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Mechanisms of Allergic Disease - Environmental and genetic determinants for the development of allergy

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Abstract:

Allergic disease can be viewed as an early manifestation of immune dysregulation. Environmental exposures including maternal inflammation, diet, nutrient balance, microbial colonisation and toxin exposures can directly and indirectly influence immune programming in both pregnancy and the postnatal period. The intrauterine microclimate is critical for maternal and foetal immunological tolerance to sustain viable pregnancy, but appears susceptible to environmental conditions. Targeting aspects of the modern environment that promote aberrant patterns of immune response is logical for interventions aimed at primary prevention of allergic disease. Defining the mechanisms that underpin both natural and therapeutic acquisition of immunological tolerance in childhood will provide insights into the drivers of persistent immune dysregulation. In this review we summarise evidence that allergy is a consequence of intrauterine and early life immune dysregulation, with specific

focus on contributing environmental risk factors occurring preconception, in utero and in the early postnatal period. We explore the immunological mechanisms which underpin tolerance and persistence of allergic disease during childhood. It is likely that future investigations within these two domains will ultimately provide a road map for the primary prevention of allergic disease.

Key words:

Preconception

Allergy

T regulatory cell

Microbiome

Micronutrient

INTRODUCTION – THE ‘DEVELOPMENTAL ORIGINS’ OF ALLERGY

Many aspects of health in later life are determined, at least in part, through environmental conditions during critical times of development in early life. The immune system is inherently responsive to the environment and ensuing patterns of reactivity may influence the development and function of many organ systems. Variations in environmental conditions during initial allergen encounter appear to modify patterns of immune response during very early life (FIGURE 1). In this context, the early environment may determine not

only the *specificity* of responses but also the *patterns* of response and with it, the propensity for both allergy and inflammation. How the developing immune system responds to the environment as a whole, and how and why responses are increasingly prone to allergic dysregulation is therefore of intense interest.

The environment can influence the risk of health and disease at any age; however the impact of adverse exposures can be more profound when organs are forming and patterns of biological response are being established[1]. This concept forms the basis of the Developmental Origins of Health and Disease (DOHaD) field, which also proposes that prevention of non-communicable diseases (NCDs) should most logically begin in early life, to improve biological reserve, and promote adaptive responses and healthy behaviours. This framework does not propose that environmental exposures *only* matter in early life. Moreover, allergic disease is likely to be only one of the many diseases associated with detrimental environmental exposures. [2]. It is important to understand how changes in allergic disease are inter-related to the increases seen in so many other inflammatory NCDs.

For some aspects of development, there are relatively fixed windows of susceptibility [3]. This is demonstrated most clearly in studies of the developing central nervous system, where intervention trials have shown that early life exposures can have dramatic effects on structural, cognitive, special sense and emotional outcomes [4, 5]. Meaningful effects have been shown for other health outcomes associated with the metabolic syndrome[6] and immune outcomes.

GENETICS AND EPIGENETICS

Twin studies indicate that a substantial component of allergic risk is inherited, with significantly higher rates of allergic diseases including asthma, food allergy[7], atopic dermatitis[8], allergic rhinitis and atopic sensitization[9] in monozygotic compared with dizygotic twins[10]. Calculated heridity (the proportion of variance due to genetic factors) for asthma ranges from 0.36 and 0.75[11]. Peanut allergy heridity is reported to be higher at 0.82[7], however data outside asthma studies are based upon smaller cohorts. Genome wide linkage and candidate gene association studies have identified more than 120 genes which appear associated with atopic diseases, but no major susceptibility genes for asthma and atopy have been identified, underlining the complex heterogeneity of heridity in allergic disorders.

Against this background, environmental exposures appear important in modifying transcription and translation of relevant susceptibility genes. Molecular alterations to DNA and chromatin which leave the underlying DNA sequence unaltered and are mitotically heritable and reversible are known as epigenetic changes. These alterations arise via multiple mechanisms including modification of histones (via methylation or acetylation), DNA methylation, or with interfering RNAs (microRNAs, small interfering RNAs and long non coding RNAs)[12].

There is strong evidence of epigenetic regulation of immune responsiveness in humans, beginning in the prenatal period. For example, expression of innate immune genes for Toll like receptors are influenced by both prenatal and postnatal microbial exposures, or

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surrogates for microbial exposure such as farm or animal contact[13]. Postnatal microbial exposures, particularly in the gastrointestinal tract, dynamically influence gene expression during infancy and beyond. Macro and micronutrient exposures, both directly and indirectly via GIT microbial bio-products (such as short chain fatty acids) are associated with epigenetic changes[14]. Altered intestinal microbial colonisation may influence risk of allergic disease through epigenetic changes at the interferon-gamma (IFN γ) locus in naïve T cells[15]. Prenatal epigenetic modifications may determine the evolving T cell phenotype. Specifically, food allergic infants are shown to have dysregulated DNA methylation at mitogen activated protein kinase (MAPK) signalling-associated genes during early CD4⁺ T-cell development, which might contribute to aberrant T cell responses associated with food allergy[16]. Such early epigenetic changes could increase the risk of sustained immune responsiveness to harmless environmental antigens through the life course.

