

Asthma and pregnancy: emerging evidence of epigenetic interactions *in utero*

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Purpose of review

Pregnancy is arguably the most critical period of developmental programming. Here, we particularly focus on the emerging paradigm that disease propensity is epigenetically determined by maternal exposures that have the capacity to activate or silence fetal genes through alterations in DNA and histone methylation, histone acetylation, and chromatin structure.

Recent findings

The most notable recent candidate to emerge in this role has been dietary folate, a methyl donor clearly associated with changes in gene expression and disease susceptibility through gene hypermethylation. Animal studies also provide the first evidence that the allergy protective effects of microbial exposure in pregnancy may be mediated by changes in methylation of Th1 genes of the offspring. There is also emerging evidence that perinatal differences in immune function of allergy-prone newborns extend beyond previously recognized differences in effector T cell (Th1/Th2) function, to also include differences in neonatal regulatory T cell (Treg) and Th17 function, and moreover, that these pathways are also epigenetically regulated.

Summary

New studies reinforce the importance of in-utero exposures (including dietary nutrients, microbial products, cigarette smoking, and certain maternal medications) in fetal immune development and in programming the susceptibility to asthma and allergic disease.

Keywords

allergic disease, asthma, cord blood, cytokines, epigenetics, fetus, folate, maternal diet, pregnancy

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Introduction

Pregnancy is a critical time for both mother and fetus. For the mother, complex physiological, metabolic, and hormonal changes have implications for the growing number of women with asthma. With the epidemic rise in allergic disease, asthma is considered the most prevalent medical condition to complicate pregnancy, affecting almost 10% of pregnancies in industrialized countries. Recognition and optimal control of the disease are important for both maternal and fetal well-being. As a major risk factor for allergic disease in offspring, the maternal allergic phenotype may have a role in perpetuating and even amplifying the allergy epidemic. For the fetus, pregnancy is arguably the most critical period of developmental programming. This review focuses particularly on factors determining fetal immune development and susceptibility to asthma and allergic disease, including new evidence that immune development is epigenetically regulated.

Maternal implications of asthma in pregnancy

Pregnancy induces a number of alterations in maternal physiology to cope with the extra demands from the fetus and placenta for nutrition, oxygen, and waste removal. These complex adaptive changes can adversely affect pre-existing maternal disease states such as asthma. Recent studies indicate that 55% of asthmatic women have exacerbations during pregnancy with the severe exacerbations being more likely in women with a history of severe disease [1]. Maternal asthma may also cause aberrant maternal adaptation during pregnancy with consequences for not only maternal disease severity, but also altered placental function, with an increased risk of preterm delivery [2], congenital malformation [3], still-birth [4], and fetal growth retardation [4].

Effect of pregnancy on maternal immune function

The fetoplacental unit releases proteins, steroids, and microparticles including exosomes and cells into the

maternal circulation, many of which have been implicated in the pregnancy-associated shift in maternal immune responses away from Th1-driven cell-mediated immunity in favor of Th2-related humoral responses. We have recently shown that pregnancy modifies maternal cytokine production with a relative reduction of Th1 responses to both environmental antigens [5] and fetal alloantigens [6]. Allergic mothers had lower IFN γ responses during pregnancy [6], and we proposed that this could influence the cytokine milieu experienced by the fetus (discussed further below). Immunosuppressive and regulatory cytokines (IL-10 and TGF- β) also play a role in maintaining maternal immune tolerance to paternal alloantigens during pregnancy. There is new evidence that these regulatory responses are initiated prior to conception, with the induction of maternal CD4⁺/CD25⁺/FoxP3⁺ regulatory cells following exposure to paternal antigens in seminal fluid [7^{**}]. Others have questioned whether previous exposure to paternal antigens (in previous pregnancies) may influence regulatory T cell (Treg) activity, although this is not yet known [8].

