The Pros and Cons of Airway Lining Fluid Composition Analysis

Allergy and asthma are common conditions with genetic and environmental contributions (1). The prevalence of allergic diseases and asthma has increased during the last few decades (2). This increase in atopic diseases may be associated with prenatal and early-life exposure to triggering factors or, conversely, lack of exposure to protective factors (2, 3). Heredity from mothers or fathers may impact development of complex diseases differently. In this context, maternal atopy is more strongly associated with a child's risk to develop atopic disease than is paternal atopy (4–6), indicating a maternal effect on the child's immunological status.

The airway mucosa of the infant is exposed to the mother's milieu before birth and to allergens, pollutants, and microorganisms after birth. In their article in this issue of the *Journal* (pp. 275–280), Følsgaard and colleagues hypothesized that maternal and paternal atopy have a differential effect on cytokines and chemokines in the upper airway mucosa of healthy newborns (7). They studied 309 healthy subjects at 1 month of age. Subjects were recruited in Sjælland (Zealand), Denmark, and enrolled in the new Copenhagen Prospective Study on Asthma in Childhood (COPSAC₂₀₁₀) cohort. Using a recently developed method (8), fluid lining mucosa of the upper airway was collected with strips of filter paper inserted into the nasal cavity. The filter had “wicking” properties, that is, efficient absorption of fluid with minimal adsorption of proteins to the paper. Thus, fluid proteins could be eluted from the paper into a defined volume, allowing the concentrations of 18 chemokines and cytokines to be estimated using multipot assays.

Results were analyzed in relation to parental atopy, defined as a history of physician-diagnosed asthma, hay fever, and/or atopic dermatitis, without an objective measurement such as IgE level or skin test for allergen-specific IgE (which is a limitation of the study). Principal component analysis indicated that in newborns with atopic mothers (with or without atopic fathers) as compared with nonatopic mothers (with or without atopic fathers), there was global down-regulation of the cytokines and chemokines. Many individual mediators (e.g., eotaxin-1, eotaxin-3, interferon γ , interleukin [IL]-1 β , IL-13, monocyte chemoattractant protein-1, monocyte chemoattractant protein-4, and tumor necrosis factor- α) were decreased when subjected to *t* test. In contrast, newborns with atopic fathers (with or without atopic mothers) were similar to those with nonatopic fathers (with or without atopic mothers). Further and importantly, there was significant down-regulation in those with atopic mothers and nonatopic fathers compared with those with atopic fathers and nonatopic mothers. Overall, the data indicate that maternal, but not paternal, atopy is associated with mediator down-regulation in the upper airway mucosa of the infant. The authors interpret this finding as representing delayed maturation of the immune response that may put the infant at higher risk to develop atopy. Further follow-up would be needed to establish if there is indeed a link between down-

regulated mediators in the upper airway mucosa lining fluid at 1 month and atopy later in life.

The minimally invasive sampling method would seem to have many advantages. In addition to minimizing discomfort, it avoids allergen challenge and nasal lavage, the confounding effect of the variable dilution of analytes during lavage, and the possibility that dilution may result in mediator concentrations lower than the detection limit of the assay (9, 10). Another strength is that the sample being studied is from a target organ of allergic disease rather than assaying cytokines and chemokines in stimulated cord or peripheral blood, as has been done previously in newborns (11–13). Further, use of principal component analysis is a strength. From variables or measurements that possibly are related, such analysis (14) extracts a few underlying, latent, uncorrelated variables, called principal components, which describe the systematic part of the variation. Principal component (PC) 1 describes the greatest part of data variation, PC2 the next greatest, and so on. In their data, the authors found that PC1 described 66% of the total variation and PC2 only a further 8%, and that there were significant differences in PC1 but not in PC2 among groups. The analyses indicated that all mediator concentrations are correlated and that the general down-regulation in newborns with atopic mothers mainly reflects one underlying inhibiting tendency.