NATURAL GENETIC VARIANTS and ALLERGIC DISEASE

The discovery that filaggrin gene variants can predispose not only to eczema, but to food allergy, allergic rhinoconjunctivitis and asthma, suggests an important role for skin barrier integrity [and/or upper airway/oesophageal mucosal integrity] in preventing the development of allergic immune responses [17]. Recent findings that filaggrin mutations are associated with down regulated E-cadherin expression, and that E-cadherin ligation on human type 2 innate lymphoid cells (ILCs) inhibits Th2 cytokine secretion may provide a critical mechanistic link between skin barrier and immune function[18]. Other genetic causes of disrupted epidermal barrier are also associated with allergic sensitisation and disease. For example, Netherton syndrome caused by dysfunction of the anti-protease gene

Kazal type 5 (SPINK5) [19] and ectodermal dysplasia [20] are both associated with allergic disease. Other natural genetic variations which predispose to allergic disease through altered immune function rather than via epidermal barrier defects include primary defects in regulatory T (Treg) cell function [21, 22], and secondary defects in Treg cell function caused by aberrant antigen presenting cell function in Hyper IgE syndrome via a defect in Signal Transducer and Activator of Transcription 3 (STAT3)[23], in Hyper IgE syndrome by Dedicator Of CytoKinesis 8 mutations[24], and in Wiskott-Aldrich syndrome[25].

TIMING OF ENVIRONMENTAL EXPOSURES AND ALLERGIC PROGRAMMING

The timing of environmental exposures has important effects for immune development, as with other organ systems. While postnatal exposure does have adverse effects, antenatal exposure has more critical toxic effects on a broader range of developing organ systems. Moreover, there are likely to be other “sensitive” periods for both the immune system and organs, such as the lungs, which are affected by allergic disease. For example, the effects of cigarette smoke exposure, independently associated with early asthma risk [26, 27], might be more profound in-utero during foetal lung and airway development with additional effects on the foetal immune system [28, 29]. Similarly, timing is emerging as important for microbial exposures. Historically, there has been a *postnatal* focus to the hygiene hypothesis and the importance of intrauterine microbial exposure has been relatively overlooked. To date, most studies have focused on promoting *postnatal* infant colonization rather than on influencing materno-foetal interactions much earlier in pregnancy [30]. It is now clear that the womb is not ‘sterile’ and that maternal microbial transfer to the offspring begins during normal healthy pregnancy [31] providing an initial antenatal source

of immunostimulation. Epidemiological studies also indicate that a 'high microbial environment' during pregnancy affords greater protection from allergy than postnatal exposure alone [32].

IMMUNE RESPONSES IN-UTERO AND EARLY LIFE

Discussion of the immune response in the peri-natal period requires appreciation of the fundamental differences to the adult. An exhaustive overview is beyond the scope of this review but a few key features should be noted. Human neonatal circulating T cells are predominantly recent thymic emigrants (RTEs) so are more akin to thymocytes than the mature naive T cells that comprise the bulk of the non-memory compartment in adults, although this decreases with age. Adults RTEs are far less abundant [33]. This and other features bestows neonatal T cells with different responses to the microenvironment including; a non-classical IL-4 program during which an unglycosylated isoform of IL-4 [34] is produced, a relative abundance of interleukin-8 producing T cells, a regulatory T cell default program within the CD4⁺ T cell compartment [35], and dampened classical Th1, Th17, and Th2 responses reflecting intact and even hyperactive early T cell signalling events that are uncoupled from downstream events such as activation of gene transcription [36]. These are presumably highly evolved strategies to limit inflammation and tissue damage during the transition from a relatively germ free intrauterine environment to a germ laden extrauterine environment as the immune system establishes tolerogenic or immunogenic antigen responses. Clearly, there is much scope for this process to be perturbed and dysregulation of these processes is postulated to underlie the increased prevalence of not only IgE-

mediated allergy but a range of other non-communicable diseases with inflammatory aetiology.

Lymphoproliferative and cytokine responses by allergen exposed umbilical cord blood mononuclear cells and their relationship to allergic disease outcomes are of longstanding interest. However, *in vitro* cord blood responses likely represent an allergen/antigen-dependent rather than a conventional antigen-specific phenomenon, with an initial burst of short-lived cellular immunity by RTEs in the absence of conventional T cell memory. These responses are generally limited in intensity and duration via the parallel activation of regulatory T cells [35]. However, irrespective of antigen-specific reactivity, the dynamics and intensity of the immune response measurable at birth likely reflect intrauterine programming and with this, the capacity to modulate the development of antigen-specific responses.

