Viral Infections, Cytokine Dysregulation and the Origins of Childhood Asthma and Allergic Diseases

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Background: The origins of asthma and allergic disease begin in early life for many individuals. It is vital to understand the factors and/or events leading to their development.

Methods: The Childhood Origins of Asthma project evaluated children at high risk for asthma to study the relationships among viral infections, environmental factors, immune dysregulation, genetic factors, and the development of atopic diseases. Consequently wheezing illnesses, viral respiratory pathogen identification, and in vitro cytokine response profiles were comprehensively evaluated from birth to 3 years of age, and associations of the observed phenotypes with genetic polymorphisms were investigated.

Results: For the entire cohort, cytokine responses did not develop according to a strict T helper cell 1 or T helper cell 2 polarization pattern during infancy. Increased cord blood mononuclear cell phytohemagglutin-induced interferon-γ responses of mononuclear cells were associated with decreased numbers of moderate to severe viral infections during infancy, especially among subjects with the greatest exposure to other children. In support of the hygiene hypothesis, an increased frequency of viral infections in infancy resulted in increased mitogen-induced interferon-γ responses at 1 year of age. First year wheezing illnesses caused by respiratory viral infection were the strongest predictor of subsequent third year wheezing. Also, genotypic variation interacting with environmental factors, including day care, was associated with clinical and immunologic phenotypes that may precede the development of asthma.

Conclusions: Associations between clinical wheezing, viral identification, specific cytokine responses and genetic variation provide insight into the immunopathogenesis of childhood asthma and allergic diseases.

Key Words: asthma, cytokines, rhinovirus, wheezing

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For many individuals, allergic and/or asthmatic symptoms begin in the first few years of life. Multiple factors are likely responsible for the development of these phenotypes. It is vital to understand the genetic (eg, immune dysregulation) and environmental (eg, respiratory tract infections) factors that interact during a critical time in the development of both the immune system and the lung regarding their ability to significantly influence the inception of allergic airway diseases in the first decade of life. This review will highlight recent findings in a high risk birth cohort designed to prospectively evaluate the contribution of both genetic and environmental factors on the inception of childhood allergic airway diseases, with a focus on early childhood viral respiratory tract illnesses.

FACTORS THAT INFLUENCE THE INCEPTION OF ASTHMA

Virus-specific, host-specific and genetic factors may be responsible for the development of asthma in childhood. Virus-specific factors include the ability to induce virus-specific IgE antibody[1–3] and the type of cytokine response to various viral peptides[4] or specific viruses.[5] Host-specific factors that seem to be important in the transient wheezing phenotype include lung function and exposure to maternal smoking, but not maternal asthma.[6] In the persistent wheezing phenotype, risk factors include exposure to passive smoke, a maternal history of asthma and an elevated serum IgE level in the first year of life and at age 6.[7,8] Genetic factors include the presence of atopy in the host and genetic polymorphisms that may influence asthmatic development.[9–11]

At present, however, the relative importance of these factors is uncertain due to a paucity of prospective longitudinal analyses. It appears that interactions between the viral,

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environmental and host factors are multidirectional and dynamic in that the atopic state can influence the lower airway response to viral infections,[7,12] viral infections can influence the development of allergy,[13–15] and interactions can occur when individuals are exposed simultaneously to both allergens and viruses.[13,16–18] Acute viral infections are associated with acute exacerbations of asthma both in children and adults[19] and can induce short- and long-term alterations in airway physiology, including increasing airway responsiveness[20] and creating abnormalities in airflow,[21] lung volumes[22,23] and gas exchange.[24]

Prospective studies have shown that respiratory syncytial virus (RSV) infections are a significant independent risk factor for subsequent frequent wheezing within the first decade of life.[25] RSV infections severe enough to require hospitalization may confer even higher longer-term risk for both asthma and allergic sensitization.[13] The mechanisms underlying these associations are unclear. However, taking into account that virtually all children have been infected with this virus by age 2 years, it can be assumed that host-dependent factors must be contributing significantly to these outcomes.[26] Some factors that have been evaluated include the immune response (both innate and adaptive) to RSV and host-related differences (gender, lung size, passive smoke exposure)[7] that may predispose an infant or child to lower airway physiologic alterations as a consequence of the infection. Finally the severity of the lower airway injury (ie, the development of bronchiolitis) may also influence the emergence of both asthma and allergic sensitization.[13]

