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Contemporaneous maturation of immunologic and respiratory functions during early childhood: Implications for development of asthma prevention strategies

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The term *asthma* refers to a spectrum of wheezing syndromes resulting from airways inflammation triggered by a range of environmental stimuli, the most important of which are aeroallergens and viruses. We describe below a model for the cause of atopic asthma in which discrete sets of developmental factors governing the postnatal maturation of the immune and respiratory systems play central and complementary roles in disease causality. Within the immune system, the relevant developmental processes involve maturation of T_H1 and associated innate immune functions that combat infection and concomitantly antagonize the early programming of T_H2-polarized immunologic memory against inhalant allergens. Within the respiratory system, the relevant developmental processes involve intensive lung growth and airway remodeling during infancy. We hypothesize that delayed maturation of T_H1-associated functions during early postnatal life increases the risk for sensitization to aeroallergens and for severe respiratory infection, resulting in airway inflammation at a crucial stage in lung development and precipitating changes in lung growth that are the harbingers of susceptibility to persistent asthma. We further hypothesize that protection of the growing lung against the effects of inflammation during infancy and early childhood has unique potential as a generic strategy for asthma prophylaxis. (J Allergy Clin Immunol 2005;116:16-24.)

Key words: *Atopy, asthma, immune development, innate immunity, T cells, dendritic cells*

Abbreviations used
APC: Antigen-presenting cell
DC: Dendritic cell
PRR: Pattern recognition receptor
RSV: Respiratory syncytial virus
RTE: Recent thymic emigrant
TCR: T-cell receptor
TLR: Toll-like receptor

Asthma is a complex disease process that derives from interactions between environmental and genetic factors that are only poorly characterized. A number of asthma phenotypes are recognized to exist in children. Although these are separable epidemiologically, it is not a simple matter to determine which phenotype is present in an individual wheezy child. The type of asthma that is likely to persist into adult life is that associated with atopy (atopic asthma). In contrast, children with the type that is mainly associated with viral infection are more likely to outgrow their symptoms. However, in both cases, the underlying mechanisms involve aberrant, excessive, or both types of immune responses against the eliciting agents, resulting in turn in inflammatory damage to airway tissues and ensuing alterations in mechanical properties, together with exaggerated responsiveness to bronchoconstrictor stimuli. Although these events might be initiated *de novo* at any stage of life, it is clear from hospitalization records that the life phase during which they most frequently occur is early childhood.[1] Moreover, the disease process persists in a significant proportion of patients for at least 10 years[1] and, in many cases, might last into adulthood.

The pathogens that drive viral-associated asthma and the aeroallergens responsible for atopic asthma elicit immune responses that are dominated respectively by T_H1 and T_H2 cytokines. In experimental systems production of these 2 classes of cytokines is mutually antagonistic,[2] reinforcing the view that these 2 forms of asthma represent related but mechanistically distinct disease entities. However, recent findings from a range of independent clinical and laboratory studies suggest an alternative hypothesis

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for the cause of asthma in childhood, notably that a significant proportion of risk for both forms of asthma derives from common-shared variations in developmental regulation of T_H1 function. These variations result in delayed maturation of T_H1 competence during infancy, thus increasing susceptibility to viral infection and its subsequent spread to the lower respiratory tract and concomitantly increasing susceptibility to programming T_H2-polarized immunologic memory against aeroallergens. Crucial to subsequent development of persistent asthma, this life phase is also a period of intensive growth and remodeling of the lung and airways, and inflammatory damage to these rapidly growing tissues through viral pathways, allergy pathways, or both alters key differentiation programs, resulting in long-lasting changes in respiratory function. Thus the ultimate development of persistent asthma can be viewed as the result of interactions between a triad of factors: environmental triggers that initiate immunologically mediated inflammation in the airways and 2 discrete sets of developmental factors that control programming of long-term response patterns to exogenous inflammatory stimuli within the immune and respiratory systems.