Affects of maternal immune changes on maternal asthma predisposition

Many pregnancy-induced changes in the maternal immune profile are also associated with the pathophysiology of asthma. We have shown that pregnancy increases the production of pro-inflammatory mediators such as RANTES, IL-6, and the cell adhesion factor soluble ICAM-1. Although this is seen in both nonasthmatic and asthmatic women [9], it suggests that pregnancy may activate inflammatory pathways associated with asthma in predisposed women and contribute to changes in maternal asthma severity. Proteomic analysis also indicates a number of circulating proteins that are specific to the presence of maternal asthma, and change with gestational age [10]. This could also potentially influence both maternal asthma and placental function. Finally, there are changes in the circulating levels and the function of granulocytes and monocytes in pregnancy, although it is not yet clear how this alters maternal susceptibility to asthma in pregnancy [11].

Affects of maternal asthma on placental immune function

As asthma during pregnancy is associated with fetal growth retardation and preterm birth [2,4], we recently examined whether maternal asthma was associated with changes in pro-inflammatory placental cytokines, such as TNF α , IL-1 β , IL-6, IL-8, and IL-5. Interestingly, we found placental expression was significantly increased for all these cytokines in pregnancies complicated by mild asthma, but only in the presence of a female fetus [12^{*}]. This was related to sex-specific differences in fetal cortisol, suggesting placental immune function is sex-

specifically regulated by cortisol in pregnancies complicated by asthma [13].

Affect of fetal sex on maternal asthma

Since 1930, there have been anecdotal and scientific reports that a female fetus is more likely to be associated with deterioration of maternal asthma [14], although no fetal-derived sex-specific factors have been identified. In the most recent population-based prospective cohort study, peak expiratory flow variability was also greater in asthmatic women pregnant with a female fetus (relative to those with a male fetus) [15], with indirect evidence that this may be mediated by noneosinophilic pathways [16].

Implications of asthma treatment in pregnancy

Although further research is needed, it is currently accepted that poorly controlled maternal asthma in pregnancy poses greater risk to mother and fetus than the effects of the medications, notably inhaled corticosteroids (ICS), required to control the disease [2,17]. Extensive review of the available literature suggests no increased risk for congenital malformations, cardiovascular defects, decreased gestational age, birth weight or birth length among infants born to women using inhaled budesonide during pregnancy compared with the general population [18]. Although there is some evidence of morphometric differences in feto-placental vascularity due to the effects of asthma and use of ICS [19], and population studies show evidence of a small increased risk of congenital malformation in children born to women with asthma [3], this was not explained by gestational exposure to asthma drugs [3]. Of some interest, there are sex-specific differences in the fetal-placental response to glucocorticoids [12^{*}]. We speculate that this is related to the regulation of glucocorticoid receptors by testosterone based on preliminary evidence of fetal sex-specific differences in placental GR α mRNA of both normal and asthmatic pregnancies [20]. Finally, although oral steroid use has been associated with preterm birth, this may reflect the effects of asthma severity, and it is currently recommended that medical management should be identical to nonpregnant women with moderate-to-severe asthma exacerbations, and that complete control of symptoms and prevention of exacerbations must remain the primary management objective [21] (as also reviewed by [17]).

Pregnancy as a critical time for fetal developmental programming

There are now many elegant models showing how environmental changes at critical times during development can profoundly alter the phenotype of genetically identical animals, although epigenetic modification (reviewed in [22]). Epigenetic changes play a key role in

activating or silencing genes by altering DNA and histone methylation, histone acetylation, and chromatin structure, as summarized in Fig. 1. These modifications determine the degree of DNA compaction and hence determine the accessibility for gene transcription, allowing genetically identical cells in a single individual to show major differences in morphology and function. This is fundamental to cell differentiation and governs all aspects of normal development. The resulting inherent plasticity allows adaptive changes that may confer evolutionary advantage according to potentially diverse conditions in the anticipated environment. Although these concepts of developmental programming are well studied in other fields, epigenetic models of allergic disease are only now beginning to emerge.

