

Progress in Understanding the Epigenetic Basis for Immune Development, Immune Function, and the Rising Incidence of Allergic Disease

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Abstract The profile of allergic disease worldwide continues to change as the number of severe IgE-mediated allergies increases. This phenomenon is thought to reflect the outcome of combined genetic/environmental/developmental/stochastic effects on immune development, but understanding this remains a challenge. Epigenetic disruption at key immune genes during development has been proposed as a potential explanation for how environmental exposures may alter immune cell development and function. This represents an emerging area of research with the potential to yield new understanding of how disease risk is modified. Here, we examine recent developments in this field that are defining new epigenetic paradigms of allergic disease.

Keywords Epigenetics · Allergic disease · Anaphylaxis · Immune development · Sensitization · IgE · DNA methylation · Histone acetylation · Gene–environment interactions · Epigenetic regulation

Introduction

The global prevalence of allergic disease is approaching pandemic proportions. A striking feature of this health crisis is the surging prevalence of potentially life-threatening anaphylactic reactions. Recent reports indicate the rates of these severe and acute allergic reactions appear to have approximately doubled

over the last 15–20 years [1–4], which is largely attributable to a rise in food-related anaphylaxis among very young children [5–7]. These trends are most apparent in Australia, the UK and the USA, and coincide with rapidly escalating rates of food allergy in these countries, which have increased over a similar time frame [8, 9]. This significant increase in the frequency of severe allergic reactions, and the more generalized rise in chronic non-communicable diseases (NCDs), has been linked to western lifestyles and urbanized environments, which are thought to be less favorable for promoting healthy immune development [10]. Understanding the causes and mechanisms of these diseases remains a key challenge, since the combined contribution of genes and environment still does not adequately explain the incidence of allergic diseases, nor their rapid increase in prevalence.

Epigenetic mechanisms are increasingly providing a new understanding of gene versus environment interactions. Modifications to the epigenome have been purported to mediate endogenous or exogenous environmental exposures on immune development [11]. These processes provide regulatory control of gene expression independently of genomic sequence, and vary in response to developmental/environmental cues and due to stochastic events. In the immune system, epigenetic control of gene expression plays a central role in development, differentiation and immune regulation [12]. Disruption of these pathways could stably alter the host immune response at the individual level, or may underscore much of the variation in the clinical manifestations of allergic diseases at the population level. The challenge now is to determine the extent to which disruption of epigenetic gene control is causally related to chronic immune inflammatory conditions, and how these pathways might explain the rising incidence of allergies and anaphylaxis. This review explores the recent progress that has been made in experimental studies of epigenetic dysregulation in allergy, and some of the clinical and epidemiological studies linking variation in epigenetic marks with disease outcomes.

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Evolutionary Perspectives of Acute IgE Mediated Immune Responses

The Th2 responses that characterize allergic disease are not inherently pathological and only cause disease when excessive or misdirected. These pathways have been conserved in the human genome as the result of evolutionary pressure over millennia, as part of the myriad of elaborate defenses against potential environmental threats. Challenging the traditional concepts that these pathways evolved to defend against helminthes, Palm and colleagues [13] have recently proposed that the very rapid ‘acute’ IgE-mediated responses are likely to have evolved to protect against a much broader, diverse range of environmental irritants, toxins, venoms, hematophagous fluids (from ectoparasites such as mosquitoes and ticks) and noxious xenobiotics and phytochemicals. The resulting IgE-mediated symptoms (sneezing, tearing, coughing, vomiting, diarrhea, and itch) all serve to rapidly expel or remove these threats, potentially explaining the urgency of response and protection from ongoing threat through host aversion and conditioned avoidance [13]. These pathways also serve to enhance tissue barrier function and repair mechanisms, which restrict penetration, prevent spread, and promote healing. When activated chronically or inappropriately, these pathways also produce the many features of allergic disease. The perspective offered by Palm and colleagues may give new insight into the diversity of ‘allergens’ (plants, mites, venoms, antibiotics, animals, and fungi) that invoke the Th2 armamentarium.

Gene–Environment Interactions Modify the Acute Allergic Response

It is clear that changing patterns of gene–environmental interactions are responsible for the rise in allergic disease and many other inflammatory non-communicable diseases (NCD), and that epigenetic effects may present a potential mechanism. However, while environmental changes must be driving this ‘pandemic’, genetic predisposition remains an important determinant of individual susceptibility. Persistence of genetic polymorphisms that have no functional disadvantages in traditional environments may specifically predispose to disease under radically altered ‘modern’ conditions. Thus, while epigenetic effects appear to mediate the predisposing effects of environmental change, these may also be differentially affected by functional genetic polymorphisms (as discussed further below). There are already many examples of gene–environmental interactions that vary according to genotype, highlighting the considerably complexity of these relationships across scores of interrelated pathways.

