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## 3rd International Immunonutrition Workshop

### Session 5: Early programming of the immune system and the role of nutrition Immunology of pregnancy

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The conceptual framework for reproductive immunology was put in place over 50 years ago when the survival of the fetal semi-allograft within an immunocompetent mother was first considered. During this time, a number of paradigms have emerged and the mechanisms receiving current attention are those related to immune tolerance, such as regulatory T-cells and indoleamine 2,3,-dioxygenase, and innate immunity, such as natural killer cells, trophoblast debris and inflammation. A key consideration is the temporal and spatial variation in any of these pathways (e.g. implantation *v.* parturition). As fetally derived trophoblasts are the semi-allogeneic cells with which the maternal immune system comes into contact, understanding the immune response to these cells is critical. There is much interest in the immunological pathways that support a healthy pregnancy and how they might be perturbed in adverse pregnancy outcomes. Additionally, there is increasing awareness that antenatal determinants of the immune function of pregnant women and their offspring have consequences for health and disease in childhood and beyond. Changes in maternal diet over recent decades coincide with the increasing prevalence of allergic and other immune-mediated diseases, and the modification of maternal diet has emerged as a strategy for disease prevention. Approaches undergoing trial at numerous sites around the world include dietary supplementation with fish oil and/or probiotics. Understanding the underlying mechanisms of any positive effect on disease outcomes should reveal further novel strategies for disease prevention.

#### Immunology: Pregnancy: Allergy: Diet: Fetal programming

Over 50 years ago, three strategies were suggested to explain the immunological survival of the fetus within the self/non-self paradigm: (1) anatomic separation of maternal and fetal tissues; (2) antigenic immaturity of the foetus; (3) lack of response by the maternal immune system (reviewed in Bilington<sup>(1)</sup>). Along with the possibility of the pregnant uterus as an immunoprivileged site, investigators in reproductive immunology have sought to identify the mechanisms that explain the ability of the immunocompetent mother to tolerate the semi-allogeneic foetus within this conceptual framework.

One of the key things to remember while studying the immunology of pregnancy is the variety of discrete compartments that must be considered: the embryonically derived placenta, the embryonically derived amnion and chorion that form the placental/fetal membranes, the maternally derived decidua (lining of uterus/endometrium

of pregnancy), amniotic fluid, the foetus itself and the systemic maternal immune function. Mammary gland development and breast milk could also come under this umbrella. It is also worth noting that temporal differences need to be considered, e.g. implantation *v.* parturition. Finally, the fetal cells that actually come into direct contact with maternal cells are the trophoblast of the placenta and fetal membranes giving two main sites of interaction to consider, that between the maternal decidua and trophoblast especially in early pregnancy, and that between syncytiotrophoblast on the surface of placental villi and cells in the maternal circulation<sup>(1,2)</sup>.

There are a few examples of impaired responses to infectious agents in pregnancy: pregnant women are more susceptible to respiratory viruses, especially influenza<sup>(3)</sup>; pregnant women are more likely than non-pregnant women to develop malaria and to have severe infection with adverse

**Abbreviations:** CTLA-4, cytotoxic T lymphocyte antigen-4; HLA, human leukocyte antigen; IDO, indoleamine 2,3,-dioxygenase; NK, natural killer; TGF, transforming growth factor; Th1, T helper 1; Th2, T helper 2.

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pregnancy outcomes including fetal and maternal death<sup>(4)</sup>. Despite this, the concept of immune suppression during pregnancy has been superseded by the current paradigm of active immunological tolerance of fetal cells by the pregnant woman. Consequently, an inadequate or perturbed tolerance response has been hypothesised to underlie adverse pregnancy outcomes such as preeclampsia<sup>(5)</sup>. A number of different immunological mechanisms have been postulated and the more prevalent of these are considered briefly herein.

### Non-classical MHC class I

Semi-allogeneic cells of embryonic origin (trophoblast) invade the maternal decidua to open up maternal uterine arteries and facilitate blood supply to the foetus. The interaction with decidual natural killer (NK) cells has a key role in this. One of the roles of NK cells is to destroy human leucocyte antigen (HLA) null cells and the expression of non-classical HLA by trophoblast is postulated to be a mechanism of evading NK cell killing. The non-classical MHC class I molecules HLA-E, -F and -G are less polymorphic than the classical HLA class I molecules and have different expression patterns: all three (as well as HLA-C) are expressed on the extravillous trophoblast that invades the maternal decidua, whereas the villous cytotrophoblast and syncytiotrophoblast found within the placenta express surface HLA-E and secrete a soluble form of HLA-G.