DYSREGULATION OF CYTOKINE RESPONSES IN CHILDREN WITH ALLERGIES AND/OR ASTHMA

Cytokine dysregulation [T helper cell (Th) 1/Th2 imbalance] plays an important role in the development of asthma.[27] Cytokine profiling of cord blood indicates that at birth, largely as a result of placentally derived Th2 trophic factors, the newborn infant's mononuclear cell response is skewed toward a Th2-like phenotype [production of interleukin (IL) 4, IL-5 and IL-13] and away from Th1 [diminished interferon (IFN)-γ production].[28] The relative nature of this Th1/Th2 imbalance may be a predictor of the subsequent development of allergic disease and/or asthma.[28–31]

Children at increased risk of developing allergic diseases and/or asthma have lower cord blood mononuclear cell generation of IFN-γ[31,32] and IL-13.[33] These children's capacity to generate normal IFN-γ responses lags behind a nonatopic control population as well.[28,34,35] This suggests a critical period in the early life of the atopic child in which target organs such as the lung may be particularly vulnerable to environmental stress factors such as viral respiratory tract infections. Mechanisms that have been considered to contribute to these observations include both a diminished production of, and response to, IL-12,[36,37] a posttranslational defect in IFN-γ secretion,[38] and genetic polymorphisms of genes coding for both IFN regulatory factor-1 (a transcription factor that also functions as a determinant of Th1 responses)[39] and IFN-γ.[9] Interestingly children at high risk of developing allergies and/or asthma based on parental histories may have Th1 cytokine response profiles that synergize with Th2 cytokines in driving the expression of atopic disease in childhood.[40]

The hygiene hypothesis proposes that the immune system of the newborn infant may be significantly influenced by the type, amount and timing of various environmental exposures and has been extensively reviewed elsewhere.[41] This may affect immunoinflammatory responses and the expression of various allergic diseases, including asthma.[42,43] In part, the increase in atopic disease such as asthma may be due to a skewing of Th2 cytokine responses from a lack of environmental exposure that would normally cause a shift toward Th1 responses.

Environmental factors known to enhance Th1 responses and associated epidemiologically with a reduced incidence of allergy and/or asthma include the following: infection with tuberculosis,[44] measles[45] and hepatitis A[46,47]; increased exposure to infections as a result of a greater number of older siblings or day-care attendance early in life[42,48]; a reduction in IFN-γ production as a result of decreased environmental endotoxin exposure; and/or a diminished response based on genetic polymorphisms for the major endotoxin receptor, CD-14.[49] Restoration of Th1/Th2 balance may also be impeded by frequent oral antibiotic administration with concomitant alterations in gastrointestinal flora.[43] Immune imprinting may actually begin in utero, with maternal influences possibly related to transplacental transfer of allergens and/or cytokines potentially contributing to Th1/Th2 cytokine balance.[50] While these observations have generated intense interest in the intrauterine/neonatal, genetic and environmental influences, conflicting results have prevented any firm conclusions at the present time.[47,51]

Cytokines that appear to be especially relevant include IL-4 and IL-13, which play critical roles in IgE antibody isotype switching and can augment fibroblast proliferation.[52] IL-13 also affects signaling pathways in airway smooth muscle[53] and in murine models causes inflammation, mucus hypersecretion, subepithelial fibrosis and airway hyperresponsiveness.[54–56] Also, IFN-γ has the potential of causing damage to airway epithelium[57] and suppressing IL-4–induced airway eosinophilia.[58] Although IFN-γ limits IL-13–induced goblet cell hyperplasia and eosinophilic inflammation, it increases the number of cells expressing high levels of antigen-presenting molecules, natural killer cells and IL-6 in the airways. Thus, the simultaneous presence of IFN-γ and IL-13 may prepare the lungs for increased responses to inhaled antigens.[59] Thus the exogenous administration of IFN-γ during viral infection significantly attenuates the development of chronic airway inflammation and physiologic dysfunction.[60] These latter observations led to the development and implementation of the Childhood Origins of ASThma (COAST) project.

