The emerging field of epigenetics provides a new frontier for understanding mechanisms underlying well-recognized gene-environment interactions. Epigenetics refers to the study of heritable changes in gene expression, caused by chemical modifications in a chromosome, which are so termed because they alter the likelihood of gene transcription but do not change the underlying sequence. This particular mechanism of gene regulation is fundamental to all aspects of growth and development, determining the flow of genomic information in a temporal and tissue-specific manner. The epigenetic program is encoded by specific histone modifications (methylation and acetylation) and DNA methylation patterns, which determine the degree of DNA compaction and thus the accessibility of genes for transcription (Fig 1). In addition to histone modifications and DNA methylation, there are also other gene regulatory networks, including microRNAs, small interfering RNAs, and long noncoding RNAs, all of which serve to control gene expression. However, these are distinct from epigenetic traits as it is unclear whether these processes are transmittable across generations. During cellular differentiation, these networks selectively alter the patterns of gene expression to allow considerable cellular diversity while the DNA code remains unchanged. In a general sense the epigenetic code provides plasticity of gene expression in response to environmental changes, allowing more rapid phenotypic adaptations across generations. As developmental changes are most profound during embryonic and fetal life, this is also arguably when the epigenetic program is most vulnerable to environmental changes. Given that these modifications are reversible and sensitive to environmental factors, they provide a mechanistic link between environmental exposures, developmental programming, and risk for disease.

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In the context of asthma and allergic disease, a substantial body of work suggests a link between epigenetic gene regulation, immunity, and physiologic development. Around the turn of this century, cellular immune studies described a role for histone and DNA methylation changes in the control of cellular immune development. The increasing rates of early childhood allergy led to the novel hypothesis that environmental changes can promote allergic predisposition through epigenetic effects on gene expression, and that this begins in fetal life.

Since then, epigenetics has become the cornerstone in the quest to understand the “developmental origins” of complex modern diseases, such as asthma and allergy.

It has already been established that maternal exposures, such as cigarette smoke, can modify both fetal lung development and fetal immune function, contributing to an increased risk of subsequent asthma and respiratory disease. Other important intrauterine exposures, including the maternal diet and microbial exposures, are also known to modify the risk of allergic disease in the offspring. Recent studies now show that these prenatal exposures (diet, microbial infections, tobacco smoke, and other pollutants)
can epigenetically activate or silence immune-related genes with substantial effects on immune programming. These new studies provide a novel framework to understand the mechanisms by which the environment influences developmental and immune programming and determines the risk for subsequent disease. The new hope is that these pathways may also provide a foundation for targeted early interventions to prevent disease.

DEVELOPMENTAL PROGRAMMING, EPIGENETICS, AND IMMUNE FUNCTION

There is now substantial evidence that early immune development is altered in children affected by asthma and allergic diseases. These alterations lead to dysregulated immunity characterized by a deficiency of interferon γ (IFN-γ) production, altered innate immunity, and deficient T regulatory cell (Treg) networks, which culminate in a propensity for uncontrolled T helper (Th) 2 immune responses. These differences are evident at birth, suggesting that prenatal factors may be particularly important in alternatively programming neonatal immunity. There is now conclusive evidence that immune development is epigenetically regulated. These mechanisms are known to control both Th1 and Th2 cell differentiation and are also a prerequisite for FoxP3 expression and Treg differentiation. There is, therefore, growing interest in exposures during pregnancy that can activate or silence these genes and alter the balance of neonatal immune responses.

During pregnancy, complex immunologic mechanisms have evolved to allow the fetal and maternal immune systems to coexist. The maternal cellular immune system adapts subtly during pregnancy to a "Th2 state" to down-regulate Th1 IFN-γ production via hypermethylation (gene silencing) of the IFNG gene promoter in CD4+ T cells at the maternofetal interface. FOXP3+ cells are also attracted to the maternofetal interface by human chorionic gonadotrophin, and recent studies now provide preliminary evidence that the FOXP3 gene is expressed at lower levels in the placentas of allergic women (and allergic infants) (S. L. Prescott, MD, PhD; A. Osei-Kumah, PhD; T. Richman, BSc; D. Martin, BSc; J. A. Dunstan, PhD; M. K. Tulic, PhD; et al, unpublished data, February 2010).

