

## REVIEW

# Environmental epigenetics and allergic diseases: recent advances

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## Clinical & Experimental Allergy

### Abstract

Significant strides in the understanding of the role of epigenetic regulation in asthma and allergy using both epidemiological approaches as well as experimental ones have been made. This review focuses on new research within the last 2 years. These include advances in determining how environmental agents implicated in airway disease can induce epigenetic changes, how epigenetic regulation can influence T helper cell differentiation and T regulatory cell production, and new discoveries of epigenetic regulation associated with clinical outcomes.

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### Introduction

The field of epigenetics provides a new look at the old debate of nature vs. nurture and reexamines the question 'What health risks are heritable?' Publication rates on epigenetics and the complex diseases asthma and/or allergy have increased rapidly over the last several years (Fig. 1). This new attention is a result of a growing recognition that epigenetic regulation has the potential to explain many mysteries in the pathogenesis of allergy, such as its susceptibility stemming from pre-natal environmental exposures, and its subsequent variable phenotype. Significant strides in the understanding of the role of epigenetic regulation in asthma and allergy using both epidemiological approaches as well as experimental ones have been made.

In this article, we will review two areas where significant advances have been made: (1) epigenetic regulation in response to environmental exposures and (2) epigenetic regulation associated with the development of asthma and allergy, both at the molecular and at the clinical level. This review will focus on new research within the last 2 years. These works include recent advances in determining how environmental agents implicated in airway disease can induce epigenetic changes, how epigenetic regulation can influence T helper (Th) cell differentiation and T regulatory (Treg) cell production, and new discoveries of epigenetic regulation associated with clinical outcomes.

### Defining epigenetic regulation

The modern definition of epigenetics is the inheritance of changes occurring in gene expression that does not depend on changes to the DNA sequence [1]. However, the term epigenetics, as coined by C. H. Waddington in the 1940s, is 'the causal interactions between genes and their products, which bring the phenotype into being' [2]. Waddington's definition initially referred to the role of epigenetics in embryonic development; however, the definition of epigenetics has evolved over time and is implicated in a wide variety of biological processes. The most common epigenetic mechanisms include DNA methylation, histone modifications, and non-coding RNAs. These molecular changes have the potential to modify the transcription of genes involved in the host response to environmental compounds, the ensuing pro-inflammatory response, or even the efficacy of pharmacological treatment.

Epigenetic modifications may be heritable across multiple generations such that pre-natal parental or grandparental exposures impact gene expression in offspring without altering DNA sequences [3]. Early seminal work using mice models performed by Cooney and colleagues and Waterland and Jirtle demonstrated this inheritance for the kinked tail (*Axin<sup>Fu</sup>*) allele and agouti-viable yellow (*A<sup>vy</sup>*) allele [4–6]. Alternatively, epigenetic modifications

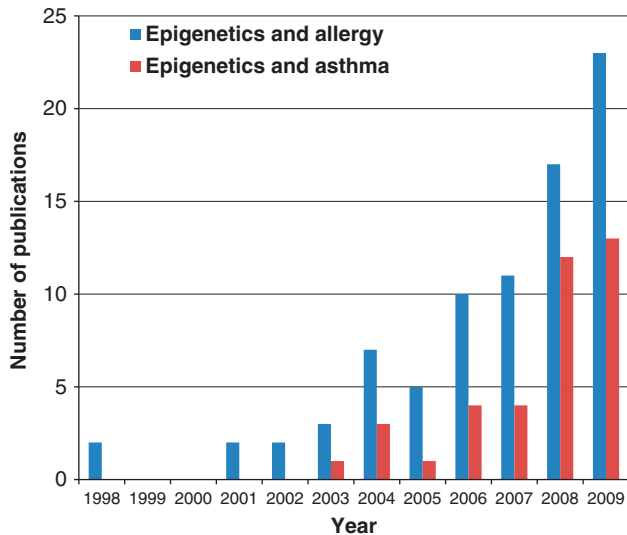


Fig. 1. Publications on epigenetics and allergy, asthma through 2009. Number of publications was determined through PubMed search using the search phrases 'epigenetics and allergy' or 'epigenetics and asthma'.

may occur post-natally, leading to sustained effects on gene transcription [7]. In other work, Weaver and colleagues found that after exposure to histone deacetylase (HDAC) inhibitors such as trichostatin A or methionine in adulthood, rats underwent reversal of early-life epigenetic programming in the hippocampus associated with glucocorticoid receptor expression and hypothalamic pituitary-adrenal and anxiety-mediated behaviours [8]. Hence, epigenetic modifications may also be reversible during certain time periods according to a model that may fit the remitting course of childhood asthma.

