

Review article

Silent mysteries: epigenetic paradigms could hold the key to conquering the epidemic of allergy and immune disease

Epigenetic mechanisms provide new insights into how environmental changes may mediate the increasing propensity for complex immune diseases such as allergic disease. There is now strong evidence that early environmental exposures play a key role in activating or silencing genes by altering DNA and histone methylation, histone acetylation and chromatin structure. These modifications determine the degree of DNA compaction and accessibility for gene transcription, altering gene expression, phenotype and disease susceptibility. While there is already evidence that a number of early environmental exposures are associated with an increased risk of allergic disease, several new studies indicate *in utero* microbial and dietary exposures can modify gene expression and allergic disease propensity through epigenetic modification. This review explores the evidence that immune development is under clear epigenetic regulation, including the pattern of T helper (Th)1 and Th2 cell differentiation, regulatory T cell differentiation, and more recently, Th17 development. It also considers the mechanisms of epigenetic regulation and early immune defects in allergy prone neonates. The inherent plasticity conferred by epigenetic mechanisms clearly also provides opportunities for environmental strategies that can re-programme gene expression for disease prevention. Identifying genes that are differentially silenced or activated in relation to subsequent disease will not only assist in identifying causal pathways, but may also help identify the contributing environmental factors.

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There has been a remarkable increase in immune-mediated diseases over the last 30–40 years, including allergic diseases and many other inflammatory immune diseases (1), with speculation that this is related to a breakdown in immune regulatory pathways (2). The multi-factorial nature of both genetic propensity and environmental change has made causal pathways difficult to identify. So far genomic approaches have failed to explain the pathogenesis or the rise in allergic disease, and there is growing focus on epigenetic changes that may play a greater role in determining phenotype than genome sequence, which has not changed during this period. It is now generally accepted that environmental change is driving this 'epidemic' and that events during early development have major effects.

Early events are critical

Extensive data from both human and animal studies indicate that events or exposures during critical stages in pregnancy can alter gene expression and potentially induce permanent changes in many physiological processes, resulting in increased disease susceptibility in

the offspring (3). There is firm evidence that these changes in gene expression are mediated through epigenetic mechanisms (4). The rapidly expanding field of epigenetics has become the cornerstone of the DOHaD hypothesis (Developmental Origins of Health and Disease) (5), which proposes that all organ systems undergo developmental programming *in utero* that predetermines subsequent physiologic and metabolic adaptations during adult life. Although this has been best studied in the context of cardiovascular and metabolic disease (6, 7), the epidemic rise in both allergic and autoimmune diseases also highlights the susceptibility of immune pathways to modern environmental influences. It is intriguing that many of the same environmental risk factors (including reduced microbial exposure, dietary changes and environmental pollutants) are implicated in both allergic and autoimmune diseases, suggesting early effects on common fundamental immune-regulatory pathways (Fig. 1). While there is clear evidence that immune function is regulated by epigenetic mechanisms (reviewed in (8)), epigenetic models that explain the rise in allergic disease are only beginning to emerge (9, 10). One of the most notable recent developments in the allergy field has been an animal model demonstrating that maternal folate