Epigenetic Mechanisms of Genomic Control

Genomic mechanisms that alter the density of the chromatin state and its accessibility for transcription factor binding can collectively be grouped as epigenetic. The most common epigenetic modifications include DNA methylation, the addition of a methyl group to cytosine–guanine dinucleotides (CpG) present in DNA which is associated with gene silencing; histone modifications, which involve the competitive action of enzymes that transfer or remove covalent modifications to histone tail domains; and non-coding RNA signaling events (reviewed in [14]). These epigenetic marks are distributed throughout regulatory regions of the genome and control gene expression in a dynamic manner, compared with the DNA sequence, which is stable over time. They determine the packaging of DNA into either transcriptionally active (heterochromatin), fully repressed (euchromatin), or partially repressed (bivalent) chromatin states. The combined pattern of accessible and inaccessible chromatin confers cell-type-specific patterns of gene expression that are reproduced during mitosis, providing a mechanism for each cell to ‘remember’ its identity, and conferring a unique ‘epigenome’ to each cell type. This additional layer of genomic information is sensitive to changes in environmental conditions allowing for adaptive changes in gene expression independently of DNA sequence. Despite this, variations in the genetic code can alter the epigenetic state at a given loci [15], and therefore it is important to note that there is some dependency between the two.

Epigenetic Processes in Early Immune Development and the ‘Developmental Origins of Disease’ Hypothesis

In the mammalian immune system, epigenetic regulation of gene expression is critical in hematopoiesis during the stratification of intermediary and terminal cell phenotypes [16, 17]. Disruption in these developmental pathways can have profound effects on normal immune development. For instance, mice deficient for the DNA methyltransferase enzymes display inappropriate terminal differentiation of hematopoietic stem cells (HSC), resulting in depletion of the progenitor pool or a myeloid bias in the composition of hematopoietic progenitors [16–18]. In humans, analogous mechanisms regulate the developmental timing and expression of immune genes. Quantitative differences in DNA methylation at key immune genes such as *IFNG* have been described in neonatal CD4⁺ cells compared with adult and infant CD4⁺ cells, and the rate of demethylation that occurs upon *IFNG* induction in neonatal CD4⁺ cells also differs compared with adult cells [19, 20]. In innate immune pathways, the rate of TLR3 induction differs in neonatal versus adult monocytes when induced to differentiate into dendritic

cells (DC), and this is attributable to relative differences in histone acetylation profiles [21]. Similarly, reduced levels of IL-12 produced by neonatal DC are associated with a repressed chromatin state compared with adult cells [22].

In pregnancy and fetal life, developmental epigenetic mechanisms are of particular interest, since disruption in these pathways may underlie the developmental origins of many immune diseases. Complex immunologic mechanisms have evolved to allow the fetal and maternal immune systems to coexist during pregnancy, including subtle adaptations of humoral, cellular immunity, and regulatory T cell function. Macrophage cells deployed at the feto–maternal interface exhibit hypermethylation at classical macrophage activation genes, and relative hypomethylation in the alternative pathway, a property that maintains an anti-inflammatory phenotype [23]. The promoter of *FOXP3* is hypomethylated in the placental tissue compared with cord blood-derived CD4+ cells, and this may be important in maintaining tolerance at this immunologically sensitive site [24].

Maternal factors such as nutrition, stress, obesity could alter the placental and/or in utero environment, and the epigenetic programming of the embryo. This could be a critical determinant of immune outcomes in the offspring, and this notion is at the heart of the developmental origins of disease hypothesis. Evidence for this is emerging: a study by Slaats et al. [25] found lower DNA methylation levels at the CD14 promoter in placentas of mothers living in rural farming environments compared with those not living on a farm. Methylation of the CD14 gene promoter was highly correlated with CD14 mRNA expression. Variations in these epigenetic pathways may underscore altered cord blood immune responses and allergic outcomes in later life [26, 27]. A case–control study of atopic dermatitis in 12-month-old infants ($n=346$) found maternal atopy, smoking, and maternal cytokines (IFN- γ , IL-13, IL17E) were associated with lower Treg numbers, quantified by measuring methylation at the *FOXP3* Treg-specific differentially methylated region [28•]. This was associated with an increased risk of dermatitis at 12 months. Whilst these findings are preliminary, they fuel speculation that maternal exposures may epigenetically activate or silence key immune genes modifying the risk for allergic disease.