#### DNA methylation

DNA methylation is an epigenetic mechanism that allows the regulation of transcription via the addition of methyl groups to the fifth carbon of the nucleotide cytosine. It is the most frequent covalent modification of DNA [9]. DNA methylation often takes place in repetitive, high-frequency CpG sites known as islands. These are regions with at least 500 base pairs and contain > 50% of cytosine and guanine nucleotides. Such regions are located at the 5'-terminal. CpG methylation inhibits gene transcription by either blocking the ability of transcription factors to bind to the recognition sites on the CpG nucleotides or by facilitating the binding of transcription-inhibiting proteins [10].

#### Histone modification

Chromatin is structured within the cell nucleus into units called nucleosomes, composed of histone octamers around which DNA is coiled. Post-translation modifications of histones by means of acetylation, methylation,

and phosphorylation are key elements in the chromatin packaging of DNA. As a result of this tight packaging, RNA polymerase II and transcription factors cannot reach their recognition sequence to turn on transcription. With acetylation, the DNA around the histone core unwinds, activators of transcription obtain access to DNA, and gene expression can then proceed [11]. Conversely, the removal of acetyl groups as mediated via HDAC induces gene silencing. HDACs operate as part of large multi-protein complexes so that when they are associated with other proteins, they can form transcriptional repressor complexes [12]. Histone methylation is another epigenetic modification that is generally associated with transcriptional repression by adding a methyl group to amino acids in a histone protein; however, methylation of some histone residues may result in transcriptional activation, such as in the case of methylation of lysine 4 of histone 3 (H3K4). In contrast, methylation of lysine 9 or 27 of histone 3 (H3K9 and H3K27) is transcriptionally repressive [13].

Histone modifications may occur in association with DNA methylation. Histone methylation differs from DNA methylation in that histone methylation directly alters chromatin structure to repress transcription, whereas DNA methylation induces transcriptional silencing through the recruitment of methyl-CpG-binding proteins. These proteins selectively recognize and bind methylated DNA to repress transcriptional activity through the recruitment of HDAC-containing complexes [14]. Regulatory elements that aid in chromatin remodelling over long distances by binding of transcription factors and formation of chromatin loops also fall into the category of post-translational histone modifications. Enhancers bind transcription factors and stimulate the formation of chromatin loops, allowing promoter-transcription factor interaction, whereas silencers make the chromatin structure inaccessible, thereby inducing opposing effects on gene transcription [15, 16].

#### Epigenetic regulation, pre-natal exposures, and asthma

So why consider epigenetic regulation in the pathogenesis of asthma? The variable natural history of asthma is determined by complex genetic and environmental influences and epigenetics may be a link to this intricate interplay. Environmental exposures during time periods when there is greater susceptibility to epigenetic regulation may be responsible for developmental plasticity. These time periods when epigenetic modifications may be more likely to occur include pre-natal, early childhood, and adolescence [17]. These time windows of susceptibility coincide with time periods when the asthma phenotype is known to be variable and changing [1, 18]. During the pre-natal period, in particular, epigenetic reprogramming

may lead to a generation of cells with a broad developmental potential [19].

In the first case (i.e. pre-natal time period), growing scientific evidence has supported a role for intrauterine environmental influences in the risk for later paediatric asthma [20, 21]. This premise has been described poignantly as the rationale behind the Barker hypothesis that postulates that organs undergo developmental programming *in utero*. This programming pre-determines the subsequent physiologic and metabolic adaptations during adult life. Thus, through epigenetic regulation, *in utero* events have the potential to program persisting disease phenotypes and also determine the subsequent risk of disease [17].

