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Maturation of immune responses at the beginning of asthma

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The prevalence of childhood asthma appears to be increasing worldwide. A critical element in the development of childhood asthma is maturation of the child's immune system. Most asthmatic children have a history of recurrent lower respiratory tract illnesses associated with airway obstruction during the first year of life. Most infants and young children who will go on to have persistent wheezing and asthma show high IgE production and eosinophilic immune responses at the time of their first viral lower respiratory tract illness. Understanding the genetic and environmental factors that regulate the maturation of the immune response during early life will greatly enhance the development of strategies for the primary and secondary prevention of asthma. (*J Allergy Clin Immunol* 1999;103:355-61.)

Key words: Asthma, respiratory syncytial virus, allergen

Several recent reports¹ suggest that the prevalence of childhood asthma is on the rise worldwide. In the United States the evidence indicates that these changes in prevalence rates are due at least in part to increases in asthma incidence,² but increases in asthma severity (and thus in disease duration) cannot be excluded. Unfortunately, our understanding of the natural history of childhood asthma is very limited, and this is particularly true in regard to those factors that determine asthma incidence. In fact, considerable advances have been made in our understanding of the mechanisms by which different stimuli (eg, infection, allergen exposure, and cold air challenge)

Abbreviations used

APC:	Antigen-presenting cell
DC:	Dendritic cell
F-protein:	Fusion protein
G-protein:	Attachment protein
LRI:	Lower respiratory tract illness
NK:	Natural killer
RSV:	Respiratory syncytial virus

can trigger asthma attacks. Much less is known, however, about the disease processes involved in the development of asthma during early childhood and on the genetic and environmental factors that regulate the onset of the disease.

I have previously proposed the unifying concept that childhood asthma is a developmental condition in which alterations in the maturation of the immune system, of the control of airway tone, and of the regulation of lung and airway growth play a crucial role.³ In this review I will concentrate mainly on the first of these aspects (ie, on the patterns of maturation of the immune response that are involved in the development of asthma). The role of lung growth as a predisposing factor for asthma has been reviewed elsewhere.⁴

THE INCEPTION OF ASTHMA

There is little doubt that the first manifestations of asthma can occur at any age. However, longitudinal studies have recently suggested that most school-age asthmatic children have a history of recurrent lower respiratory tract illnesses (LRIs) associated with airway obstruction during the first years of life.² The relation between these LRIs and the subsequent development of asthma remains controversial. Wheezing LRIs in early

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life occur mostly in concomitance with viral infections, especially those caused by respiratory syncytial virus (RSV),⁵ and are very common, affecting up to 30% of all children.⁶ Moreover, for the majority of children with wheezing LRIs during the first 3 years of life, symptoms remit during childhood.⁶ It could thus be argued that the LRI-asthma association is spurious and that acute responses to viral infection in early life are not the harbingers of chronic asthma of the school years. Several recent findings both in humans and in animal models strongly contradict this conclusion.

It is now apparent that there may be different patterns of immune response to RSV and other viruses during LRIs in infancy. We recently showed that children who had wheezing LRIs during the first 3 years of life and who were still wheezing at the age of 6 years (persistent wheezers) had total serum IgE levels at the time of their first acute LRI that were higher than those obtained during convalescence (approximately 30 days later).⁷ No such acute changes in total serum IgE were observed in children who also had wheezing LRIs in early life but who were not wheezing at the age of 6 years (transient wheezers). This is in spite of the fact that the etiology of the first LRI was very similar in both groups, with almost two thirds of all agents isolated being RSV, and the age at which the first LRI occurred was also very similar (approximately 1 year). We also noticed that transient wheezers showed a marked acute eosinopenic response to the LRI, a response that was similar to that observed in children with nonwheezing LRIs. This eosinopenic response to acute infection has been known for decades, but the factors that determine it are not well understood.⁸ Interestingly, persistent wheezers showed no such response, and as a consequence, the number and percentage of circulating eosinophils during the acute LRI was significantly higher among persistent wheezers than among transient wheezers.⁷ This clearly suggested 2 different patterns of immune response to viruses, with persistent wheezers showing the kind of pattern (IgE- and eosinophil-mediated) believed to be characteristic of allergic asthma.