It is now clear that both the maternal allergic phenotype and maternal environmental exposures in pregnancy critically determine the risk of subsequent infant allergic disease (Fig. 2). Perhaps one of the most notable recent developments has been the emergence of a model that clearly demonstrates that maternal diet (folate intake) in pregnancy can alter allergic predisposition of offspring through epigenetic mechanisms [23^{••}] (as discussed further below). There has been an urgent call for more studies to explore this new epigenetic paradigm for asthma and allergy pathogenesis [24^{••},25[•]].

Fetal immune development: new evidence of epigenetic control?

Although there has been long standing interest in the regulation of neonatal Th1/Th2 polarization and how this determines subsequent immune development, there is now growing focus in the role of other T cell populations including Tregs and pro-inflammatory (Th17) cells in this process of maturation. Furthermore, there is also good evidence that all of these pathways are under epigenetic regulation (as recently reviewed by Jansen *et al.* [26]).

Methylation of the gene promoter is the main epigenetic mechanism controlling Th1 expression. Reduced IFN γ expression in immature neonatal CD4⁺ T cells (compared with adults) is associated with hypermethylation (gene silencing) of the IFN γ promoter [27]. More recent studies indicate that Treg function is also impaired in the neonatal period compared with adults [28,29], and that epigenetic change (demethylation) is also prerequisite for FOXP3 expression and Treg differentiation [30,31]. There has been growing speculation that factors that increase gene methylation may increase the risk of disease by silencing pathways (Th1 and T regulatory differentiation) that normally inhibit Th2 allergic differentiation [24^{••},32]. In a landmark study, maternal supplementation with folate (a dietary methyl donor) resulted in hypermethylation (suppression) of regulatory genes in lung tissue, development of allergic airways disease and associated systemic allergic responses [23^{••}], raising

Figure 1 Environmental factors can have epigenetic influences on DNA and histone methylation and histone acetylation during early development to alter gene transcription, the resulting phenotype and disease predisposition

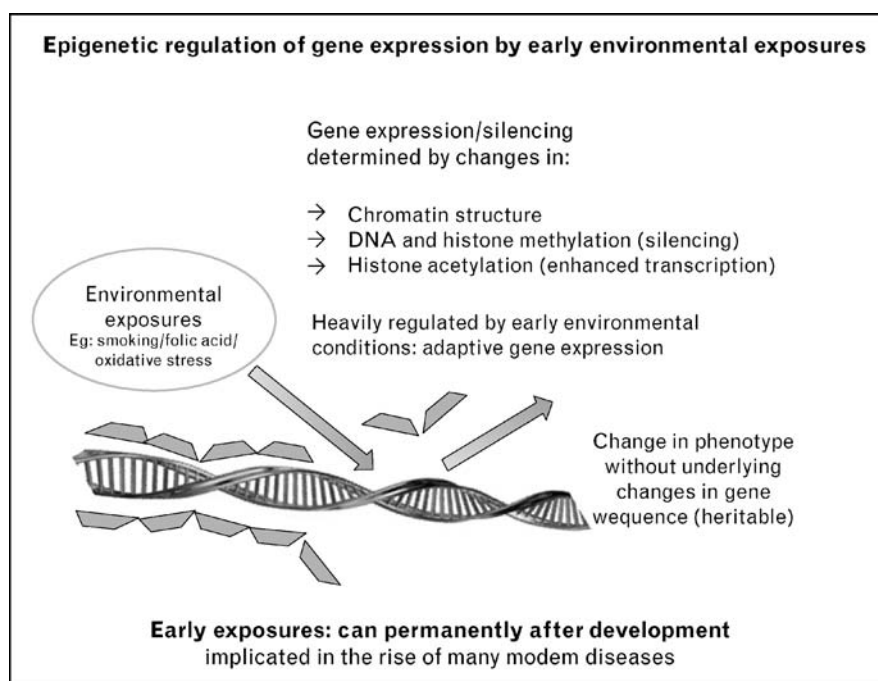
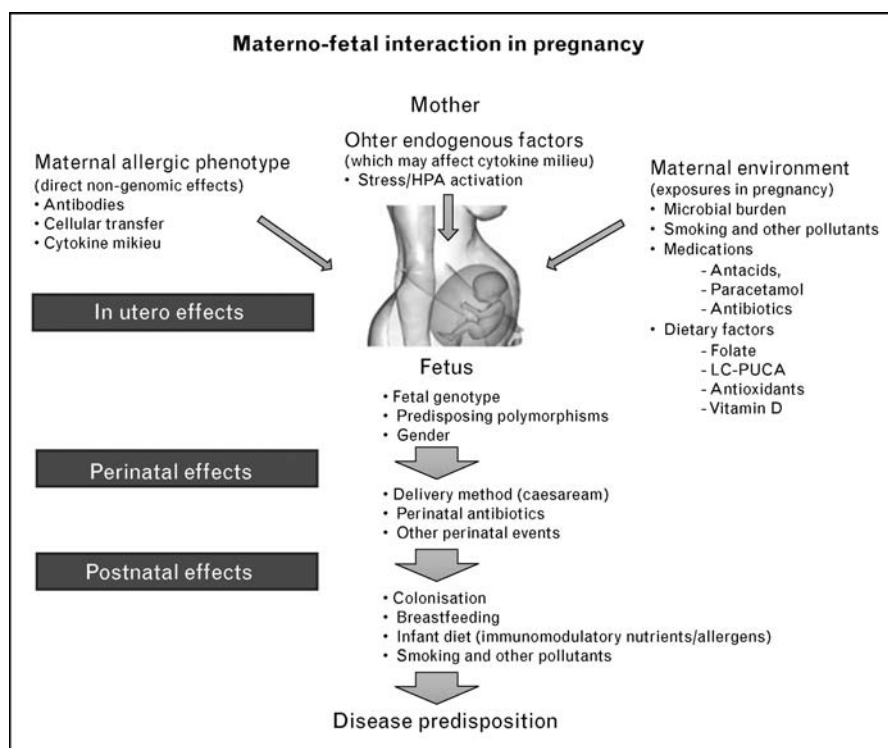