In conclusion, the authors have made the novel observation that there is general down-regulation of cytokines and chemokines in the upper airway mucosa lining fluid in infants with symptomatic atopic mothers, likely mainly due to one underlying factor. The mechanism behind this maternal effect on the child's immunological status during pregnancy or first month of life remains unresolved. Possible explanations or hypotheses include exposure to allergens *in utero* (15), transfer of some factors or signals from the mother to the fetus, and/or a situation in which atopic mothers have different environmental or microbial exposures than nonatopic mothers during pregnancy or in the child's infancy. Allergic mothers may also avoid certain foods and have a different diet or nutrition than nonallergic mothers. Future studies seem warranted to address whether any of these possibilities explains the observed effect and to explore the significance of the effect.

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Birth Cohorts in Childhood Asthma: Lessons and Limitations

The role of rhinovirus infection as a major trigger of asthma exacerbations in the preteens was recognized over 15 years ago, when molecular diagnostic methods were first used to study children with asthma in a community setting (1). A viral precipitant was identified in 80% of asthma exacerbations in this age group, and rhinovirus was the most common culprit. The Childhood Origins of Asthma (COAST) study, an important birth cohort inaugurated over a decade ago, has investigated the role of rhinovirus at an earlier age. Results from this cohort, published in 2008, suggested that rhinovirus might also have a role in the etiology of asthma in young children at high risk of asthma and allergic disease (2). More than 90% of children with wheezing episodes associated with rhinovirus infection at the age of 3 years in this cohort would go on to have protocol-defined asthma at age 6 years. The risk of later asthma with rhinovirus wheeze was greater than that seen with respiratory syncytial virus (RSV). Furthermore, rhinovirus wheezing episodes appeared to be a more important risk factor than allergic sensitization.

A further report from the COAST study, in this issue of the *Journal* (pp. 281–285), seeks to establish which comes first—wheezing episodes due to rhinovirus or allergic sensitization (3). If allergic sensitization leads to rhinovirus wheeze, which in turn predicts asthma, in high-risk infants, then primary prevention of allergic sensitization might prevent asthma. Of course, the converse (rhinovirus wheeze leads to allergic sensitization) might also be true. Jackson and colleagues report data on 285 children at high risk of asthma and allergic disease, due to parental asthma or allergy. Sensitization status was assessed annually using allergen-specific IgE, and each wheezing episode was investigated for a viral precipitant by nasal lavage and polymerase chain reaction. The investigators conclude that sensitization precedes wheeze and therefore that sensitization may have a causal role in the development of viral wheeze. Once again, the roles of rhinovirus and RSV were compared. Sensitization led to an increased risk of rhinovirus-induced wheeze but not wheeze associated with RSV.

So what has been the outcome of previous interventions to prevent allergic sensitization and wheeze in high-risk infants? Although some trials of allergen reduction strategies have shown significantly reduced allergic sensitization (4, 5), paradoxically, increased sensitization has also been shown (6). A systematic review of multifaceted interventions in infancy has shown a significant reduction in parent-reported wheezing in children aged 7–8 years (7). Multifaceted interventions may include advice that is appropriate for all infants, such as exclusive breast feeding for the first 6 months of life and avoiding passive smoke exposure. However, other aspects of these interventions are intrusive and expensive, for example acaricides, occlusive mattresses, use of exclusion diets for breast-feeding mothers, and disposing of pets. Unfortunately, less complex interventions (such as occlusive bedding alone) do not seem to be effective in the primary prevention of wheezing illness (7).

What do these findings mean for the clinician? The findings of epidemiological research in young children with wheezing illness have proven difficult to apply to clinical practice. The definitions used in birth cohort studies are often unhelpful to the clinician. For example, the landmark Tucson cohort used the term “transient early wheeze” to describe children who had wheezing at 3 years but in whom these symptoms had resolved by 6 years (8). It is incorrect to assume that transient early wheeze is equivalent to clinical definitions such as “episodic viral wheeze” proposed by the recent European Respiratory Society taskforce (preschool children who wheeze intermittently but who are well between episodes) (9). Yet these clinical definitions are more helpful in predicting the likely response (or nonresponse) to treatment. Unfortunately, there are few effective treatments for episodic viral wheeze. Recent studies have demonstrated that, in contrast to asthma, oral steroid treatment is ineffective for acute episodes of episodic viral wheeze (10). Preventative treatment with montelukast gives modest protection against wheezing episodes (11), and inhaled steroids offer none (12).

Similarly, the children with rhinovirus wheezing described in the current report from the COAST study are not the children