Ongoing Postnatal Exposures and the Accumulation of Epigenetic Variation Over Time

In mature cells, epigenetic pathways also play a central role in processes such as regulation of leukocyte differentiation, survival, proliferation, and regulation. Disruption in these epigenetic pathways is transmissible to daughter cells during mitosis, potentially resulting in the accumulation of substantial epigenetic variation within tissues over time. This could

potentially modify the individual's immune response and mediate 'threshold-effects' of disease. For example, there is evidence that T cells can epigenetically trans-differentiate into alternative lineages. Polarized CD4+ T-helper cells exhibit sub-type-specific methylation patterns at cytokine genes [29]; however, they retain bivalent epigenetic marks at the genes encoding the 'master regulator' transcription factors (*Tbx21*, *Gata3*, *Runx3*) [30]. This allows for considerable T cell plasticity in the face of changing circumstances, and may be a key mechanism in fine-tuning immune responses. There have been recent reports of intermediary T-helper cell phenotypes including distinct populations IL-5-producing and IL-5-deficient Th2 cells, which are associated with differences in active (H3K4me3) and repressive (H3K27me3) histone marks about the IL-5 promoter [31]. Similarly, ex vivo populations of intermediary Th1/Th17 phenotypes that produce both IFN- γ and IL-17 have also been observed, and these contain a unique profile of DNA de-methylation at both the IFN- γ and IL-17 promoters compared with single-positive counterparts [32]. Whether these populations represent true intermediary phenotypes, or whether they represent transient CD4+ cells that have undergone incomplete epigenetic reversion or heterogeneity within a defined population, is still uncertain. Regardless, accumulation of epigenetically variable cell populations within a tissue over time could modify an individual's inflammatory response, and may underscore much of the individual variation in response to therapies. There is evidence of accumulation of Th1/Th17 cells in patients with Crohn's disease and arthritis [33] suggesting a potential biologically plausible role in the chronic inflammatory response.

There is also evidence of epigenetic fine-tuning of immune responses in other cell populations, like Tregs. In patients that have undergone dual sublingual oral immunotherapy, CD45RO+Treg cells lose methylation at the *FOXP3* locus, and this correlates with a stably expressing FOXP3 phenotype [34•]. Interestingly, antigen-naïve (CD45RA+) Tregs in the same patients are unaltered over the treatment period, indicating highly specific effects. Clinically, this correlates with reduced allergen responsiveness, IgE levels, and basophil activation; however, it is unclear how long these populations persist, and whether they require constant exposure to maintain the epi-phenotype.

New Epigenetic Insights into Allergic Disease from Experimental Models

Determining whether or not epigenetic variation associated with immune outcomes represents true cause, or simply effect, is difficult to address in human studies. Furthermore, it is difficult to establish whether these effects may persist over subsequent generations. In this regard, experimental models continue to provide the most convincing evidence of

epigenetically pathways causally related to the allergic phenotype. Some of the recent animal work has demonstrated that, during allergen sensitization/challenge, an increase in DNA methylation at the *IFNG* promoter in CD4⁺ cells is associated with a decrease in IFN- γ production, and enhanced airways inflammation [35]. The silencing of the *IFNG* locus may also involve the action of histone methylase SUV39H1, a key enzyme involved in chromatin organization. Disruption of SUV39H1 alters the ratio of methylated to acetylated histone H4K9 and subsequent binding of HP1 α to the promoters of Th1 genes, enforcing maintenance of the Th2 lineage and concomitant repression of Th1 cells [36]. Interestingly, in both these studies, chemical inhibition of DNA methyltransferase or SUV39H1 rescued the inflammatory phenotype and lung pathology. There is also evidence that such effects can persist across generations when allergen exposure occurs during pregnancy. An interesting animal study demonstrated that grand-offspring (third generation progeny) of mothers sensitized and challenged with high dose *Aspergillus fumigatus* in early pregnancy exhibited lower methylation at the IL-4 promoter, higher IgE, and airways eosinophilia in response to allergen challenge compared with counterparts exposed during late pregnancy [37].

There has also been progress in understanding the mechanism through which the microbiota regulates intestinal immune function. Using mice raised in germ-free conditions, Xue et al. [38] demonstrated that the microbiota down-regulates expression of miR-10a which targets the IL12/IL23-p40 molecule in intestinal DC and may contribute to the maintenance of intestinal immune homeostasis. This is of interest as a potentially important mechanism related to the hygiene hypothesis in the development of allergic disease. Collectively, these functional studies provide compelling leads to investigate in human populations, and provide encouraging evidence for therapeutic strategies that modify epigenetic marks.