Neonatal immunity reflects these maternal events, with reduced Th1 function and relative dominance of Th2 activity, with underlying epigenetic changes driving these patterns of gene expression. This has fueled speculation that factors that increase gene methylation may increase the risk of disease by silencing pathways (Th1 and Treg differentiation) that normally inhibit Th2 allergic differentiation and propensity for allergic airways disease.

EARLY EVENTS AND EPIGENETIC REGULATION OF AIRWAY DEVELOPMENT

Asthma and allergic respiratory disease are the culmination of both local epithelial dysfunction and generalized Th2 atopic propensity. Although systemic proclivity for allergy is a major contributor to allergic airways disease, there is accumulating evidence that local mucosal expression of disease occurs through independent processes in Th2-predisposed individuals. Local epithelial-mesenchymal interactions are now believed to play a critical role in asthma pathogenesis, and a number of early life influences are believed to contribute to abnormal local tissue function in response to inflammation (Fig 2).

In the antenatal period, maternal environmental exposures have the capacity to influence lung development. There is very strong evidence that maternal smoking in pregnancy has adverse effects on fetal lung development and asthma risk. This includes increased responsiveness to methacholine, smooth muscle layer thickness, and collagen deposition. Altered DNA methylation patterns have been observed in several genes in buccal cells from children exposed in utero to tobacco smoke, which may be a likely mechanism for increased risk for disease. Similarly, maternal infections and the use of antibiotics have been associated with an increased risk for allergic airways disease, as have specific aspects of maternal diet, maternal stress levels, and exposure to pollutants, all of which have been associated with epigenetic changes (as discussed in the “Specific Environmental Exposures Can Modify Epigenetic Programming and Susceptibility to Allergic Airways Disease” section).

After birth, environmental exposures also influence local events in the developing airways. Viral infection, particularly with respiratory syncytial virus, is one of the strongest postnatal associations with asthma. Wheezing lower respiratory infection in the first year of life is a strong risk factor for asthma at 6 years of age in both nonatopic (OR, 4.1; P < .0005) and atopic (OR, 9.0; P < .0005) children. This strongly suggests that viral-induced lower airway inflammation during the early period of postnatal development can have profound long-term effects that are more marked than effects of inflammation at later ages.

Currently there is very little evidence about whether early-life infection with respiratory syncytial virus is associated with epigenetic changes that might predispose to the development of a systemic Th2 immunologic profile. There is some evidence that adenoviral
infections increase the expression of inflammatory genes via surface coat proteins, which are capable of interacting with histone modifiers, \(^{35}\) and play a role in the pathogenesis of COPD. \(^{36}\) In general, infection with viral agents has also been associated with DNA methylation events in the host. \(^{37}\) Although the development of better animal models will clarify the issue, it is likely that early viral infections may induce epigenetic changes in inflammatory pathways, which synergize with allergen sensitization, to contribute to the development of a Th2-biased asthmatic phenotype. \(^{38}\)

**SPECIFIC ENVIRONMENTAL EXPOSURES CAN MODIFY EPIGENETIC PROGRAMMING AND SUSCEPTIBILITY TO ALLERGIC Airways Disease**

Environmental changes that have been epidemiologically linked with the allergy epidemic, including microbial burden, \(^{39,40}\) dietary changes, \(^{41,42}\) and environmental pollutants, \(^{15,43,44}\) have also been shown to have effects on fetal gene expression and immune function. \(^{5,10,45,46}\) Moreover, emerging epigenetic effects of these exposures \(^{7,13,14,16,47}\) provide a new mechanism for the observed effects on gene expression. These new findings suggest that these exposures can induce stable epigenetic changes in gene expression, which can be passed to offspring and subsequent generations (Fig 2).

