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Antenatal Risk Factors, Cytokines and the Development of Atopic Disease in Early Childhood

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SUMMARY. Atopic diseases are complex entities influenced by an array of risk factors including genetic predisposition, environmental allergens, antenatal exposures, infections and psychosocial factors. One proposed mechanism by which these risk factors contribute to the development of atopic disease is through alterations in the production of T helper type 1 (Th1) and type 2 (Th2) cytokines. The objectives of this review are to discuss antenatal exposures that are associated with pediatric atopic diseases, to discuss the influence of the intrauterine environment on neonatal immune responses, to provide an overview of the Th1 and Th2 pathways and how they relate to atopic disease, and to summarize our current understanding of the association between cytokine responses in cord blood and the development of atopic disease in early childhood.

A. INTRODUCTION

With the recent rise in atopic disease prevalence, it would be beneficial for clinicians to become familiar with research advances made in the area of pediatric atopic disease pathogenesis. Atopic diseases -- asthma, allergic rhinitis, atopic dermatitis, and food allergy -- are complex entities with an array of risk factors that may be categorized into genetic predisposition, environmental allergens, antenatal exposures, infections, and psychosocial factors (Figure 1).

Genetic predisposition is central to the development of atopic disease as shown by the increased disease prevalence among first-degree relatives of affected individuals and those with a positive family history for atopic disease;[1-3] by monozygotic versus dizygotic twin studies;[4] and by the identification of numerous chromosomal linkages, single nucleotide polymorphisms, and haplotypes that are associated with an increased risk for atopic disease or biomarkers of atopy, such as serum IgE levels.[5-7] Atopic diseases are inherited as complex diseases involving the interplay of as many as 20 separate genes. Evidence of gene-environment interactions demonstrates that the environment has a modifying effect on the expression of certain genes in atopic disease.[8 9]

The environment and infectious diseases impact the development of atopic disease, and recently have received a great deal of attention in view of the recent upsurge in atopic disease prevalence. It is unclear whether infections alter actual disease risk; however, respiratory syncytial virus (RSV) and rhinovirus infections are associated with an increased likelihood of having subsequent wheezing and childhood asthma.[10 11] Supporters of the controversial "hygiene hypothesis" attribute the increased prevalence of atopic disease in the Western world to a relative decrease in infectious diseases associated with trends that include, but are not limited to, smaller family size, an increased emphasis on hygiene and the widespread use of antibiotics.[12 13] This theory is supported by evidence showing a decreased risk of atopic disease where there is an increased exposure of young children to microorganisms, including an increased exposure to endotoxin in the first several months of life,[14] daycare attendance in infancy,[15] living with older siblings,[12 15] living on

a farm,[16] and early pet exposure.[17 18] Additional studies, but not all,[19] have shown an association between antibiotic use in early life and an increased risk for asthma or atopy later in childhood.[20 21]

Pediatricians are generally familiar with genetic predisposition and many of the postnatal exposures associated with atopic disease. However, antenatal exposures associated with the development of atopic disease and recent advances in atopic disease pathogenesis may not be in the purview of the general pediatrician or practitioner. This review focuses on the role of the intrauterine environment and antenatal exposures in the development of atopic disease in early childhood. The objectives of this review are to discuss antenatal exposures that are associated with pediatric atopic diseases, to discuss the influence of the intrauterine environment on neonatal immune responses, to provide an overview of the T helper type 1 (Th1) and Th2 pathways and how they relate to atopic disease, and to summarize our current understanding of the association between cytokine responses in cord blood and the development of atopic disease in early childhood.

