

Immunopathology of Allergy and Asthma in Childhood

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WHAT DO WE KNOW?

Episodic wheeze or cough, with nonasthmatic conditions ruled out, has in the past been the most commonly used criterion for defining childhood asthma with skin prick testing or specific IgE analysis used to determine whether it is associated with an IgE-mediated allergy. Despite obvious limitations, it has become evident that the pathophysiology of atopic asthma in children is similar to that in adults. Initial observations of the tissue remodeling that occurs in asthma were made by examining lung biopsies from children who had mild asthma and children who had died of asthma (1). Subsequent studies have revealed that the pathology of such tissue is reminiscent of that in the asthmatic adult and is characterized by narrowing of the airways, loss of epithelium, and cellular infiltration dominated by eosinophils and mast cells.

Examination of bronchoalveolar lavage (BAL) fluid collected from children revealed that levels of soluble intercellular adhesion molecule 1 (sICAM-1) are higher in children with asthma compared with children with other airway diseases such as cystic fibrosis (2). Epithelial cell numbers are also significantly increased in subjects with asthma versus control subjects (3) and in infant wheezers (no delineation between asthmatic and nonasthmatic children was made) versus control subjects (4), while eosinophil numbers are increased in BAL fluid from children with asthma versus infant wheezers (viral associated) and nonwheezing control subjects (3, 5). In one of these studies a nonbronchoscopic procedure was used to collect BAL fluid from children undergoing surgery under a general anesthetic (5). Further studies from this group showed that eosinophil cationic protein (ECP) levels were significantly higher in BAL fluid collected during asymptomatic periods from subjects with atopic asthma than in normal control subjects (6). This suggests that in the airway eosinophils in children with atopic asthma are activated even in the absence of ongoing clinical symptoms. In contrast, histamine (a mast cell-derived product) levels were raised in both subjects with atopic asthma and individuals with virus-associated wheeze versus control subjects.

Analysis of biopsy samples obtained before the onset of clinical symptoms and/or diagnosis of asthma revealed that children who later developed asthma (2–5 yr after the biopsy was taken) had considerably higher numbers of eosinophils in the lamina propria compared with children who did not develop asthma. The children who developed asthma also had significantly greater thickening in subbasement membrane collagen (7).

Observations such as those described above indicate that the airways of the child with allergic asthma undergo pathological changes similar to those of the adult with allergic asthma. One of the important issues is whether these inflammatory changes precede the onset of clinical symptoms. As in-

flammation is present both during and in the absence of symptoms, it is probable that airway inflammation predates the first manifestation of clinical symptoms. Understanding the development of both the respiratory tract and the immune system before the onset of inflammation will yield insight into the initiation and progression of airway disease.

It has become evident that the initiation of asthma-associated airway pathology arises as a complex interaction between genetics and the environment, with the latter currently most amenable to manipulation for controlling disease development. The best example of an environmental influence is the association between maternal smoking and respiratory function in infancy. Infants exposed to passive cigarette smoke during the first year of life have more lower respiratory tract infections (8), and infants whose mothers smoked during pregnancy have reduced lung function in the neonatal period (9). The effects of maternal cigarette smoking on the respiratory tissue of the infant have been identified. Examination of respiratory tissue from infants of smoking mothers who died from sudden infant death syndrome (SIDS) demonstrated increased inner airway wall thickness (which would exaggerate airway narrowing) compared with infants of nonsmoking mothers who also died of SIDS (10).

Examination of the consequences of passive smoking for the infant indicates that the most important time for environmental influences may be during fetal life and the first year after birth. Potential early life influences on subsequent disease development have been identified. A number of epidemiological studies suggest that enhanced fetal growth may be related to an increased risk of asthma and atopy in childhood (11). However, systematic studies investigating the underlying mechanisms have yet to be undertaken. Two areas of particular interest in this context are lung growth and maturation of the immune system.