Figure 2 This illustrates the endogenous and exogenous maternal factors that may influence the developing immune system of the fetus and influence disease predisposition



questions about the role of maternal folate supplementation in the allergy epidemic [25*].

Another major mechanism of epigenetic regulation is histone acetylation. Exposures that inhibit histone deacetylase (HDAC) such as oxidative stress enhance NFκB-driven transcription of inflammatory genes [33], and upregulate Th2 cytokine (IL-13, IL-5) and GATA3-mediated T cell responses [34,35]. There is also emerging evidence that the Th17 lineage may be regulated through similar epigenetic mechanisms [26]. Notably, one recent study [36] has shown that human Treg can differentiate into Th17 cells and that this plasticity is also epigenetically determined through histone/protein deacetylase activity. Although the developmental role of this lineage is not clear, it is notable that Th17 function is also impaired in neonates compared with adults [29] and that there are significant correlations between Th17 and Th2 function [29,37*].

There is now intense interest in patterns of early gene expression that lead to allergic disease, and environmental factors that may be associated with this.

The effects of maternal atopy and other endogenous maternal factors on fetal immune development

The mother provides the first environment for the developing fetus, and in addition to transmitting effects of her

own environment (discussed below), she also exerts direct immunologic effects. This is likely to occur as a result of variations in the cytokine milieu at the materno-fetal interface and trafficking of maternal antibodies, cytokines, cells and as yet unidentified factors across the placenta. As noted above, we have identified differences in the patterns of response of allergic women to fetal antigens [6], suggesting effects on the cytokine milieu at the materno-fetal interface. Although animal models are likely to be different from the human situation, it is notable that maternal allergen-specific IgG through the placenta or breast milk mediates tolerance in the offspring and that IFNγ is critical for this process [38,39]. Another intriguing observation of as yet unclear significance is that substantial numbers of maternal cells cross the placenta to reside in fetal lymph nodes, inducing the development of CD4⁺CD25^{high}FoxP3⁺ Tregs that suppress fetal reactivity to maternal antigens [40]. This antigen-specific tolerance induced in utero persists at least until early adulthood and may be active in regulating immune responses after birth [40].