**Maternal Diet in Pregnancy**

Modern dietary changes appear to be providing less tolerogenic conditions during early immune programming and may also provide important avenues for preventing disease. Modern diets differ in many aspects from more traditional diets, with more processed and synthetic foods and less fresh fish, fruits, and vegetables. These changes in nutrients have been implicated in the increase in asthma and allergic disease, including the decline in polyunsaturated fatty acids, \(^{10}\) soluble fiber, \(^{48}\) antioxidants, \(^{49}\) and other vitamins, \(^{50}\) based on recognized immunologic effects as well as epidemiologic associations.

In one of the first epigenetic models of allergic disease, the role of folic acid in the pathogenesis of asthmatic airways disease has recently come to the forefront. This model was based on the capacity of folate to epigenetically modify gene expression through its role as a dietary methyl donor for DNA. \(^{51}\) Supplementation of pregnant mice with a high-folate diet resulted in altered gene methylation patterns...
and decreased transcriptional activity in the lung tissue of progeny mice with increased airways hyper-reactivity, airways eosinophilia, and production of chemokine and inflammatory macrophage proteins.\textsuperscript{14} One of the genes implicated was runt-related transcription factor 3, which several independent studies have indicated has a protective role in airways disease through the induction of FOXP3\textsuperscript{+} Tregs.\textsuperscript{32} This has been followed by reports in humans\textsuperscript{53,54} linking folic acid supplementation during pregnancy with increased risk of asthma and respiratory disease in infants. However, until this is confirmed and the mechanisms more closely studied it is premature to make changes in the current practice aimed at using folate to prevent neural tube defects. This highlights the urgent call for more studies in this area. These studies should be considered in the context of other related dietary nutrients, such as vitamins B2, B6, B12, methionine, and choline, which may also be implicated through their effects on folate-mediated one-carbon metabolism.

**Microbial Exposure In Utero**

Although postnatal exposures are recognized as the largest source of direct microbial exposure to the developing infant, it is becoming clearer that the epigenetic influences of bacteria begin in utero. In humans, exposure to a high microbial burden in rural farming environments is protective against asthma and allergy.\textsuperscript{40} New studies show that nonpathogenic microbial strains (Acinetobacter lwoffi) isolated from these farming environments can induce epigenetic effects when administered to pregnant animals and protect the offspring from experimental postnatal asthma (H. Renz, MD, personal communication, May 2010). This effect depends on increased expression of IFN-\gamma mediated by an increase in H4 acetylation of the IFN-\gamma promoter. Notably, these effects were abolished by inhibition of histone acetylation following ganciclovir treatment. Human studies also show that allergy protection by in utero microbial exposure is associated with enhanced neonatal Treg function, FOXP3 expression, and associated epigenetic effects (demethylation) of the FOXP3 gene.\textsuperscript{16} Based on these and other observations, it has been recently proposed that microbial exposures may epigenetically modify the patterns of immune gene expression during critical periods of early development and contribute to allergic predisposition.\textsuperscript{67}

**Exposure to Tobacco Smoke and Air Pollutants**

Oxidative stress produced by exposures such as cigarette smoke and air pollution can have significant epigenetic effects by altering nuclear factor \(\kappa\)B (NF-\(\kappa\)B) activation or by histone modification and chromatin remodeling of proinflammatory genes. As a potent source of oxidative stress, cigarette smoke contributes to reduced histone deacetylase activity, which results in differential activation of NF-\(\kappa\)B and the expression of proinflammatory cytokines IL-6 and IL-8 in peripheral lung tissue.\textsuperscript{55} NF-\(\kappa\)B is ubiquitous in cells and can induce histone modifications that activate or silence inflammatory genes and other signal-transduction pathways. In pregnancy, the induction of inflammatory genes can influence placental function and fetal programming.\textsuperscript{56} Oxidative stress from traffic exhaust particles can also have epigenetic effects in pregnancy. Studies in mice demonstrated that exposure to diesel exhaust particles augmented the production of IgE following allergen sensitization (Aspergillus fumigatus) through hypermethylation of IFNG and hypomethylation of the IL4 locus.\textsuperscript{15} Of note, Perera et al\textsuperscript{57} recently reported that high levels of maternal exposure to traffic particles correlate with methylation of the acyl coenzyme A synthetase long-chain family member 3 and with the development of asthma symptoms in children.