B. ANTENATAL EXPOSURES ASSOCIATED WITH PEDIATRIC ATOPIC DISEASE AND CORD BLOOD BIOLOGIC ASSAYS SHOWING EVIDENCE OF NEONATAL ANTIGEN-SPECIFIC IMMUNITY

The importance of the intrauterine environment in atopic disease pathogenesis is supported by data showing a greater influence of maternal over paternal atopy on disease risk in the offspring[1 2] and multiple antenatal risk factors for pediatric atopic disease.[2 22-24] A number of maternal health characteristics and behaviors during pregnancy are associated with pediatric atopic disease in the offspring. These include low maternal parity,[25] respiratory and genitourinary infections,[23 26] cigarette smoking,[22 27-29] and antibiotic use during pregnancy,[30] a proxy for maternal infection. At birth, risk factors for the atopic disease that may reflect intrauterine exposures include higher gestational age,[2] low birthweight and prematurity,[22] and delivery by cesarean section.[31-33]

Beyond the epidemiologic associations found between certain antenatal exposures and the subsequent development of atopic disease in offspring, there are biologic measures that show evidence of neonatal

T-cell and B-cell immune responses to antenatal exposures. Cord blood biologic assays among offspring demonstrate evidence of neonatal immune responses to antigens.[34 35] At birth, in vitro allergen-stimulated T cell or cord blood mononuclear cell (CBMC) proliferation occurs in response to a variety of environmental allergens, including beta-lactoglobulin, birch pollen, bovine serum albumin, cat fur, cockroach, house dust mite (HDM), mouse, ovalbumin and timothy grass pollen.[34 36-39] Some investigators believe that these responses indicate that neonatal immune responses, thought to be naïve, may in fact be influenced by antenatal exposures,[37] but others have challenged the specificity of such studies.[40] Antigen-specific B cell responses to maternal antigen exposures during pregnancy have also been described. Following administration of tetanus vaccine to pregnant women, investigators have detected antigen-specific immunoglobulin M (IgM) production in cord blood.[41] In addition, measurable total and allergen-specific IgE in cord blood provide evidence that isotype class switching occurs in response to in utero allergen exposure.[35]

There are a number of plausible mechanisms by which in utero sensitization may occur. One possibility is that the antigen or processed peptide to which the mother is exposed during pregnancy reaches the placenta where antigen presentation occurs. In support of this mechanism, the dust mite antigen, Dermatophagoides pternissinus 1 (Der p 1), has been detected in amniotic fluid and umbilical cord blood, introducing the possibility of transplacental passage of antigens.[42] In addition, antigen presenting cells (APCs) have been detected in the placenta and have been shown to facilitate antigen-induced T cell proliferative responses.[43]

C. OVERVIEW OF TH1 AND TH2 CYTOKINES AND HOW THEY RELATE TO ATOPIC DISEASE

In this section, we provide a simplified overview of Th1 and Th2 cytokines and how they relate to atopic disease. In an effort to better understand disease pathogenesis and to identify biologic measures to predict atopic disease and potential therapeutic modalities to treat disease, recently much attention has been focused on Th1 and Th2 cytokine production and their counter-regulatory actions. A general understanding of Th1

and Th2 cytokines is necessary to understand how cord blood cytokines relate to pediatric atopic disease development.

Th1 and Th2 lymphocytes, thought to be the differentiated progeny of a population of naïve lymphocytes, are defined by the cytokines that they produce. Table 1 describes major Th1 and Th2 cytokines. To a large extent, Th1 and Th2 pathways -- influenced by cytokines, other immunologic cells, and transcription factors -- are thought to be counter-regulatory (see Figure 2). The Th1 pathway is essential for cell-mediated immunity and occurs in response to some bacterial infections. The Th2 pathway, essential for all humoral immunity, is thought to play a major role in atopic disease (see Figure 3), with multiple studies showing an association between the atopic phenotype and elevations of Th2 cytokines in the sera and the bronchoalveolar lavages of affected individuals.[44-46]