Another way to consider pre-natal epigenetic regulation is as a mechanism underlying the heritable component of allergic disease that is not attributable to the DNA sequence alone [22]. Gene expression may be determined by its parental origin. This functional inequality of expression between two parental alleles of a gene is defined as genomic imprinting. The mechanism of imprinting is complex and not completely understood; however, the 'imprint mark' may be a parental-specific methylation of CpG-rich domains that silences gene transcription and is established during gametogenesis. These imprinted marks on a gene, however, must be erased in the germline when transmitted through individuals of the opposite sex, but maintained during somatic cell division [23, 24]. Imprinting has been implicated in embryogenesis. For example, Barton and colleagues showed that eggs with only the maternal or the paternal genome can only develop up to the blastocyst stage [25]. This finding suggests that the developing embryo needs both the maternal and the paternal genomes so that if the maternal copy of a gene has been imprinted and is inactive, there will still be an active paternal copy of the gene [26].

Gene expression in asthma and atopy may also be influenced by imprinting of parental genomes such that the genetic pre-disposition to allergic disease can be silenced by DNA methylation when transmitted from the father but expressed when transmitted by the mother. Some epidemiological literature supports this phenomenon. For example, Litonjua et al. [27] and colleagues reported that the risk for childhood asthma increased with a maternal, but not a paternal history of asthma. Furthermore, in a meta-analysis by Lim and colleagues, maternal asthma predisposes offspring to disease more so than paternal asthma [28]. Liu and colleagues found that maternal, but not paternal, total IgE levels significantly correlated with elevated IgE levels in cord blood at the age of 6 months [29]. However, in a more recent work, Ferreira and colleagues investigated the role of global DNA methylation patterns in this 'maternal effect' of atopy, namely the greater predominance of asthma or allergy if the mother, as opposed to the father, is atopic. In their essentially negative study, they found that the AluSp

element (a CpG-rich stretch of DNA) in the  $\beta$ -chain of the IgE receptor gene was hypermethylated across all children regardless of the atopic status of the parents or the children [30, 31]. Hence, the findings that support imprinting as the explanation for the disproportionate inheritance of atopy from the mother are mixed, and more correlative than mechanistic.

Intrauterine exposures causing epigenetic regulation may also affect asthma risk. Recent epidemiological support for this premise was suggested by Li and Colleagues. Using retrospective questionnaires, they demonstrated that pre-natal exposure to maternal tobacco smoking was associated with lower pulmonary function and increased asthmatic symptoms in childhood. Moreover, combined maternal and grandmaternal smoking during pregnancy was associated with an even greater risk of childhood asthma, suggesting a multi-generational heritability for asthma [32].

#### *Pre-natal folate and risk of atopy and asthma*

One feature of the intrauterine environment that has been implicated in later childhood risk for atopy is pre-natal diet and folate intake specifically. Folate is a relatively large source of methyl donors and pre-natal supplementation may alter DNA methylation and consequently gene expression. In mice, the importance of a pre-natal diet high in methyl donors was demonstrated by Hollingsworth and colleagues, who found that this diet (combined with soy, also known to alter DNA methylation) given to mice during gestation and weaning was associated with greater airway hyper-reactivity, airway eosinophilic inflammation, chemokines, and IgE production in the offspring. The phenotype was also associated with greater levels of DNA methylation and RNA and protein suppression of runt-related transcription factor 3 (*Runx3*), a gene associated with silencing of *CD4* during T cell lineage decisions [33, 34]. Consistent with Hollingsworth, Whitrow et al. [35] found that women who received supplemental folic acid during late pregnancy as opposed to early supplementation, as measured by retrospective consumption questionnaires and interviews, had children with a greater risk of physician-diagnosed asthma. However, Håberg and colleagues reported that the use of folic acid supplements in pregnancy during the first trimester, also measured by retrospective questionnaires, was associated with a slight increase in the risk of early respiratory infections and wheeze [36]. Their differing findings with regard to the timing of folate exposure during pregnancy may be explained by differences in reporting of folate intake as a bi-categorical (yes/no) value and also a shorter follow-up (18 months vs. 5.5 years). Nonetheless, they suggest that a critical window within pregnancy during which folate exposure can epigenetically influence the subsequent risk for asthma may be important to investigate prospectively and should be confirmed in more human studies. In other work, Steegers-Theunissen and colleagues