HLA-G is the most extensively studied non-classical MHC class I molecule and the role of HLA-G in modulating the maternal immune response to semi-allogeneic cells of fetal origin is well established<sup>(6,7)</sup>. Soluble and membrane forms of HLA-G are derived by the alternative splicing of HLA-G transcripts. HLA-G interacts with inhibitory receptors on leucocytes and mediates numerous effects: it modulates NK cell activity<sup>(6,7)</sup>; stimulates high levels of anti-inflammatory cytokines, such as transforming growth factor (TGF)- $\beta$ 1, from mononuclear phagocytes<sup>(8)</sup>; induces Fas/Fas ligand-mediated apoptosis of CD8+T and CD8+NK cells and inhibits antigen-specific cytotoxic activity of CD8+T-cells<sup>(9)</sup>. Since the immunomodulatory role of HLA-G in pregnancy was identified, there has been much interest in its potential as either a therapeutic or diagnostic tool in transplant immunology, autoimmunity and cancer<sup>(10)</sup>.

The study of HLA-E and HLA-F was hampered initially by the observation that the expression of these appeared to be restricted to inside the cell. Surface expression of HLA-E has now been found to be dependent on the presence of peptides from other HLA class I molecules. This complex of HLA-E with HLA class I molecule-derived peptides, especially from HLA-G and HLA-C, interacts with either activating or inhibiting CD94/NKG2 on NK cells to modulate NK cell activity. There is little knowledge about HLA-F and the peptides that it binds have not been identified<sup>(11)</sup>.

### Natural killer and natural killer T cells

NK cells have a role in the control of implantation and transformation of the uterine vasculature by trophoblasts,

on which blood supply to the foetoplacental unit depends<sup>(12)</sup>. There is an accumulation of NK cells in the maternal decidua in the first and second trimester with a reduction in the third trimester<sup>(13,14)</sup>. About 30% of cells in first trimester decidual tissue are leucocytes and up to 70% of these are CD56<sup>bright</sup>CD16-NK cells. This contrasts with the peripheral blood where CD56<sup>dim</sup>CD16+NK cells dominate<sup>(13)</sup>. The CD56<sup>bright</sup> population of NK cells accumulates at the implantation site and when activated these cells secrete an array of cytokines including macrophage inflammatory protein 1 $\alpha$ , granulocyte/macrophage-colony stimulating factor, TNF $\alpha$ , interferon- $\gamma$ , TGF- $\beta$  and leukaemia inhibitory factor, with minimal impact on cytotoxicity. CD56<sup>bright</sup> NK cells express high levels of CD94 and their accumulation at the implantation site has been postulated to be driven by HLA-E/G expression on extravillous cytotrophoblast that acts as a homing receptor. Alternatively, a cascade of cytokine production at the implantation site has been postulated to cause NK cell differentiation.

Interest in natural killer T (NKT) cells at the materno-fetal interface stems from their immunoregulatory properties. As their name suggests, NKT cells have the features of NK and T-cells: like T-cells they express a T-cell receptor, but this is invariant<sup>(15)</sup>; unlike T-cells they are not restricted by MHC class I or class II molecules, but recognise glycolipids in the context of the MHC-like molecule CD1d. Trophoblasts within first trimester decidua express CD1d and 0.48% of CD3+T-cells in the decidua have an NKT phenotype; this represents a 10-fold higher concentration compared to blood. Decidual CD4+NKT cells have a T helper 1 (Th1) bias and produce granulocyte/macrophage-colony stimulating factor, whereas those in the peripheral blood are T helper 2 (Th2)-biased<sup>(16)</sup>.