PROGRAMMING OF T-CELL MEMORY AND DEVELOPMENTAL REGULATION OF IMMUNE COMPETENCE IN EARLY LIFE

The defining feature of the adaptive arm of the immune system is the phenomenon of immunologic memory, a process shaped by evolution to enhance the effectiveness of immune defenses on subsequent reinfection with individual pathogens. The strengths of such a mechanism in resistance to infection (ie, increasingly rapid and more intense responses with repeated pathogen challenge) are also the basis for immunopathology in allergic disease. In this context any errors in immunologic programming resulting from initial misclassification of ubiquitous airborne allergens as pathogens that merit expression of specific T_H2-polarized immunity are reiterated repeatedly at subsequent exposures, resulting in cumulative damage to airway tissues.

The initial programming of memory against aeroallergens typically occurs during early childhood,[3] and the outcome of this process (as discussed below) can determine the allergen responder phenotype and hence susceptibility to diseases such as atopic asthma into adulthood. The functional competence of the immune system during this period is accordingly likely to be a crucial determinant of susceptibility to allergic sensitization, and the nature of the factors that regulate immune function in early life are only partially understood. A brief synopsis of our current understanding of the functional status of the 2 major arms of the immune system during this period follows.

Adaptive immune function in early childhood

The reduced functional capacity of the adaptive immune system at birth is well recognized and has been variously attributed to deficiencies within the T-cell system itself, within the antigen-presenting cell (APC) compartment responsible for provision of activation signals to T cells, or both.[4,5] Circulating T-cell numbers are increased in infancy relative to later life,[6] and there is a high proportion of expressed surface markers, such as CD1[7] and CD38,[8] which are characteristic of functionally immature recent thymic emigrants (RTEs). A significant proportion additionally coexpresses both CD4 and CD8,[9,10] which is also characteristic of immature T cells or T cells that have been recently activated. However, very few express classical activation markers, such as CD25, CD69, or CD154.[9]

T cells from neonates and infants also display reduced capacity to express sustained responses to *in vitro* stimuli. Initial *in vitro* proliferation rates of these cells after stimulation are higher than those of corresponding cells from adults[11,12]; however, overall cell growth in response to mitogens such as PHA slows after 48 to 72 hours, reflecting the greater susceptibility of immature T cells to apoptosis.[8] Moreover, capacity for activation through the T-cell receptor (TCR) is also reduced.[13] T cells from this age group are also highly susceptible to induction of anergy after stimulation, which has been ascribed to reduced IL-2 production[14] and deficient Ras signaling.[15] Other reported signaling deficiencies in infant T cells have been reported in relation to Lck,[16] protein kinase C,[17] and nuclear factor κB.[18]

A range of effector functions are reportedly deficient in T cells during infancy. These include provision of T-cell help for B-cell antibody production[19] and target cell cytolysis.[20] These functional deficiencies are clearly the result of a combination of developmental factors, the most important of which are likely to be reduced expression on postactivated immature T cells of CD40 ligand[21,22] and reduced overall capacity to produce cytokines.[23-27] Of particular relevance to this review is the markedly diminished capacity of infant T cells to produce IFN-γ, which is seen in PBMC cultures stimulated with APC-dependent stimuli[28] and with isolated infant T-cell clones stimulated with APC-independent stimuli.[23] Recent studies indicate that selective attenuation of IFN-γ gene expression in T cells *in utero* and in early postnatal life is due largely to hypermethylation of CpG sites in the proximal promoter, which results in reduced capacity to transcribe IFN-γ–specific mRNA.[29-31]

The latter finding accounts, at least in part, for the generalized T_H2 polarity that is the hallmark of the fetal and infant immune system.[32] This is further consolidated by reduced IL-12 production by innate immune cells[33] and altered responsiveness to secreted IL-12[34] coupled with hyperresponsiveness to IL-4[35] by T cells in these age groups.

Collectively, these developmental deficiencies provide at least a partial explanation for the relatively poor performance of infant T cells in *in vitro* T cell–cloning systems[23] and for the inefficient generation of T-cell memory *in vivo* after natural infection[36] or immunization.[37] However, as detailed below, developmental

deficiencies in the T-cell system are mirrored by those in the innate immune system, particularly in the APC populations that regulate T-cell activation.