found that periconceptional folic acid use of the mother was related to increased methylation of the insulin-like growth factor 2 (*IGF2*), a critical and widely expressed embryonic growth factor of the child. Furthermore, maternal S-adenosylmethionine (an endogenous source of methyl groups formed from demethylated methionine) concentrations were correlated positively with *IGF2* methylation of the child's peripheral blood DNA [37]. These findings suggest that pre-natal dietary folate may act as an epigenetic regulator of gene expression and ultimately may be associated with altered disease risk.

#### *Pre-natal environmental exposures and asthma risk*

A second intrauterine environmental exposure widely implicated in later paediatric asthma is air pollution. Pre-natal environmental tobacco smoke (ETS) exposure has been associated with decreased pulmonary function and a greater risk for developing asthma in both children and adults [38–41]. Wu and colleagues showed that the mice exposed to sidestream smoke during earlier periods of gestation had significantly elevated methacholine responses for lung resistance, decreased lung compliance, increased substance P innervation in tracheal smooth muscle, and elevated levels of nerve growth factor in BAL [42]. In humans, our group showed that at the age of 2 years, more difficulty in breathing and probable asthma were reported among children jointly exposed to pre-natal polycyclic aromatic hydrocarbons (PAHs) and post-natal ETS [43]. As described below, data on how these intrauterine exposures may confer a greater risk for allergy and the role of epigenetic regulation are just beginning to emerge.

#### **Epigenetic regulation of the effects of environmental exposures implicated in allergy, asthma: the latest evidence**

There is a growing body of literature implicating epigenetic mechanisms in the host response to several environmental exposures implicated in the development of atopy or asthma. For example, considerable work has been carried out recently on the induction of epigenetic changes following exposure to air pollution. For one, our group found that concomitant exposure of mice to diesel exhaust particles (DEP) and allergen *Aspergillus fumigatus* augmented IgE production potentially by inducing DNA hypermethylation at selected CpG sites in the interferon (IFN)- $\gamma$  promoter and DNA hypomethylation in the interleukin (IL)-4 promoter. The extent of altered methylation correlated with IgE production [44]. While the bulk of non-coding DNA may not be conserved between humans and mice, sequence comparisons reveal islands of highly conserved non-coding sequences (CNS). This has been seen at the site located approximately 5 kbp 5' of the transcription start site (TSS) for the IFN- $\gamma$  gene and also at the -53 CpG and -190 CpG of the IFN- $\gamma$  promoter in CD4

cells [45, 46]. However, Janson and colleagues found interspecies differences in the methylation status of the IFN- $\gamma$  promoter in naïve CD4 T lymphocytes. In humans, the IFN- $\gamma$  promoter displays a high level of methylation in naïve CD4 T lymphocytes, but becomes demethylated with Th1 differentiation, whereas in mice, the IFN- $\gamma$  promoter region of naïve CD4 T lymphocytes is demethylated [47].

In other work, Cau and colleagues documented altered chromatin modification following exposure to DEPs. In their experiments, exposure of human bronchial epithelial cell lines to DEPs increased acetylation of histone H4 associated with the cyclooxygenase (COX)-2 promoter. This led to the post-translational degradation of HDAC1 and recruitment of histone acetyltransferase (HAT) p300 to the COX-2 promoter, ultimately activating COX-2 gene expression [48]. In a recent human cohort study by Baccarelli and colleagues [49, 50], exposure of adults to diesel and fine particulate matter (PM)<sub>2.5</sub> was associated with DNA demethylation in repetitive elements such as LINE-1, an indicator of global methylation. Similarly, Tarantini and colleagues studied the short-term (after 2 consecutive days off from work in a steel production plant) and long-term (after 3 consecutive days of work in a steel production plant) effects of PM<sub>10</sub> exposure on both global and asthma candidate gene [inducible nitric oxide synthase (iNOS)] DNA methylation in humans. They reported that short-term PM<sub>10</sub> exposure may be associated with DNA demethylation of the iNOS promoter, and long-term PM<sub>10</sub> exposure was associated with global DNA demethylation estimated in Alu-repeated elements (another indicator of global methylation) [51]. Our group showed that higher transplacental exposure to PAH was associated with altered DNA methylation of acyl-CoA synthetase long-chain family member 3 (ACSL3), possibly a novel asthma candidate gene [52]. Combined, these studies implicate epigenetic regulation, including both DNA methylation and histone modification, in the individual response to exposure to air pollution, and diesel, PM, and PAH specifically. Finally, a recent study by Breton and colleagues reported that pre-natal tobacco smoke was associated with lower DNA methylation for AluYb8 repetitive elements in children. Interestingly, pre-natal exposure was associated with lower LINE-1 methylation in the GSTM1 null children but higher methylation in the GSTM1-present children. This work suggests a novel interaction between exposure and genotype influencing susceptibility to DNA methylation [53].