### T helper cytokine profiles

Since the observation that Th2 cytokines are normally produced at the materno-fetal interface, the hypothesis that established pregnancy (i.e. excluding implantation and labour) is associated with a Th2-biased immune response has dominated the reproductive immunology literature<sup>(17-19)</sup>. This is despite the lack of any really convincing data from human subjects and the observation that mice lacking expression of the main Th2 cytokines IL-4, IL-5, IL-9 and IL-13 (i.e. quadruple knockouts) do not have fertility problems (no differences in litter sizes and all offspring healthy) indicating that these cytokines are not essential for fetal survival even during allogeneic pregnancy<sup>(20)</sup>. It must be noted that progesterone-induced blocking factor induces a Th2 cytokine profile<sup>(21,22)</sup>. Progesterone-induced blocking factor is a feature of normal human pregnancy and can be measured in urine from pregnant women; the lack of progesterone-induced blocking factor predicts spontaneous pregnancy termination and neutralising endogenous progesterone-induced blocking factor leads to pregnancy termination in mice<sup>(23,24)</sup>.

At the outset of the study of Th1/Th2 balance in pregnancy the focus was T-cells themselves but the materno-fetal interface itself is relatively devoid of T-cells. Thus, the paradigm has evolved to encompass type 1 and type 2

cytokines irrespective of their cell source which can include NK cells<sup>(25–27)</sup> and the gestation-associated tissues themselves<sup>(28)</sup>. An appreciation of the temporal and spatial variation in the dominance of either type 1 or type 2 cytokines has also emerged. For example, NK cells and interferon- $\gamma$  are pivotal to remodelling the uterine vasculature during implantation<sup>(26,27)</sup>. Conversely, in a study using surface markers of Th1 (IL-18 receptor) and Th2 (ST2L) in maternal peripheral blood, NK cells rather than CD4+ and CD8+T cells were found to have a bias to a type 2 phenotype, a bias lost with preeclampsia<sup>(25)</sup>.

Overall, there is now an appreciation of the need for balanced type 1/type 2 responses depending on the tissue site and the stage of pregnancy. More recently the role of the innate immune response and inflammation has become of interest. Given that IL-4, IL-10 and IL-13 are anti-inflammatory cytokines<sup>(29)</sup>, they might serve to prevent an overactive inflammatory response; the type 1/type 2 paradigm should be re-evaluated from this perspective.

### Innate immunity

Innate immunity/inflammation is now considered to have a physiological role in implantation, the maintenance of pregnancy and parturition<sup>(26,27,30)</sup>. The innate immune response also contributes to adverse outcomes in fertility and pregnancy, including tubal infertility associated with *Chlamydia trachomatis*, infection-associated preterm delivery and preeclampsia<sup>(31,32)</sup>. There is extensive evidence of a heightened systemic inflammatory response in pregnant women<sup>(33)</sup>, for example, increased leucocyte count and increased levels of C-reactive protein and other acute phase proteins<sup>(34)</sup>. This might be balanced by increased circulating levels of anti-inflammatory molecules such as TGF- $\beta$ 1<sup>(35)</sup> and IL-4<sup>(36)</sup>.

The enhanced systemic inflammatory response during pregnancy has been postulated to be mediated by particulate debris or soluble products derived from the placenta, which enter the maternal circulation. Cell-derived microparticles occur commonly in the circulation, but the levels of these are increased with normal pregnancy being derived from numerous cell types, especially the syncytiotrophoblast. These placenta-derived microparticles are postulated to drive normal systemic inflammation in pregnant women and to be further increased in preeclampsia<sup>(2,37)</sup>. These microparticles might also have a role in down-modulated T-cell function during pregnancy. Syncytiotrophoblast microparticles can be found attached to circulating monocytes and unbound in the circulation from the second and increasing into the third trimester. When isolated *in vitro* from the placenta, they can induce an inflammatory response in non-pregnant women<sup>(31)</sup>. From animal studies, it has been shown that the presentation of alloantigens coincides with the detectable release of placental debris from around mid-gestation<sup>(38)</sup>.

There is much interest in identifying molecules and signalling pathways that might link the so-called type 2 bias of pregnancy with the innate immune function. Recently, it was shown that the immunoregulatory molecule T-cell Ig and mucin domain (Tim)-3 which has been suggested to

modulate the Th1/Th2 balance and have a role in the innate immune response is up-regulated on monocytes but not T- and B-cells in the peripheral blood of pregnant women<sup>(36)</sup>. Tim-3 was found to enhance innate immunity and Th1-associated anti-pathogen responses in pregnancy; blocking Tim-3 impaired phagocytosis, signalling via lipopolysaccharide, T-cell proliferation and interferon- $\gamma$  production. The IL-4 signalling pathway has a role in the up-regulation of Tim-3 expression on monocytes<sup>(36)</sup>. Abnormalities in Tim-3 expression were also observed in women suffering miscarriages at 12–20 weeks of pregnancy.

