Editorial

Should more be done during pregnancy to reduce allergies in children?

Maria C Jenmalm*

"The allergy epidemic must be counteracted by research identifying successful preventive measures ... early-life events occurring during critical windows of immune vulnerability can have a long-term impact on immune development."

Allergic diseases have become a major public health problem in affluent societies. Asthma, the most common childhood chronic disease, has a major impact on both the physiological and psychological wellbeing of young children, as well as on socioeconomic costs due to hospital admittance, treatment costs and parental sick leave. The allergy epidemic must be counteracted by research identifying successful preventive measures. The timing of such interventions is critical, as early-life events occurring during critical windows of immune vulnerability can have a long-term impact on immune development [1]. Thus, factors influencing the early education and maturation of the immune system are especially important for subsequent allergy development [1]. The immunological interaction between the mother and her offspring during gestation is close [1,2]. Maternal atopy is a stronger risk for allergy development than paternal atopy [3], indicating that prenatal maternal immune-mediated signals can shape immune development in the offspring. An increasing body of evidence suggests that allergy-preventive measures may need to start during pregnancy.

Allergic diseases are characterized by inappropriate immune responses to innocuous foreign proteins known as allergens. Atopy is defined as a personal and/or familiar tendency to produce IgE antibodies to allergens (i.e., become sensitized). Atopic individuals show excessive Th2-like responses to allergens, including production of IgE-inducing IL-4 and IL-13, as well as eosinophilia-enhancing IL-5 and IL-9 [4]. During the early phase of the IgE-mediated allergic reaction, allergen crosslinking of IgE antibodies on mast cells triggers the release of inflammatory mediators [4]. Cytotoxic mediators from eosinophils are important in the late-phase reaction, causing chronic inflammation [4].

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*Division of Inflammation Medicine, Department of Clinical & Experimental Medicine/AIR pl 10, Faculty of Health Sciences, Linköping University, SE-581 85 Linköping, Sweden; Tel.: +46 10 103 41 01; Fax: +46 13 13 22 57; maria.jenmalm@liu.se

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Allergic diseases include atopic eczema, bronchial asthma, allergic rhinoconjunctivitis and immediate types of urticaria and food allergy. The so-called 'allergic march' typically begins with the development of IgE antibodies to food allergens, accompanied by symptoms of atopic eczema and food allergy. The IgE reactivity to food allergens usually declines after infancy and tolerance to those allergens develops. Later in childhood, inhalant allergen sensitization develops together with asthmatic symptoms, while the onset of allergic rhinoconjunctivitis is usually seen from an early school age [1].

Although allergic diseases have a hereditary component, changes in the genotype cannot explain the rapid increase in allergy prevalence. Thus, loss of protective factors or appearance of risk factors in the environment may contribute to the increasing prevalence of these diseases. A reduced microbial pressure, resulting in insufficient induction of T cells with regulatory and/or Th1-like properties to counteract allergy-inducing Th2 responses, may underlie the allergy epidemic [5–8]. Most studies investigating the underlying mechanisms have focused on postnatal microbial exposure [1,8].

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However, an increasing body of evidence from studies carried out both by our group and by others suggests that the maternal microbial environment during pregnancy can program the immune development of the child [1,8,9]. Thus, in experimental murine models, maternal treatment with microbial products such as the commensal *Acinetobacter lwoffii* during gestation attenuates allergic sensitization and airway inflammation in the offspring [10]. In addition, epidemiological studies indicate that maternal exposure to a farm environment during pregnancy protects against allergic sensitization and disease, whereas exposure during infancy alone has a weaker effect or no effect at all [8,11]. Continued enhanced postnatal microbial exposure may be required for optimal allergy protection [11]. Furthermore, in human allergy intervention studies, probiotic supplementation to the mother during pregnancy, as well as to her baby postnatally, may be important for preventive effects [1]. Thus, a preventive effect on atopic eczema has primarily been demonstrated in studies carried out both by our group and by others where probiotics were given both pre- and post-natally [1,9,12,13], whereas studies with either postnatal or prenatal supplementation only failed to prevent allergic disease [1,14]. Prenatal probiotic supplementation was not given until 36 weeks of gestation in any of the studies [1,9,12–14]. If prenatal microbial exposure is vital for the preventive effect, supplementation should perhaps be started from the second trimester of pregnancy, when circulating fetal T cells have developed [1].