Unfortunately, there is very little information on the nature of the immune response to RSV infection at the airway level in infants with different wheezing outcomes later in life. Everard et al⁹ studied total IgE and tryptase concentrations in bronchoalveolar lavage fluid of infants who were intubated because of respiratory failure during LRI and who were thus at high risk for the development of persistent wheezing.¹⁰ Several, but not all, of these infants showed either measurable IgE or mRNA for IgE in the lavage fluid, and tryptase was detectable in 11 of 12 samples, with absolute values that were raised compared with those observed in control samples. Azevedo et al^{11,12} have shown that alveolar macrophages from wheezing infants are activated *in vivo* to release larger amounts of eicosanoids, TNF- α , and thromboxane B₂ compared with nonwheezing control subjects, but the relation between these findings and the subsequent development of asthma remains elusive. Stevenson et

al¹³ studied the cellular constituents of bronchoalveolar lavage fluid in children of different ages with atopic asthma, nonasthmatic atopy, and viral-associated wheeze. They found that eosinophil counts were much higher in children with atopic asthma than in the other 2 groups.

Further work specifically focusing on the airway immune response to viral infection in infants and young children in relation to the subsequent development of persistent wheezing is urgently needed.

DETERMINANTS OF ACUTE RESPONSE TO LRI IN EARLY LIFE

What determines the patterns of response to acute viral LRI in early life is not well understood and has also been scantily studied in humans. Recently, Román et al¹⁴ studied the nature of immune responses to confirmed RSV infections in children who were hospitalized for these infections before age 15 months. When compared with control subjects, children infected with RSV showed decreases in CD8⁺ T cells and in activated suppressor/cytotoxic (CD8⁺/CD25⁺) T cells. Cultured PBMCs from RSV-infected children showed lower IL-4 and lower IFN- γ responses than those observed in control children, but the deficits in IFN- γ responses were much more marked than those for IL-4, thus resulting in significant decreases in IFN- γ /IL-4 ratios for RSV-infected children when compared with control subjects. The authors attributed these results to a functional depression of CD4 T cells in RSV-infected children and speculated that this depression would be more severe for T_{H1}-like cells.¹⁴ It is important to stress, however, that these results were obtained using a population of children hospitalized with RSV. This subpopulation has been shown to be much more prone to be allergic and to later have asthma¹¹ than children seen with RSV-associated LRIs as outpatients.⁶ Moreover, because T-cell subtypes were not directly studied, it is possible that IFN- γ and/or IL-4 may have been produced by cells other than CD4⁺ T cells (eg, natural killer [NK] or CD8⁺ T cells), which are also known to produce these cytokines (see below).

Further caution regarding the role of T_{H2}-like cells in determining RSV-mediated lung disease is suggested by work based on animal models. It is reasonable to postulate that if the predominance of a T_{H2}-like response is responsible for acute LRI associated with RSV, then suppression of such responses should decrease the severity of disease in infected animals. Experiments testing this hypothesis were facilitated by the discovery that 2 RSV gene products are the most important in determining the immune response in mice: the attachment (G) protein and the fusion (F) protein.¹⁵ BALB/c mice sensitized to G-protein expressed by recombinant vaccinia virus had a T_{H2}-driven lung eosinophilia with local production of IL-4 and IL-5 on subsequent rechallenge with the virus.¹⁶ Sensitization with F-protein, however, produced a T_{H1}-like immune response with no eosinophilia after viral infection.¹⁶ On the basis of these results, mice sensitized to G-protein were treated with IL-12, a known potent

suppressor of T_{H2} -like reactions, at different times between sensitization and rechallenge with RSV.¹⁷ These IL-12-treated mice showed significant increases in IFN- γ -producing cells and decreases in IL-4- and IL-5-producing cells during rechallenge, and lung eosinophilia was markedly decreased.^{15,18} However, not only was illness associated with RSV not eliminated, but it was increased in this model. These experiments strongly suggest that reversing T_{H2} -mediated responses may not be sufficient to eliminate RSV-mediated respiratory disease in susceptible individuals.

