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Interactions between respiratory tract infections and atopy in the aetiology of asthma

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Interactions between respiratory tract infections and atopy in the aetiology of asthma. P.G. Holt, P.D. Sly. ©ERS Journals Ltd 2002.

ABSTRACT: The prevalence of asthma, in particular atopic asthma, has markedly increased in recent years. Accumulating evidence suggests that environmental factors associated with allergic sensitization and exposure to microbial stimuli during infancy and early childhood, are associated with these changes in prevalence. However, considerable controversy surrounds the role of microbial agents, as evidence has been presented for both positive and negative effects in this context.

The review below focuses upon interactions between immune competence during infancy, the development of T-helper (Th)1-polarized *versus* Th2-polarized memory against inhalant allergens, and susceptibility to virus infection. In particular, recent finding are highlighted which suggest that delayed postnatal maturation of Th1 function is associated with increased risk for early postnatal sensitization to inhalant allergens, and also with risk for viral bronchiolitis during infancy.

Variations in the kinetics of postnatal maturation of T-helper 1 function may in part be attributable to polymorphisms in the CD14 gene, which influence host responsiveness both to bacterial as well as viral stimuli.

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It is evident from a wide range of studies carried out in different geographical areas, that the prevalence of allergic diseases, and in particular atopic asthma, has increased markedly over the last 2–3 decades. Moreover, the increases become evident in successive birth cohorts during early childhood. It is also generally accepted that "diagnostic shift" is not a significant factor in these changes. Accordingly, given the timeframe over which the changes have occurred, the responsible factor(s) must be environmental, presumably interacting with one or more susceptibility genes.

The search for the relevant genetic and environmental factors continues, and a variety of potential candidates have been identified. Prominent amongst these are factors related to respiratory infections, particularly those occurring in childhood. Paradoxically, these infections have been invoked both as potential protective factors in relation to asthma/allergy development, and as triggers of asthma exacerbations in atopic asthmatics. The mechanisms by which these effects may be mediated are incompletely understood; however, recent findings suggest some intriguing new possibilities, which are discussed in this review.

T-cell immunity to inhalant allergens: the basis of variations in responder phenotype within the adult population

The epithelial surface of the upper and lower respiratory tract are continuously exposed to an array of pathogenic and nonpathogenic airborne antigens, and the induction and local expression of local T-cell immunity must accordingly be tightly controlled, in order to avoid the pathological consequences of chronic T-cell-driven inflammation. While it is highly plausible that resistance to airborne pathogens is a direct function of the speed of mobilization and the intensity of expression of local innate and adaptive immune mechanisms in the lung, the converse situation appears more complex. In particular, it is not clear how the immune system avoids continually responding to airborne antigens,

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such as pollens, which are intrinsically nonpathogenic, whilst being both highly immunogenic and ubiquitous in the natural environment.

The classical explanation for this discrimination has envisaged the mucosal immune system mounting localized secretory immunoglobulin (Ig)-A responses against such antigens. This IgA "blanket" has been perceived to function as a protective exclusion barrier to minimize the penetration of inert antigens below epithelia, thus preventing effective systemic sensitization of the immune system. However, the results of studies arising from the introduction of T-cell cloning technology into this field have necessitated a radical revision of these ideas. Notably, it is now clear that active T-cell immunity to common nonpathogenic airborne antigens is essentially universal within the adult human population, and that qualitative aspects of these immune responses determine relevant clinical outcomes (if any).

Thus, the majority of individuals contain populations of recirculating memory T-cells in their peripheral blood which recognize pollen antigens (and many other such proteins present in indoor and outdoor dusts), and in doing so are triggered to release low levels of cytokines such as interferon (IFN)-γ, which are characteristic of the T-helper (Th)1 pattern of T-cell immunity [1, 2]. Such subjects produce only low levels of allergen-specific IgG antibodies, and manifest no clinical symptoms upon exposure. In contrast, a minority of subjects contain T-memory cells, which respond to the same antigens *via* release of Th2-associated cytokines such as interleukin (IL)-4, IL-5 and IL-13 [1, 2], often in conjunction with IFN-γ (*i.e.* Th0-like; [3, 4]).

