

The relevance of allergen exposure to the development of asthma in childhood

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Sensitization to 1 or more of the common indoor allergens has been consistently associated with asthma among children and young adults (odds ratios for asthma, 3-18). For dust mite and cockroach allergens, there is a dose response relationship between domestic exposure and sensitization. Given that allergen provocation can induce many of the features of asthma, the findings strongly suggest that there is a causal relationship between allergen exposure in the home and asthma. However, it remains unclear at what time the critical exposure occurs (ie, in infancy or later) and what role allergen exposure has played in the increasing prevalence and severity of asthma. Objective evidence of an immune response to allergens is generally not present until after 2 years of age. Viral infections play several different roles in asthma in childhood. In infancy, respiratory syncytial virus infection can induce bronchiolitis and set up recurrent wheezing over the next few years. However, the risk factors for this are maternal smoking and small lungs at birth, rather than allergy. By contrast, the role of rhinovirus in precipitating attacks in children and young adults is strongly associated with allergy. Thus the likely scenario is that allergen exposure over the first few years of life induces sensitization (ie, T_{H2} cells and IgE antibodies). Continuing exposure can maintain inflammation in the nose and lungs. However, many other factors contribute to wheezing such that there is no simple relationship between allergen exposure and asthma. Nonetheless, it is clear that the changes that have increased asthma have acted on allergic children. (*J Allergy Clin Immunol* 2000;105:S503-8.)

Key words: Asthma, allergens, viral infections, infancy

Because all plants and animals produce enzymes and other proteins, it is inevitable that humans are exposed to a wide range of foreign proteins that are potentially immunogenic. For many of these noninfectious sources, exposure consists of inhaling small quantities repeatedly over a period of months or years; in keeping with results in mice, the most common immune response to this form of exposure is immediate hypersensitivity (ie, IgE antibodies and T cells of the T_{H2} type). Given the consistent evidence that inhaled allergens play an important role in chronic asthma among school-aged children, it becomes very important to understand when immune responses to inhalants occur and how they interact with other factors that are involved in the development of asthma. Infants

Abbreviation used

BHR: Bronchial hyperreactivity

RSV: Respiratory syncytial virus

and children are also exposed to foreign proteins in their food, and immune responses to these may influence both the development of lung disease and subsequent responses to inhaled allergens.

Traditionally, the best recognized and best understood inhaled allergens were those derived from pollens. However, it has long been recognized that house dust extracts could produce positive skin tests. Over the last 15 years, the contents of house dust have become well defined. More important, it has become clear that it is sensitization to indoor allergens that is most strongly associated with asthma.¹ In many humid countries, allergens derived from dust mites are by far the most important cause of sensitization, and much of the epidemiologic and immunologic data relate to mites (Table I).¹⁻³ Evaluation of the immunologic data has been made possible by the availability of purified allergens from dust mites that allow measurement of specific antibodies and evaluation of T-cell responses *in vitro*.^{4,5} It is also possible to measure dust mite allergens in the environment with the use of monoclonal antibody-based immunoassays.⁶ Thus we can consider 3 separate but related areas: (1) the nature of the immune response, (2) the relationship between exposure and immune responses to mite allergens, and (3) the relevance of this immune response to the development of asthma.

THE NATURE OF THE IMMUNE RESPONSE TO ALLERGENS

In almost all epidemiologic studies, sensitization to allergens has been determined by skin tests or serum assays for IgE antibodies. However, the immune response also includes IgG, IgG4, and IgA antibodies. These antibodies are most reliably detected with radiolabeled purified allergens. Following children from birth, antibodies only become detectable around 2 years of age.^{2,7} In general, IgG antibodies are detected first, but IgE can be detected in the serum of many atopic children by the age of 4 years.^{2,8} With ELISA assays, rather different results have been obtained. In some of these studies, IgG antibodies can be detected in a large proportion of sera and can be detected very early in life. It has even been suggested that IgG antibodies detected in chord sera

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Supported by NIH Grant AI20565 and U19-AI34607.