Maternal allergy is a recognized risk factor for infant allergic disease, leading to extensive investigation of differences in neonatal immune function in the cord blood of infants deemed at high risk versus low risk of disease based on maternal disease. Other studies have investigated this longitudinally and also provided

mounting evidence of presymptomatic differences in newborns that later develop allergic disease. Of these, immaturity of Th1 function has been the most consistent observation (reviewed previously in [41]). However, there is now emerging evidence that allergic disease may also be associated with attenuated Treg function [37•,42•] and differences in innate immunity [43••]. Our studies have shown increased neonatal inflammatory responses following Toll-like receptor (TLR) activation in high-risk neonates (and those who go on to develop allergic disease), but others have reported no changes in high-risk infants [37•]. A number of other novel markers have been identified in relation to allergic risk (maternal allergy) or subsequent allergic disease. This includes eotaxin, which was noted to be higher in cord blood of asthmatic women [44], and lower levels of induced CD30 expression on CD4⁺ T cells in high-risk neonates [45]. Higher levels of other proallergic markers in cord blood have also been associated with increased development of allergic disease in the first 2 years of life, including Th2-associated macrophage-derived chemokine [MDC (CCL22)] levels [46]. We have also noted that expression of critical T cell signaling molecules (protein kinase-C zeta) vary in relation to both subsequent allergic disease and therapeutic interventions to prevent disease [47].

All of these pathways need to be explored further as predictive markers, pathogenic factors and possible future therapeutic targets.

Effects of maternal environmental exposure in pregnancy on fetal immune development and allergic outcomes

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. Although there is clear evidence that immune function is regulated by epigenetic mechanisms (as above and reviewed in [48]), epigenetic models to explain the rise in allergic disease are only beginning to emerge [24••,25•]. Here we explore the key maternal environmental factors that may be implicated (as also summarized in Fig. 2).

Maternal diet in pregnancy

Variations in maternal nutrition in pregnancy are well known to influence developmental programming and disease risk in many organ systems [22], and a range of maternal dietary factors in pregnancy have been implicated in immune development and allergic risk (recently reviewed by [49]). This includes evidence that common dietary 'patterns', such as the 'Mediterranean diet' may protect against wheezing in early childhood [50,51], although this has not been seen in all studies [52•]. Other

studies have looked at the effects of 'specific' dietary nutrients, and of these, the epigenetic effects of dietary folate have attracted the greatest recent interest, as discussed above [25•]. Although the significance in humans is not yet clear, one recent study [53] has reported that folate supplements in pregnancy are associated with increased childhood wheezing. This emerging new paradigm of allergy pathogenesis warrants urgent investigation.

Other specific nutrients implicated in either the prevention or pathogenesis of allergic disease include polyunsaturated fatty acids (PUFAs), antioxidants, and a range of specific vitamins and micronutrients (recently reviewed by [49]). Of these, n-3 PUFAs are the best studied in both observational studies and randomized controlled trials (reviewed in [54]). New studies suggest long-term benefits (asthma reduction) following fish oil supplementation in pregnancy [55•], as well as effects on neonatal immune function [56•]. There has been more reluctance to undertake intervention studies with other nutrients proposed to be protective against allergic disease (such as vitamins E, A, and D), as they also have the potential to increase disease and other adverse effects (as reviewed by [49]). At this stage, it is too early for specific dietary recommendations to prevent allergic disease.