Attenuation of innate immunity and APC function in childhood

The innate immune system is an ancient form of host defense against infection, which relies on a limited number of pattern recognition receptors (PRRs) to identify conserved molecular patterns expressed by microbial pathogens. Secreted PRRs, such as CD14 or LPS-binding protein, bind to microbes and facilitate their destruction by phagocytosis or the complement system. Toll-like receptors (TLRs) induce antimicrobial genes and inflammatory cytokines within a variety of cells while activating dendritic cells (DCs) to initiate adaptive immune responses. Importantly, different microbial stimuli differentially activate distinct signaling pathways within DCs, thereby inducing distinct classes of immune responses. The extent to which these mechanisms develop during early childhood remains largely undefined but is likely to have important implications for the development of allergic sensitization.

Neonatal monocytes are less responsive than adult cells to bacterial lipopeptides, double-stranded RNA and LPS that act through TLR-2, TLR-3, and TLR-4, respectively,[38-40] but show a normal response to an imidazoquinoline compound that acts through TLR-7 and TLR-8.[39] The reduced responsiveness to LPS stimulation might be a consequence of reduced expression of the adapter protein MyD88 in neonatal cells.[38] Little is known at this stage regarding changes in TLR expression or function during infancy and childhood.

An increasing body of data indicates that there are major differences in APC function between neonates and adults. Cord blood monocytes show impaired chemotaxis and capacity to synthesize TNF-α but normal phagocytosis compared with that seen in adult monocytes.[41,42] DCs in the normal placenta preferentially induce T_H2 differentiation by naive T cells,[43] although it is not clear whether the influence of placental DCs persists into postnatal life. At birth, there is a modest reduction in circulating DC numbers relative to that seen in adults,[44] with many neonatal DCs appearing relatively immature, with lower levels of intercellular adhesion molecule 1, class I MHC, and II MHC expression.[44,45] Cord blood appears to contain relatively more plasmacytoid DCs than myeloid DCs,[46,47] which is in contrast to the situation in adults, in whom myeloid DCs predominate. In healthy children there is a decrease in the numbers of circulating plasmacytoid DCs from birth to approximately 5 years of age,[48] whereas numbers of myeloid DCs remain relatively constant.

Neonatal DCs exhibit reduced antigen presentation compared with their adult counterparts,[44,45] and it is clear that some of the deficiencies in neonatal T-cell function can be attributed to immaturity in APC function.[49,50] Thus although neonatal T cells proliferate relatively poorly when activated by allogeneic neonatal DCs, lymphoproliferation reaches adult levels when activated by allogeneic adult DCs.[45,51] Neonatal APCs lack the capacity to deliver T_H1-polarizing signals to T cells,[4,50] although this can be overcome with the use of potent adjuvants.[52-54] The capacity to synthesize the bioactive form of IL-12, a key T_H1-polarizing cytokine, is reduced at birth and matures relatively slowly during childhood, with adult levels of synthesis not reached until adolescence.[33] A similar deficit in capacity for type I IFN production has been reported at birth,[47,55,56] although how production of this key antiviral cytokine changes with normal childhood development is unclear.

DELAYED POSTNATAL DEVELOPMENT OF IMMUNE COMPETENCE AND RISK FOR DEVELOPMENT OF ATOPIC DISEASE

As noted above, early childhood represents the period during which allergen-specific T-cell memory generation is initiated and potentially consolidated into long-term response patterns.[6,57] Earlier studies that identified allergen-responsive T cells in cord blood[58] suggested that this process might in fact be initiated *in utero* through transplacental leakage of allergen. However, recent evidence from our laboratory indicates that the responding T cells in cord blood are functionally immature RTE-bearing TCRs that are structurally different from those in mature T cells. These RTEs are immunologically naive but can nevertheless recognize allergens through low-affinity/low-specificity TCR interactions, becoming transiently activated and producing a burst of cytokines before death by apoptosis.[59] However, an additional intriguing finding from this study was that a byproduct of this nonspecific allergen recognition process was activation of previously quiescent T-regulatory cells.[59] Follow-up studies currently in progress suggest that programming of long-lived T-memory cells with conventional high-affinity TCRs generally commences in the second half of the first year of life (our unpublished observations), and the time of commencement of T-memory programming is highly variable within the overall population. During this same period, background maturational changes are also occurring within the innate and adaptive arms of the immune system, which are aimed at redressing the T_H2 imbalance that, as noted above, is the hallmark of the fetal immune system.[32]