Immune responses at birth - Old paradigms re-examined

Allergen-specific IgE has been detected in cord blood in many studies (e.g. [37]) and it is generally held that IgE does not cross the placenta [38] . However, over 50% of infants with specific IgE at birth no longer have detectable IgE at 1 year of age [37], so the relevance of this early “allergic” signal is unclear. Given the presence of IgE in amniotic fluid and breast milk and the expression of IgE receptors within the fetal gastrointestinal tract there might be an evolutionary role for maternal to fetal signalling via IgE that relates to the maternal parasite burden that is now dysregulated[39].

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Elucidating the mechanisms underpinning allergic sensitisation and inflammation operated initially within the Th1/Th2 paradigm. Effector CD4⁺ T cell cytokine responses tend to be lower in neonates with allergic disease risk and/or subsequent allergic outcomes and not just for IFN γ [40] possibly reflecting reduced downstream expression and activity of key T cell signalling molecules in atopy predisposed neonates [41]. However reduced IFN γ and delayed maturation of “Th1” type responses is one of the most commonly reported phenomenon in neonates at risk of subsequent allergic disease [40] (FIGURE 2).

The capacity of antigen presenting cells (APC) such as dendritic cells to “educate” T cells depends on the local microenvironment, as this determines cell surface co-stimulatory molecule expression and cytokine output which in turn determines the effector phenotype of antigen-specific T cells. Therefore, dendritic cells link the innate and adaptive immune responses. The innate immune compartment is generally hyper-responsive in newborns with allergic disease risk and/or outcomes [42, 43] and this is down-regulated post-natally in allergic children while being up-regulated in non-allergic children [43, 44]. For example, Papua New Guinean (PNG) neonates have APCs with an activated phenotype that are less responsive to inflammatory challenges than those from Australian born neonates, [45] supporting the notion of a functional spectrum at birth from traditional environment (lowest) to Western/non-allergic and then Western/allergic (highest).

Mucosal surfaces in adults and children typically contain a vast network of dendritic cells well positioned to capture antigen from the environment. At birth, the lungs, in contrast to the gastrointestinal tract, are relatively devoid of dendritic cells and numbers increase over the first year of life particularly in response to viral infection. Viruses have many

immunomodulatory effects that outlive active infection and might impact on disease pathogenesis [46]. Deficiencies in the immune surveillance properties of lung dendritic cells might underpin the development of airways inflammation and asthma [47]. Some of the changes in immunological responsiveness described in offspring with allergic risk and/or outcomes could impact on the development of vital immune surveillance functions in the airways.

Regulatory T cells

Treg cell undoubtedly play an important role in tolerance. Allergic disease can be viewed as a breakdown in tolerance, and their importance in allergic disease is illustrated by the allergic manifestations disorders in which Tregs are absent or non-functional. Infants with mutations in the FOXP3 gene (IPEX syndrome) develop neonatal onset severe atopy and autoimmune disease requiring bone marrow transplant for survival [22]. Similarly, infants with Dedicator of cytokinesis 8-deficiency (DOCK8), who also manifest in infancy with severe eczema, anaphylaxis to food allergens (in addition to their immuno-compromise) have impaired suppressive activity of circulating Treg cells [48].

During normal pregnancy there is an expansion of maternal peripheral blood Treg cell pool in both humans and mice [49, 50]. Studies of human newborn Treg function (typically via depletion studies or supplementation assays) generally support an association between reduced function at birth and allergic outcomes. For example, suppressor function of newborn Treg cells who developed egg allergy are reduced [51] and post-natal changes in

Treg numbers and/or immunosuppressive function are inversely related to allergy phenotypes in infancy [52]. Whereas the turnover and suppressor function of non-atopic infant's Treg cells appears to increase with age, there is a delay in this process in atopic infants [53]. Unlike APCs, where there seems to be a spectrum related to lifestyle westernization, this does not seem to be the case for Treg cells [54].

Lower frequency of circulating Treg cells at birth are linked to environmental exposures such as maternal smoking and maternal allergy and these low numbers are associated with increased risk of atopic sensitisation and the development of atopic dermatitis in early childhood [55, 56]. A farming lifestyle can modify Treg numbers favourably [57] and genetic polymorphisms, e.g. IL-10, can influence the transcription of Treg cell markers [58]. Polymorphisms in FOXP3 are reported to be associated with both increased susceptibility and protection from allergic disease in cohort studies[59-61], and asthma discordance in monozygotic twin studies is linked to epigenetic modification of the FOXP3 locus [62].