The dichotomous cytokine secretion patterns were originally defined in the murine system [5], and the extrapolation of this Th1/Th2 paradigm to the human system is not universally accepted. In particular, it has been pointed out that the hallmark of Th1 immunity in the mouse is delayed type hypersensitivity (DTH), and yet there is no evidence of allergen-specific DTH in nonatopics who ostensibly contain Th1-polarized allergen-specific Th memory cells [6]. In relation to this debate, the authors have recently employed alternative measures of T-cell immunity to analyse qualitative aspects of Th memory to allergens in humans. The authors have demonstrated [7] that during the initial phase of antigen-specific reactivation, Th memory cells exhibit characteristic patterns of expression of transcription factors (TF), which mirror those seen during cytokine-driven polarization of naive cells down the Th1 or Th2 differentiation pathways. Notably, Th2 differentiation is associated with upregulation of expression of the TF GATA-3, while Th1 differentiation is associated with a reciprocal pattern of active downregulation of the GATA-3 gene. An identical dichotomy in GATA-3 expression patterns is seen when peripheral blood CD4+ T-cells from atopics and nonatopics are stimulated *in vitro* with common inhalant allergens, such as house dust mite [7], providing independent confirmation of the applicability of the Th1/Th2 paradigm to humans.

In relation to the aetiology of atopic asthma, the central issue is then: how are these dichotomous Th memory patterns programmed?

Allergen-specific T-helper memory programming in childhood

A variety of evidence indicates that initial priming of the naive immune system against inhalant allergens occurs during early life, in many cases during late gestation. This conclusion is based on a series of studies from independent laboratories [8–12] demonstrating the presence of cells in cord blood mononuclear (CBMC) specimens, which proliferate in response to allergens. Additionally, a recent study from the authors′ group involving deoxyribonucleic acid genotyping of T-cell clones derived from such cultures has confirmed the foetal origin of the responder cells [13].

It is not clear from these studies whether initial Th-cell priming occurs *via* contact with native allergen, which is transported across the placenta, or *via* stimulation with cross-reacting antigens. However, tracking these responses postnatally in a series of cross-sectional and prospective studies from several labs (*e.g.* [14–16]) has revealed a characteristic pattern of "response maturation", which segregates atopics from nonatopics. A clear example is demonstrated in recent results from the authors′ laboratory. Firstly, these show that initial low-level responses in CBMC are dominated by Th2 cytokines [13], but are subsequently modified during infancy and early childhood. Thus, Th2 reactivity is steadily boosted in children who progress to early expression of atopy symptoms [14] and/or skin-prick test positivity to inhalants [17, 18]; whereas, these responses are diverted ("immune deviated") towards a Th1-like pattern in the nonatopics. This Th memory generation process in many individuals appears to be complete by the end of the preschool or early school years, potentially locking individuals into lifelong patterns of allergen responsiveness. However, as discussed later, this memory generation process is not the sole determinant of clinical responder phenotype, in particular in relation to asthma, as only a subset of children sensitized to inhalant allergens go on to develop persistent wheeze [19].

Genetic *versus* environmental determinants of allergic sensitization: the significance of microbial exposure in early life

The genetic basis for susceptibility to allergic respiratory disease is the subject of intensive research internationally. It is acknowledged that a variety of genes are likely to be involved in this highly complex disease. However, some key elements in the process can be deduced from studies focusing on issues relevant to Th1/Th2 responsiveness in atopics and nonatopics.

Literature from the 1970s, relating primarily to expression of severe atopic disease (especially atopic dermatitis) in childhood, contains a range of independent reports suggesting that various aspects of

adaptive immune function in affected subjects was deficient, relative to healthy aged-matched controls. More recently, the authors' laboratory has sought to extend these findings to allergic disease in general. The key findings, based upon quantitative T-cell cloning studies and cytokine analyses on extensive panels of isolated clones, indicate that an important element of genetic susceptibility to atopy involves delayed postnatal maturation of Th1 function [20]. The present authors showed that the capacity of CD4+ Th-cells from infants at high risk (HR) of atopy to produce both Th1 and Th2 cytokines was reduced relative to their low risk (LR) counterparts; however, the reduction in IFN-γ was greatest, resulting in a relative Th2 "skewing" of responsiveness in the HR children. These findings have since been extended to CBMC in confirmatory studies from a range of independent laboratories [21–26].