### Regulatory T-cells

Recent years have seen resurged interest in T-cell populations with immunoregulatory or suppressive properties. Initially identified by their ability to suppress autoimmune disease, regulatory T-cells also have been shown to modify a broad range of immunological activities associated with inflammatory and infectious diseases, cancer and transplantation<sup>(39)</sup>. Abnormalities in the activity of these cells have been implicated in susceptibility to many conditions with underlying immune aetiology. Several types of regulatory T-cells have been described and these can be subdivided broadly into natural and inducible regulatory T-cells. To date, only natural CD4+ regulatory T-cells that can be identified by the expression of CD25 and low expression of CD127 (IL-7 receptor  $\alpha$  chain)<sup>(40)</sup> have been studied in pregnancy. CD4+/CD25+/CD127<sup>lo</sup> regulatory T-cells express FoxP3 which has been described as the master regulator of development and function of this population<sup>(41)</sup>, and their functional properties have been attributed variously to CTLA-4 (cytotoxic T lymphocyte antigen-4), TGF- $\beta$  and IL-10, but this remains an area of ongoing investigation and controversy.

In mice and human subjects, CD4+CD25+T-cell numbers increase in early pregnancy and decline from mid-gestation to reach levels equivalent to the non-pregnant state by term<sup>(42–44)</sup>. As CD25 is also a marker of T-cell activation, it is imperative that other phenotypic or functional data be available to clarify that the CD4+CD25+T-cell population being studied is regulatory. CD4+CD25+T-cells able to suppress autoimmune and allogeneic responses are expanded in the spleen, blood and various lymph nodes of pregnant mice. The absence of these cells leads to immunological rejection of the foetus in allogeneic but not syngeneic pregnancies<sup>(43)</sup>. In human subjects, an increase in CD4+CD25+T-cells in peripheral blood in the first and second trimester was described, but without additional phenotypic/functional data it was unclear whether this represented increased numbers of regulatory or activated T-cells<sup>(44)</sup>. In a recent study, where a more definitive phenotyping strategy was used, it was found that CD4+CD25+CD127<sup>lo</sup>FoxP3+T-cells were reduced in the second trimester and that expression of FoxP3 itself was lower with pregnancy. The function of this population in pregnant women was comparable to that in non-pregnant women<sup>(45)</sup>.

CD4+CD25+T-cells are relatively abundant in first trimester decidua (about 20% of decidual CD4+T-cells v. about 8% of CD4+T-cells in peripheral blood of pregnant

women) and they respond poorly to T-cell stimulation and have suppressive activity that is cell-contact dependent<sup>(46)</sup>. The numbers of CD4+CD25+T-cells in both decidua and peripheral blood in early pregnancy are decreased in spontaneous abortion<sup>(46)</sup>. As in peripheral blood, there is a decrease in the relative abundance of these cells by term to about 14%. As many of these express CTLA-4, OX40 and glucocorticoid-induced TNF receptor-related protein, they are likely to have regulatory function<sup>(44)</sup>.

The expansion of CD4+CD25+T-regulatory cells occurs very early in pregnancy and declines in both the decidua and blood by term. Therefore, it has been suggested that the expansion in these cells is not driven by exposure to fetal alloantigen. This has led to interest in the impact of pregnancy-related hormones on these cells and both estradiol and progesterone have been implicated<sup>(45,47)</sup>. However, in another animal study, CD4+CD25+T-regulatory cell numbers were greater in allogeneic *v.* syngeneic pregnancies with the number of male offspring in the litter having a positive impact on numbers of these cells suggesting a role for fetal alloantigens<sup>(48)</sup>.

### Indoleamine 2,3,-dioxygenase

Indoleamine 2,3,-dioxygenase (IDO) is the first and rate-limiting enzyme in tryptophan degradation. IDO degrades the indole moiety of tryptophan, serotonin and melatonin, and initiates the production of neuroactive and immunoregulatory metabolites known as kynurenines. This leads to local depletion of tryptophan and increased kynurenines both of which affect T-cell proliferation and survival: tryptophan starvation inhibits T-cell activation, and the products of tryptophan catabolism regulate T-cell proliferation and survival<sup>(49–51)</sup>.