More recent experimental studies in calves and mice suggest that NK and CD8+ cells also play an important role in the immune response against RSV. Taylor et al¹⁹ showed that calves depleted of CD8+ T cells had prolonged nasopharyngeal excretion of RSV, prolonged viral isolation from lung washings, and increased severity in pneumonic consolidation when compared with control animals. Calves treated with anti-CD4 antibodies also showed increased pneumonic consolidation, although viral clearance was not delayed.²⁰ Mice primed with a recombinant vaccinia virus expressing the RSV-G protein and in which a strong CD8+ T-cell epitope from RSV-matrix protein had been inserted showed suppression of eosinophil recruitment into the lungs and suppression of T_{H2} -like cytokine production on subsequent challenge with RSV.²¹ Sensitization to RSV-F glycoprotein in CD8+ T-cell deficient mice resulted in marked pulmonary eosinophilia on subsequent rechallenge with RSV¹⁷ in contrast to the usual mononuclear infiltrate, with no eosinophilia reported for wild-type mice.¹⁶ Thus work in both calves and mice strongly suggest that CD8+ T cells control T_{H2} -like responses and T_{H2} -driven eosinophil recruitment into the lungs during RSV infection.

Very recent data also suggest that NK cells are the most abundant IFN- γ -producing cells encountered in lung tissues during the early phases of acute RSV infection in mice.¹⁸ Moreover, NK cytotoxicity and IFN- γ production correlated with increased numbers of lung CD8+ T cells, and mice with few NK cells had low CD8+ T cells and subsequently had lung eosinophilia.¹⁸

The relevance of this information obtained in animal models to RSV infection in human infants is unknown. Recent evidence suggests that, much like the case for CD4+ T cells, subtypes of CD8+ T cells and of NK cells are also identifiable by the kind of cytokines they release on stimulation. So-called T_{C1} CD8+ T cells produce T_{H1} -like cytokines such as IFN- γ , whereas T_{C2} cells produce T_{H2} -like cytokines such as IL-4. Lysates of resting CD8+ peripheral blood cells obtained from subjects with mild atopic asthma have been shown to contain more IL-4 than those of healthy nonatopic subjects, but IFN- γ levels were similar in both groups.²² CD8+ cells stimulated with concanavalin A showed significant increases in IFN- γ production but no changes in IL-4 production, and this effect was observed in both asthmatic and nonasthmatic subjects. The authors concluded that CD8+ T cells are an important source of IL-4 and other T_{H2} -like cytokines and that this capacity is acquired

in vivo.²² Peritt et al²³ cultured human NK cells in the presence of either IL-12 or IL-4. NK cells grown in the presence of IL-4 (NK-2-type cells) differentiated into cell populations producing IL-5 and IL-13, whereas those cultured with IL-12 produced IL-10 and IFN- γ (NK-1-type cells). Although it is not known whether NK-2 cells exist in vivo in humans, IL-5-producing NK cells contribute to peritoneal eosinophilic infiltration in a mouse model of allergic inflammation.²⁴

Because IFN- γ expression in NK cells precedes lung CD8+ T-cell recruitment during RSV infection in mice,¹⁸ and CD8+ T cells appear to control the nature of CD4+ responses (see above), it is tempting to speculate that the nature of the NK-cell response to RSV may be the link between RSV-associated LRIs in early life and the subsequent development of asthma. This hypothesis requires further empirical support.

SENSITIZATION TO AEROALLERGENS IN EARLY LIFE AND THE DEVELOPMENT OF ASTHMA

Our work⁶ and that of others¹⁰ has clearly shown that infants and young children with RSV-associated LRIs who will go on to have persistent wheezing are much more likely to become sensitized to local aeroallergens than transient wheezers. The factors that predispose children at risk for asthma to have specific IgE against asthma-related allergens are beginning to be elucidated. Recent seminal studies by Prescott et al²⁵ have contributed very important new data to our understanding of these complex processes. It has been known for several years that children who either have a family history of allergies²⁶ or who will go on to become sensitized to local aeroallergens²⁷ have a diminished capacity for secretion of T_{H1} -like cytokines such as IFN- γ when compared with control subjects. This finding has suggested that delayed maturation of T_{H1} -like immune responses during early life may be an important marker of an inherited predisposition to allergic disease.²⁸ Prescott et al²⁵ showed that the characteristic allergen-induced cytokine responses by cord blood mononuclear cells comprise IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, suggesting a generalized skewing toward the T_{H2} -like phenotype at this age. It was also shown that IL-10 and IL-13 were exclusively produced by CD4+ T cells and IL-6 by non-CD4 cells in this system, whereas the cellular origin of the other cytokines was not elucidated.²⁵