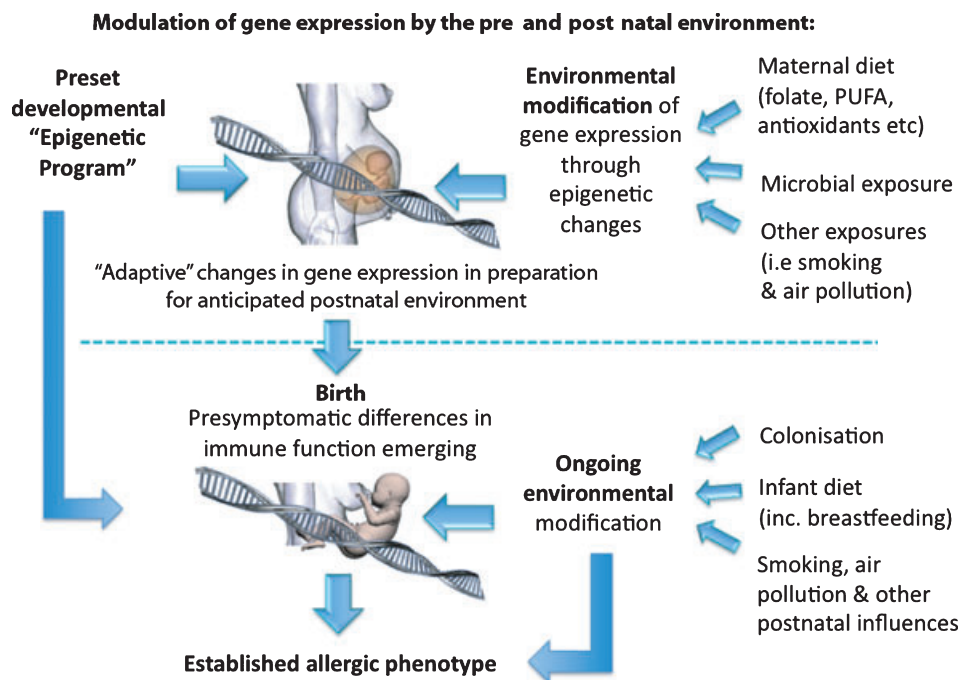


Figure 1. A range of factors in the prenatal and postnatal environmental have the capacity to alter epigenetic programming and gene expression during early development. This may have implications for the developing phenotype and subsequent disease predisposition.

intake in pregnancy can alter allergic predisposition in offspring through epigenetic mechanisms (11) (as discussed further below). This has led to an urgent call to explore this new epigenetic paradigm further in the pathogenesis of asthma and allergy (9, 10).

Epigenetic modifications determine development, differentiation and phenotype

Epigenetic processes govern all aspects of normal development, and are fundamental to achieving the cellular differentiation and diversity required by complex organisms. Changes in the methylation of DNA and histones, and histone acetylation alter the degree of DNA compaction and accessibility for gene transcription, regulating gene expression in a temporal and tissue-specific manner (12). This allows genetically identical stem cells to produce a range of lineages with major differences in morphology and function. The resulting changes in gene expression are subsequently passed to somatic daughter cells during mitosis. These intricate events appear to be orchestrated by a still poorly understood epigenetic program mediated by complex RNA signalling networks (13–15).

The main processes which modulate chromatin architecture and establish epigenetic memory, operate through the recruitment of histone modifiers and DNA methyltransferases (16):

Chromatin modulation: Eukaryotic chromosomes consist of DNA packaged in a highly ordered way around histone proteins forming a nucleosome. These

nucleosomes are further packaged into dense chromatin fibres, which exist as either transcriptionally silent heterochromatin, or transcriptionally active euchromatin. Nucleosome repositioning is the mechanism by which the DNA is made available for transcription, and allows the binding of polymerases to promoter sequences within the DNA. The nucleosomal subunits of chromatin contain histone tail domains which are unstructured and are the target sites of posttranslational modifications including lysine acetylation, lysine and arginine methylation, serine and threonine phosphorylation, ubiquitination and sumoylation (reviewed in (17)). It is thought that these modifications affect either the charges on the histone proteins thereby causing repulsion and opening up the chromatin structure, or by permitting binding by other DNA associated proteins. In this way the repositioning of heterochromatin into euchromatin, and nucleosome-nucleosome associations are regulated (17). The placement of these modifications is orchestrated by a variety of site-specific enzymes such as histone acetyltransferases (HATs), histone deacetylases (HDACs) and histone methyltransferases (HMTs) (18). In general terms, removal of acetyl groups by histone deacetylase (HDAC) generally leads to gene silencing, whereas acetylation by histone acetyl transferase (HAT) opens chromatin structure for enhanced gene transcription (18). Methylation of histone residues can affect nucleosome interaction with other proteins. Generally histone methylation leads to transcriptional repression, however in some cases methylation of some lysine and arginine residues may lead to activation.