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0091-6749/2000 \$12.00 + 0 1/0/99979

TABLE I. Structural and functional properties of indoor allergens

Source	Allergen	Molecular weight (kd)	Function	Sequence
House dust mite				
<i>Dermatophagoides spp.</i>	Group 1*	25	Cysteine protease	cDNA
	Group 2*	14	Epididymal protein	cDNA
	Group 3	~30	Serine protease	cDNA
	Der p 8	26	Glutathione-S-transferase	cDNA
<i>Euroglyphus maynei</i>	Eur m 1	25	Cysteine protease	PCR
Mammals				
<i>Felis domesticus</i>	Fel d 1*	36	Uteroglobin	PCR
<i>Canis familiaris</i>	Can f 1*	25	Taste perception	cDNA
<i>Mus musculus</i>	Mus m 1	19	Pheromone-binding protein	cDNA
<i>Rattus norvegicus</i>	Rat n 1	19	Pheromone-binding protein	cDNA
Cockroach				
<i>Blattella germanica</i>	Bla g 2*	36	Aspartic protease	cDNA
	Bla g 4	21	Calycin	cDNA
	Bla g 5	23	Glutathione-S-transferase	cDNA
<i>Periplaneta americana</i>	Per a 1	20-25	Unknown	cDNA

*Monoclonal antibody-based ELISA.

From Platts-Mills TAE, Vervloet D, Thomas WR, Aalberse RC, Chapman MD (Co-Chairmen). Indoor allergens and asthma: Third International Workshop, Cuenca, Spain. J Allergy Clin Immunol 1997;100:1-24. By permission.

reflect an immune response in utero.⁹ Solid-phase assays such as ELISA have 2 problems. First, the nonspecific background is related to the total serum level of the isotype being measured. This makes sensitive measurement of IgG or IgG1 antibodies difficult or impossible. By contrast, measurements of IgG4 or IgE antibodies are more reliable. Second, because the antigen is fixed, solid phase assays will detect antibodies of lower affinity than those detected with fluid phase assays. In addition, the solid phase has a wide range of proteins so that the IgG antibodies that are binding to proteins other than those recognized by IgE antibodies may be detected. Thus results for IgG antibodies with ELISA need to be interpreted with caution.

Evidence for T-cell responses to allergens was obtained as early as 1973. Most, but not all, studies reported that individuals with allergy had specific T cells although individuals with no allergy made little or no response. These studies have always been most convincing when purified allergens have been used.^{10,11} Once it became possible to measure cytokines, it was clear that T cells from individuals with allergy produced cytokines in vitro. Subsequently, it became clear that T cells from individuals with allergy make cytokines typical of T_{H2} responses (ie, IL-4 and IL-5).¹² By contrast, when allergen-specific T-cell clones can be derived from individuals with no allergy, they generally produce IL-2 and IFN- γ .^{12,13} It is now undoubted that circulating T cells of the T_{H2} type are characteristic of the response to dust mite allergens among individuals with allergy. However, there are confusing reports about the timing of the response. In some studies, T-cell proliferation in response to mite extracts has been detected in chord blood, suggesting that the first recognition of allergens occurs in utero.^{9,14} However, these responses are not detectable at 6 months of age, and it remains to be estab-

lished that the cells that proliferate are part of an immune response. Some of these studies have used a serum-free medium (Aim V), which may allow nonspecific effects of the antigen preparation used. Studies with the Aim V medium that used other antigens showed very marked inhibition of both proliferation and cytokine production.¹⁵ A further problem with interpreting the data on responses in infants comes from the report that similar responses are found with the chord blood of infants whose mothers were not exposed to significant mite allergen during pregnancy.¹⁶ It is unlikely that sufficient mite allergen can be absorbed and cross the placenta when the quantity inhaled per day is only 10 to 100 ng, and it is not conceivable that the fetus makes an immune response when the mother is exposed to a quantity of antigen that is not associated with immune responses in children.

THE RELATIONSHIP BETWEEN EXPOSURE AND IMMUNE RESPONSE

Intuitively, it is obvious that individuals will only experience an immune response if they are exposed to sufficient antigen. However, accurate definition of these parameters depends on being able to measure exposure. The measurement of allergens in house dust became possible when the allergens were purified and has become easier and more consistent with monoclonal antibodies.⁴⁻⁶ We can now measure representative allergens derived from dust mites, cockroach, cat, and dog.^{4-6,17} In addition, the relationship between airborne levels of each allergen and floor or reservoir concentrations has been defined.^{1,18,19} However, this does not amount to an accurate measurement of the quantities inhaled by an infant or child over a prolonged period of time. This caveat also applies to avoidance measures, because their success is