Maternal microbial exposure in pregnancy

Although postnatal exposure is likely to play a greater role in immune maturation, animal studies clearly demonstrate that in-utero (maternal) exposure to both pathogenic [57] and nonpathogenic microbial products [58] can prevent allergic outcomes in the progeny. In humans, a new study from New Zealand provides further evidence that in-utero exposure to a farming environment (as a surrogate for high microbial burden) protects against the development of childhood eczema, asthma and allergic rhinitis – an effect that was independent of postnatal exposure [59]. This is consistent with earlier German studies in which prenatal exposure to high microbial burden in farming environments has been associated with altered expression of innate immune genes, reduced risk of allergic disease in the progeny [60], and more recently, modification of cord blood allergen-specific IgE levels [61]. New studies possibly have provided the first evidence animal model to show microbial exposures alter fetal immune development through epigenetic mechanisms (with altered methylation of the IFN γ promoter). R. Teich, M. Conrad, R. Ferstl, S. Brand, N. Blümer, Ö.A. Yildirim, C.C. Patrascu, A. Hanuszkiewicz, R. Schneider-Stock, S. Akira S, *et al.* (manuscript in preparation) used a bacterial strain (notably *Acinetobacter lwoffii*) present in very high quantities in farming environments in an animal model to assess possible allergy-protective effects. Prenatal intranasal administration of these apathogenic bacteria to pregnant animals significantly reduced airways inflammation in the progeny.

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. This may be the first study to show that microbial exposures alter fetal immune development through epigenetic mechanisms. In humans, intervention studies using microbial products are mainly limited to probiotics. Although there is some evidence that these products may reduce the risk of eczema [62], this may be strain dependent [63] and it is not clear whether this effect is the result of prenatal or postnatal supplementation (or both). In one study [64], the administration of probiotic bacteria during the final weeks of pregnancy was associated with an increase in cytokine (IFN γ) detection in cord blood; however, another study [65] that performed much more comprehensive investigation of immune function found no effects on any aspect of neonatal cellular profile of function.

Maternal smoking and exposure to other environmental pollutants

There are very convincing data that maternal exposure to cigarette smoking in pregnancy has many adverse effects on the fetus, including effects on lung function as asthma risk. Several more recent studies [66–68] have provided further evidence that maternal smoking contributes to an increased risk of asthma. Although the prenatal versus postnatal effects of smoking are difficult to separate, previous studies have suggested that the effects are greater *in utero* [69]. In contrast, one recent (albeit much smaller) Japanese study [68] reported that postnatal exposure was more strongly associated with the risk of infant wheeze than prenatal exposure. The relationship with other allergic diseases and sensitization has been less clear. The results of a 10-year prospective study (of 1314 children) [70] indicate that maternal smoking is a strong risk factor for allergic sensitization as well as asthma, but only in children of allergic parents.

It is logical to explore the role of modern environmental pollutants in the recent allergy epidemic, as these have only appeared in the last century and many have immunological effects [71]. It is also notable that prenatal exposure to these organic pollutants has been implicated in both autoimmunity and allergic disease, as expertly reviewed by Hertz-Picciotto *et al.* [72]. This is a difficult area to study in humans, on whom only correlative studies are possible.

Effects of maternal medications in pregnancy

One of the most fascinating and novel observations of this year has been the findings of a large-scale study [73^{••}] showing exposure to acid-suppressive medications in pregnancy was associated with an increased risk of developing childhood allergy. Although this is the first evi-

dence in humans, it does follow animal studies showing similar effects. The mechanisms are not clear, although it has been proposed that these medications may interfere with the normal denaturation and proteolysis of ingested antigens potentially increasing the both levels and immunogenicity.

In the last year, a series of publications [74^{••},75–77] have focused attention on the use of paracetamol in pregnancy as a risk factor for childhood asthma, supporting earlier observations [78]. As paracetamol depletes antioxidant glutathione levels, it has been implicated in the development of both abnormal lung function and immune function. Although these effects are not large [74^{••},75–77], this potential risk factor is avoidable and requires further investigation.

The increasing use of antibiotics is another factor potentially affecting infant microbial colonization. The effects of perinatal antibiotics on allergic outcomes will be assessed as part of a large-scale study aimed at looking at the long-term effects of maternal antibiotic prophylaxis for premature rupture of membranes [79].