THE COAST PROJECT

The COAST project began in 1998 to evaluate the relationships among viral infections (and other environmental factors), cytokine dysregulation (both from an immunologic and genetic perspective) and the development of allergic

diseases and/or asthma in a high risk birth cohort based on parental histories of asthma and/or allergies. A 2-hit hypothesis was proposed initially, stating that the presence of both cytokine dysregulation and experiencing viral infections at critical developmental periods would be required for the inception of childhood asthma. The methods used to ascertain these relationships have been previously described.[61–64]

Briefly 289 children were recruited at birth if at least 1 of their parents was atopic (at least 1 positive aeroallergen skin test) or had been diagnosed by a physician with asthma. Wheezing illnesses were thoroughly documented and nasal secretions for viral respiratory pathogen identification were examined both by culture and polymerase chain reaction.[65,66] Also, in vitro cytokine response profiles (IFN-γ, IL-5, IL-13, IL-10) to a variety of mitogens and antigens were evaluated annually, beginning at birth.[63]

Etiology, Frequency and Severity of Respiratory Tract Illnesses in the First 3 Years of Life. A total of 1668 nasopharyngeal wash specimens was obtained during infancy.[66] The likelihood of viral identification was related to the severity of illness, as measured by the symptom score (Fig. 1, $P < 0.0001$). Viral identification occurred in 78% of the severe (score ≥10), 70% of the moderate (score 5–9) and 63% of the mild illnesses (score 1–4) (Fig. 1). Virus was recovered from 66% of the wheezing illnesses. Viral recovery from scheduled well visits (score 0) was 31% based on culture results of all well samples and reverse transcriptase-polymerase chain reaction for rhinovirus (RV) performed on randomly selected culture-negative well samples.

During infancy, a variety of respiratory viruses were recovered from children with each illness severity (Fig. 1), RV being the most common. Of the 566 moderate to severe illnesses, only 7 (1%) required hospitalization. The viruses identified during these illnesses included RSV (n = 3), RV (n = 1), RV + echovirus (n = 1), influenza A (H3N2) (n = 1) and RSV + influenza A (H1N1) (n = 1). There were 99 moderate to severe RSV illnesses, 51 (51.5%) of which were wheezing respiratory illnesses. There were 258 moderate to severe RV illnesses, 60 (23.3%) of which were wheezing respiratory illnesses.[66]

Developmental Cytokine Response Patterns and the Expression of Biologic and Clinical Markers of Atopy. Cytokine response profiles were initially evaluated from a developmental perspective in the first year of life to examine relationships among immunologic maturation and the expression of biologic and clinical markers of atopy. Phytohemagglutin (PHA)-stimulated cytokine response profiles at birth and at 1 year of age were compared with blood eosinophil counts and total and allergen-specific IgE levels (dust mites, cat, egg, *Alternaria* species, peanut, milk, dog) at 1 year of age and at the development of atopic dermatitis and food allergy. For the cohort as a whole, cytokine responses did not evolve according to a strict Th1 or Th2 polarization pattern. PHA-stimulated cord blood cells secreted low levels of IL-5