TABLE II. Sensitization as a risk factor for asthma (symptomatic BHR)

Country	Study	Dominant Allergen	Odds ratio	Pollen	Article
United Kingdom	Prospective	Mite (cat)	19.7*	NS	Sporik et al ²
New Zealand	Prospective	Mite (Asp)	6.6*	NS	Sears et al ⁵⁰
Sweden	Population	Cat, dog	3.9*	Birch [‡]	Ronmark et al ⁵¹
Australia	School	Mite	≥ 10.0*	NS	Peat et al ²⁰
United States					
Virginia	School	Mite (cat, cockroach)	6.6*	NS	Squillace et al ³
Atlanta	Acute (ER)	Mite, cockroach	8.2*	NS	Call et al ²⁸
Arizona	Prospective	Alternaria	0	NS	Halonen et al ⁵²
New Mexico	School	Cat, dog	6.2 [‡]	NS	Sporik et al ⁵³

**P* < .001.

[‡]32.

usually judged on the basis of 1 or 2 measurements of the concentration of allergen in reservoir samples. Despite the inaccuracy of exposure measurements, a clear dose response relationship between exposure to dust mite allergens and sensitization has been established. Results of this kind have been obtained by comparing houses within communities in Australia, England, or Germany^{2,20,21} or by comparing different climatic zones (ie, Briancon versus Marseilles in France; or Charlottesville, Va, versus Los Alamos, NM, in the United States).^{3,22,23} The main conclusions for dust mite are clear. In communities where the mean group I mite allergen concentration in bedding is more than 2 µg/g of dust, dust mite sensitization will be common among atopic individuals and will be significantly associated with asthma (Table II). In Germany, Kuehr et al²⁴ confirmed that the threshold for atopic children was approximately 2 µg. By contrast, for "nonatopic" children (ie, those children who had no positive skin tests), sensitization was unlikely to occur at concentrations less than 40 µg/group I allergen/g. The threshold values for exposure can be seen simply in relation to sensitization or in relation to the risk of asthma among atopic individuals.

Understanding the timing of the immune response is important because it defines the time at which avoidance measures, or other interventions, would have to be performed to influence these responses. The only convincing data relates to avoidance measures in the first year of life.⁸ Those studies strongly suggest that exposure in the first year of life can influence responses. Our own data from the prospective study found that measurements of mite allergen in children's bedrooms during the first 2 years of life predicted subsequent sensitization. In addition, measurement of exposure at age 1 to 2 years was a better predictor of asthma at age 11 years than measurements of exposure at age 11 years.² In that study, very high levels of exposure in the first year were also associated with earlier onset of disease. Peat et al^{20,25} in Sydney have found similar data supporting the importance of early exposure to high levels of mite allergen. Overall, there is good evidence that early exposure to dust mite allergens is a significant factor in sensitization and the development of asthma. However, whether this needs to occur within some defined period of time or is cumula-

tive over the period up to the time when immune responses are detectable is not clear. Although it seems clear that early exposure to high concentrations of allergen is important, it is still difficult to define the age at which the critical events in this response occur. In prospective studies, some children do not experience skin tests, antibodies, or symptoms until after the age of 5 years.²

For allergens other than dust mite, the dose response relationship to sensitization is less well defined. This is in part because the studies have not been done, but also because exposure is influenced by decisions of the inhabitants and because there are very different patterns of distribution. The quantities of animal dander or cockroach antigens are in large part influenced by whether the occupants choose to allow these animals to share the house. For cat and dog allergens, it is clear that allergen remains airborne so that there is a different relationship between reservoir measurements and inhaled allergen.¹⁸ The particles carrying cat and dog allergen are "sticky" and are transferred extensively from houses with a cat to other houses or to school.^{26,27} It is possible to identify communities where very few of the families have pets and where sensitization to these allergens is not significantly associated with asthma. Thus in several recent studies on African American children living in poverty, sensitization to cat allergens was not found to be a risk factor for asthma. The mean concentration of allergen found in dust from those houses (ie, <1 µg Fel d 1/g dust) could be said to define a threshold below which sensitization to cat allergen is unlikely.²⁸⁻³¹

Allergens derived from the German cockroach are an important source of sensitization in areas where infestation is common.²⁸ However, it is not clear at what age sensitization occurs, nor where the main exposure occurs. In most infested houses, the highest concentrations are found in kitchens, and this may be the best site to sample to define whether a house is infested.³⁰ On the other hand, the recent National Cooperative Inner City Asthma Study found that cockroach allergen in children's bedrooms was an important predictor of hospitalization.³¹ The results allow the conclusion that in communities where less than 5% of the houses have more than 10 units Bla g 2/g dust, sensitization to cockroach allergens will be rare and will not be associated with asthma.