The Th2 dominance seen in individuals affected by atopic disease has led many researchers to believe that environmental and infectious exposures, including antenatal exposures, may modulate disease risk through alterations in the balance between the Th1 and Th2 cytokine pathways. A number of exposures associated with an enhanced Th2 response and a reduced Th1 response are also associated with atopic disease. For example, maternal cigarette smoking during pregnancy, a known risk factor for childhood asthma, is associated with increased levels of the Th2 cytokine, interleukin (IL)-13, and decreased levels of the Th1 cytokine, interferon (IFN)-γ, in cord blood.[47] Respiratory syncytial virus (RSV) infection, a risk factor for wheezing as noted above is also associated with a Th2 response.[48] Similarly, in some studies, early antibiotic use in infancy is associated with an increased risk for atopic disease,[20 21] and it is also associated with a Th2-dominant response in mice.[49] On the other hand, exposures associated with an enhanced Th1 response are associated with a reduced risk of atopic disease. Specifically, Th1-inducing infections, such as *Mycobacterium tuberculosis* and hepatitis A virus, are associated with a reduced risk of atopic disease among affected individuals.[50 51]

The relationship between Th-1/Th-2 inducing exposures and atopic disease is not always straightforward, and there are studies that question these associations. While one study showed that Th1-

inducing immunization with bacillus Calmette-Guérin (BCG) was associated with a reduced risk of atopic disease, another study showed that it was not.[50 52] Similarly, though Shaheen et al showed that individuals with a history of Th-1 inducing measles infection had a lower likelihood of atopy than those without measles,[53] a later study showed that measles infection was associated with a higher risk of atopic disease.[54] Other studies show that Th1 and Th2 pathways are not always counter-regulatory. For instance, in mice, Hansen et al showed that the production of the Th1 cytokine, IFN- , was insufficient to counteract the effects of IL-4 and IL-5.[55] Instead of attenuating Th2 cell-induced airway hyperreactivity and inflammation, Th1 cells actually caused severe airway inflammation.

The intriguing associations between some Th-1 inducing exposures and a reduced risk of atopic disease, Th-2 inducing exposures and an increased risk of atopic disease, and the counter-regulatory actions of the Th1 and Th2 pathways for many years have provided an immunologic basis for the hygiene hypothesis. In addition, the Th1/Th2 paradigm has prompted a great deal of research that has broadened our understanding of atopic disease pathogenesis. Though the relationship between cytokine responses and atopic disease is complex and not fully understood, we know that cytokines play an important role in atopic disease.

D. CURRENT UNDERSTANDING OF THE ASSOCIATION BETWEEN CYTOKINE RESPONSES IN CORD BLOOD AND THE SUBSEQUENT DEVELOPMENT OF ATOPIC DISEASE

In the search for predictive biologic markers for atopic disease, investigators recently have explored the relationship between cord blood and peripheral blood mononuclear cell (CB and PBMC) cytokine responses to mitogen and antigen stimulation and the subsequent development of atopic disease. Table 2 provides a sample of studies from major research groups around the world that have substantially contributed to this body of work. The most consistent finding from these and related studies has been that individuals who develop atopic disease and those who have a positive family history of atopy even in the absence of disease have lower levels of the Th1 cytokine, interferon-γ (IFN-γ), at birth when compared with their unaffected counterparts.[3 36 39 56 57] IFN-γ levels produced in response to allergen-stimulated CBMCs have been shown

to be inversely related to cord blood IgE levels,[56] a marker with high specificity but low sensitivity for atopic disease.

