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Session 5: Early programming of the immune system and the role of nutrition
Allergic disease: understanding how *in utero* events set the scene

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Events and exposures in pregnancy can have critical effects on fetal development with lasting implications for subsequent health and disease susceptibility. There is growing interest in how modern environmental changes influence fetal immune development and contribute to the recent epidemic of allergy and other immune disorders. Rising rates of allergic disease in early infancy, together with pre-symptomatic differences in immune function at birth, suggest that antenatal events play a predisposing role in the development of disease. A number of environmental exposures in pregnancy can modify neonatal immune function including diet, microbial exposure and maternal smoking, and there is emerging evidence from animal models that these factors may have epigenetic effects on immune gene expression and disease susceptibility. Furthermore, functional genetic polymorphisms also alter individual vulnerability to the effects of these environmental exposures, highlighting the complexity of gene–environmental interactions in this period. All these observations underscore the need for ongoing research to understand the pathogenesis and rising incidence of disease in the hope of better strategies to reverse this.

Allergic disease: Pregnancy: Cord blood: Cytokines: Microbial exposure: Antioxidants: Fatty acids: Folate: Allergy prevention

While the spotlight is on the early postnatal period for understanding the events that lead to oral tolerance, it is becoming clearer that the scene is set much earlier in development. Now extensive data from both epidemiological and experimental studies indicate that gene–environmental interactions during pregnancy can induce permanent changes in physiological processes and disease susceptibility[1] by altering gene expression and disease predisposition through epigenetic mechanisms[2]. This has been the foundation of the newly established field of Developmental Origins of Health and Disease[2]. Although this has been best studied in the context of cardiovascular and metabolic disease, the epidemic rise in both allergic and autoimmune diseases also highlights the susceptibility of immune pathways to modern environmental changes. Moreover, the rising rate of disease in early infancy is further evidence that early events must have a critical role. A recent animal model has provided the first evidence that alterations in the maternal diet in pregnancy can alter the risk of allergic airways disease in the offspring through epigenetic changes in gene expression[3]. The mother provides the first environment for the developing fetus, and this review explores the range of maternal factors that may influence fetal immune development including both exogenous environmental exposures and endogenous factors.

Immune development and regulation in pregnancy

Human lymphocytes derived from the yolk sac appear in the liver within several weeks of conception. By 10–12 weeks of gestation, they are evident in the thymus[4] and

Abbreviations: Th1, T-helper cell type 1; Th2, T-helper cell type 2; Treg, regulatory T-cells.

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show responsiveness to mitogen stimulation[5] and allogeneic graft *v*. host reactivity[6]. Thymocytes appear to be capable of binding antigens from 20 to 22 weeks gestation, and allergen-specific responses have been also recorded as early as 22 weeks gestation[7]. However, while many groups have demonstrated that cord blood mononuclear cells can respond to environmental allergens, there has been ongoing debate about whether these reflect conventional memory responses as they do not correlate well with either maternal exposure or subsequent development of allergic disease (reviewed in Holt[8]). There is some evidence that these responses reflect a default response by recent thymic emigrants to first antigen encounter, which also leads to the activation of regulatory T-cells (Treg)[9]. At birth, cytokine production is dominated by T-helper cell type 2 (Th2) cytokines[10] and many aspects of neonatal immune function are still immature including antigen presenting cells, T-helper cell type 1 (Th1) and pro-inflammatory Th17 effector T-cells and Treg function[11]. It has been proposed that postnatal microbial exposure provides an essential source of immune stimulation for all of these pathways and protection from allergic diseases[11]. There is therefore intense interest in factors which influence the patterns of perinatal immune function and their subsequent regulation.

Immune development is under epigenetic regulation

Epigenetic regulation is fundamental to cellular differentiation and all aspects of normal development. Specifically, changes in the methylation of DNA and histones, and histone acetylation regulate gene expression by altering the DNA compaction and accessibility for gene transcription[12]. There is clear evidence that T-cell differentiation is under epigenetic control[13], including Th1 and Th2 differentiation[14–18], FoxP3 expression and Treg differentiation[19,20] and Th17 differentiation[21].