The effects of delivery method

The last year has also seen a flurry of reports consistently suggesting that nonvaginal birth (caesarean section) is associated with an increased risk of asthma, as another possible contributor in the hygiene hypothesis. This includes two meta-analyses [80,81], a large-scale population study of more than 1.8 million children in Norway [82^{••}], a registered case–control study in Finland [83], and a smaller but well characterized US cohort [84], all with similar findings. It has been suggested that a stronger association with emergency than elective sections [82^{••}] may be due to other factors associated with high-risk deliveries such as interpartum antibiotics [85].

Maternal allergen exposure in pregnancy

The longstanding debate over the role of allergen exposure *in utero* has returned to the forefront again recently. This has partly been the result of a series of reports detecting allergen-specific IgE, to both food and inhalant allergens, in cord blood [86[•],87[•]]. However, levels correlated more closely to maternal specific-IgE levels [86[•]] than subsequent infant levels [87[•]], suggesting that this may be of maternal origin [88]. Although both food and inhalant allergens have been detected in cord blood, together with clearly measurable fetal allergen-specific cellular responses, the significance of these is unclear, particularly because these do not have the characteristics of ‘normal’ memory responses and they do not correlate well with either maternal exposure or subsequent postnatal risk of allergic disease (reviewed in [88]).

In the clinical setting, many international expert bodies have independently concluded that there is insufficient evidence to justify the continued use of allergen-restrictive diets in either pregnancy or early infancy [89–91]. Although restrictive recommendations have been in place in many allergy-prone industrialized countries, there is no clear evidence that they prevent allergic disease, and yet further reports that delayed allergen exposure may actually increase the risk of allergy [92]. New studies also reinforce that maternal consumption of foods (including peanut) during pregnancy or lactation are not risk factors for specific allergy in childhood [93,94,95•].

Maternal stress in pregnancy

The hypothalamic–pituitary–adrenal (HPA) axis and immune system are closely linked because adrenal glucocorticoid production modulates inflammatory responses. The placental immune system is also partially regulated by glucocorticoids. Pregnancies associated with inflammatory diseases that activate the HPA axis are also associated with upregulation of placental Th1 cytokines and poor fetal outcomes. As indicated above, our data suggest that the effects of maternal asthma on placental immune function are specifically regulated by cortisol, but that this is dependent on infant sex [12•]. We speculate that this may have lasting implications for subsequent HPA and immune function in childhood. This is based on previous animal studies showing that other early physical stressors (exposure to endotoxin) have long lived effects on both HPA function and immune function in the offspring, again with sex differences [96].

Although there are also recognized associations between psychological stress and immune function, the effects of maternal mood and psychological stress in pregnancy on fetal immune development are not well understood. We recently observed that newborns of mother with depressive symptoms in pregnancy have higher spontaneous cytokine production including (IL-6 and IL-10) and higher stimulated cytokine responses to both bacterial antigens and allergens [97]. Further studies are needed to determine the significance of this.

Conclusion

The physiologic, metabolic, and immunologic changes that occur in pregnancy may have consequences for the growing number of women affected by asthma. Recognition and adequate treatment are important for maternal health and to reduce the risk of stillbirth, fetal growth retardation, and other fetal morbidity. Based on the current evidence, we speculate that in-utero events have a more critical influence on perinatal immune function than genomic inheritance. First, maternal (rather than paternal) allergy is a major determinant of neonatal Th1

function and disease susceptibility, suggesting direct maternal effects. Second, there is a wealth of evidence that antenatal exposures (such as maternal diet, microbial exposure, and smoking) can modify neonatal immune function. Third, the now epidemic rates of disease suggest that environmental changes are unmasking pre-existing high rates of genetic propensity. Finally, and perhaps most notably, there is clear evidence that environmental exposures in pregnancy has epigenetic effects on fetal gene expression, thereby altering disease predisposition. Identifying genes that are differentially silenced or activated in relation to subsequent allergic disease and asthma will assist in identifying causal pathways, and help identify the contributing environmental factors.

References and recommended reading

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 483–484).

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