What determines this generalized T_{H2} bias in the newborn is a matter of speculation. It is likely that allergens cross the placenta and are thus presented to immunocompetent cells in exceedingly small amounts. Presentation of such small amounts of antigen during the neonatal period is known to strongly skew immune responses toward T_{H2} -like cytokines in mice.²⁹ Animal models have also suggested that the fetomaternal immune interphase is dominated by T_{H2} -like cytokines,³⁰ and this has been teleologically explained by the potential detrimental effects of T_{H1} -like cytokines on the outcome of pregnan-

cy.³¹ It is plausible to surmise that under placental influences the fetus may also develop preferential T_{H2}-like responses to external antigens, although this paradigm is not universally accepted.³²

MATURATION OF THE ANTIGEN- PRESENTING CELL SYSTEM AND T_{H1}/T_{H2}-LIKE RESPONSES

Studies of immune responses to antigens by cord blood mononuclear cells show that under the right experimental conditions, newborn T cells are capable of mounting an adequate T_{H1}-like response.³³ The low capacity of infant T cells to produce IFN- γ is thus not mainly caused by an intrinsic defect of these cells. It is therefore possible that the defect responsible for the sluggish T_{H1}-like responses in early life may reside in other cells, and a suitable candidate appears to be the antigen-presenting cell (APC) system.

Normal mature APCs selectively induce differentiation of T_{H1}-like effector and memory cells by secreting IL-12.³⁴ In newborns, however, the T_{H1}-selective mechanism is apparently inefficient, and little IL-12 is produced at the time of antigen presentation.³⁵ This immaturity is not apparently caused by a decrease in the rate of mRNA transcription for either of the 2 chains (p40 and p35) that compose the IL-12 p70 heterodimer. In contrast, the half-life of p40 mRNA has been shown to be 3 times lower in cord blood mononuclear cells than in adult PBMCs.³⁶ Thus in the absence of efficient T_{H1}-polarizing stimuli, antigens that are first presented by the APC system during this immature phase may be associated with the production of predominant T_{H2}-like memory cell clones. Persistence of these clones could predispose to T_{H2}-like responses after subsequent encounters with these antigens.

Among APCs, dendritic cells (DCs) are known to be crucial in the control of primary immune responses by APCs.³⁶ These cells are generated in the bone marrow, but they migrate to peripheral tissues (including the airway epithelium) and are initially specialized in the local acquisition of antigen. They then migrate to local lymph nodes, where they modify their phenotype and specialize in antigen presentation.

The factors that determine the maturation of APCs in early childhood may thus be decisive in establishing the mature, T_{H1}-like responses and thus in protecting against early allergic sensitization. As explained earlier, CD8⁺ T_{C1} cells (and perhaps NK-1 cells) mediate the process of immune deviation away from the default T_{H2}-like response during viral infection. T_{C1} cells secrete large amounts of IFN- γ when stimulated with antigen *in vitro* and thus may stimulate APCs (eg, DCs) to produce IL-12 because IFN- γ is a potent adjuvant in IL-12 production by DCs *in vitro*.³⁷ In this context genetic factors that delay the development of mature IFN- γ responses by NK cells or CD8⁺ T cells may also delay IL-12 responses by APCs and thus prolong the period during which default T_{H2}-like responses are predominant. Environmental fac-

tors may also be crucial. Viral infections that preferentially stimulate NK-1-type responses may contribute to the maturation of APCs and skew the immune reaction toward the T_{H1}-like phenotype, whereas the contrary may occur for viral infections that elicit weak NK responses¹⁸ or putative NK-2-type responses.²³

The most extensively studied factors contributing to the maturation of APCs are, however, those derived from bacteria or bacterial compounds. LPS is a potent stimulator of DC maturation both *in vivo*³⁸ and *in vitro*.³⁹ It has been shown that LPS can induce substantial IL-12 production by DCs.³⁷ On the basis of these observations, Holt et al⁴⁰ have proposed that exposure to LPS and other bacterial wall products (perhaps from gut flora) may be a necessary step for the postnatal maturation of APCs in general and of the DC system in particular.