Changes in the number and/or function of circulating Treg cells might reflect developmental pressures on the thymus [53] with the underlying mechanism related to expression of microRNAs, global post-transcriptional regulators of gene and thereby protein expression, with miR-223 particularly implicated to date [63].

Other cell subsets

Recently there has been a growing appreciation of the contribution of other cell subsets to allergic disease susceptibility. These subsets include Th17 cells, T follicular helper (Tfh) cells, regulatory B (Breg) cells, and ILCs. Neonates make a negligible IL-17 response [64] and the

ability to make IL-17 increases dramatically prior to 3 months of age [65]. Various peri-natal exposures, such as farming, and genetic predisposition might control early Th17 maturation [66] but for at least the first 12 months of life the infant displays a propensity to develop Treg rather than Th17 cells [65]. Historically, IL-4 derived from Th2 cells has been linked to class switching by B cells for IgE production but the possibility that IL-4-producing T follicular helper (Tfh) cells support class switching and/or IgE affinity maturation has emerged [67]. However, very little is known about the activity of these cells in early life.

Similarly, little is known about B regulatory cells and ILCs during this critical window of immune programming. Cytokine production by B cells including IL-10, IL-35 and IL-17 can regulate immune responses, especially via IgG4 [68]. ILCs lack lineage markers but are a rich source of multiple cytokines and are involved in tissue homeostasis but might also shape the microenvironment during first antigen exposures. From animal models IL-5- and IL-13-producing ILC2 subset [69] has emerged as involved in priming type 2 adaptive immune responses to inhaled allergens [70, 71] but not to food antigens [72]. Evidence that rhinovirus infection in neonatal but not adult mice drives the production of IL-25 and expansion of ILC2s [73] further suggests these cells might have a critical role in the development of allergic asthma in particular. Group 2 ILCs have been demonstrated in umbilical cord blood and while there was a sex difference in their abundance, with newborn boys having more than newborn girls, there was no association with allergic outcomes in the small series of samples studied [74]. Cytokine outputs were not studied and it will be of interest to investigate potential functional differences in these cells and allergic disease outcomes.

Placental microclimate and immune programming

Not only does the placenta regulate the traffic of proteins, lipids, glucose, and other molecules from the maternal to the foetal circulation, it provides a rich milieu of immunoregulatory cytokines and growth factors to which the foetus is exposed. This provision continues post-natally via the breast milk. Cytokines such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) link environmental exposures of haematopoietic and non-haematopoietic cells to the maturation of allergy effector cells and may be as critical in early priming and establishment of skewed immune reactivity in early life. For example, TSLP, along with basophils, might have a role in early life sensitisation to food via the skin [75]. TSLP is found in breast milk [76] and IL-33 in the placenta [77], so levels of and response to endogenous levels of these critical cytokines in the peri-natal period might have potent immunomodulatory effects. Immunoregulatory differences are evident in the placenta of infants who develop allergic disease [78] and the passage of mediators from the maternal to the foetal circulation might differentially impact on haematopoietic, especially eosinophil/basophil, progenitor phenotype and function of the newborn [79].

While the intrauterine environment is relatively germ free and there is a known association between intrauterine infection and preterm birth, the normal healthy materno-fetal interface is by no means sterile. Traditional microbiological approaches used decades ago revealed microorganisms in 13% of placentas that could not be explained as contamination [80]. Fluorescence in situ hybridisation (FISH) has shown bacteria within the fetal membranes [81] and whole genome shotgun metagenomic sequencing has revealed a rich placental microbiome composed of non-pathogenic commensal microbiota [82].

Bifidobacterium and *Lactobacillus* DNA have been isolated from placenta irrespective of mode of delivery and in the absence of membrane rupture[83]. Thus maternal transmission of commensal bacteria appears to be a normal part of pregnancy [31, 84] and might modulate placental provision of the immunoregulatory mediators discussed above.

Exposure to inflammation in utero and early life

Patterns of microbial [85] and nutritional exposures (including breastfeeding) [86] in early life influence the dynamics and baseline measures of inflammation in later life[85]. Level of C-reactive protein (CRP) (a measure of inflammation) is a major predictor of NCDs and 'all cause' mortality in later life [87-89]. Even in childhood, higher CRP is associated with a greater risk of allergic sensitization, especially to foods [90]. Thus rising rates of obesity and the associated metabolic changes might be another factor in the recent increase in food allergy. Higher baseline CRP levels measurable in high-income countries [85], reflect the rising propensity for inflammation with 'modern' lifestyles, nutrition, and declining microbial diversity – the same risk factors as for allergy.