IDO has been implicated in the induction of immune tolerance during many immunologically mediated events, including pregnancy. The treatment of pregnant mice with a pharmacologic inhibitor of IDO-induced maternal T-cell-mediated rejection of allogeneic but not syngeneic fetuses<sup>(52)</sup>. Inhibition of IDO is also associated with T-cell-dependent, antibody-independent complement deposition and haemorrhagic necrosis at the materno-fetal interface in allogeneic but not syngeneic pregnancies<sup>(53)</sup>. The timing of fetal loss coincided with the up-regulated expression of IDO<sup>(52)</sup>. IDO can be detected in day 6 human blastocysts and then throughout pregnancy in syncytiotrophoblast, extravillous cytotrophoblast, placental macrophages and the fetal membranes. Down-regulated phytohaemagglutinin-stimulated proliferation of peripheral blood mononuclear cells by conditioned medium from first trimester placental and decidual cultures is IDO-dependent<sup>(54)</sup>. Much of the interest in IDO and pregnancy has focused on the capacity of placenta-/decidua-derived IDO to suppress maternal T-cell reactivity directed at paternal alloantigens. An alternative hypothesis has been put forward, focusing on the potential effects of the products of tryptophan degradation (i.e. kynurenines) that include the removal of oxygen radicals, opening of blood vessels and increased production of NAD<sup>(55)</sup>.

There is a link between IDO and regulatory T-cells<sup>(49)</sup>. CD4+CD25+T-cells induce tryptophan catabolism by

dendritic cells and a tolerogenic phenotype in a CTLA-4-dependent manner<sup>(56)</sup>. Similarly, expression of IDO by dendritic cells and macrophages in maternal decidua is up-regulated via CTLA-4, and this has been suggested to indicate that CTLA-4 expressing CD4+CD25+ regulatory T-cells that infiltrate the decidua in early pregnancy induce IDO expression by decidual dendritic cells/macrophages expressing the appropriate counterligand (B7 family members CD80 and CD86)<sup>(57)</sup>.

### Complement

Activation of complement is a strategy for destruction and elimination of pathogens, but can also damage host cells and tissues. The maternal circulation and the placenta contribute to a fully active complement system at the materno-fetal interface. The regulation of complement during pregnancy is critical in preventing maternal-mediated damage of the placenta and foetus. Deficiency in a negative regulator of complement activation (Crry) resulted in embryonic lethality, due to a failure to control complement. This was associated with complement deposition and inflammation, including an extensive polymorphonuclear granulocyte infiltrate in the foetoplacental unit<sup>(58)</sup>. There is no direct human counterpart of Crry, but other regulators of complement activation such as decay accelerating factor (CD55), CD59 and membrane co-factor protein (CD46), are expressed extensively in the human placenta. Syncytiotrophoblasts that form the outermost layer of the chorionic villous tree of the placenta and are in direct contact with maternal blood express all three regulatory proteins and these are known to be functional<sup>(59)</sup>. Currently, there is particular interest in additional roles for CD46 in reproduction because of its expression on the inner acrosomal membrane of spermatozoa and the observation that CD3/CD46 cross-linking on T-cells induces an IL-10 producing regulatory T-cell population<sup>(60,61)</sup>.

### Fetal origins of adult disease

Since Barker first hypothesised on the fetal origins of adult disease there has been a wealth of scientific literature published that relates to this. The essence of this hypothesis is that during fetal development, the organs and systems of the body go through periods when they are plastic and sensitive to the environment. This leads to physiological or morphological changes associated with health and disease in adulthood<sup>(62)</sup>. The original model was proposed for the group of diseases including CHD, stroke, high-blood pressure and type-2 diabetes. The general concept has now been extended to encompass the early life origins of other diseases, such as allergy, and now includes the development of disease in childhood.

Antenatal exposure to dietary components, cigarette smoke, microbial products and many others have been implicated to have a role in the development of the fetal immune system with knock-on effects for health in childhood and beyond. How these exposures impact on maternal systemic immune function during pregnancy, immune function at the materno-fetal interface and fetal immune development are relatively understudied. Recent years

have seen an interest in the effect of these exposures on epigenetic modification of chromatin structure enabling heritable gene regulation without changing the nucleotide sequence. This occurs particularly via the modification of histone tails that alter the interaction between histones and DNA. These modifications include acetylation, methylation, phosphorylation, poly-ADP ribosylation and ubiquitination<sup>(63)</sup>. For example, chronic maternal consumption of a high-fat diet in primates is associated with increased acetylation of candidate genes and altered gene-specific expression in fetal hepatic tissue<sup>(64)</sup>.