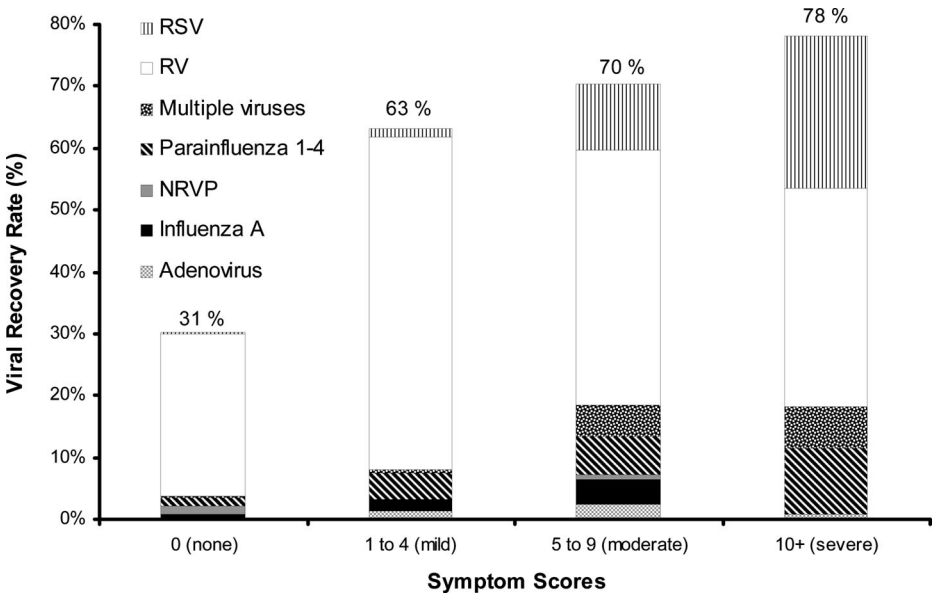

FIGURE 1. Likelihood of viral identification increases with more severe illnesses. Nasopharyngeal mucus samples were collected at scheduled clinic visits (2, 4, 6, 9, 12 months of age) or during acute respiratory illnesses. Symptoms were scored based on the following scoring system: fever (≥100°F) = 1 point; cough: mild = 1 point, moderate = 2 points, severe = 3 points; rhinorrhea: mild (suction 0–4 times/d or wipe every 2 hours or less) = 1 point, moderate to severe (suction ≥5 times/d or wipe ≥1 time/h) = 2 points; hoarseness = 1 point; duration of illness >4 days = 1 point; apnea = 3 points; wheezing = 5 points; retractions = 5 points; tachypnea = 5 points; cyanosis = 5 points. If the symptom score was ≥5, signifying a moderate to severe upper and/or lower respiratory infection, a sick visit was scheduled and nasal lavage was performed and processed as described. NRVP indicates nonrhinovirus picornavirus. Reprinted with permission from *J Allergy Clin, Immunol.* 2005;116:571–577.[66]

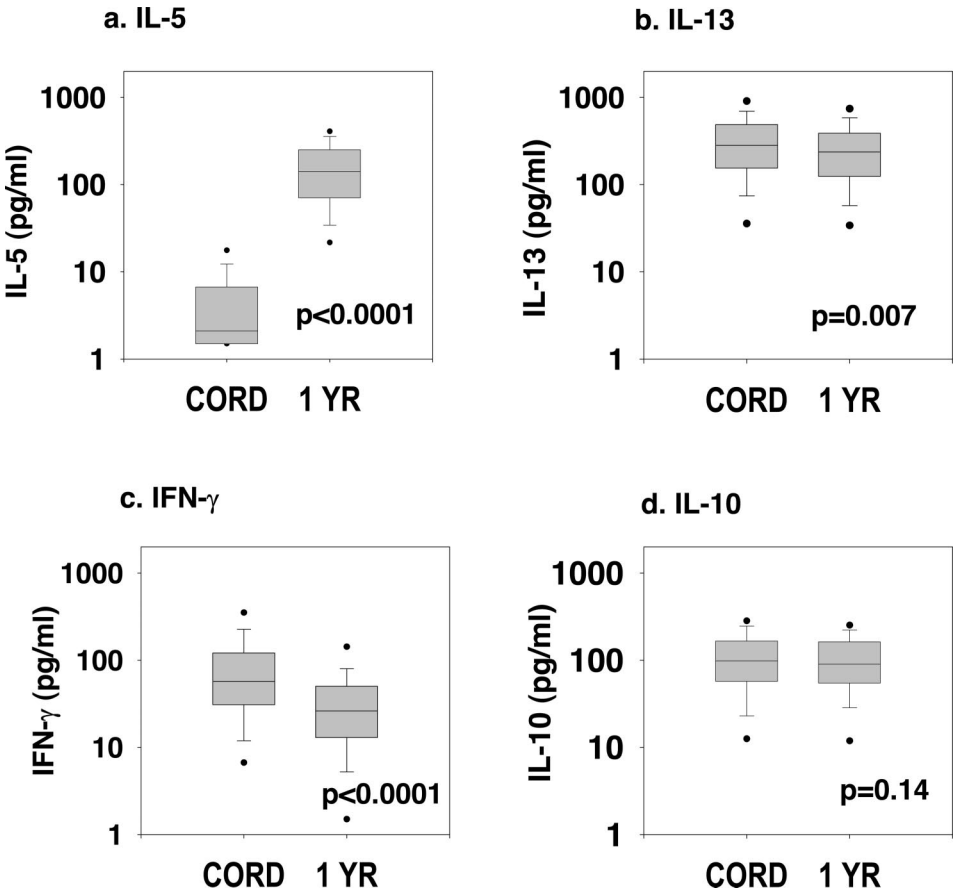

FIGURE 2. Interval changes for cytokine response profiles from birth to 1 year for the entire cohort. The box represents the 25th and 75th percentiles, with medians indicated by horizontal lines, and the 5th and 95th percentiles represented by filled circles. Reprinted with permission from *J Allergy Clin Immunol.* 2003;11:740–746,[63] with permission.