The maternal phenotype, including both obesity and allergy, is increasingly recognised to play a role in foetal programming. Rising rates of maternal obesity, a chronic inflammatory state, in pregnancy can influence foetal programming to contribute to the rising rates of inflammation and immune dysregulation in the next generation [91-94]. Similarly, maternal allergy is a stronger determinant (than paternal allergy) of allergic risk [95-97], and neonatal Th1 IFN γ responses [98] suggesting direct materno-foetal interactions *in utero*.

METABOLOMICS AND IMMUNE PROGRAMMING

The same hormones that regulate appetite, fat storage and metabolism also regulate immune function. Leptin is a major hormone involved in fat metabolism, and levels are 5 times higher in severely obese people compared with their lean counter-parts [99]. In animals, there is clear evidence that leptin mediates immune and metabolic adaptations that conserve energy in the face of food scarcity, whereas relative abundance of food enhances immune activation and inflammatory responses, an effect achieved by infusing leptin [100]. A member of the IL-6 family, leptin enhances both innate and adaptive immune responses [101] [102, 103] inducing proliferation and cytokine secretion by naive and memory T cells [104, 105]. These changes contribute to increased inflammation with excessive weight gain, and are consistent with higher leptin levels in both allergy [106] and autoimmunity [107, 108].

The intra-cellular pathways that link metabolism and immunity, and that might determine the phenotype of innate and adaptive effector cells in allergy and other diseases are under intensive investigation. Understanding these will likely shape the immune programming paradigm over the coming years. For example, pro-inflammatory effector cells such as M1 macrophages, neutrophils and Th17 cells support their effector functions by ramping up ATP generation through increased glycolytic flux [109]. Nuclear receptors, such as vitamin D receptor, peroxisome proliferator-associated receptor γ (PPAR γ) and retinoic acid receptor, link lipid metabolism to gene expression and program many aspects of innate and adaptive immune activity [110]. This includes retinoic acid-dependent development of FOXP3+

regulatory T cells within the gut [111] and inhibition of pro-inflammatory cytokine production by monocytes and macrophages via activation of PPAR γ [112] .

MICROBIOME AND IMMUNE PROGRAMMING

The mucosal immune system must coordinate and integrate environmental signals to determine immunologic or tolerogenic outcomes upon antigen exposure. This is arguably the dominant factor driving maturation of Th1 and regulatory immune responses in the postnatal period (FIGURE 3). Declining biodiversity globally has been postulated as a contributing factor to the increasing prevalence of allergic and other chronic inflammatory NCDs [113]. Observations of altered gut microbiota composition in infants who developed allergic disease spawned numerous studies of the use of probiotics and prebiotics to “restore” an evolutionary normal allergy protective gut microbiota and these have been of variable success. However, emerging evidence suggests that it is the immune system, especially within the gastrointestinal tract, that determines the composition of the local commensal flora. For example, FOXP3⁺ Treg cells can facilitate this through inflammation and regulation of IgA to control host-microbiota symbiosis [114]. Therefore, differences in microbiota that are reported to be associated with the development of allergic disease might reflect pre-existing differences in gut mucosal immune function programmed via environmental and genetic interactions during peri-natal development. The exogenous supply of various immunomodulatory molecules, e.g. soluble CD14 [115] , first via the amniotic fluid and then post-natally via the breast milk might be critical determinants of immune function and thereby the composition of the microbiota within the gastrointestinal tract. Despite emerging data on the role of maternal commensal flora composition during

pregnancy in shaping immune function of the offspring, we are far from understanding the complexity of this interplay in humans.

There is growing awareness of the interplay between nutrition and the microbiome, particularly the effects of specific nutrients such as vitamin D and soluble dietary fibre (oligosaccharides). In addition to their *direct* immunomodulatory effects these nutrients also appear to modulate the microbiome, and relative deficiencies are implicated in the rising rates of immune and inflammatory diseases in general [116, 117].

Alteration of the microbiome by dietary supplementation

Probiotics

The use of probiotic bacteria has been investigated more extensively than that of prebiotics. Collectively probiotic studies have shown protection from developing eczema, although the findings are not consistent (reviewed in [118] [30]). Probiotics, in human intervention trials to date, have been instigated in the final weeks of gestation, rather than in early pregnancy. Probiotics have been shown to significantly alter expression of innate TLR-related genes in the placenta and in the foetal gut [119], and influence cord blood serum cytokines[120]. Given the likely role of the maternal microbiome in pregnancy for both immune and metabolic homeostasis [121], and the knowledge that immature lymphoid follicles emerge in the foetal gastrointestinal tract from around 16 weeks of pregnancy [122] it is more logical to intervene much earlier in pregnancy [123, 124]. However the known association between inflammation and adverse pregnancy outcomes, suggests that the use of a

proinflammatory probiotic supplement during early pregnancy may also carry risks, which require further careful evaluation.