DNA methylation: Perhaps the most robust and well-studied mechanism of epigenetic transcriptional regulation is that of DNA methylation, mediated by members of the DNA methyltransferases family (DNMT). DNMTs confer covalent methyl modifications to CpG islands in the promoter regions of deoxyribose nucleic acid. This mechanism is thought to act through two ways: either sterically inhibiting the binding of the transcriptional machinery, or by acting as donor binding sites for methyl-CpG-binding-domain proteins (MBDs) that may recruit histone modifiers (19). Typically the modification of DNA by DNMTs confers transcriptional repression, while hypomethylation of gene promoter regions is often associated with increased transcriptional activity (20), however, these modifications may have the reciprocal effect in mammalian processes such as dosage compensation (17). The overall result is changes to the transcriptional activity of particular genes that are stable, heritable and modifiable in response to developmental and environmental cues (21).

Following fertilization both the sperm and the oocyte combine genomes and extensive demethylation and remethylation of the genomes ensue, which is thought to operate in order to erase previous paternal imprints and re-establish sex-specific imprints (22). This wave of epigenetic reprogramming is thought to restore totipotency to the egg however; some genomic loci either fully or partially escape epigenetic reprogramming during *in utero* development (22, 23). DNA and chromatin modifications influence each other during development, whereby histone methylation can help direct DNA methyltransferases, and DNA methylation may serve as a template for rebuilding histone modifications after replication. This process occurs through common SET domain-containing proteins that both specifically methylate histones, as well as recruit DNA methyltransferases. In general it is thought that targeted gene silencing, and epigenetic reprogramming, occur by modifications in histone residues, followed by changes in DNA methylation (24).

These postsynthetic modifications of DNA and/or DNA associated proteins, as well as transcriptional and posttranscriptional modifications to RNA species bring about major variations in gene expression. New insights into the role of noncoding RNAs in modulating the epigenetic network suggest these mechanisms are highly conserved (14), and the plasticity of such networks are probably engineered to facilitate adaptation (17). Closer to implantation, the reprogrammed zygote undergoes *de novo* methylation and it is from this time, that critical environmental exposures can result in the acquisition of new epialleles either through insufficient erasure of epigenetic marks during gametogenesis, or changes in methylation patterns after implantation. There has been speculation this may be associated with the persistence of fetal genes characteristic to atopy and asthma (25).

Evidence that immune development is under epigenetic regulation

It is well established that T-cell differentiation is under epigenetic control through changes in DNA/histone methylation and/or histone acetylation [as recently reviewed by Jansen et al. (19)] providing marked plasticity in T cell development. This allows adaptive change but also confers vulnerability to environmental and development events which can alter patterns of early T cell differentiation through these pathways. While there has been long standing interest in the regulation of neonatal T-helper type 1 (Th1)/Th2 polarisation in the perinatal period and how this determines subsequent immune development, there is now growing focus in the role of other T cell populations including Tregs and proinflammatory (Th17) cells in this process of maturation. Epigenetic mechanisms have been shown to regulate both Th1 and Th2 differentiation (26–30) and changes in promoter methylation are a prerequisite for FoxP3 expression and Treg differentiation (31) (32).

Th1 differentiation: Methylation of the IFN- γ gene promoter is the main epigenetic control of Th1 expression, and is essential for determining both lineage commitment and subsequent developmental regulation of gene expression. Several studies have reported that promoter regions in the IFN- γ gene are hypermethylated in naïve CD4+ T-cells and undergo progressive demethylation, associated with increased transcriptional activity, during Th-1 lineage commitment (33–36). This is almost always accompanied by silencing (DNA methylation) of IFN- γ in Th2 cells (19). Following lineage commitment, developmental control of IFN- γ expression occurs by the same epigenetic processes. It has been previously established that reduced IFN γ expression in neonatal CD4+ T cells (compared with adults) is associated with hypermethylation (gene silencing) of the IFN γ promoter (34). Postnatal maturation of CD4+ T-cells is accompanied by progressive demethylation about the IFN- γ promoter, corresponding with upregulated IFN- γ transcriptional activity implying an age-dependent postthymic maturation of CD4+ T-cells, mediated by epigenetic control of IFN- γ (33). These observations have led to speculation that factors that increase gene methylation may increase the risk of disease by silencing pathways (Th1 and T regulatory cell differentiation) that normally inhibit Th2 allergic differentiation (10, 25).