Airway smooth muscle completely coats the branching epithelial tubules early on in lung development (53 d of gestation) and by this time there is an abundant neural plexus ensheathing the smooth muscle (12). Formation of new alveoli proceeds at a rapid rate during fetal development and the first 2 yr of life. The immune system is also developing at this time, so inappropriate development of either the respiratory tract or immune system during fetal and/or early postnatal life may be associated with abnormal function and disease.

It is becoming clear that compromised immune function(s) in early life is associated with an increased propensity for the development of atopy, which, in turn, is a major risk factor for asthma. In particular, it has been known for several years that infants (13) and neonates (14–16) at high risk of atopy have a reduced capacity to produce the helper T cell (Th) type 1 cytokine interferon γ (IFN- γ) compared with their low-risk counterparts. Furthermore, it has been suggested that this may compromise their ability to develop normal patterns of Th1-like immunity against inhaled allergens (17). There is also evidence that T cells from high-risk subjects may also produce reduced levels of some Th2 cytokines (13, 18). However, prospective studies indicate that the development of atopic symptoms by the age of 2 yr is associated with progressive up-

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regulation of Th2-like immunity to inhalants, particularly in high-risk subjects, whereas those who remained symptom free developed a more Th1-like response pattern (18). Earlier studies also indicate that infants at high risk of atopy have additional defects in serum opsonization (19) and in secretory IgA production (20), suggesting a generalized low-level deficiency in immune function.

WHAT DO WE NEED TO KNOW?

Predicting Which Children Will Develop Asthma

Is it possible to predict which children will develop atopy and/or asthma as early as during pregnancy? Family history will provide some indication of a child's potential risk but it is not completely predictive. A number of groups are looking at placental function and components of the amniotic fluid with respect to subsequent development of allergic disease, including asthma. However, it will be many years before such studies yield any useful methods for predicting at-risk children.

Umbilical cord blood may also offer a useful sample source for analysis. It has already been demonstrated that mononuclear cells in cord blood from children at risk of allergic disease (i.e., with a family history), and especially those who develop allergic disease, exhibit reduced IFN- γ production in response to mitogens or antigens (14–16). In fact, this response extends to a range of cytokines, including interleukin 13 (IL-13) and IL-6 (16). Long-term follow-up of many studies may determine if there is an association between immunological function at birth and allergic asthma. However, in those studies identifying an association between reduced cytokine production and allergic disease during the first few years of life, there is always some overlap between the group with allergic disease and the allergy-free group. Thus, cord blood measurements may prove useful only in predicting disease outcome if other disease markers are investigated. However, we need to determine if the assessment of antigen-specific reactivity at birth provides us with information about the antigen that will trigger subsequent disease.

Of great interest is the development of techniques for directly assessing respiratory function in infants. This presents the investigator with a number of technical problems associated with sedation and subject cooperation. Further problems are created by the lack of standard equipment and reference data. Despite this, numerous groups are developing techniques to enable direct assessment of respiratory function by a variety of commonly used procedures (21–23). Will simultaneous assessment of immune function and respiratory function in early life have more predictive power?

An alternative to directly measuring respiratory function is to identify markers of airway inflammation that are accessible via noninvasive techniques. In this context, expired air may offer a useful sample source. The most likely candidate to be measured in expired air is nitric oxide (NO) (24). It is also of interest to note that there is a direct correlation between resting levels of exhaled NO in children with atopy and the number of inhalant allergens to which they respond with a positive skin prick test (SPT) (25). The collection of exhaled air is a relatively simple technique for children as young as 5 yr of age, but it becomes more difficult with younger children, although studies measuring NO in preterm infants have been published (26).

Measurement of markers of inflammation in peripheral blood currently offers the best noninvasive means of making the required assessments. Currently, of the markers most commonly measured in subjects with active asthma and during disease provocation, those indicative of eosinophil activation are

found most frequently. In children airway function correlates with circulating markers of eosinophil activation (27); however, longitudinal studies remain to be completed.

Can the Progression from Childhood Allergy and/or Early Wheeze to Persistent Asthma Be Predicted?