Low levels of IFN-γ may represent impaired Th1 pathway function, immature development of the Th1 pathway, early destruction of Th1 cells[58] and/or dominance of Th2 immune responses. Some investigators have speculated that the relative lack of Th1 cytokine expression in newborns at risk for atopic disease may be due to APC immaturity and an inability to release IL-12, the major induction cytokine for the Th1 pathway. The initially low levels of IFN- γ seem to extend beyond the neonatal period as children who develop atopy also lack the normal Th1 response to BCG vaccination in infancy.[50] Also, children at increased risk for atopic sensitization have an attenuated production of the Th1 cytokine, IFN- γ, in early infancy.[59]

Levels of Th2 cytokines produced in response to antigen-stimulation of CBMCs among children who develop atopic disease, however, are less consistent than the low levels of IFN-γ that have been found. Among individuals who are at high risk for or develop atopic disease, investigators have shown a Th2 dominant response as shown by elevated levels of IL-5,[60 61] while others have shown lower levels of Th2 cytokines, such as IL-13.[62]

Some reports suggest that cytokine responses change during early childhood, and that such changes differ for non-atopic individuals when compared with atopic individuals. For non-atopic individuals, studies have shown a decline in the Th2 cytokine IL-4 and an increase in production of the Th1 cytokine IFN-γ in the first 2 years in response to house dust mite (HDM) antigen-stimulated mononuclear cells.[63] At the same time, children who develop atopic disease show an up-regulation of Th2 cytokines, including IL-5,[62] IL-9, and IL-13 in response to allergen or mitogen stimulation of PBMCs in the first 2 years of life,[64] but the exact timing of this Th2 skewing is unknown.

There is some evidence that the majority of tested newborns, independent of their risk for atopy, have Th2-skewed responses to common environmental allergens, with cord blood elevations of cytokines,

including IL-4, -5, and –13.[64] This Th2 skewing is thought to reflect the Th1/Th2 state of the mother as women toward the end of pregnancy are believed to be Th2 dominant as a means of maintaining the pregnancy and protecting the fetus against the toxic effects of Th1 cytokines, such as IFN-γ.[65] Lending strength to this idea, some recent studies have shown a correlation between maternal and neonatal cytokine profiles.[66 67] It is thought by some that non-atopic individuals shift from being Th2 skewed at birth to being more Th1/Th2 balanced, and that atopic individuals instead show an up-regulation for Th2 and continue to be Th2-skewed thereafter. Whether the ultimate cytokine profiles of an individual are influenced by antenatal or post-natal exposures, or a combination of the two is unknown.

This review would be incomplete without mentioning some of the limitations to the current literature on cord blood cytokines and their relationship to pediatric atopic disease. Research in this area is limited by the operational definition of disease with some investigators comparing cytokine profiles of children with and without atopy, with the latter defined by a positive family history, positive skin prick testing (SPT) or radioallergosorbent testing (RAST), or some combination of these, rather than actual symptomatic, clinical disease. There are also inherent limitations regarding the techniques used for measuring cytokines. For example, certain cytokine levels, including IL-4 and IL-9, and at times IFN- , are often below detectable limits in cord blood by standard enzyme-linked immunosorbent assay (ELISA) methodology and have, therefore, been based on semi-quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) testing for specific messenger ribonucleic acid (mRNA). CBMCs and PBMCs may not represent the immunologic cells found in the airways or lungs, and cytokines responses from these cells may not reflect those in the target tissues.

E. CONCLUSIONS

Rapid progress has been made in our understanding of how neonatal cytokine production relates to the subsequent development of atopic disease; however, the exact determinants of neonatal cytokine profiles are not well-understood. Studies that relate antenatal sociodemographic and health conditions with cord blood

cytokine profiles are just beginning. Increasingly we have begun to recognize not only the importance of antenatal exposures on the subsequent risk for atopic diseases, but also postulate the mechanisms by which such relationships occur. In particular, cytokine profiles in cord blood appear to alter in response to antenatal exposures, and may predict later development of allergic disease. Though children with atopic disease seem to demonstrate a Th2 dominance once they are diagnosed with allergic disease, it is not clear when this Th2 dominance develops but it is believed to develop in early childhood. Despite limitations that affect the overall generalizability of work in this area, an increasing prevalence of atopic disease, along with growing evidence that antenatal factors contribute to disease development, calls for further research in the area of neonatal cytokines and their impact on the development of childhood atopic disease.

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