Another potential allergy prevention strategy is perinatal ω-3 polyunsaturated fatty acid supplementation [15,16]. A typical modern western diet may contain a ten-times higher ratio of ω-3:ω-6 fatty acids than traditional diets a century ago, due to decreased fish consumption but also an increased intake of vegetable oils rich in ω-6 fatty acids [17]. Furthermore, modern industrialized animal husbandry is associated with a high ω-6 fatty acid content in, for example, meat and eggs [17]. The ω-6 polyunsaturated fatty acid arachidonic acid gives rise to eicosanoids including prostaglandin D_2, prostaglandin E_2 and leukotriene B_4, with significantly higher inflammatory potential than the ω-3-derived 3-series prostaglandins and 5-series leukotrienes [17]. Furthermore, a low ω-3 polyunsaturated fatty acid intake may be associated with decreased formation of ω-3-derived resolvins and protectins, which reduce and actively resolve inflammation in animal models, although their anti-inflammatory potential in humans is not yet well studied [17]. While postnatal supplementation with fish oil rich in ω-3 during infancy has failed to prevent allergy development, administration of fish oil to mothers during pregnancy and lactation has shown promising allergy-preventive effects in high-risk families [15,16]. Thus, findings from both probiotic and ω-3 supplementation studies support the concept that maternally mediated signals during perinatal critical windows of immune vulnerability may affect immune development in the offspring.

Interestingly, while the allergy-preventive effects of maternal fish oil supplementation during pregnancy and lactation seemed to be strongest in offspring of nonatopic mothers [15], the preventive effects in our study, in which *Lactobacillus reuteri* was administered to mothers from gestational week 36 and to infants during the first year of life, were strongest in the children of atopic mothers [9]. An enhanced effect of maternal and infant probiotic supplementation in the children of atopic

mothers could suggest that modulatory influences on perinatal immune-mediated signals are particularly important in the presence of maternal allergic inflammation [1]. The more pronounced preventive effect of ω-3 supplementation in nonatopic compared with atopic mothers on infant allergy development [15] and the Th1/Th2 balance [18] has been suggested to be due to a disturbed polyunsaturated fatty acid metabolism in atopic mothers. As the effects of probiotic and fish oil supplementation seem to vary by maternal atopic status, a combined perinatal supplementation strategy may be more successful. Furthermore, probiotic and ω-3 polyunsaturated fatty acid administration may act synergistically at the cellular level via immunoregulatory [1] and anti-inflammatory [17] mechanisms, respectively. We are now testing these possible synergistic effects in a new double-blind placebo-controlled randomized study, PROOM-3, which has been recruiting pregnant women since February 2012 (for details see [101]). Four hundred and eighty pregnant mothers from high-risk families will be included in the study at the 20th week of gestation. They will be randomized to four study groups. One group will receive placebo for ω-3 and *L. reuteri*, the second will receive ω-3 supplementation and placebo regarding *L. reuteri*, the third will receive *L. reuteri* and placebo regarding ω-3 and the fourth group will receive both ω-3 and *L. reuteri* supplementation. ω-3 capsules will be given to mothers during pregnancy and lactation, while *L. reuteri* oil drops will be given to the mothers during pregnancy and to the children during their first year of life. The children will then be followed to 2 years of age; the primary outcome being IgE-associated eczema, and secondary outcomes will include maternal and infant immunomodulation. Thus, in this study the effect of longer exposure to probiotics during pregnancy and postnatally on allergy prevention can be evaluated, as well as synergistic effects between probiotics and ω-3 supplementation. Furthermore, the allergy-preventive effect of perinatal ω-3 supplementation reported by Furuhjelm *et al.* [15] may be confirmed by PROOM-3, which is an independent study. Safety is, of course, of utmost concern in any study evaluating perinatal treatment. Probiotic and ω-3 supplementation studies during pregnancy, including studies on prevention of preterm delivery, have previously been well tolerated, with low risks of side effects [15,19,20]. Although beyond of the scope of this article, the allergy-preventive effects of prenatal supplementation with vitamin D, which has immunomodulatory properties, are also currently being investigated [16].

Environmental exposures during pregnancy may alter gene expression in the offspring via epigenetic mechanisms, heritable changes in gene expression occurring without alterations in the DNA sequences [21]; a kind of cellular memory. Epigenetic modifications determine the degree of DNA compaction and accessibility for gene transcription, thus resulting in changes in gene expression that are subsequently passed to somatic daughter cells during mitosis [21]. The main processes modulating DNA accessibility to establish epigenetic memory occur via post-translational histone modifications and methylation of DNA CpG dinucleotides [21]. DNA methylation, associated with transcriptional repression, is more rigid than histone modifications, with DNA methyltransferases conferring covalent methyl modifications upon evolutionary conserved regulatory gene elements; CpG islands [21]. The methylation pattern is thus preserved with high fidelity through cell divisions, assuring preservation of cellular inheritance [21].