This maturation process is driven by contact with microbial stimuli not present within the fetal environment[3] and is mediated through specific PRRs, such as CD14 and the TOLL receptors.[60] It appears feasible that signaling through these PRRs might be one of the central mechanisms by which microbial exposure can potentially protect against atopic sensitization in early life,[3] as conceptualized in the hygiene hypothesis.[61]

It is now recognized that the kinetics of postnatal maturation of T_H1 function are highly variable within the overall population, and in particular, the process is sluggish in children at high genetic risk of allergy.[3,23] Of note, this association is restricted to the $CD4^+$ T-cell

compartment; T_H1 function exemplified by IFN-γ response capacity in $CD8^+$ T cells displays a different developmental pattern and is less constrained during infancy relative to the $CD4^+$ T-cell compartment.[31] Moreover, our recent findings in a prospective study on high-risk children indicate that high-level IFN-γ production by neonatal $CD8^+$ T cells is associated with early allergic sensitization,[62] indicating marked differences between the contribution of the 2 T-cell compartments to atopy pathogenesis.

It is well accepted that there are differences in APC function between atopic and nonatopic adults.[63-65] The issue of when and under what circumstances these differences in APC function first become apparent remains to be elucidated. Variations in APC function might antedate the onset of allergic disease or alternatively might only arise as a result of allergic inflammation. In adults the numbers of circulating plasmacytoid DCs are greater in atopic than in healthy subjects,[66,67] whereas initial reports in children suggest the reverse might be true, with lower numbers of plasmacytoid DCs in children with asthma compared with in healthy control subjects.[68] Further longitudinal studies of DC numbers and function are clearly necessary to determine whether deficits in DC maturation during early childhood antedate important clinical outcomes, including allergen sensitization, development of asthma or eczema, and the risk of respiratory infections.

Variations in the functional capacity of other potential APC populations might also contribute to the risk of allergic sensitization. In particular, the capacity of neonatal monocytes to respond to external stimuli through upregulation of HLA-DR expression has been shown to reflect patterns of *in vitro* allergen-specific immune responsiveness in the overall PBMC compartment.[69] Consistent with this finding, HLA-DR expression on cord blood monocytes from individuals who manifest clinical allergic disease by age 2 years is significantly reduced relative to the population at large, suggesting relative functional immaturity at birth.[69] Reduction in capacity to synthesize IL-12 might also identify a group of children at increased risk for the development of atopy.[70,71]

The attenuation of $CD4^+$ T_H1 function in high-risk infants might be explicable by polymorphisms in the PRR genes *CD14* and *TLR2*, which have recently been identified.[72,73] These receptors are important regulators of T_H1 function, in particular production of IFN-γ and IL-12. During the initial stage of T_H-memory generation, these cytokines play key roles in driving and stabilizing the initial maturation of T_H1 clones.[2] Hence any inherent constraints on T_H1 cytokine production during this early stage of T-cell memory generation would favor default to the T_H2 pathway, thereby skewing immune responses against aeroallergens and resulting in allergic sensitization.