#### **Epigenetic regulation and T cell changes related to the allergic immune response: the latest evidence**

##### *T helper 1 vs. T helper 2 cells*

The role of Th2 polarization in allergic disease has been well studied since Mosmann et al.' [54] seminal reports in

mice in 1986. Despite this abundance of research, it still remains unclear what molecular processes are important for the persistence of T cell polarization, especially *in vivo*. Previous studies have examined the role of intrauterine cytokine environments in transplacental transmission of T cell polarization. For example, using a mouse model, Hamada and colleagues reported that post-natal ovalbumin (OVA) challenge induced airway hyperresponsiveness (AHR) in the offspring of mothers sensitized to OVA prenatally, whereas no effect was seen in the offspring of non-sensitized mothers. Furthermore, post-natal challenge to unrelated allergens induced a similar response in the offspring of OVA-allergic mothers, suggesting that maternal inheritance of an asthma-like phenotype may be allergen independent. Importantly, treatment of asthmatic mothers with a neutralizing anti-IL-4 antibody before conception abrogated this response [55]. This finding associates IL-4 and potentially other intrauterine cytokines as a mediator of maternal inheritance of susceptibility to AHR, at least in mice. The role of epigenetic regulation in the intrauterine cytokine environment has yet to be demonstrated.

Recent advances have been made in the understanding of the regulation of levels of methylation of CpG sites within the IFN- $\gamma$  promoter. Jones and colleagues documented that during Th2 polarization, the IFN- $\gamma$  promoter undergoes *de novo* methylation, most notably in the CpG located at the -53 position proximal to the TSS, which is highly conserved across species. DNA methylation at this site prevents transcription factor (c-Jun, CREB) binding required for IFN- $\gamma$  gene expression during Th1 polarization [46]. White and colleagues also demonstrated that under Th1-polarizing conditions, CpG sites -295, -186, and -54 upstream to the TSS of the IFN- $\gamma$  promoter in neonatal CD4<sup>+</sup> T cells were demethylated, whereas under Th2-polarizing conditions, the majority of these sites remained methylated, again providing an epigenetic mechanism for possible suppression of the IFN- $\gamma$  locus in Th2-polarized cells [56]. Recent advances have also focused on chromatin remodelling of the IFN- $\gamma$  promoter. Zhang and colleagues found that nucleosomes at the IFN- $\gamma$  gene promoter undergo repositioning to develop a more open topography, which creates new access to transcription factor binding in Th1 cells; this occurs in a STAT4-dependent, T-bet-independent manner [57]. The activation of STAT4 allows for histone acetylation to accumulate at the conserved regions of the IFN- $\gamma$  promoter, facilitating STAT4 transcription factor binding, thereby committing naïve T cells towards a Th1 lineage and not that of Th2. These results suggest that IFN- $\gamma$  transcription is determined in part by the balance of the activity of histone acetylases and deacetylases recruited to the IFN- $\gamma$  promoter. However, the IFN- $\gamma$  promoter can also acquire repressive epigenetic modifications, such as histone methylation, particularly in developing Th2 cells. For

example, Chang and Aune found that methylation of lysine 9 of histone 3 (H3K9) was sustained in Th1 cells, but rapidly extinguished in developing Th2 cells, whereas lysine 27 of histone 3 (H3K27) became methylated during Th2 differentiation. Both events occurred via STAT6- and GATA-3-dependent mechanisms [58].