Th2 differentiation: GATA-3 mediated chromatin remodelling at the Th2 cytokine locus (*IL4/IL5/IL13/RAD50*) is essential for Th2 lineage commitment (37). The mechanics of this process are not fully understood but involve changes in permissive histone modifications following T-cell receptor (TCR) signalling, and concomitant silencing (DNA methylation) of IFN- γ (38). It has recently been demonstrated that GATA-3 is sufficient and absolutely necessary to induce these epigenetic modifications and stabilize Th2 cell growth (37).

The use of trichostatin A, a global inhibitor of HDAC activity, induces alterations in the Th1/Th2-associated cellular responses from CD4⁺CD45RO⁺ (memory) infant T-cells, skewing recall responses toward a Th2 phenotype (39). Moreover, exposures that inhibit HDAC such as oxidative stress and histone deacetylase inhibitors enhance NFκB driven transcription of inflammatory genes (40), and upregulate Th2 cytokine (IL-13, IL-5) and GATA3-mediated T cell responses (39). Bronchial biopsies from untreated asthmatics possess greater levels of histone acetyltransferase and lower levels of HDAC activity. Notably, these levels are reversed following treatment with inhaled steroids (41), suggesting therapeutic pathways also involve altering epigenetic status (at least in part). This is supported by pharmacological studies demonstrating that the regulation of TNF-α induced expression of important inflammatory chemokines such as eotaxin and MCP-1 occurs by histone H4 acetylation in human airway smooth muscle (42). These pathways appear to be driven by NFκB transcription of inflammatory genes associated with airways disease.

These observations have led to speculation that factors that increased gene DNA methylation and endogenous HDAC activity may increase the risk of disease by silencing pathways (Th1 and T regulatory cell differentiation) that normally inhibit Th2 allergic differentiation (10, 25).

Treg differentiation: More recent studies indicate that Treg function is also impaired in the neonatal period compared with adults (43–45), and that epigenetic changes (DNA demethylation) are also a prerequisite for FOXP3 expression and Treg differentiation (31, 32). Several regulatory elements exert locus control over FOXP3 expression and influence the development of regulatory populations, including putative FOXP3 promoter regions, TGF-β sensor regions and Treg-specific differentially methylated regions. FOXP3 Tregs and conventional CD4⁺ T-cells display differences in promoter methylation. CpG islands in the FOXP3 promoter of Tregs are virtually devoid of methylation marks, and these are albeit weakly methylated in naïve T-cells (31, 46). Following stimulation, T-cells acquire further methylation in the FOXP3 promoter, thus preventing the induction of gene transcription and stabilizing the development of an effector phenotype.

Th17 differentiation: There is also emerging evidence that the Th17 lineage may be regulated through changes in histone acetylation epigenetic mechanism (31). Notably, one recent study has shown that human Treg can differentiate into Th17 cells and that this plasticity is also epigenetically determined through histone/protein deacetylase activity (47). Although the developmental role of this lineage is not clear, it is notable that Th17 function is also impaired in neonates compared with adults (45) and that there are significant correlations between Th17 and Th2 function (44, 45). It therefore is likely that epigenetic processes similar to those involved

in Th1/Th2 commitment also control Th17 cell fate determination.

Other cell types: While the majority of research has focused on T-cells as effectors of the immune response there is little doubt that a variety of cell types and cellular processes are regulated by genes under epigenetic control, including the tissue specific assembly of Ag receptor genes in B and T cells (48) and macrophage activation (49). Given dynamic and complex nature of immune regulation it is likely that epigenetic processes play a key role in a broad range of immune-related processes (50).