Evidence for Environmental Effects on Epigenetic Regulation

Epigenetic modifications have already been shown to be sensitive to environmental exposures including diet, exposure to pollutants, and cigarette smoke. This plasticity of the epigenome is essentially the ‘holy grail’ of complex disease biology as it links environmental exposures with variations in gene expression, and potentially with disease outcomes. Diet-induced epigenetic changes and their association with health, particularly in utero, has been the subject of much current research. The extent to which nutrition can modify the established epigenetic landscape is an active area of research, and there is hope that dietary intervention may

represent a cost-effective strategy to maintain a healthy epigenome. Vitamin D is one such dietary component under current scrutiny. Recent studies have implicated a role for vitamin D and the vitamin D receptor (VDR) in the induction of genes that are involved in inflammation, cell proliferation, and apoptosis. There is also evidence that vitamin D insufficiency in pregnancy is linked with food allergy in childhood [39–41]. Whether these effects are mediated through epigenetic events is still unclear, although the vitamin-D receptor (VDR) itself is regulated by epigenetic events. Unliganded VDR is bound to a repressor complex, which recruits histone de-acetylase to chromatin. Binding of 1,25(OH)₂D₃ dissociates the repressor complex promoting binding to target genes [42]. As yet, the epigenetic changes associated with vitamin D effects on immunity have not been characterized, although 1,25(OH)₂D₃ has been shown to repress IL-12 expression via the recruitment of a histone deacetylase complex to the IL-12B transcriptional start site [43]. This may represent an important pathway with clear modifying effects on genes known to control the polarization of T-helper cells [44]. Studies in this area are ongoing and likely to reveal exciting epigenetic effects on immune regulation.

Folate is another key dietary component implicated in epigenetic disruption and adverse fetal outcomes [45]. A number of recent studies have provided evidence to suggest high maternal folate intake in pregnancy may be linked to a higher incidence of immune-related conditions including respiratory illness (asthma, infections) and eczema [46, 47]. Animal models also suggest that excessive folate disrupts DNA methylation and gene expression in the developing fetus, with links to allergic outcomes [48]. This has clear public health implications and has warranted immediate investigation in humans using well-characterized cohorts; these studies are currently underway.

The direct effects of high-dose folate exposure on DNA methylation are still unclear. Perhaps the first study to investigate this reported that periconceptional supplementation with 400 μ g/day was associated with differential methylation at the *IGF2* gene in infants, and this was linked to low birth weight, providing suggestive evidence of excessive folate, altered DNA methylation, and adverse fetal outcomes [49]. A more recent study of 173 obese women postmenopause found high serum folate level correlated with DNA methylation measurements at repeat LINE-1 elements in the genome, and several immune markers including IgA levels and CD4/CD8 ratio [50].

Understanding the complexity of nutritional effects on epigenetic profiles is indeed challenging, as the timing of these exposures may be critical [47]. The question of which cell types are relevant to study, and whether individual dietary components should be studied in isolation or together is not well defined. A recent randomized control trial of

maternal micronutrient supplementation (folate, zinc, vitamins A, B, C, D) conducted in the Gambia, where seasonal variation in nutrition affects fetal growth, reported sex-specific effects of maternal micronutrient supplementation on cord blood DNA methylation levels at *IGF2* and *GTL-2* [51]. Interestingly, these differences did not persist at 9 months; however, the small sample size in this study was a limiting factor, and the use of mixed populations of cells might also have reduced the ability to detect differences at 9 months.

A recent study conducted among American mother–infant pairs investigated the association between maternal intake of a range of methyl-donor nutrients taken during the first or second trimester, with LINE-1 DNA methylation measurements in first and second trimester maternal blood, and cord blood at birth [52]. It was found that, in combination, maternal intake of methyl-donor nutrients, taken either periconceptionally or during the second trimester, was not associated with LINE-1 DNA methylation measured in first trimester ($n=830$), second trimester ($n=671$) or cord blood ($n=516$) [52]. Betaine intake was inversely associated with cord blood LINE-1 methylation, but the data suggested an interaction with cadmium, which itself was associated with first trimester methylation, and inversely associated with cord blood methylation, highlighting the vast complexity of these combined nutritional effects. Further to this, a specific association between periconceptual choline and cord blood methylation levels was reported in males only.