The main epigenetic mechanism controlling Th1 expression is methylation of the interferon-γ gene promoter. This is hypermethylated (i.e. underexpressed) in neonatal CD4+ T-cells and shows progressive demethylation by adulthood[22]. Changes in methylation (demethylation) are also prerequisite for FOXP3 expression and Treg differentiation[19,20]. Another major mechanism of epigenetic regulation is histone acetylation. Removal of acetyl groups by histone deacetylase generally leads to gene silencing, whereas acetylation by histone acetyl transferase opens chromatin structure for enhanced gene transcription[23]. Exposures that inhibit histone deacetylase such as oxidative stress up-regulate Th2 cytokine (IL-13 and IL-5) and GATA3-mediated T-cell responses[23,24]. The Th17 lineage also appears to be regulated through similar epigenetic mechanisms[13,21].

These insights have logically led to interest in factors which may promote allergic propensity by increased histone acetylation (Th2 promotion) and/or increased gene methylation (Th1 and Treg silencing)[25,26]. As discussed further later, the first evidence of this comes from an animal model in which maternal folate supplementation (a dietary methyl donor) resulted in hypermethylation (suppression) of regulatory genes and the development of allergic disease in the offspring[3]. At this stage, the implications in human subjects are not clear, but this provides a platform for investigating epigenetic pathways as a mechanism for gene–environmental interactions in allergic disease.

Altered patterns of immune response begin to emerge in fetal life

There have been numerous studies showing pre-symptomatic differences in the immune responses of newborns who later develop allergic disease (reviewed in Prescott and Clifton[27] and Prescott[28]). This was initially thought to largely reflect inherited genetic risk. However, the epidemic rise in allergic disease has raised the alternative hypothesis that this may be due to more complex alteration in immune gene expression conferred by gene–environmental interactions *in utero*. Thus, at least some of the environmental effects driving the rise in allergic disease may begin *in utero*, and the differences in neonatal immune function may be the first signs of this increasing allergic predisposition.

A relative immaturity of neonatal Th1 immune function has been one of the clearest and most replicated neonatal associations with allergic disease[29–31]. Although Th1 responses are generally suppressed at birth under the Th2-dominant influence of pregnancy, this appears to be more marked in neonates with allergic predisposition or subsequent allergic disease[29–31]. Other aspects of neonatal effector T-cell function may be impaired in this population[10]. More recently, there has been emerging evidence that allergic disease is also associated with attenuated neonatal Treg function[32,33] and differences in innate immunity[34–37]. A number of other neonatal markers have been identified in relation to allergic disease (reviewed in Prescott and Clifton[27]), though none of these has so far been shown to be of accurate predictive value. Further research is needed to understand the functional significance and the possible contribution to the disease pathogenesis. It is possible that that impaired Th1 and Treg function may contribute to a reduced capacity to suppress Th2 responses in the early postnatal period; however, this is likely to be oversimplistic. While there has been long-standing speculation that dysfunction of antigen presenting cells and innate immunity may contribute to the apparent immaturity of Th1 activity, there is still only indirect evidence to support this[38,39]. Furthermore, while some groups have shown that markers of innate activity (such as Toll-like receptor function or expression) are lower in neonates at risk of allergic disease[35,37], we have shown the opposite[34]. While this needs to be examined further, collectively these observations do suggest that differences in neonatal immune function confer increased susceptibility to subsequent postnatal environmental influences and contribute to an evolving allergic phenotype.

Evidence that maternal environmental factors can modify fetal immune development

While there is a hereditary component of allergy, only environmental change can account for the rapid rise in

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disease. There is growing evidence that maternal environmental exposures including dietary factors[40,41], cigarette smoke[42,43] and microbial exposure[36,44] can modify neonatal immune responses.

Maternal dietary influences on immune development

Maternal nutrition is critically important for most aspects of fetal development, including the immune system. Complex dietary changes with progressive industrialisation have been implicated with the rise of allergic disease. As with other exposures, nutritional changes are likely to have more profound effects on pregnancy when the organ systems and physiological responses are developing. Many dietary nutrients have recognised immunomodulatory properties and plausible biological mechanisms of influence[45]. This includes PUFA[46], antioxidants and other vitamins[47]. Of these, PUFA are among the most extensively studied in this context. A declining intake of anti-inflammatory *n*-3 PUFA (found in oily fish) has been implicated in the rise in allergic disease[48], and a series of studies have shown a protective relationship between maternal *n*-3 PUFA consumption in pregnancy and subsequent infant allergic disease[49–54], though not all were significant after allowing multiple comparisons[55]. Several intervention studies using fish-oil supplementation in pregnancy have also suggested protective effects against allergic disease in early childhood[40,56] and a long-term (16-year) follow-up study showed a reduction in subsequent asthma[57]. A number of other studies are currently in progress to hopefully assess this more definitively.