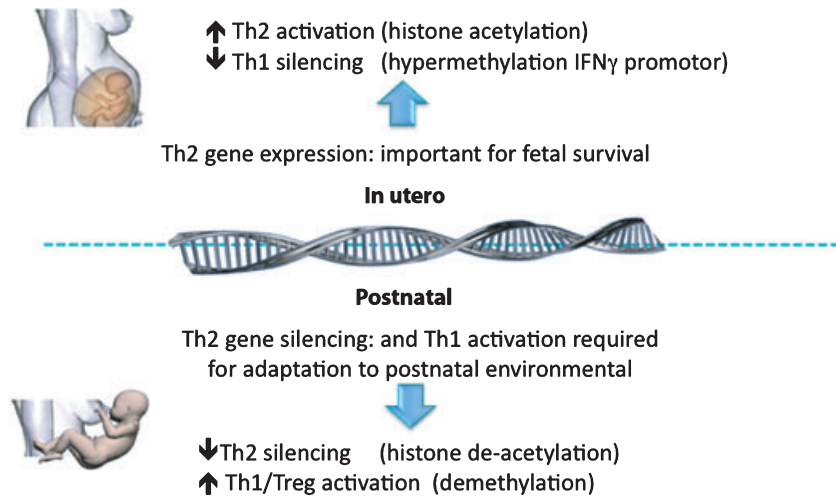
Developmental differences in gene expression of allergy prone infants

The clear epigenetic regulation of T cell development makes it highly likely that the maladaptive T cell differentiation in allergic disease is associated with failure of epigenetic control. Consistent establishment of the immune-related epigenetic profile is essential to prevent inappropriate gene expression during periods of developmental change. Genes that prevent fetal rejection in pregnancy (notably Th2 associated pathways) are highly conserved for survival in this period, but are likely to be under fierce epigenetic control thereafter (Fig. 2). We propose that recent environmental changes are influencing epigenetically regulated genes and driving the persistence of Th2 immune responses. Notable differences in the immune responses of allergic and nonallergic children are evident at birth suggesting that *in utero* environmental exposures have the capacity to alter epigenetic programming. Additional postnatal environmental cues that are normally important for modulating the expression of these genes and driving Th1-mediated immunity may also be lacking in modern environments (51–53) (Fig. 2).

One of the most consistent findings in atopy-prone individuals is the diminished capacity to mount Th1-mediated IFNγ responses at birth, coupled with a delayed postnatal maturation of Th1 immunity (reviewed previously in (54)). Epigenetic modification in pregnancy may also explain the consistently stronger effects of maternal (than paternal) allergy on both Th1 function (55) and allergic disease, and potentially the rise in allergic disease with successive generations.

There is emerging evidence that infants deemed to be at high risk (HR) of allergic disease (based on their familial history) also display differences in both Treg (44, 56) and innate immune function (57). Schaub et al. recently reported that HR infants have reduced numbers, markers and function of Tregs compared with LR infants, based on maternal atopy (44). We have also shown preliminary evidence of altered Treg function in infants who subsequently develop allergic disease (56). This has led to conjecture that greater immaturity of both Th1 and Treg function may increase the risk of inappropriate persistence of a Th2 immune phenotype. Whilst the effects of allergic risk

Developmental changes in TH1/TH2 gene expression are epigenetically determined and under additional environmental influence



Optimal epigenetic regulation may depend on environmental stimulation:
If environmental cues suboptimal → inappropriate persistence of perinatal gene expression

Figure 2. This illustrates broad concepts of developmental change in the epigenetic regulation of T cell differentiation using the Th1/Th2 effector as an example. There is growing speculation that environmental changes may be influencing these processes, and that the rise in allergic disease may be linked to suboptimal environmental exposures during this early period.

(maternal atopy) on the development of innate immunity is less clear, with a number of conflicting observations (44, 45, 58) the evidence nonetheless suggest very early (presymptomatic) developmental differences in innate immune gene-expression in atopy prone individuals.

That these differences are detectable at birth further highlights the likelihood of early alterations in epigenetic programming of allergy prone infants.