Previous work has also focused on the regulatory elements of the IFN- $\gamma$  locus. For example, Lee and colleagues reported that Th1 cells display DNase I hypersensitivity and histone modifications at CNS1, whereas Th2 cells do not. This finding indicates that this region is more permissive to transcription in Th1-differentiated cells compared with Th2 cells [45]. Hatton and colleagues found that CNS22 is particularly susceptible to permissive histone modifications and selective T-bet binding, and subsequently functions to increase IFN- $\gamma$  expression in Th1 cells. The deletion of CNS22 resulted in decreased IFN- $\gamma$  expression in Th1 effector cells, cytotoxic T lymphocytes and natural killer cells [59]. These findings implicate CNS22 as an epigenetic regulator of IFN- $\gamma$  expression. Furthermore, Shnyreva and colleagues reported that histone acetylation in the IFN- $\gamma$  promoter, intronic regions, CNS1, and CNS2 increased as naïve T cells differentiated into IFN- $\gamma$ -producing effector CD8<sup>+</sup> and Th1 T cells, but not into Th2 T cells [60]. In other work, Schoenborn and colleagues performed a large-scale epigenetic profiling using DNA methylation analysis and DNase hypersensitivity site mapping of the IFN- $\gamma$  gene. They found that regulatory elements such as CNS -54 and +46 functioned mainly as boundary elements, where DNA sequences prevent promoter-enhancer interactions. In contrast, more proximal CNSs such as CNS -34, -22, and -6 functioned to increase the transcription of the IFN- $\gamma$  gene [15].

DNA methylation also plays an important role in the control of Th2 cytokine expression and stabilization during Th cell development. Most notably, when naïve T cells are activated under Th2-polarizing conditions, demethylation occurs at the IL-4 gene promoter [16, 61]. More recently, investigations have focused on the epigenetic regulation of IL-13 expression. Webster and colleagues reported that in naïve CD4<sup>+</sup> T cells, DNA hypomethylation was limited to the distal promoter of IL-13; however, with Th2 differentiation, the proximal IL-13 promoter was hypomethylated preferentially. Furthermore, histone H4 acetylation levels and hypersensitivity sites at the IL-13 proximal promoter were considerably higher in Th2 cells [62]. These results suggest that differential IL-13 expression may depend on the epigenetic control and accessibility of the proximal promoter in Th2 cells.

In other work, Kim and colleagues characterized demethylation events for the Th2 cytokine locus control region rad50 hypersensitive site 7 (RHS7), an area where IL-4, IL-5, and IL-13 expression is regulated [63]. Under

Th2-polarizing conditions, *RHS7* becomes demethylated in a STAT6-dependent manner in CD4<sup>+</sup> T cells. This demethylation appears to require a signalling contribution from both the IL-4 receptor and CD28, whereas GATA3-mediated signalling also involving demethylation appears to be insufficient for this remodelling [64]. Additionally, Chong and colleagues found that loss of the proximal enhancer of the CD4 gene (E4p) in immature CD4<sup>+</sup>CD8<sup>+</sup> thymocytes resulted in unstable expression of CD4 in mature T cells. However, the deletion of E4p in cells already committed to the Th cell lineage resulted in stable CD4 expression. Transcriptionally permissive histone modifications of histone 3, such as acetylation and trimethylation, required initial E4p enhancer activity. These findings suggest that E4p is an epigenetic regulator of CD4 expression [65]. Dispirito and colleagues found that the level of diacetylation of histone 3 increased as naïve CD8<sup>+</sup> T cells developed into T memory cells, thereby implicating epigenetic regulation in CD8 differentiation [66]. In other recent work, Hughes and colleagues reported that paradoxically, DNA methylation may be associated with transcriptional permissiveness, particularly in areas of increased promoter CpG island density. Using DNA methylation and gene expression analysis, they found that 27% of methylated genes (55 genes) in primary human CD4<sup>+</sup> T cells are expressed. Of the methylated, but transcriptionally expressed genes, methylation peaks were located approximately nine nucleosomes away from the TSS and had higher maximum CpG island densities within promoter sequences of target genes. In contrast, methylated and transcriptionally repressed genes were located only six nucleosomes away from the TSS and had a relatively lower maximum CpG island density [67]. This finding may be a consequence of the poor recruitment of methyl binding proteins and ultimately HDACs secondary to the longer distance from the TSS; however, this has yet to be demonstrated. This alteration of repressive DNA methylation patterns at key CpGs may play a role in T cell polarization. In summary, recent advances in epigenetic regulation have provided considerable insight into Th cell lineage commitment, although our knowledge is still relatively limited in the area of *in vivo* changes.