Prebiotics

The low-fibre, high-fat 'western' diet is associated with adverse changes in gut microbiome, altered gut barrier function [116], increased systemic endotoxin and low-grade TLR-mediated systemic inflammation with increased CRP, IL- β 1, TNF, and IL-6. So far limited studies of prebiotics in the postnatal period in man, have shown favourable effects on colonisation and eczema reduction either alone [125] or in combination with probiotics [126]. To our knowledge, the only RCT to use prebiotics in pregnancy was too small to definitively assess immune effects on the foetus or clinical outcomes, but did achieve favourable changes in maternal gut microbiota, [127]. Observational studies in human pregnancy show that high fibre diets are associated with reduced risk of preeclampsia and dyslipidaemia.

Animal models using dietary supplements high in fibre and probiotics provide clear evidence that gut microbiota modulate immune programming. Manipulation of the microbiome has been shown to modulate not only animal models of allergic disease [128] and autoimmunity, but also the rates of obesity, cardiovascular and metabolic effects (reviewed in [129, 130]). This has generated obvious interest in the role of 'prebiotic' oligosaccharides to promote favourable colonisation and reduce inflammation. In humans, prebiotic fibre selectively stimulates growth of beneficial gut microbiota, particularly bifidobacteria but

also lactobacilli, and short-chain fatty acid (SCFA) fermentation products that mediate direct anti-inflammatory effects[131]. This promotes intestinal integrity, and reduces systemic endotoxin and antigenic load in experimental models. SCFA (including acetate, and butyrate and propionate) have recognised epigenetic effects [132], playing a critical role in systemic metabolic function, and stimulating regulatory immune responses [116] with anti-inflammatory effects evident in distal tissues such as the lung [133]. In pregnant animal models, prebiotics alter colonization and metabolic homeostasis [134], and reduce eczema-like inflammation in offspring [135].

Microbiome and pathogen interactions

While declining microbial diversity appears to play a role in the changing predisposition to disease, specific pathogens may still have an etiological role by inducing tissue damage (including lung development), disrupting barrier function and altering local immune responses. In particular, viral respiratory infection, particularly with respiratory syncytial virus (RSV), is one of the strongest postnatal associations with allergic airways disease (reviewed in [46]). Wheezing lower respiratory infection in the first year of life is a powerful risk factor for subsequent asthma in both non-atopic and atopic children. It is plausible that either early life infections such as RSV may “cause” or promote an atopic asthma phenotype, or that immune dysregulation decreases capacity to effectively clear virus, thus making atopic children more symptomatic and susceptible to viral induced wheeze. There is evidence for both possibilities. For example, early life respiratory viral respiratory infections may retard lung growth and respiratory function, substantially increasing the risk of asthma, particularly against a background of inhalant sensitisation. Acute respiratory infection is

associated with a burst of Type 1 IFN production in infected airway tissue, resulting in local up-regulation of the FcεR1 complex on the surface of adjacent airway dendritic cells. In the presence of specific IgE, this results in markedly enhanced allergen processing and activation of Th2 memory cells. The resulting systemic IL-4/IL-13 signal is sensed by bone marrow precursors, promoting upregulation of FcεR1 on immature DC before they are released to traffic to lung and airways, thus perpetuating FcεR1 expression in the airways. As infection progresses a build-up of Type 1 IFN is important in terminating IL-4/IL-13-mediated upregulation of FcεR1 expression. Children with more severe asthma exacerbation after viral infection have reduced Type 1 IFN production, suggesting that an underlying deficiency in capacity to generate Type 1 IFN. This might increase risk of sustained Th2 responses and severe symptoms following infection, and of more chronic airways disease.

Alternatively, it has been shown that allergic sensitisation preceded onset of viral associated wheeze in the COAST study [136]. Moreover, reduced “intrinsic” interferon responses may inhibit virus-induced interferon responses via enhanced cross linking of FcεR1 on plasmacytoid dendritic cells [137]. Overall viral infection in the context of pre-existing sensitisation and impaired Type 1 IFN production is likely to play an important role in the pathogenesis of allergic airway disease. Similarly, other organisms, such as *Staphylococcus aureus* may play a role in the development of allergic skin disease in children with pre-existing defects in skin barrier and/or immune function.

On the other hand, some organisms previously considered only as pathogens might have a protective role against allergy and other immune-mediated diseases. The 'loss' of these organisms, might be indicated in the rising rates of these diseases. One such example is *H. pylori*. In traditional environments *H. pylori* colonisation is almost ubiquitous from around 4-6 months of age, and persists unless eradicated with antibiotic treatment. These bacteria are emerging as important initiators of immune regulation in the upper gastrointestinal tract [138], inhibiting autoimmune and allergic T cell clones and protecting against allergies, asthma, and inflammatory bowel diseases [139] particularly with early colonization [140-142]. The immunomodulatory effects of attenuated or killed *H. pylori* strains and their capacity to reduce and prevent the symptoms of allergic disease are currently under exploration.