Gene-environmental interactions are mediated through epigenetic mechanisms

Epigenetic processes allow adaptive developmental changes in response to potentially diverse conditions in the anticipated environment. Environmental changes that can influence DNA/histone methylation and histone acetylation have the capacity to induce adaptive changes in gene expression and phenotype. This plasticity is demonstrated in many elegant models showing how variations in the maternal environment can profoundly alter the phenotype of genetically identical offspring, though epigenetic effects (reviewed in (5)). In the context of allergic disease, a number of antenatal exposures [such as maternal diet (59), microbial exposure (60), and smoking (61)] have been shown to modify neonatal immune function and disease risk. More recently, several models have specifically demonstrated that at least some of these exposures mediate developmental effects through epigenetic changes (discussed further below).

Significantly, because epigenetic changes are transmitted with each cell division, patterns of early gene expression have lasting implications for subsequent development and disease risk. Moreover, the effects are heritable across generations with additional implications for offspring (11). This novel concept has lead to the recent recognition that exposures or events experienced by one generation have implications for the development and phenotype of subsequent generations.

Although *in utero* events arguably have the greatest potential to determine subsequent phenotype, ongoing postnatal exposures are also clearly important in governing evolving phenotype (Fig. 1). In this context, changes in gene expression induced by postnatal exposures could account for the spontaneous expression of new phenotypes, discordance among monozygotic twins, and variations in the onset and clinical patterns of a number of disease states (21). It is possible that similar events could explain the enormous variation in the evolution and expression of the allergic phenotype.

Candidate environmental factors in the allergy epidemic: evidence of epigenetic effects

The recent rise in immune disease is a clear indication that environmental changes are having effects on key developmental pathways. Population studies indicate a number of environmental and lifestyle changes are implicated in the rise of allergic disease. These include a reduction in

'natural' Th1/Treg-trophic microbial signals caused by cleaner environments (the hygiene hypothesis) (62), complex changes in dietary composition and a number of other lifestyle effects such as smoking (61) and exposure to pollutants (63) and toxins (64). The potential relevance of these factors is further highlighted as many also have clear effects on functional gene expression in key immune pathways in early life (61, 65–67).

Evidence that cleaner environments have epigenetic effects on gene expression

A decline in both infectious exposures (62) and changes in the composition of normal intestinal commensals (68) have been implicated in the rise in allergic disease and autoimmunity (1). Although postnatal exposure is likely to play a greater role in immune maturation, both human and animal studies clearly demonstrate that *in utero* (maternal) exposure to both pathogenic (69) and nonpathogenic microbial products (70), can prevent allergic outcomes in the progeny. A recent study has demonstrated that maternal exposure to farming environments during pregnancy may induce higher numbers of Tregs with attenuated function in cord blood (71). Notably, farm environment exposure consistently produced a trendwise increase in DNA methylation at the Treg-specific differentially methylated region (TSDR), suggesting an epigenetic mechanism by which maternal exposure to specific stimuli may induce enhanced immunoregulatory function in offspring.

Investigation of these protective farming environments led to the identification of bacterial strains (notably *A. lwoffii*) that are present in very high quantities in this unique environment. Prenatal intranasal administration of this apathogenic bacteria to pregnant animals provided an allergy-protective effect to the progeny, with significant reduced airways inflammation (Teich R, Conrad M, Ferstl R, Brand S, Blümer N, Ö Yildirim A et al., submitted). There were significant differences in the ontogeny of Th1 IFN γ production in the offspring of exposed mothers, which were directly related to changes in methylation of the IFN γ promoter.

These may be the first studies to show that microbial exposures alter fetal immune development through epigenetic mechanisms.

In the postnatal period microbial exposure is essential for normal immune maturation. There has been building speculation that early colonisation facilitates demethylation of the IFN γ gene, thereby increasing Th1 response capacity and reducing the risk of Th2 allergic responses (72). This is supported by indirect *in vitro* evidence that microbial products induce changes in epigenetic regulation of pro-inflammatory and anti-microbial genes, which retain distinct patterns of histone acetylation and methylation marks in their promoters after endotoxin challenge (73). Neonatal CD4⁺ T-cells have been shown to undergo a higher degree of demethylation about the IFN γ promoter compared with adult cells when coaxed

down the Th1 lineage pathway *in vitro* (33), suggesting that neonatal Th1 priming may be necessary for less restrained IFN γ expression in later life. Thus, low microbial burden (reduced Th1 signals) in the early postnatal environment may lead to persistently higher methylation (gene silencing) of the IFN γ promoter and increased risk of the allergic phenotype (72). Better understanding of these pathways may provide targets for more effective allergy prevention strategies.