Prenatal environmental exposures may alter gene expression via epigenetic mechanisms, aiming to induce physiological adaptations to the anticipated postnatal environment, but potentially also increasing offspring disease susceptibility. This 'developmental origins of health and disease' hypothesis was originally proposed by David Barker [22]. Although the importance of fetal programming has mostly been studied in cardiovascular and metabolic disease, this hypothesis is also very attractive in the context of environmentally influenced immune-mediated diseases. The maternal microbial environment during pregnancy may program the immune development of the child, via epigenetic mechanisms, regulating appropriate maturation of innate immunity [8,10] and T helper cell and Treg responses [5–7]. Th1, Th2 and Th17 differentiation is under epigenetic control and human Treg commitment requires demethylation of the *FOXP3* promoter [23].

The epigenetic regulation of childhood immune development by maternal microbial exposure is probably induced via changes in maternal immune regulation [10], as there is a

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close immunological interaction between the mother and her offspring during pregnancy [1,2]. The placenta allows a crosstalk between maternal stimuli, possibly induced via microbial stimulation of maternal Toll-like receptors, and fetal responses [10]. As fetal T cells have developed during the second trimester of gestation, maternal signals may then direct the immune cell lineage commitment of the offspring during a critical developmental period when the epigenetic program is highly susceptible to environmental influences [1]. During pregnancy, the fetal–maternal interface is characterized by high levels of Th2-like cytokines and enrichment of Tregs, diverting the maternal immune response away from damaging Th1-mediated immunity [1]. The association of cord blood IgE levels and neonatal IFN-γ production with maternal but not paternal atopic heredity may depend on an even stronger Th2 deviation in atopic compared with nonatopic pregnant women [1,3,24]. As the cytokine milieu shapes the T helper differentiation, particularly during naive compared with established responses [4], the neonatal immune system is Th2 skewed [1,25]. We have shown an even more marked neonatal Th2 skewing in infants later developing allergic disease [2,25], possibly due to prenatal epigenetic effects via maternal immune regulation that may be possible to redress by enhanced microbial exposure (e.g., via probiotic supplementation) during pregnancy. The Th2 bias of the newborn should then develop toward a more balanced immune phenotype, including maturation of Th1-like responses [6] and appropriate development of Treg responses [7]. In farm studies, contact with multiple animal species during pregnancy is positively correlated with Treg function and IFN-γ production at birth, and with innate immune receptor expression at birth and during childhood [7,8]. A failure of Th2 silencing during maturation of the immune system may underlie development of Th2-mediated allergic disease [25]. Appropriate microbial stimulation, both pre- and post-natally, may be required to avoid this pathophysiological process [11].

In this respect, the gut microbiota is quantitatively the most important source of microbial stimulation, possibly providing a primary signal for the maturation of a balanced postnatal innate and adaptive immune system [1,26,27]. Our immune system has probably evolved as much to manage and exploit beneficial microbes as to fend off pathogens [1]. The gut microbiota differs during the first months of life in children who later do or do not develop allergic disease [27,28], and the diversity of the microbiota may play an important role in regulating allergy [27] and mucosal immune development [26,29]. To what extent the maternal gut microbiota composition influences that of her offspring is not yet fully clear. Differences in microbiota composition depending on delivery mode do indicate a mother–child transmission of microbiota during vaginal delivery [1]. However, due to the vast complexity of the gut microbiota, more detailed basic microbial ecology studies, now made possible by advances in DNA sequencing technologies [27], in clinically and immunologically well-characterized children and their mothers, are needed. Also, the way in which the maternal gut microbiota impacts the development of the microbiota of the child, in addition to its effects on immune maturation during infancy, needs further investigation.

In conclusion, the maternal microbial and dietary environment during pregnancy may program the immune development of the child. Prenatal environmental exposures may alter gene expression via epigenetic mechanisms, aiming to induce physiological adaptations to the anticipated postnatal environment, but potentially also increasing disease susceptibility in the offspring. Efficacious preventive measures, required to combat the allergy epidemic, may be identified by determining how the immune interaction between mother and child is influenced by perinatal microbial and dietary factors.

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Website

101 Can Supplementation With Lactobacillus Reuteri and Omega-3 Fatty Acids During Pregnancy and Lactation Reduce the Risk of Allergic Disease in Infancy? (PROOM-3). http://clinicaltrials.gov/ct2/show/NCT01542970