#### *Diet and the early life origins of allergic disease*

The concurrent decrease in the consumption of saturated fat and increase in that of polyunsaturated fat parallels changes in allergic disease prevalence. Similarly, the prevalence of obesity in western populations has increased concurrently with asthma and other allergic diseases. These observations have piqued interest in a link between dietary factors, obesity and allergic disease. Of particular interest is the maternal intake of various dietary components during pregnancy. Maternal Zn intake during pregnancy has been inversely associated with wheezing to 2 years of age<sup>(65)</sup>, asthma ever and current asthma at 5 years of age<sup>(66)</sup>. Low maternal vitamin E intake during pregnancy has been associated with increased proliferative responses by cord blood mononuclear cells<sup>(67)</sup>, increased likelihood of wheezing to 2 years of age<sup>(65,68)</sup> and an increased likelihood of asthma/wheezing symptoms during the first 5 years of life<sup>(66)</sup>. The child's own nutrient intake was not associated with any of the outcomes measured.

Folate supplementation before and during early pregnancy has been advised since the demonstration that folic acid supplementation during this time dramatically reduces the risk of neural-tube defect in newborns. More recently, folic acid supplementation during early pregnancy has been suggested to increase the risk of asthma, wheezing and respiratory disease<sup>(69)</sup>. The physiological effects observed in an animal model were associated with epigenetic effects, and post-natal supplementation of the pups' diets did not induce the same physiological effects<sup>(70)</sup>. An epidemiologic study in over 32 000 children found a small but significant increase in the relative risk for wheezing, lower respiratory tract infections and hospitalisation associated with such infections during the first 18 months of life with maternal intake of folic acid and cod liver oil<sup>(71)</sup>. In contrast, serum folate levels in children aged 2 years and older were inversely associated with high total IgE levels, atopy and wheeze<sup>(72)</sup>. This study did not address the issue of maternal supplementation and the observation is in keeping with that from the mouse study<sup>(70)</sup> that it is folate exposure during pregnancy and the associated epigenetic effects that might have a negative impact on allergic disease.

#### **Nutritional approaches to modulating immune function in pregnancy and the peri-natal period**

Nutritional supplementation during pregnancy offers the most acceptable way of potentially modulating the immune

function of the mother and her offspring and thereby preventing diseases with origins in fetal life. Studies of maternal supplementation with vitamin D, fish oil or probiotics dominate this field.

#### *Vitamin D*

Many women are deficient in vitamin D both before and during pregnancy. This has raised concerns for development of the child (e.g. optimal skeletal development) and has been linked to increasing prevalence of allergic diseases such as asthma<sup>(73)</sup> and type-1 diabetes<sup>(74)</sup>. The provision of adequate vitamin D to pregnant and breastfeeding women is a leading public health issue in many countries and randomised controlled trials of vitamin D supplementation are underway at numerous sites. This raises concerns that excessive vitamin D might have its own health consequences. Elevated maternal vitamin D in late pregnancy was not associated with the child's body size, intelligence, psychological or cardiovascular health at 9 months and 9 years of age<sup>(75)</sup>. Despite the epidemiological evidence linking reduced vitamin D with increasing prevalence of allergy, elevated maternal vitamin D in late pregnancy was associated with increased risk of eczema at 9 months and asthma at 9 years of age<sup>(75)</sup>. Thus, high-maternal exposures have little impact on the parameters of general health, but high levels might have a negative impact on allergic diseases. This observation is supported by studies in mice<sup>(76)</sup> and other studies of supplementation in infancy and increased risk of atopic disease in adulthood<sup>(77)</sup>. Larger studies are required as are studies to clarify the impact on immune function at birth of low and high levels of maternal vitamin D. A key consideration is the source of vitamin D in the pregnant woman's diet as maternal vitamin D intake from food is negatively related to the risk of asthma and allergic rhinitis at 5 years of age, but supplements alone were not associated with any outcome<sup>(78)</sup>.