The present interpretation of these findings [27], based on the literature relating to the immunology of pregnancy, is that the process involving the normal transition of adaptive immune function from the foetal Th2-polarized state, to the more balanced but Th1-dominant state characteristic of the mature immune system, progresses more slowly in HR than in LR children. Furthermore, it can be hypothesized that as a result of this delay, HR children will be at heightened risk of occasional "failures" in the immune deviation process which underlies the repolarization of neonatal Th2-skewed responses to allergens towards the Th1 pattern characteristic of nonatopics [27].

The precise kinetics and nature of the mechanism(s) underlying the postnatal maturation of Th1 function are not completely understood, but recent findings in several areas are shedding fresh light on the process. Firstly, experimental studies in mouse models have demonstrated the important role of promoter methylation in silencing of the IFN-γ gene in Th2 cells [27], and recent studies from the authors' laboratory (P.G. Holt, TVW Telethon Institute for Child Health Research, Perth, Western Australia, personal communication) suggest that hyperactivity of a comparable mechanism in human foetal naive CD4+ T-cells may be responsible for their reduced capacity to transcribe the IFN-γ gene, relative to their adult CD4+ counterparts. Secondly, other previous studies from animal models [28] indicate that postnatal maturation of immune function in mammals is driven by contact with microbial stimuli not usually encountered during foetal life, and that an important part of this stimulation is directed selectively towards Th1 functions [29]. Accordingly, the authors have proposed that one of the factors underlying the differences in kinetics of the Th1 maturational process may involve genetically determined variations in capacity to respond to these microbial stimuli in early life [26]. One example of this may underlie the findings of SHIRAKAWA *et al.* [30], which indicate that failure to generate long-term Th1-polarized memory in response to bacille Calmette-Guérin immunization during infancy, is associated with heightened risk for subsequent development of atopy and asthma.

A second example of a pathway, which is likely to be of major importance in this context, involves the CD14 gene encoding the high affinity receptor for bacterial lipopolysaccharide (LPS). It is highly likely that LPS, by virtue of its capacity to stimulate Th1-trophic IL-12 production *via* soluble CD14 dependent mechanisms, plays a key role in postnatal upregulation of Th1 function, and the authors have hypothesized that normal gastrointestinal bacterial flora may play a key role in this process [31]. Recent findings from MATRICARDI *et al.* [32] suggest that gastrointestinal pathogens potentially exert similar effects.

The present authors have additionally collaborated with BALDINI *et al.* [33] in identification of a polymorphism in the CD14 gene, which is associated with reduced levels of soluble CD14 in serum, decreased Th1 function, and increased intensity of atopic sensitization. This polymorphism may have additional implications, given the recent report demonstrating a key role for CD14 in mediating the innate immune response to respiratory syncytial virus (RSV) [34]. It is feasible that further polymorphisms in genes related to the recognition of microbial (particularly viral) stimuli await discovery.

In this regard, it is also pertinent to note the recent reports suggesting that exposure to LPS in airborne dusts during childhood appears to be inversely related to sensitization to inhalant allergens [35, 36]. A potential explanation for these findings involves the known stimulatory effects of LPS on dendritic cell (DC) populations in the airway mucosa (discussed later).

The apparent dualistic effects of respiratory tract infection on asthma and allergy

It is clear from studies in school children that respiratory viral infections are potent triggers of asthma exacerbations, in particular in atopic asthmatics [37]. It is also evident that lower respiratory tract infections during infancy, in particular those which occur at a level of severity sufficient to trigger wheeze, are associated with increased risk for subsequent development of persistent airway hyperresponsiveness (AHR; [38, 39]). In relation to acute exacerbations, while the precise mechanisms remain to be defined, it is likely that the onset of wheeze is at least partially the result of the summation of airway tissue damage resulting from the host response to the infection, together with that stemming from ongoing Th2-mediated immuno-inflammatory responses to inhalant allergens.