Conversely, another American study examined the effects of the intake of interrelated dietary components, stratified by a ‘prudent’ diet (high intake of vegetables and fruits), or ‘Western’ diet (high intake of meats, grains, dairy, oils, and potatoes), in 149 participants aged between 45 and 75 years [53]. The authors reported that the prudent dietary pattern was associated with reduced methylation at LINE-1 elements in whole leukocyte DNA. The disparity in the findings of these different studies may be due to differences in the source populations and sampling frames, since the latter study was conducted in a mixed population of older individuals. Furthermore, LINE-1 methylation may not be a very informative epigenetic marker as it simply provides readout of overall levels of DNA methylation, and ignores the distribution of DNA methylation marks across important regulatory regions of the genome.

Advances in Clinical Studies of Epigenetics and Allergic Disease

Perhaps the most challenging, yet promising, area of epigenetic research is to identify disease-associated changes in epigenetic marks that may be informative about the underlying disease pathology. A number of challenges face these

population-based studies, including determining which cell types in the blood or tissue are likely to be affected, demonstrating causality, understanding which regions of the epigenome are important for regulatory function, and dealing with the dynamic nature of epigenetic marks which fluctuate over time. Convenient access to peripheral blood nevertheless makes this an attractive prospect for studies aimed at identifying novel disease candidates and predictive epigenetic signatures.

Air pollution has been linked to epigenetic modulation and may play a role in regulating the expression of genes important in inflammation and asthma. Previous work in this area has demonstrated that exposure to polycyclic aromatic hydrocarbons during pregnancy is associated with altered DNA methylation at several genes [54], and that diesel exhaust particles in combination with allergen exposure can alter methylation at T-helper genes and in vivo production of IgE [55]. A recent cross-sectional study compared peripheral Treg cells from children living in areas of high (Fresno, California, USA) versus low (Stanford, California, USA) ambient annual air pollution. Functional Treg suppression and chemotaxis was impaired in asthmatics from Fresno, which was associated with increased *FOXP3* methylation and reduced *FOXP3* expression [56].

There is evidence that the chromatin structure is altered in patients with asthma. Ito et al. have previously shown an abnormal ratio of HAT/HDAC activity in bronchial biopsy specimens from asthmatic patients, an effect which was reversed in patients taking inhaled steroids [57]. Cosio and colleagues have now recently investigated HAT and HDAC activity in alveolar macrophages and in whole PBMC from asthmatic patients. Similar to the findings from Ito et al., they report increased HAT activity and reduced HDAC activity in alveolar macrophages, but not PBMC, from patients with asthma compared with controls, which was associated with increased transcription of inflammatory proteins, and reversible on treatment with corticosteroids [58].

These cell-specific differences have been reported in other clinical studies and highlight the importance of studying epigenetic aberrations in situ. Liang studied monocytes from patients with atopic dermatitis (AD) and found them relatively deficient in DNA methylation at the *FCER1* promoter compared with healthy controls [59]. This correlated directly with mRNA expression and surface expression of the FcεR1γ protein. Han and colleagues instead found no differences in DNA methylation genome-wide in CD4+ from patients with AD compared with controls. Interestingly, the same study reported distinct hypomethylation across 26 regions of the genome, and elevated methylation at 121 genes in CD4+ from patients with psoriasis, indicating that aberrant methylation in CD4+ cells may affect T cell function in patients with psoriasis, but not with AD [60].

As mentioned previously, sequence polymorphisms have been associated with localized changes in DNA methylation, and there is a need to consider these in the context of epigenetics. A recent Spanish study of a Caucasian population of adults with allergic asthma ($n=262$) and healthy controls ($n=251$) investigated genetic polymorphisms and DNA methylation in CD19+ B cells [61]. The study reported a significant association between the allergic asthmatic phenotype and the presence of the -197 T>C and the -613C>T polymorphisms in the prostaglandin D2 receptor. DNA methylation was also altered in CD19+ B cells from allergic asthmatics, and the -613C>T polymorphism was significantly associated with differential methylation in the asthmatic population (-163CC=84.2 % methylation; -613CT=42.5 % methylation) but not the control population. Similar sequence-related effects on methylation have been reported in type-2 diabetes [62].

Conclusions

Progress continues to be made in uncovering new epigenetically regulated pathways responsive to changes in the environment, which are relevant to the etiology of allergic diseases. Whilst many of these still constitute preliminary evidence, some of these findings have been reproducible in population-based studies. The stability of epigenetic effects in vivo still needs to be determined; however, the emerging evidence suggests that some of these effects may persist across generations. There are also questions remaining as to whether observed differences in the epigenome are on the causal pathway to disease, and it is likely that well-designed longitudinal studies will be needed to determine this.

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