**Ingested Persistent Organic Pollutants With Epigenetic Effects**

Organic products of industry and agriculture (including polychlorinated biphenyl compounds, organochlorine pesticides, dioxins, and phthalates) contaminate modern homes, food, clothing, and water sources, accumulating in human tissue with age. Although they have immunosuppressive effects at high doses in humans,\textsuperscript{58} low levels of contamination may more selectively inhibit Th1 immune responses\textsuperscript{60} and favor allergic Th2 immune responses through their “estrogenic” hormonal activity. Some of these products have been readily measured in breast milk, cord blood, and placental tissue, highlighting the potential to influence early development.

Notably, many of these and other contaminants have more recently been associated with epigenetic effects (reviewed in Reference 60), including effects on global DNA methylation patterns at the low-dose exposure found in the ambient environment.\textsuperscript{61} We have recently measured persistent organic pollutants (particularly organochlorine pesticides) in 94% of adipose samples from mothers undergoing caesarean section and 62% of breast milk samples.\textsuperscript{62} Although we observed that levels have declined since the 1970s, this does not exclude a role in the intervening increase in allergic disease, particularly since the effects may not be apparent for several generations. This is a difficult area to study in humans, in whom only correlatative studies are possible, but these “modern” exposures should remain an important consideration in the increase of modern diseases.
Other Factors That May Modify Early Gene Expression and Disease Risk

In the context of asthma and allergic disease, the maternal phenotype is particularly important as a well-recognized risk factor for infant disease. Maternal asthma and allergic status has a much stronger effect than paternal allergy on both allergic disease and Th1 IFN-γ production by the neonate. We have also shown that maternal allergy modifies immune interactions between mother and fetus and reduces Th1 activation to the human leukocyte antigen mismatch of fetal alloantigens. This may affect the cytokine milieu at the materno-fetal interface and could be implicated in the attenuated Th1 responses commonly observed in infants of atopic mothers. Foreseeably, the increase in maternal allergy may also be amplifying the effect of other environmental changes.

Many other changes in the intrauterine environment can have direct effects on placental gene expression and potentially phenotypically modify the offspring. Preeclampsia, corticosteroid use, and stress have been all associated with epigenetic changes in gene expression, placental immune function, growth retardation, and congenital defects. Maternal stress has an important role in placental gene expression since adrenal glucocorticoid production through the hypothalamic-pituitary-adrenal axis modulates inflammatory gene expression, with recognized effects on glutathione metabolism and DNA methylation. Inflammatory disease during pregnancy can also alter placental immune function through cortisol production in a sex-specific manner. Our recent studies also indicate that early induction of innate inflammatory genes in the neonate (including IL-1β and tumor necrosis factor α) is strongly related to the subsequent development of allergic disease. These inflammatory mediators, which are important for immune programming, induce histone modifications and may be responsible for alternatively programming neonatal immunity.

CONCLUDING REMARKS

Effective prevention strategies are the ultimate goal in reversing the allergy epidemic. This will require a better understanding of environmental drivers, target genes, and mechanisms of early life immune programming. Preliminary studies strongly suggest that early events can have a defining influence on subsequent immune development and allergic susceptibility, and that many of these effects are mediated by epigenetic modifications. Emerging epigenetic paradigms in allergic disease are likely to provide further novel insights into the mechanisms and pathways through which the early environment can harness to modify immune development and prevent allergic disease.

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