Additional sequelae of attenuated T_H1 function in high-risk subjects during infancy also appear likely. These include reduced capacity to respond to vaccines, including diphtheria, tetanus, acellular pertussis,[37] pneumococcal polysaccharide,[74] and BCG.[75] More importantly, attenuation of T_H1 function is associated with increased susceptibility to upper respiratory tract viral infections, such as respiratory syncytial virus (RSV), and their subsequent spread to the lower respiratory tract,[76,77] the control of which is strongly T_H1 dependent. In this regard we have recently shown in a prospective cohort study of 130 infants that slow postnatal maturation of IFN-γ response capacity was associated with acquisition of cellular immunity to RSV, the latter being a surrogate marker of infection.[37] More direct evidence for such an association has recently been provided in a larger cohort study on 285 children in whom low-level IFN-γ response capacity at birth was associated with increased risk for respiratory viral infection during the first year of life.[78] A comparable association has also been described in relation to low-level IL-12 in cord blood.[79] It is also pertinent to note the recent animal model studies from Culley et al[80] demonstrating that infection with RSV in the early postnatal period during which T_H-cell function is maximally T_H2 skewed elicits a comparably T_H2-skewed host defense response against the virus, including the programming of T_H2-polarized immunologic memory, which can promote airway eosinophilia on subsequent reinfection.

INTERACTIONS BETWEEN ATOPY AND INFECTION IN ASTHMA PATHOGENESIS

Respiratory viral infection is a well-recognized trigger of asthma exacerbations in schoolchildren, particularly those with established atopy.[81] Additionally, viral respiratory infections in infancy that spread to the lower respiratory tract and trigger wheeze are associated with increased risk for asthma over at least the ensuing preschool years,[82] suggesting a possible role for early infections in asthma initiation.

Sensitization to inhalant allergens per se has long been recognized as an important risk factor for asthma development. However, recent observations suggest that the asthma risk is magnified if sensitization manifests first in very early childhood.[83,84] It is also pertinent to note that the asthma risk associated with wheezing lower respiratory tract infection during infancy is maximal in subjects who also have atopy.[85] A recent prospective study from our group on a large birth cohort demonstrated that nonatopic children with 2 or more wheezing lower respiratory tract illnesses during their first year displayed a 4-fold risk of asthma at age 6 years, and this increased to 9-fold risk in atopic subjects (Fig 1).[84,85] Significantly, wheezing lower respiratory tract illnesses did not increase the risk for atopy development per se, indicating that although these factors are potentially synergistic, they operate through distinct causal pathways.

POSTNATAL DEVELOPMENT OF LUNG FUNCTION: A CRITICAL EARLY WINDOW FOR LONG-TERM DAMAGE?

Episodes of airways inflammation of sufficient intensity to trigger wheeze are relatively common during infancy.

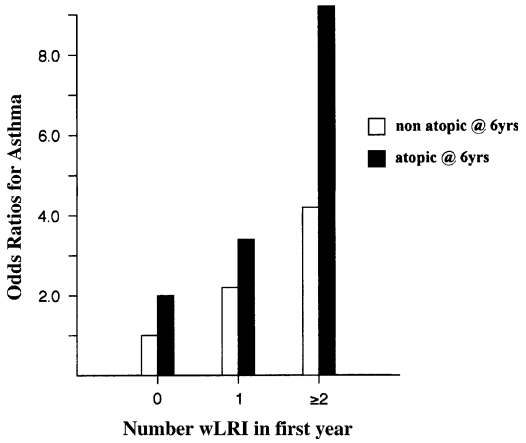

FIG 1. Asthma risk in 6-year-old children in relation to atopy and early wheeze. Data are derived from a prospective cohort study by Sherrill et al,[84] showing the number of wheezing lower respiratory tact illnesses *(wLRI)* in the first year of life.