As noted above, early sensitization to inhalant allergens is a strong risk factor for subsequent development of atopy during childhood. However, population-based studies suggest that although up to 40% of young children manifest SPT reactivity to one or more inhalant allergens, only about one-quarter of these individuals develop asthma that persists into adolescence and/or adulthood (28). Current indications are that inflammation-driven airway remodeling comparable to that observed in adults with asthma is also a hallmark of asthma development in childhood (7). As with adults this is associated with the presence of activated circulating CD4⁺ T cells producing Th2 cytokines (29–32), and it has been suggested that the subset of SPT-positive children who develop persistent asthma are those with the most intense and prolonged Th2 responses (33).

Why this should be restricted to a small subset of SPT-positive subjects remains to be determined, but there may be several reasons for this. First, excessive production of NO within the airway mucosa may be a contributing factor: NO inhibits T cell activation via reversible dephosphorylation of intracellular signaling kinases (34) but in doing so “selects” for Th2 cells via differential inhibition of Th1 cells (35). Differences in airway macrophage function, at least in animal models, have been shown to profoundly influence the intensity of local T cell responses after aerosol challenge (36), and appropriate BAL studies of children, in which the focus is on alveolar macrophages, may provide useful new information in this context. There is also increasing evidence that “directed” migration of T cells to specific tissues via expression of tissue-specific homing molecules (addressins) may be an important factor in the pathogenesis of many inflammatory diseases. A notable example of this is expression of the skin-selective addressin cutaneous lymphocyte-associated antigen, in relation to effector T cells in atopic dermatitis (37). More recently attention has been drawn to the potential role of the mucosal addressin $\alpha 4\beta 7$ which directs T cells to the respiratory and gastrointestinal tracts (38).

An additional possibility is that covert variations in the balance between different classes of Th2 cytokines may be important determinants of pathogenicity, a potential example being the relative contribution of the antiinflammatory cytokine IL-10 to individual T cell responses. IL-10 production is reportedly deficient in adults with atopic asthma (39), and the intensity of SPT reactions in atopic 6 yr olds is inversely related to the magnitude of their allergen-specific IL-10 responses (40). There is also increasing interest in the potential contribution of Th1 cytokines, particularly IFN- γ , to the development of chronic allergic diseases in humans (41), and this has been intensified by findings implicating Th1 cells in the pathogenesis of asthma in the murine model (36). In this context it is pertinent to note that peripheral blood mononuclear cells (PBMCs) from a significant proportion of SPT-positive 6-yr-old children coproduce high levels of IFN- γ and Th2 cytokines in response to allergen stimulation *in vitro* (40).

Thus the key questions to be addressed are as follows:

- What determines who develops an allergy to inhalant antigens?
- How early is inflammation of the airways occurring?
- Are there measurable inflammatory markers in young children that can be used to predict those who will go on to develop asthma?

- What is the relative contribution of infection and allergy to airway inflammation at different ages?
- Can exhaled air be obtained routinely from young infants, and are the levels of NO in such samples useful in identifying those children who already have inflammation of the airways before the onset of clinical symptoms? Is measurement of H₂O₂ useful in this respect?
- Why is allergic asthma restricted to a subset of SPT-positive children? Can methods be developed to predict which atopic children will eventually develop persistent atopic asthma?
- How early do eosinophils and CD4⁺ cells exhibit the phenotype associated with allergic asthma?
- Can the immune response be manipulated to prevent progression to persistent disease in children identified as at risk?

HOW CAN WE ACHIEVE THIS?

1. To address the difficulties associated with obtaining biopsy, induced sputum, or BAL samples from the airways of children, a unified effort is required by the groups that archive such material. Thus a multicenter approach is required. This also necessitates that a common method be used for the collection, processing, storage, and analysis of samples. Also, uniform clinical methods must be used to assess disease development in children.

2. Prospective studies should be conducted to study inflammatory markers/functions likely to contribute to the initiation and/or progression of disease. Ideally, these studies should commence at birth (earlier if possible) and monitor the child to the age of 5 yr or beyond. These studies need to be clinically driven but require a multidisciplinary input so that, for example, respiratory function, immunological responses, and biochemical markers are assessed in parallel in each child.

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