#### *Fish oil*

Decreased *n-3:n-6* long chain PUFA in the diet have been suggested to contribute to increased atopic disease in westernised countries<sup>(79)</sup>. There are many published trials of *n-3* fatty acid supplementation in children and adults with mixed results. More recently, there has been a shift to supplementation during pregnancy with anticipated immunological effects evident at birth and a positive effect on the development of allergic disease in early infancy and beyond. Fish oil supplements during pregnancy have the desired effects on the *n-3:n-6* fatty acid profile of the newborn<sup>(80)</sup>, but the perceived health benefits of fish oil to the pregnant women themselves remain uncertain<sup>(81)</sup>. Maternal fish oil supplements do have functional consequences for the mother and the newborn<sup>(82,83)</sup>; however, in the newborn this might be a generalised down-regulation of immune function<sup>(83,84)</sup>. Supplementation during pregnancy also alters the immunomodulatory profile of breast milk with postulated benefits for the development of the infant's mucosal immune system<sup>(85)</sup>. Maternal supplementation with fish oil tends to have a beneficial effect on

the development of allergic disease by the child. However, the size of any effect might relate to the duration of supplementation during pregnancy, the dose of *n*-3 fatty acids used with higher doses having the most dramatic effects, and the atopic status of the mother<sup>(84,86,87)</sup>.

### Probiotics

Studies on peri-natal supplementation with probiotics stem from observations of altered gastrointestinal microflora associated with and preceding the development of allergic disease in infancy<sup>(88)</sup>. These disease prevention studies typically take the form of maternal supplementation in the last few weeks of pregnancy and then supplementation of the infant for a variable period of time once it is born, though studies with only maternal or infant supplementation have been reported. There are many groups around the world undertaking such studies and many of these are summarised in a recent review of this field<sup>(89)</sup>. The outcomes of these studies are variable and the longer term impact of peri-natal supplementation as an allergic disease prevention strategy remains to be determined.

While any effects of probiotics relate to the species used and the population studied, one intriguing aspect of these studies is that supplementation to the pregnant woman for the last few weeks of pregnancy might be the critical component<sup>(90)</sup>. This has led to studies investigating the impact of maternal supplementation with probiotics on immune parameters and gastrointestinal microbiota at birth and in early infancy. Maternal supplementation with *Lactobacillus rhamnosus* strain GG does not lead to colonisation of the infant with the probiotic strain, despite mothers being colonised at the delivery of the baby, but affects intestinal colonisation of the infants: infants of *L. rhamnosus* strain GG-exposed mothers were more colonised with species belonging to *Bifidobacterium longum* group strains, indicating early beneficial effects on the infant's gastrointestinal microflora<sup>(90)</sup>. Maternal supplementation with *L. rhamnosus* or *Bifidobacterium lactis* was associated with changes in some immunological mediators in the umbilical cord plasma (e.g. increased interferon- $\gamma$ ) and breast milk (increased TGF- $\beta$ 1, but not IL-6, IL-10 or TNF $\alpha$  levels in breast milk collected 3–7 d postpartum)<sup>(91)</sup>. The effects of maternal supplementation with probiotics on TGF- $\beta$  content of breast milk are variable: lower TGF- $\beta$ 2, but no difference in TGF- $\beta$ 1 in colostrum of women given *Lactobacillus reuteri*<sup>(92)</sup>; higher TGF- $\beta$ 2, but no significant difference in TGF- $\beta$ 1 in breast milk collected 3 months postpartum from women taking *L. rhamnosus*<sup>(93)</sup>. Again, strain-specific effects that might differ with the length of exposure to the probiotic of interest or time since the exposure was stopped need to be taken into consideration.

The mechanisms of any effect of maternal supplementation with probiotics on immune function and allergic disease risk in the newborn await elucidation. Of interest, supplementation of adults with *L. rhamnosus* strain GG affects the antigen-specific immune responses of those adults, but maternal supplementation does not influence the same antigen-specific immune responses by her newborn<sup>(94)</sup>. Therefore, understanding the underlying

mechanisms requires studies of pregnant women and their newborns rather than extrapolation from studies in healthy non-pregnant adults.

### Summary

A number of immunomodulatory pathways contribute to the success of pregnancy with attention currently focused on regulatory T-cells, IDO, NK cells and the innate immune system. The impact of antenatal variation in the immune function on the development of the fetal immune system and thereby health and disease in childhood and beyond is of increasing interest. Changes in the maternal diet over recent decades coincide with the increasing prevalence of allergic and other immune-mediated diseases<sup>(95,96)</sup>. The modification of maternal diet has therefore emerged as a strategy for disease prevention.

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