(2.1 pg/mL), moderate levels of IFN-γ (57.4 pg/mL) and greater amounts of IL-13 (281.8 pg/mL). From birth to 1 year, IL-5 responses dramatically increased, whereas IL-13 and IFN-γ responses significantly decreased (Fig. 2). Reduced cord blood secretion of IL-10 and IFN-γ was associated with subsequent sensitization to egg. In addition, there was evidence of Th2 polarization (increased IL-5 and IL-13 levels) associated with blood eosinophilia and increased total IgE levels by 1 year of age. These findings demonstrated that cytokine responses in children at high risk of developing allergic diseases and/or asthma change markedly during the first year of life and provided further evidence of a close relationship between Th2 skewing of immune responses and the incidence of atopic manifestations in children.[63]

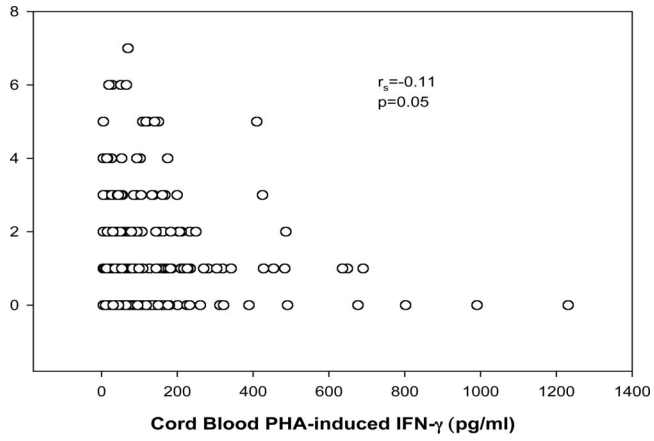

FIGURE 3. Relationship of cord blood PHA-induced IFN-γ secretion to the number of viral infections in the first year of life. The data demonstrate an inverse correlation between cord blood PHA-induced IFN-γ secretion and the number of viral infections documented by positive culture or RV-specific PCR from nasal lavage samples obtained when children had symptom scores of 5 or higher; n = 285. Reprinted with permission from the *American Journal of Respiratory and Critical Care Medicine* and the authors.[65]

Cytokine Response Patterns and Viral Infections During Infancy. The relationships between cytokine response profiles and the frequency and etiology of viral infections during infancy was also evaluated in the COAST cohort.[65] There was an inverse correlation between cord blood PHA-induced IFN-γ responses of mononuclear cells and the number of moderate to severe viral infections during the first year of life ($r_s = -0.11$, $P = 0.05$ (Fig. 3); vigorous secretion of IFN-γ was associated with fewer infections. This correlation was stronger ($r_s = -0.27$, $P = 0.028$) among children who both attended day care and had a sibling and thus had the greatest exposure to other children. There was no significant correlation between symptomatic viral infections during infancy and other PHA-induced responses (IL-5, IL-10, IL-13) from cord

blood cells. Day-care attendance and/or siblings significantly increased the likelihood of contracting RSV (1.5- to 1.6-fold increase) and RV (1.8- to 2.1-fold increase), and increased the risk of RV-induced wheezing (14–18% versus 2%, $P = 0.011$). These data suggest that neonatal IFN-γ responses may influence antiviral activity or may represent a marker of antiviral immune maturation. Conversely it was also found that the frequency of viral infections in infancy increased mitogen-induced IFN-γ responses at 1 year of age. These findings support the hygiene hypothesis and its prediction that increased numbers of infections in early childhood could influence Th1/Th2 immunologic balance.[65]

Etiologic Relationship of Infantile Viral Respiratory Illnesses with Later Childhood Wheezing. The etiology and clinical severity (moderate to severe respiratory tract illness with or without wheezing) of culture and/or polymerase chain reaction-confirmed respiratory tract infections during infancy and their relationship to persistent wheezing in the third year of life were also evaluated in the COAST children.[66] Risk factors for third year wheezing were passive smoke exposure [odds ratio (OR), 2.1]; older siblings (OR 2.5); allergic sensitization to foods at 1 year of age (OR 2.0); any moderate to severe respiratory illness without wheezing during infancy (OR 3.6); at least 1 wheezing illness with RSV (OR 3.0), RV (OR 10) and/or non–RV/RSV pathogens (OR 3.9) during infancy. When viral etiology was considered, first-year wheezing illnesses caused by RV infection were the strongest predictor of subsequent third-year wheezing (OR 6.6; $P < 0.0001$). Moreover, 63% of infants who wheezed during RV seasons continued to wheeze in the third year of life compared with only 20% of all other infants (OR 6.6; $P < 0.0001$). These data demonstrate that infants who wheeze with respiratory infections during RV seasons, even though their illnesses are not severe enough to warrant hospitalization,[67] should be considered at high risk for subsequent persistent wheezing. As such, they should be closely monitored prospectively during early childhood so that therapy can be instituted appropriately and expeditiously.[66]