THE RELEVANCE OF THE IMMUNE RESPONSE TO ALLERGENS TO THE DEVELOPMENT OF ASTHMA IN CHILDHOOD

Among school-aged children, sensitization to 1 or more of the major indoor allergens is the dominant risk factor for asthma defined as symptomatic bronchial hyperreactivity (BHR).^{3,23,32} In keeping with the view that these proteins cause asthma, inhalation of allergens in a bronchial challenge can produce both immediate and late falls in FEV₁ values. Furthermore, the late responses are accompanied by eosinophil infiltration and increase in BHR.³³ Children and young adults who are taken out of their houses and moved to "allergen free" sanatoria or hospital rooms, experience improvement in symptoms, reduced medication requirements, and decreased BHR.¹ Thus it is highly likely that current inhalation of allergens in houses plays a role in the symptoms that are so commonly experienced by individuals with allergy. Analysis of the quantities of allergen inhaled and the size of mite particles suggests that natural exposure is not sufficient to induce acute bronchospasm and may play a much more important role in maintaining chronic BHR. There are many other factors that can contribute to symptoms and BHR, and the problem is to understand how they interact with the response to allergens either in the development of asthma or in producing exacerbations.

Viral infection in early childhood has been proposed to have 2 quite opposite effects. Respiratory syncytial virus (RSV) is an extremely important cause of bronchiolitis in the first 2 years of life.^{34,35} All children are infected with RSV within the first 2 years, and they make an immune response whether they have lung symptoms or not. Thus bronchiolitis with hospitalization is the severe end of the response to this virus and appears to be related to the size of the lungs at birth. Welliver et al³⁴ suggested that RSV infection in early childhood predisposes to subsequent development of asthma. The assumption is either that the infection changes the lungs in some way or that RSV infection can influence subsequent immune responses to other antigens. The young children who are admitted to hospital with RSV infection do not have measurable antibodies to inhalant allergens or peripheral blood eosinophilia at the time when they are hospitalized.^{35,36} On the other hand, some of them have high concentrations of eosinophil cationic protein in their nasal secretions.³⁷ In mouse models of RSV infection, the immune response to different viral proteins determines whether the animals make an eosinophilic response to subsequent infection with the whole virus.³⁸ The problem is that prospective studies have given very different figures for the percentage of the children with bronchiolitis who subsequently experience the development of asthma. In addition, most groups have not been able to measure significant IgE antibody to RSV antigens in these children. At present, it seems that symptomatic RSV infection is not a precursor of allergic asthma in more than a small proportion of cases. However, the immune response to RSV may be important either as an influence on the immune system or as an indi-

cation of the immune response that the child will make to other antigens.

In an entirely different model, it has been suggested that viral and other infections in early childhood might be protective against the subsequent development of asthma.³⁹ This hypothesis was developed to explain the striking decrease in asthma among later members of large families compared with first or only children.⁴⁰ However, in many communities large families are rare, and a protective effect of early infection does little to explain the high prevalence of asthma in US cities. Although it is undoubtedly attractive to suggest that early infection could stimulate T_{H1} responses and thus influence subsequent responses to inhalant allergens, neither the epidemiologic evidence nor detailed mechanism for this hypothesis is available. One of the major changes in life style over the last 35 years has been the widespread use of antibiotics. However, the argument would have to be that antibiotics prevent a T_{H1} response to bacteria, and this predisposes to T_{H2} responses. Again, direct evidence for such a mechanism is lacking. This type of model would propose that the primary cause of the increased prevalence of asthma was progressive increase in allergic responses. There are many reasons for thinking that this is not what has happened.