In the last 12 months, dietary folate has become one of the most topical dietary nutrients in this area. As a dietary methyl donor, there has been established interest in the broader context of epigenetics; however, the recent pro-allergic effects demonstrated in an animal model have extended this interest into the field of allergic disease, and for the first time provided an epigenetic model for these immune disorders. As indicated earlier, supplementation with folate in pregnancy induced hypermethylation (silencing) of regulatory genes in lung tissue, and was associated with the development of allergic airway disease and systemic allergic responses[3]. This effect was also transmitted epigenetically to subsequent generations. This has been followed by reports in human subjects[58,59] linking folic acid supplementation during pregnancy with increased risk of asthma and respiratory disease in infants. There have been links between folate status in the postnatal period and allergic disease, but if anything, folate was protective[60]. At this stage, the significance of these findings is not yet clear. At this stage, it is premature to change current practice based on animal studies and preliminary epidemiological reports, but there is now an urgent call for further studies to address this now pressing question[61].

Most other nutrients linked with allergic disease in epidemiological studies have not been investigated in intervention studies. Maternal antioxidant vitamins have been associated with differences in neonatal immune function[41], as well as reduced risk of possible allergic outcomes including recurrent wheezing (vitamin C[62,63], vitamin D[64], vitamin E[65,66] and Zn[66]), and eczema (vitamin E[67]). However, these correlative findings are not consistent between studies and concerns have been raised about the potential for paradoxical or adverse effects of antioxidants on allergic disease[68]. This is based on speculation that antioxidant supplementation could promote Th2 differentiation by inhibiting oxidative stress[68]. At this stage, there is no place for specific dietary supplementation in pregnancy for allergy prevention, although this may be included in future strategies once these relationships are more fully understood.

Maternal microbial exposure

The decline in the level and diversity of microbial exposure is a leading candidate in the allergy epidemic. While focus has been on the role of postnatal microbial exposure, animal studies clearly demonstrate that *in utero* (maternal) exposure to both pathogenic[69] and non-pathogenic microbial products[70] can inhibit the development of allergic phenomena in the offspring. In human subjects, maternal exposure to high microbial burden in German farming environments has been associated with altered expression of innate immune genes and reduced risk of allergic disease in the children[36]. Similar protective effects of farming environments have also been observed in New Zealand[71]. This effect was independent of postnatal exposure in both studies[36,71]. In what may be another example of epigenetic regulation, there is preliminary evidence that an apathogenic microbial strain isolated from the German farming environment can mediate allergy-protective effects by epigenetic changes[72]. Intranasal administration of this strain (*Acinetobacter lwoffii*) to pregnant mice was associated with significant effects on the ontogeny of splenic CD4+ Th1 interferon-γ production in the offspring of exposed mothers. These differences were directly related to epigenetic changes in the interferon-γ promoter[72]. This supports notions that microbial exposure may modify foetal gene expression and provides a potential epigenetic mechanism. Intervention studies using microbial products in human pregnancy are mainly limited to probiotics[73]. Although there is some evidence that these products may reduce the risk of eczema[74], there is wide heterogeneity in study protocols and findings between studies. This appears to be species-dependent[75] and although there has been speculation that antenatal supplementation may explain the beneficial effects in some studies[76], this is also not consistent[73]. Furthermore, while one study suggested that probiotic bacteria during the final weeks of pregnancy was associated with an increase in cytokine (interferon-γ) detection in cord blood[77], another more comprehensive investigation found no effects on any aspect of neonatal immune function[78]. At this stage, the role of probiotics in the prevention of allergic disease is still unclear and no specific recommendation can be made.

Maternal allergen exposure

Although early allergy prevention strategies focused on allergen avoidance, there is little clear evidence that changes in food or inhalant allergen exposure in pregnancy

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are responsible for the rise in allergic disease. Moreover, there is no clear evidence that restrictive dietary recommendations actually prevent allergic disease. In contrast, there are a growing number of reports that an attempt to avoid or delay allergen exposure may actually increase the risk of allergic sensitisation[79,80]. Many international expert bodies have independently concluded that there is insufficient evidence to justify the continued use of these allergen-restrictive diets in either pregnancy or early infancy[81–83].