ROLE OF CD14 IN THE MATURATION OF APCs

The interaction between LPS and APCs is primarily mediated by CD14.⁴¹ CD14 is a glycoprotein that is present in tissues in 2 forms: a membrane-bound form (mCD14) and a soluble form (sCD14). mCD14 is mainly found in the surface of monocytes and is anchored to the cell membrane by way of glycosyl-phosphatidylinositol.⁴² sCD14 lacks the glycosyl-phosphatidylinositol anchor and is quite abundant in the blood serum. Both mCD14 and sCD14 appear to have a variety of functions, but relevant to this discussion is their role as the high-affinity receptor for LPS⁴² and other structurally related components of microbial pathogens.⁴³ Mature DCs do not express mCD14 but can be activated in a dose-response manner by sCD14-bound LPS.⁴⁴

Interestingly, CD14 can also bind arabinofuranosyl-terminated lipoarabinomannan, a glycolipid present in the cell wall of mycobacteria that has been shown to stimulate mononuclear phagocytes to produce IL-12.⁴⁵ It is tempting to speculate that the postulated inverse relation between tuberculosis infection in childhood and asthma and allergies⁴⁶ may be attributable to mycobacterial-induced maturation of the APC system and, consequently, of the T_{H1}-like response.

The crucial role of CD14 as a receptor for several bacterial signals that could be involved in the maturation of the APC system in early life prompted our group to study genetic factors that could be involved in the regulation of CD14 production. Further stimulus for this investigation came from findings by several groups (including ours) that total serum IgE levels,⁴⁷ bronchial hyperresponsiveness to histamine,⁴⁸ and circulating eosinophil levels⁴⁹ showed evidence of genetic linkage with markers in chromosome 5q31-33, the precise region where the locus for the CD14 gene (together with many other potential asthma-related genes) has been mapped. We thus screened the promoter region for the CD14 gene for variants and were able to localize a previously unidentified single nucleotide polymorphism (a C-to-T transition) in position -159 from the transcription

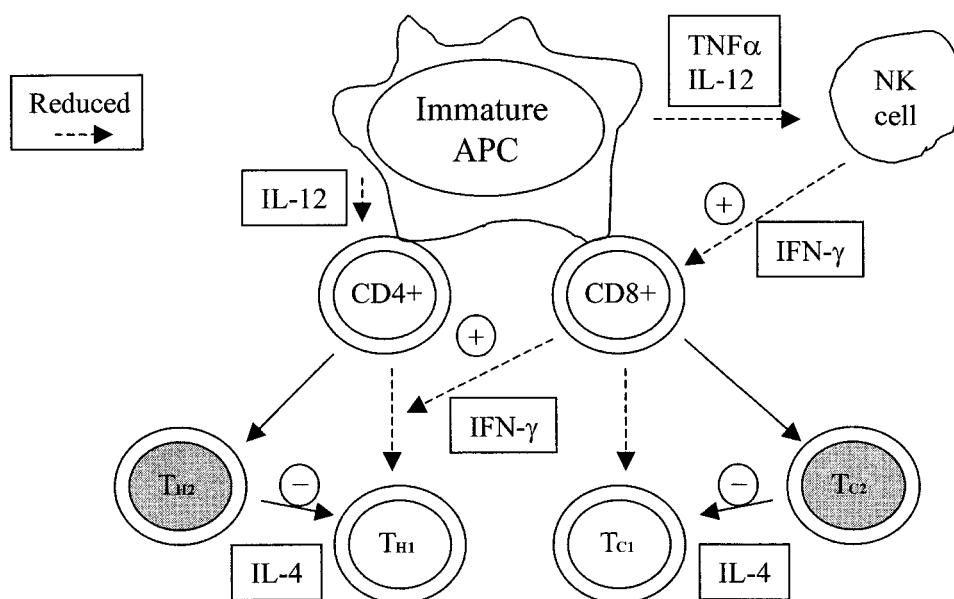


FIG 1. Theoretical development of T_{C2}/T_{H2} -like responses. In an immature APC system antigen presentation without IL-12 promotes the development of T_{H2} -like and T_{C2} -like effector cells.