New evidence that nutrition change can result in epigenetic reprogramming

Variations in maternal nutrition in pregnancy are well known to influence developmental programming and disease risk in many organ systems (5). A range of other maternal dietary factors in pregnancy have been implicated in immune development and allergic risk, including polyunsaturated fatty acids (PUFA), antioxidants and a range of specific vitamins and micronutrients (recently reviewed by (59)).

One of the most notable recent developments in the field of allergic disease is the recognition that dietary folate intake in pregnancy can specifically alter the allergic predisposition of offspring through epigenetic mechanisms (as discussed above) (9). Diet-derived methyl donors have the capacity to alter patterns of CpG methylation *in utero*. Studies from the murine agouti model demonstrate a direct link between dietary methyl donors consumed by dams, and increased loci-specific methylation of the agouti retroviral element in F1 progeny with permanent changes in phenotype (74). In a recent landmark study in mice, maternal supplementation with folate, a dietary methyl donor, led to the differential methylation of 84 loci in lung tissue, the development of allergic airways disease and associated systemic allergic responses (11). Importantly, this effect was transmitted to subsequent progeny mice. While the significance of this is not yet clear in humans, one recent study has reported that folate supplements in pregnancy are associated with increased childhood wheezing (75). There has been an urgent call for more studies to explore this new epigenetic pathway of asthma and allergy pathogenesis (9, 10).

Collectively, these observations raise the strong possibility that, as with other aspects of human health and disease (6, 76), nutritionally induced epigenetic changes may also be important in the development of allergic diseases. Nutritional exposures are also likely to be a source of on-going epigenetic influence in the postnatal period.

Other environmental exposures

A number of other early environmental exposures have been associated with an increased risk of allergic disease or altered immune effects on the fetus, including maternal medications in pregnancy (59, 77), smoking (61, 67) and other pollutants. Of these, cigarette smoke exposure has

been already recognised as a potentially important adverse exposure capable of inducing epigenetic changes in gene expression (18, 78). Although not specifically explored in this context, maternal smoking in pregnancy could feasibly also modify fetal gene expression through epigenetic pathways. Epigenetic effects could be consistent with the well-recognised long-term adverse effects of *in utero* cigarette smoke exposure.

Exposure to inhaled pollutants has been demonstrated in human and animal models to alter methylation patterns in several genes (79–81). Of particular interest is the finding that exposures to inhaled traffic pollutants during pregnancy has been associated with changes in DNA methylation, and higher parental reporting of early childhood asthma symptoms (79). A mouse model of airways hyper-responsiveness was used to demonstrate that increased total IgE resulting from inhaled diesel exhaust particles was significantly associated with hypermethylation in the IFN- γ promoter, and hypomethylation of IL-4 (81). Together these data suggest that oxidative stress from air pollutant exposure may modulate the behaviour of inflammatory genes via epigenetic changes in DNA methylation.

Conclusion

Concepts of epigenetic regulation have advanced the understanding of how the environment can induce changes in gene expression, phenotype and disease predisposition. Although this is best studied in other systems, several emerging models suggest that this may be similarly relevant in the development of allergic disease, which is also clearly initiated very early in development. Further studies are urgently needed to determine the human relevance of animal models identifying environment-induced changes in gene methylation in the aetiological of allergic disease. The challenge of ongoing research will be to identify precise networks that regulate gene expression, and to identify the key environmental exposures driving the susceptibility to disease. The inherent plasticity conferred by epigenetic mechanisms clearly also provides opportunities for environmental strategies that can re-programme gene expression for disease prevention. This is fundamental to the ultimate goal of reversing the epidemic rates of immune-mediated disorders.

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