### Treg

There is growing evidence supporting the role for Treg cells and the immunosuppressive cytokines they produce, as mechanisms by which allergen-specific immunotherapy and healthy immune responses to allergens are mediated [68]. Treg cells are capable of suppressing effector cells and therefore are likely play a role in allergen tolerance by harnessing allergen-induced inflammation in early processes such as sensitization [69]. Specifically, Treg cells contribute to the control of allergen-specific immune responses by suppressing mast cells,

basophils, eosinophils, and antigen-presenting cells that support the generation of effector Th2 and Th1 cells and by suppressing allergen-specific IgE, IgG4, IgA production, or both [68].

Epigenetic mechanisms controlling Treg development are just beginning to be explored. Studies have focused previously on FoxP3, a central control element essential for Treg development and function. Epigenetic changes are a prerequisite for FoxP3 expression and Treg differentiation. Recently, Floess and colleagues documented that Tregs induced by TGF- $\beta$  display incomplete demethylation despite high FoxP3 expression. Upon restimulation in the absence of TGF- $\beta$ , these Tregs lose both FoxP3 expression and suppressive activity. These results suggest that the expression of FoxP3 must be stabilized by epigenetic modification to allow the development of a permanent suppressor cell lineage [70]. Polansky and colleagues also found that inhibition of DNA methylation by azacytidine, even in the absence of exogenous TGF- $\beta$ , promoted *de novo* induction of FoxP3 expression. In contrast, *in vitro* methylation of Treg-specific demethylated region diminished FoxP3 transcriptional activity. This work demonstrates that epigenetic regulation in this region is critical for the establishment of a stable Treg lineage [71]. Janson and colleagues examined the methylation profile of the FoxP3 promoter in the commitment towards the Treg lineage. They found that human CD4<sup>+</sup>CD25<sup>hi</sup> Tregs displayed a demethylated FoxP3 promoter in contrast to CD4<sup>+</sup>CD25<sup>lo</sup> T cells that were partially methylated. Moreover, stimulated CD4<sup>+</sup>CD25<sup>lo</sup> T cells transiently expressed FoxP3, but remained partially methylated [72]. This characterization of the unique FoxP3 promoter methylation in Tregs suggests that a demethylated pattern is a prerequisite for stable FoxP3 expression and a suppressive phenotype. Much more research is required to illuminate the epigenetic markers needed to establish a lineage commitment towards that of Tregs and its role in atopic disease.

### Epigenetic regulation and clinical allergic disease

To date, only a few papers have examined the clinical consequences of epigenetic modifications on asthma and allergic disease. For example, our group found that the 59-CpG island methylation status of *ACSL3* is not only associated with pre-natal PAH exposure but is also significantly associated with a parental report of asthma symptoms in children before the age of 5 years. *ACSL3* belongs to the *ACSL* family of genes that encodes key enzymes in fatty acid metabolism [52]. It has been speculated that hypermethylation of this gene in Th cells or lung tissues may diminish fatty acid utilization and  $\beta$ -oxidation energy production. This in turn may influence the membrane phospholipid composition, with associated anti-inflammatory effects in asthma. However, it is