Maternal smoking

Maternal cigarette smoking in pregnancy has many adverse effects on the fetus, including effects on lung function and asthma risk. While there are documented effects on neonatal immune function[41–43], the relationship with other allergic sensitisation has been less clear. Regardless of this, the avoidance of cigarette smoke is an unequivocal recommendation in view of the many toxic effects on the fetus.

Other maternal exposures

A range of other maternal exposures could potentially influence fetal immune development. Firstly, the use of a number of medications in pregnancy has been associated with an increased risk of childhood asthma. The most consistent relationship has been seen with paracetamol, with a series of independent studies[84–87] supporting the initial reports[88]. Documented depletion of antioxidant glutathione has been proposed as a mechanism of effect on immune function and lung development. Another notable relationship has been a recent large-scale study showing that acid-suppressive medications in pregnancy are associated with an increased risk of developing childhood allergy[89]. Although the mechanisms are unclear, it does follow animal studies showing similar effects.

Secondly, modern environmental changes have been associated with the appearance of many new chemicals and persistent organic pollutants. A number of these products of industry and agriculture have been linked with immune disorders[90] because of oestrogenic (proTh2) properties, which have earned them the title of 'hormone imposters'. Although environmental measures have helped reduce the level of exposure, we have detected pesticides in 94% of maternal abdominal fat samples (collected at caesarean section) and 62% breast milk samples, albeit at very low levels[91]. The effects of these and other modern environmental exposure on immune development is still unclear and difficult to investigate, but should not be ignored as potential contributors to the rise in modern diseases.

Endogenous influences in pregnancy

In addition to the external environment, endogenous factors may also influence development during pregnancy. Our recent studies suggest that maternal allergic status is associated with a relative reduction of Th1 responses to both environmental allergens[92] and fetal alloantigens[93], and modified expression of cytokine genes in the placenta[94]. We propose that this could influence immune development by modifying the cytokine milieu experienced by the fetus. Direct maternal effects are also in keeping with the observation that maternal is a greater risk factor than paternal allergy.

The placental immune system is also partially regulated by glucocorticoids, and there is evidence that activation of the hypothalamic–pituitary–adrenal axis is also associated with up-regulation of placental Th1 cytokines and poor fetal outcomes. Animal studies show that other early stressors (exposure to endotoxin) have long-lived effects on both hypothalamic–pituitary–adrenal function and immune function in the offspring[95]. It is certain that the effects of physical and psychological stress in pregnancy on immune development need to be investigated further.

Variations in genetic predisposition add a further dimension of complexity

All of these interactions need to be viewed in the context of genetic predisposition. Functional polymorphisms confer variations in susceptibility to both disease and the effects of environmental exposures. For example, in the context of high bacterial exposure, polymorphisms in microbial recognition pathways (Toll-like receptor 2) confer protection from allergic disease, but this relationship is not seen in a low microbial burden environment[96]. Thus, the effects of genetic polymorphisms may only be relevant in certain environments. These complex interactions could obscure potentially important causal pathways and could account for the many inconsistencies between studies. There are now recognised functional genetic polymorphisms in many other pathways, which could modify the biological effects of other environmental exposures including PUFA[97], folate[98] and cigarette smoke[99]. This has highlighted the need for new research approaches to further explore these complexities.

Summary and conclusions

With the advent of the Developmental Origins of Health and Disease hypothesis[1], pregnancy is now widely recognised as a critical time for developmental programming, when the scene is set for future patterns of health and disease. New technologies and the discovery of epigenetic regulation has provided mechanisms for how environmental exposures can alter gene expression and influence the evolving phenotype[2]. Extensive environmental changes have been implicated in the epidemic rise of allergy and other immune disorders, and there is now emerging evidence of how environmental factors may modify fetal immune development. A deeper understanding of these pathways will hopefully reveal both the pathogenesis of these diseases and the reasons for the rise in prevalence. This in turn may lead to more effective strategies for disease prevention. Complex multi-factorial genetic and environmental interactions may ultimately translate to individualised early interventions tailored and targeted according to genetic predisposition. Although future developments are difficult to predict in this rapidly

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evolving field, events in pregnancy should remain a research priority.

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