MATERNAL AND INFANT MICRONUTRIENT EXPOSURE

There is significant interest in whether sufficiency-of or supplementation-with dietary micronutrients and their timings influence allergic disease outcomes. Potential detrimental effects of folate were first highlighted in a mouse model exploring the effects of maternal supplementation with a variety of methyl donors, including folate, on the development of lung disease in the offspring [143]. In humans, several observational studies have associated folic acid supplements in *late* pregnancy with an increased risk of childhood asthma [144] and eczema [145]. Again, exposure timing appears important, as a recent meta-analysis found no evidence of increased asthma risk with *early* peri-conceptual folic acid supplementation used to prevent neural tube defects (NTD) [146]. Increasing use of supplements in the pre-conception and pregnancy period can result in many women

consuming far in excess of the required recommended amounts, beyond the period of NTD risk [145]. The implications of this need to be further investigated, in light of potential adverse effects on allergy and asthma risk.

Animal models suggest a key role for Vitamin A in development of antibody responses and Treg cells, and human studies suggesting important effects on lung development [147] [148, 149]. *In-vitro* Vitamin D has relevant immunomodulatory effects [150] but there are many contradictory reports on allergy and asthma outcomes related to insufficiency of vitamin D. In general, observational cohort and cross-sectional studies which have reported an association between low maternal vitamin D and increased infant atopy (including eczema, wheeze and allergen sensitization) have been based on estimates of vitamin D by dietary questionnaires, whereas those studies that have measured maternal serum vitamin D levels in late pregnancy or in cord blood have generally not reported the same association [151]. A 2011 meta-analysis [152] (which included no randomized controlled trials) concluded there was weak evidence that high maternal dietary vitamin D and E intakes during pregnancy were protective for the development of wheezing outcomes. Two large RCTs are underway to investigate the potential for Vitamin D supplementation in pregnancy to influence atopic outcomes (VDAART and ABCvitD, NCT00920621 and NCT00856947). Small trials, examining prenatal or infant supplemental of vitamin D and A have either failed to find an affect [153, 154] or have reported increased [155] not decreased sensitization rates in supplemented infants.

Other nutritional exposures such as dietary n-3 PUFA have favourable effects on the developing immune system both *in utero* [156] and in the postnatal period [157], but achieving favourable n-3/n-6 PUFA balance earlier in development might have greater potential to reduce the burden and risk of allergic disease [158, 159]. Recent animal models have shown that the protective effects of n-3PUFA supplementation on food allergen sensitisation are dependent on Treg cells [160].

MATERNAL AND INFANT EXPOSURE TO ALLERGENS

Detailed examination of the literature concerning allergen avoidance, early introduction of allergens and complementary feeding and risk of allergic disease is largely beyond the scope of this review. However, emerging evidence suggests that maternal peanut, tree nut, milk and/or wheat consumption before and/or during pregnancy may decrease the risk of both food and non-food related allergic disease [161-163]. This might reflect the association of dietary diversity in early life with reduced allergic outcomes [164, 165]. Overall there is increasing evidence from observational cohort studies that delayed introduction of allergic foods (beyond 4-6 months) does not appear to reduce the risk of allergic disease (predominantly eczema and food allergy) in infants either with family history of allergic disease or from unselected cohorts. These studies however are all subject to some degree of reverse causation and influence from unknown confounders; as such results from randomised controlled trials examining this question in the UK, Australia, Europe and Japan are eagerly awaited. A 2012 Cochrane review [166] did not recommend avoidance of allergenic foods in the diet of pregnant women for the purpose of primary prevention of allergic disease.

MECHANISMS OF TOLERANCE IN ALLERGIC DISEASE

The key mechanisms of immune tolerance to potential allergens are extra-thymic. There is likely to be considerable overlap in mechanisms, and a degree of redundancy, as studies suggest that more than one mechanism is important in the regulation of allergy. For example murine studies of oral tolerance demonstrate that high dose enterally-applied antigen leads to deletion of the relevant T cell clone(s) whereas repeated low dose enterally-applied antigen leads to induction of Treg cells[167].