unknown how these changes directly airway influence inflammation, and future studies will need to validate whether ACSL3 is a novel asthma gene. Nonetheless, this is the first study to examine the effects of pre-natal exposure to ambient air pollutants on DNA methylation patterns in genes potentially associated with the asthma phenotype of the offspring. Another cohort study by Kwon and colleagues examined the relationship between CpG methylation and IL-4 gene expression before and after allergen stimulation in human CD4<sup>+</sup> lymphocytes from sensitized hosts. They found that after combined *Dermatophagoides pteronyssinus*/*Dermatophagoides farinae* stimulation, hypomethylation in the IL-4 promoter increased among asthmatics. The concentration of IL-4 was strongly correlated with the extent of hypomethylation [73]. In a prospective cohort study by White and colleagues, *ex vivo* IFN- $\gamma$  promoter methylation was reduced in CD8<sup>+</sup> T cells, but not in CD4<sup>+</sup> T cells from atopic children [56].

Finally, Su and colleagues examined epigenetic regulatory functions in the maintenance of Th1 and Th2 immunity in children by *ex vivo* inhibition of endogenous HDAC activity. They found that increasing cellular acetylation via administration of trichostatin, a HDAC inhibitor, to peripheral blood mononuclear cells, and the corresponding decreasing HDAC activity, shifted immune responses towards the Th2 phenotype with increased production of IL-13 and IL-5 and increased expression of GATA-3 [74]. These results suggest that endogenous HDAC was important in preventing pre-established cytokine responses from deviating towards excessive Th2 polarization. Furthermore, other work by Su and colleagues demonstrated that *ex vivo* HAT activity was increased and HDAC activity was reduced among children with allergic asthma relative to atopic non-asthmatic controls. The intensity of cellular histone acetylation increased progressively with increasing bronchial hyperresponsiveness, thereby potentially implicating epigenetic regulatory control over varying atopic asthmatic phenotypes [75].

Epigenetic modifications, including DNA methylation, are dynamic. For example, Tarantini and colleagues found that short-term exposure to PM<sub>10</sub> is associated with decreased methylation of the iNOS gene promoter over 3 days [51]. Furthermore, Baccarelli et al. [50] reported that exposure of adults to diesel and PM<sub>2.5</sub> over 7 days (using moving averages) was associated with DNA demethylation in repetitive elements such as LINE-1, an indicator of global methylation. In contrast, Bjornsson and colleagues reported that 8–10% of individuals on average had an absolute change in global DNA methylation of >20%. These results were found in two distinct populations over a duration of 11–16 years [76]. While the majority of individuals did not show significant changes in this study, there was familial clustering of longitudinal DNA methylation changes, thereby implicating a genetic component to methylation stability. Additionally, Christensen and

colleagues studied methylation patterns in 1413 CpG loci associated with 773 genes in 10 human tissue types. They found that loci in CpG islands were more likely to gain methylation within the 10 years of study follow-up compared with loci outside of CpG islands that were more likely to demethylate. These methylation changes were found consistently across tissue type [77]. While these findings may not best demonstrate the dynamic nature of these changes, it does demonstrate some plasticity of epigenetic modifications and furthermore implicates age-related losses of epigenetic patterns. Histone modifications are also believed to be dynamic and associated with even shorter term changes to gene transcription compared with DNA methylation [78]. However, future studies are needed to validate this notion.

## Conclusion

The accumulated evidence has undoubtedly solidified the case for implicating epigenetics as a mediator of a complex gene by environment interactions relevant to the development of asthma and allergic diseases. Advances have been made linking air pollution and ETS exposure with atopy via epigenetic mechanisms. Furthermore, considerable strides have been made implicating epigenetic mechanisms in T cell differentiation, especially at the IFN- $\gamma$  locus. However, much more research is still needed, particularly to delineate the role of epigenetics in the lineage commitment of Treg cells and also to define the clinical consequences of such epigenetic alterations. Despite the acceptance of epigenetic regulation in the pathogenesis of complex diseases and the rapid increase in publications, the extent of environmental epigenetics in the pathogenesis of asthma and allergies is just being realized. Longitudinal cohort studies are needed to examine the time course and time period of susceptibility to epigenetic regulation following environmental exposures and their contribution to allergic disease. Ultimately, an individual's epigenome early in life may be a helpful biomarker in determining later risk of asthma and atopy and initiating an early intervention. Despite these challenges, the future holds exciting promise.

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