Genetic Polymorphism Modification of the Development of Atopy and Asthma. Based on the importance of family history of atopy on the development of wheezing and asthma,[68] genetic variation plays a key role in the development of atopy and asthma. Interactions between environmental exposures (such as day care and viral infections) and genotype may be of particular importance. By using the COAST cohort, variations in genotypes were evaluated in the context of clinical and immunologic phenotypes that may precede the development of asthma.[10] Various polymorphisms were associated with changes in cytokine and IgE levels. For example, the *237Gly* allele of the high affinity IgE receptor β chain (FCER1B) and a silent substitution in the nitric oxide synthase 2A gene were associated with reduced IL-13 cord blood levels. Also, the *TGFB1-509T* allele was associated with increased RSV-related wheezing in the first year of life.[64]

In a recent study, 72 polymorphisms in 45 genes were evaluated for gene-environment interaction effects on the development of immune responses in the first year in the COAST cohort. The goal of the study was to determine if the effect of day care exposure on immune responses, which has been associated with decreased atopy rates in unselected cohorts,[69] would be influenced by a child's genotype. Several polymorphisms showed significant interactive effects on Th2 cytokine response profiles during the first year of life. For example, children with the *Asp/Asp* genotype at the nitric oxide synthase *3_298* locus who did not attend day care had the highest levels of Th2 cytokine responses, whereas the lowest Th2 responses occurred in the children with the *Asp/Asp* genotype who attended day care.[10] For this polymorphism, the significant effects of day care were independent of viral illnesses. This finding was consistent with the findings of another study that demonstrated a protective effect of the *Asp* allele.[70] Conversely the interactive effects of day-care exposure with the *FCE1β Glu237Gly* genotype on IL-5 cytokine responses and the *IL4RA Ile50Val* genotype on IFN-γ responses did appear to be related to more viral infections in children exposed to day care. These results demonstrate the importance and complexity of gene-environment interactions influencing the development of the immune system.

SUMMARY

Thus far, the results of the COAST project provide evidence of a bidirectional interaction between immune responses in early life and the development of wheezing illnesses and atopic phenotypes. Associations between specific cytokine responses and clinical wheezing have provided novel insights into the immunopathogenesis of viral respiratory illnesses in early life. The relationship between developmental immune responses and the evolution of wheezing phenotypes in the COAST children continues to be longitudinally evaluated to determine whether specific patterns of immune responses and/or gene by environment interactions in early childhood modulate the risk of developing physician-diagnosed asthma as the children reach their sixth birthday and beyond.

DISCUSSION

Question: Could you explain gene by environment interaction?

Robert F. Lemanske Jr., MD: Gene by environment interactions are very complicated. According to the current data on asthma genetics, it can be assumed that they are related to environmental effects; that is, the gene expresses itself differently depending on the environment.

Our research has found some interesting results with pets, showing that if you own a dog, you're protected from the development of allergic sensitization and atopic dermatitis. But if you have a cat, the results are not the same and it doesn't seem to help.

Looking at gene by environment interactions, we specifically focused on the CD-14 locus, which is a co-receptor for endotoxin. We found that if the children are TT homozygous and have a dog, their protection is much larger than if they're hetero- or homozygous CC. So, gene by environment is complex, but it's kind of where the field is going, so we're going to continue further research to gain more insight.

Question: Are you collecting information at day care about environmental exposures in addition to viruses?

Robert F. Lemanske Jr., MD: Yes, we are. We're doing some of that, but perhaps more important is what we've already done. We have gone into the homes of these children, and we've gotten vacuum samplings. Our outcomes did not find a relationship with endotoxin and protection to allergic sensitization, etc.

Results from other groups confirm the lack of a strong relationship with endotoxin. Therefore, there is probably something else in the dirt or the dust that is responsible for some of these immune response alterations. We're involved in ongoing research on the subject.

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