Perhaps the most common example of acquisition of “natural” tolerance to a pre-existing allergic disorder is the observed spontaneous resolution of food allergy in childhood. Reported rates of resolution of clinical IgE-mediated food allergy vary depending on type of food, age of cohort, and the population, but it is generally estimated to be around 65-80% for staple foods such as cow’s milk, wheat, soy and egg and 10-20% for peanut and tree nuts. Natural acquisition of tolerance in IgE-mediated food allergy is perhaps not surprisingly associated with reduction in allergen-specific IgE production, decreased basophil activation, increased allergen-specific IgG4, and induction of Treg cells [168] [169, 170]. Many of these parameters when measured at initial clinical assessment have been associated with an ability to predict likelihood of subsequent tolerance. Similarly, successful immunotherapy is frequently reported to be associated with induction of Treg cells [171] [172], regulatory CD27(+)CD35(+) dendritic cells [173] and increased allergen specific IgG4. Recent reports suggest that regulatory B cells might differentiate those with sensitisation alone compared with clinical allergy [174]. Thus, it is plausible that the same immunological

processes and key elements involved in induction of tolerance (either by nature or by man) may drive protection from allergic disease in early life.

TOWARDS A TOLERANT FUTURE

Just how to achieve more 'tolerogenic' conditions in early life to prevent allergic disease is a 'billion dollar' question, and is likely to have cost savings of this magnitude. Reducing risk factors for inflammation are the logical targets in prevention, including optimising microbial diversity, nutrition and micronutrient status, while reducing adverse pro-inflammatory exposures such as smoking and other pollutants, psychological and oxidative stress. Other lifestyle changes and altered infant rearing practices may be able to drive significant primary allergy prevention when there is sufficient high level evidence to endorse them. This gives a broader perspective to strategies such as probiotics, prebiotics, breastfeeding, omega-3 polyunsaturated fatty acids (n-3 PUFA) and other anti-inflammatory nutrients for allergy prevention. Lessons from natural and therapeutic induction of tolerance in established allergic disease highlight the importance of regulatory cells within the T cell, B cell, and possibly the dendritic cell, compartments. Manipulating the early life environment to optimise these regulatory pathways provides a promising and necessary avenue for primary prevention strategies. As many of the mechanisms of interest have broader biological impact including neurodevelopmental [175] and cardiovascular outcomes improved understanding of early life determinants of allergic disease will be of benefit for the wider NCDs epidemic.

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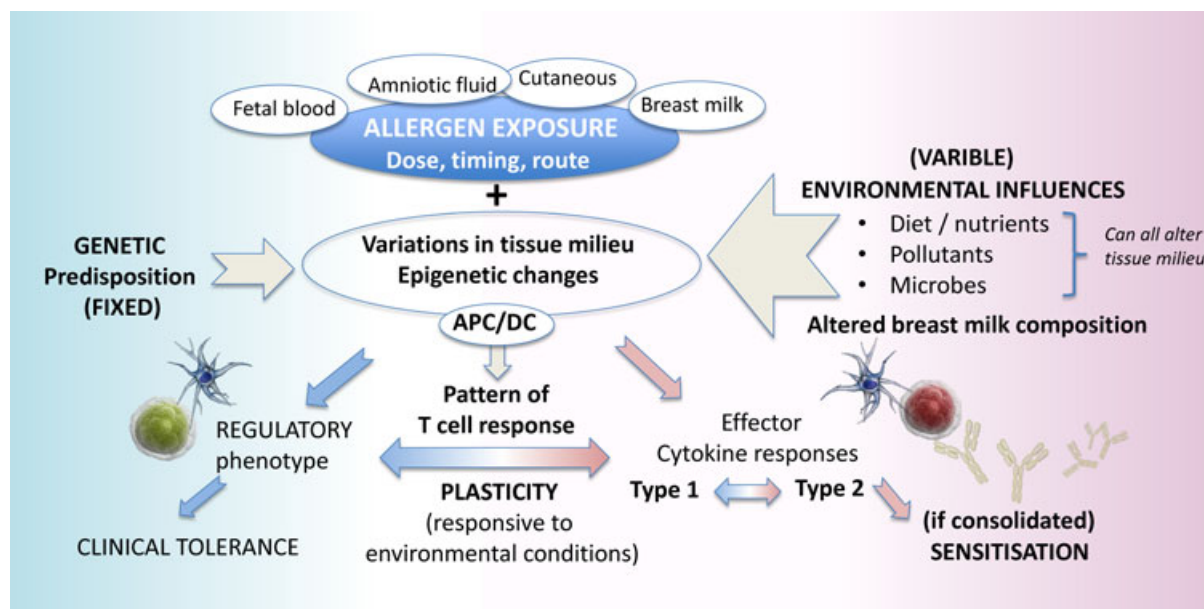
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- **Increased inflammatory** innate responses
- **Differences in effector T-cell function**
 - Immature Th1 function
 - Differences in T cell signalling
 - Epigenetic differences
- **Differences in regulatory T-cell function**