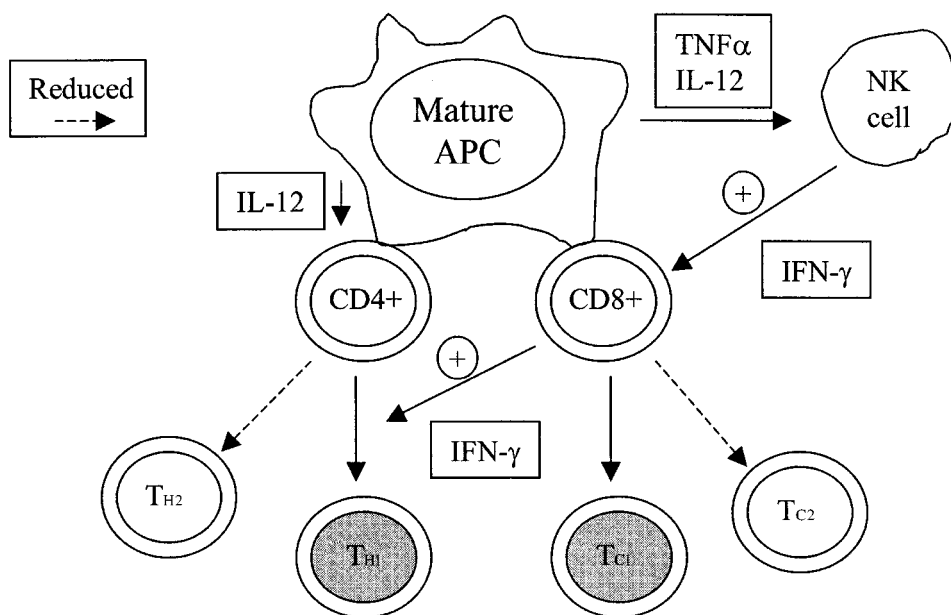


FIG 2. Theoretical development of T_{C1}/T_{H1} -like responses. A mature APC system favors the development of T_{H1} -like and T_{C1} -like responses through the presentation of antigen in conjunction with IL-12.

initiation site, with each allele present in approximately half of all chromosomes.⁵⁰

When compared with homozygotes for the C allele, school-age children who were homozygous for the T allele showed significantly higher circulating levels of sCD14. Serum sCD14 levels, on the other hand, were directly correlated with $IFN-\gamma$ responses and inversely correlated with IL-4 responses to mitogens by PBMCs. Total serum IgE levels were significantly lower in

homozygotes for the T allele when compared with carriers of the other 2 genotypes (homozygotes for C and CT heterozygotes), and this was especially noticeable and significant among children with positive skin prick test responses to local aeroallergens.⁵⁰ These results thus suggested that genetic influences may control circulating levels of sCD14 and by this mechanism modulate T_{H1}/T_{H2} balances and IgE synthesis. We are at present assessing both the role of the CD14/-159 polymorphism

in CD14 transcription and the developmental regulation of CD14 expression and APC maturation in subjects with different CD14/159 genotypes. Because CD14 expression in monocytic cell lines is known to be strongly upregulated by exposure to LPS,⁵¹ this system may be the first molecular model of gene-by-environment interaction in the development of IgE (and presumably asthmatic) responses during childhood.

A schematic summary of the hypothetical mechanisms described above is presented in Figs 1 and 2.

CONCLUSIONS

Most infants and young children who will go on to have persistent wheezing and asthma show high IgE production and eosinophilic immune responses (both in the airways and in circulation) at the time of their first viral LRI. They also have early IgE-mediated responses to local aeroallergens. Understanding the genetic and environmental factors that regulate the maturation of the immune response during early life will greatly enhance the development of strategies for the primary and secondary prevention of asthma.

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