children experiencing family violence. These observations also suggest that the early-life environment could have a long-lasting effect on the development of complex disease and subsequently reprogram biological responses to physiologic stress (Table II). Other studies in rats suggested that low levels of maternal nurturing showed higher cytosine methylation of a single CpG site in the glucocorticoid receptor promoter compared with low methylation of the same CpG sites in pups receiving high levels of maternal care. This change occurs specifically in the hippocampus¹⁰³ and shapes lifelong patterns of stress responses in pups.

The methylation change could thus be considered a contributing factor to potentiate the stress response and, subsequently, posttraumatic stress disorder, thereby increasing asthma risks. Prospective studies would help resolve many open questions in this regard. Also unclear is whether the reported changes in genetic variation or CpG methylation are functionally relevant, resulting in altered gene expression and subsequent modification of the allergic phenotype.

Conclusion

Epigenetic modifications, including both DNA methylation and histone acetylation, play an important role in regulation of different cellular functions, including regulation of inflammatory responses, DNA repair, and cell proliferation and differentiation. Some of these modifications are potentially reversible and might be considered targets for new medications and treatments for different diseases, including allergic conditions. Furthermore, various epigenetic modifications have been reported during pregnancy and early life and might be useful as biomarkers of disease development and disease risk. However, many questions remain on the role of different epigenetic regulator mechanisms in various areas. More clinical studies are needed to unmask the

exact mechanism of epigenetic modifications and their role in disease development. Other questions relating to the regulation of gene-specific epigenetic modifications and control of the events through the underlying enzymatic machinery are still open. Reversibility and stability of these effects also require further attention together with the question about the inheritance of different epigenetic marks.

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