The clearest role of viral infections in asthma is as a cause of exacerbations among patients with chronic asthma. The evidence for this comes from observations on a cohort of children, from case control studies on children seen in an emergency room, and from experimental studies on viral infection of patients with allergy.^{35,36,41,42} From most of these studies, it appears that infection with rhinovirus or corona virus in conjunction with allergen exposure or in a patient with inflammation induced by allergen exposure can precipitate an acute exacerbation. These exacerbations are accompanied by a marked increase in nasal eosinophils and cytokines, which may well parallel events occurring in the lungs. Recent evidence following up acute episodes has shown that both rhinovirus expression as judged by PCR and nasal eosinophil cationic protein become negative over the weeks after an acute episode.⁴³ When studies on rhinovirus were performed on children under 2 years of age, there was no correlation between PCR evidence of rhinovirus infection and acute episodes of asthma.³⁶ Thus it is unlikely that rhinovirus plays a role in the early development of asthma. On the other hand, rhinovirus plays an important role in the development of symptomatic asthma among individuals with allergy after the age of 2 years.

CONCLUSIONS

There is clearly a major genetic component to the development of allergic disease, and this appears to apply to both IgE antibody responses and the development of asthma.⁴⁴ By the age of 13 years, there is also a strong association between asthma and IgE antibodies to one or more indoor allergens. If we take a simple analysis, asth-

ma could have become more common because of an increase in indoor allergens leading to increased sensitization or to a change in immune responsiveness such that the same or similar exposure induces more individuals to become sensitized or that some other effect has altered the threshold for wheezing among individuals with allergy. There are many ways in which changes in domestic houses (eg, increases in temperature and furnishings and decreased ventilation) could have increased exposure to indoor allergens. However, this effect does not appear to be sufficient to explain the increase in asthma. In addition, there are several situations where the real change appears to be an increase in bronchial reactivity or wheezing among individuals with allergy.^{45,46} Thus it becomes important to understand not only the factors influencing the immune response but also those circumstances that can trigger the development of inflammation and wheezing in an allergic individual. Several studies have focused on the importance of early exposure to allergens (ie, the first 2 years of life). In most of those studies, the monitoring of exposure is restricted to 1 or 2 samples of reservoir dust. Thus at present, it is not possible to distinguish between the effect of exposure in the first 6 months or year and those of cumulative exposure over the first 3 years of life. Convincing evidence about immune responses (ie, skin tests or IgE or IgG antibodies) generally does not appear until after 3 years of age. Although there are some data suggesting that prenatal or very early postnatal exposure may influence subsequent events, none of these results are sufficiently persuasive. In particular, the data on proliferative responses from chord blood lymphocytes or increased allergic responses among children born in certain seasons of the year do not add up to a convincing argument for sensitization in utero or early life. Seasonality is also seen with food allergens, and the proliferation to mite has been seen with chord blood from children living in areas with low levels of mite exposure. It is also clear that continued exposure to mite allergens can influence sensitization among school-aged children and even among adults.²⁴

Understanding the relationship between these immune responses and the development of asthma remains difficult. Unfortunately, the prospective data does not show a simple temporal relationship. Indeed, some children who are highly allergic to mite allergens at age 11 years were first reported to wheeze before they experienced the development of skin tests or serum antibodies.^{2,7} Episodes of viral-induced wheezing could occur before the development of allergic responses, and the immune response would be seen as the reason for persistence. Some children experience the development of positive skin tests before they start wheezing. This raises the question, which may be really important, as to what makes the difference between children with allergy and children with allergy with wheezing. Recent studies suggest that a large part of the increase in asthma can be ascribed to a progressive increase in wheezing among children with allergy. Many different factors have been identified that can increase inflammatory responses to

allergen. These include diesel particulates, ozone, rhinovirus, and β -2-agonists. However, it is clear that the increase is strongly related to western society.^{1,47} Indeed, it appears that asthma remains rare among children in those countries where walking is the normal means of transport and parasitic infections remain common.⁴⁵⁻⁴⁷

In conclusion, there are several elements about asthma that are very well defined, but the events in the first 2 years of life are not. The relative importance of RSV infection, bacterial infections with or without antibiotics, allergen exposure, and nutrition remain unclear. In the development of the progression of asthma after age 2 years, allergen exposure, sensitization to indoor allergens, and intercurrent viral infections are all important. But, again, the details are not clear, and the role of these in the progressive increase in asthma in western society is less clear. It remains a major objective of research to understand the reasons for the increase in asthma among children with allergy because it is unlikely that we will achieve healthy children unless the real cause of the increase is made a target for treatment.^{48,49}

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