In many cases this appears to be mainly a consequence of small airway size, and predisposition to this form of wheeze is accordingly transient and disappears with lung growth.[86] However, a recurrent theme in many of the studies cited above is the inference that in a subset of susceptible children inflammatory damage to airway tissues can have deleterious long-term consequences if the tissue-damaging events are initiated during infancy.[1] Why should this be? We argue that although the newborn immune system must program future response profiles to meet challenges that are not present in the fetal environment, the newborn respiratory system must likewise adapt to the demands of the outside world, which includes setting appropriate response thresholds to exogenous stimuli. This adaptive process within the respiratory system includes gross structural changes associated with general physical growth, which increase airway diameter and thus progressively lower risk for intermittent physical obstruction during local inflammation. More subtle processes are also involved, including alveolarization and accompanying changes in airway epithelia, which continue to progress for at least 2 years after birth. Moreover, on the basis of animal studies, it appears likely that neural control of airway smooth muscle and irritant receptor systems is also established during this same life period through local growth and differentiation of nonandrenergic noncholinergic nerves.[87] Comparable structure-function changes are also occurring contemporaneously in the parenchymal lung compartment, and abnormalities in parenchymal mechanics acquired during this period are postulated to contribute to overall lung function changes in subsequent asthma.[88]

Several recent findings support the general postulate that structure-function changes initiated during the early postnatal period can become imprinted into the long-term functional phenotype of the respiratory system.

The most direct supportive evidence comes from studies on infants that show tracking of respiratory function[89]; that is, when respiratory function is expressed as population *z* scores, subjects identified as having low respiratory function are likely to remain low as they grow over the next few years. Similar studies have demonstrated that respiratory function also tracks from childhood into adult life.[90] In addition, infants born to smoking mothers have reduced lung function at birth, and this reduced function tracks into adolescence.[91,92] Stein et al[82] have shown that RSV infection in early life is a major risk factor for wheezing at 6 years of age and is also associated with long-term persistence of low baseline lung function, which is fully correctable with inhaled bronchodilator. The most plausible interpretation of these data is an increase in their airway smooth muscle tone related to RSV infection in early life. These findings collectively suggest that phenotypic changes, which are induced in lung and airway tissues as a result of acute inflammatory insults during infancy might, analogous to the memory response in the immune system, become programmed into the long-term functional phenotype of the respiratory system.

It is additionally noteworthy that excessive deposition of extracellular matrix proteins below the airway epithelial basement membrane, a structural abnormality now recognized as one of the hallmarks of chronic asthma in the adult,[93] has recently been reported in biopsy samples from young children,[94] suggesting that these changes might also be initiated unexpectedly early in life.

A HYPOTHETICAL SCHEMA FOR THE CAUSE OF CHILDHOOD ASTHMA

Fig 2 illustrates the central elements of this model. On the basis of the available literature, we propose 2 major causal pathways for airway inflammation in early life comprising immunologically mediated damage to airway mucosal tissues driven respectively by host responses to viral antigens or to aeroallergens. The schema envisages that genetic variations in kinetics of postnatal maturation of T_H1 competence underlie variations in risk for entry into either (or both) of these pathways. It is of interest that high genetic risk in this context is defined by positive atopic family history,[23] and this subgroup now comprises up to 45% of the overall population in first-world countries.

In this schema the baseline risk for subjects who enter either of the pathways is transient airways inflammation, which generally results in (at worst) episodic wheeze. In a smaller subset of subjects, the levels of inflammatory damage in the airways will be sufficient to interfere with ongoing differentiation of local tissues, resulting in altered lung functions (eg, expression of airways hyperresponsiveness), and in this subgroup wheeze will be more persistent. In the worst-case scenario, depicted as convergence of these pathways in the same individual, the parallel streams of inflammatory tissue damage interact synergistically, promoting disease progression to full-blown persistent asthma.

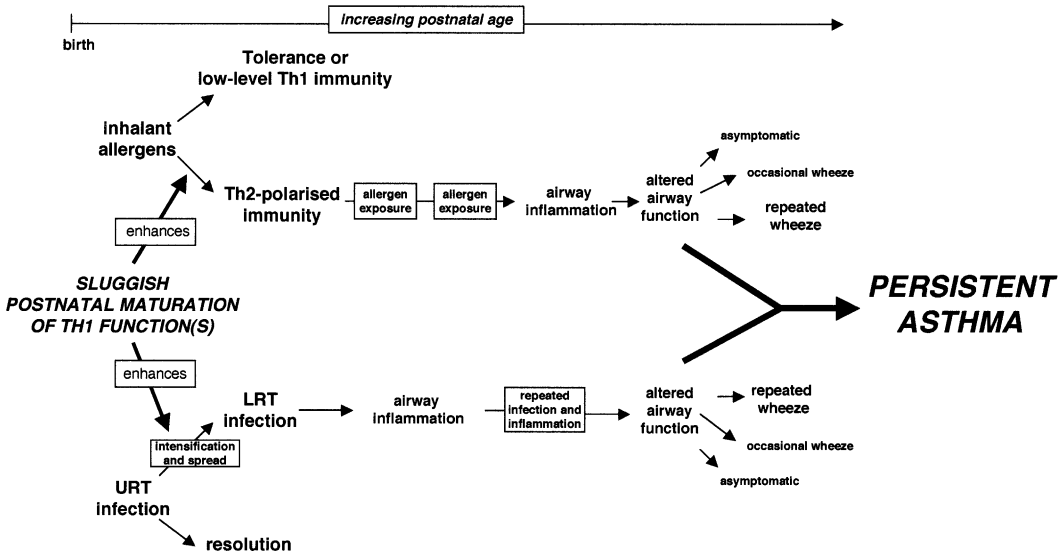

FIG 2. Interactions between airway tissue damage in early life caused by viral infections and inhalant allergy in asthma etiology. As detailed in the text, low T_H1 competence during infancy is associated with increased risk for respiratory infection and respiratory allergy, which might interact synergistically, as shown in Fig 1. *URT,* Upper respiratory tract; *LRT,* lower respiratory tract.

TESTING THE HYPOTHESIS: POTENTIAL CLINICAL TRIAL STRATEGIES FOR ASTHMA PREVENTION

At the core of this hypothesis is the concept of a critical window in early life during which immunologic and respiratory response phenotypes are most commonly programmed, and if the concept is correct, damping of the cycles of early viral or allergy-mediated damage in at-risk subjects would facilitate transit through this life phase without development of persistent disease. With respect to the allergy pathway, we have previously suggested early targeting of allergen-specific T_H2 memory development as a strategy for asthma prophylaxis.[95,96] The recent success reported in prevention of progression from allergic rhinitis to asthma in children as young as 6 years through immunotherapy[97] represents an important precedent for this approach, which, on theoretic grounds, might be even more effective in younger children in whom allergen-specific T_H2 memory is less well established and hence more susceptible to downregulation. A multicenter clinical trial protocol to test this approach in high-risk children is currently under development by the authors in collaboration with the National Institute of Health Immune Tolerance Network.

A second alternative for targeting the allergy pathway is reduction of the effect of allergy-driven inflammation on the growing lung, either through the early use of steroids[98] or more specific T_H2 antagonists, such as anti-IL-5.[99,100] A recent review[101] summarized the effects of inhaled steroids in nearly 2000 children, including a substantial number of preschool-aged children. The authors concluded that although treatment with inhaled steroids reduces symptoms and is generally safe, if used in low doses, it is uncertain whether either early introduction or long-term administration prevents development of irreversible airway obstruction. This alternative approach involving the use of selective T_H2 antagonists, such as anti-IL-5, anti-IgE, or both, has proved disappointing in relation to treatment of established atopic asthma, possibly because chronically damaged airways are hyperresponsive to a much broader range of triggering stimuli than the aeroallergen-virus combination that dominates the clinical spectrum in early life. Our recent studies in children have demonstrated a much stronger and more consistent association between allergen-induced IL-5 production, eosinophilia, or both and current asthma symptoms than is reported in adults.[102] In our view this justifies further trials with this class of drugs, although in younger age groups, with the aim of halting progression from intermittent asthma to chronic disease. These will require additional long-term safety data on the use of such drugs in young children, which should be achievable within a reasonable time frame if a systemic approach is adopted.

Targeting the viral pathway is more problematic but might hold the ultimate key to this puzzle. Respiratory syncytial virus is responsible for the majority of serious bronchiolitis occasioning hospitalization in early life, and a successful vaccine would potentially have a major effect on asthma development within the population at large, but a safe vaccine remains to be developed.

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