One factor which contributes significantly to the confusion surrounding the relationship between respiratory infections and childhood asthma is the lack of clear phenotypic descriptions of asthma in children. The old adage that "all that wheezes is not asthma" almost certainly applies here. At least three distinct wheezing syndromes can be recognized in children, each of which have different risk factors and may have different prognostic implications. These syndromes have been called by various names, but a consensus appears to be forming around the terms

"transient infantile wheeze", "viral-associated wheeze" and "atopic asthma". Whether these three conditions are different parts of a single disease spectrum or represent different diseases remains to be established.

Wheezing in the first year of life is common, with 30% of infants having at least one wheezy episode in some series [40]. Transient infantile wheeze describes a condition where the infant wheezes during the first year or two, but not later. This syndrome appears to be more common in infants born to mothers who smoke and to young mothers and is not associated with an increased prevalence of atopy later in life. Viral-associated wheezing is very common in young children in the preschool and early school years. Many studies have shown an increased prevalence of wheezing during childhood following RSV infection in infancy, but none show an increase in the prevalence of atopy in these children. Recently, STEIN *et al.* [41] have demonstrated that RSV infection in early life is a risk factor for wheezing up to 6 yrs of age, but that this risk has gone by 13 yrs. Again, the children in that study who had RSV infection in early life did not have an increased prevalence of atopy at 13 yrs. Viral-associated wheeze may be responsible for the majority of wheezing episodes in young children and may represent the majority of doctor-diagnosed asthma occurring in this age group. If this is the same syndrome as that described by STEIN *et al.* [41], it may also be responsible for the widely-held view that many children "grow out of" their asthma.

In relation to the longer term effects of infections occurring in infancy, it has been hypothesized that severe airway inflammation during this critical phase of rapid lung growth can initiate phenotypic changes in airway tissues, which are amplified during subsequent growth and differentiation, resulting eventually in expression of AHR [38, 39]. It has also been hypothesized that certain viruses, in particular RSV, have the potential to selectively stimulate "bystander" Th2 responses [42, 43], and thus amplify the development of Th2-polarized immunological memory against inhalant allergens. However, these effects may be transient, given the results of a recent long-term follow-up study, which failed to detect a link between early RSV infection and long-term expression of atopy [41].

Conversely, it has also been suggested that other respiratory infections, either due to the time at which they occur or to their particular localization and/or level of severity, may have opposite long-term effects, and instead protect against allergic sensitization and/or asthma development [44–47]. For example, American children, who develop more than one nonwheezing lower respiratory tract infection before the age of 3 yrs, display a marked reduction in atopy prevalence [48], and Estonian schoolchildren display very low allergy prevalence, but experience more prolonged and intense respiratory infections than Western European children in whom allergy is frequent [49]. One plausible mechanism for such effects involves infection-mediated stimulation of airway mucosal DC populations, which regulate the Th1/Th2 balance in immune responses to inhaled antigens [50]. These DCs rely absolutely upon the receipt of appropriate inflammatory cytokine signals, and/or stimulation from microbial products, such as LPS or viral nucleic acid, in order to upregulate their production of IL-12, which they utilize in driving Th-cell responses down the Th1 differentiation pathway [51]. In immunocompetent adult experimental animals, airway DC networks are highly responsive to viral/bacterial stimulation [52], but this and related T-cell regulatory functions are attenuated in infant animals [53, 54] and require prolonged stimulation to promote functional maturation. A recent study suggests that human airway DC populations are normally at a similar low baseline activity state during infancy, and they may upregulate to a significant degree in response to severe infection [55]. Furthermore, this may represent a mechanism by which certain infections in early life could help to tip the balance towards development of Th1 as opposed to Th2 immunity to ubiquitous aeroallergens. One prediction of such a model would be that such potentially "protective" infections may be restricted to those which are localized to the mucosae of the upper airways, which represent the principal sites for the deposition of inhaled allergens.

At present, these conflicting notions appear difficult to reconcile. However, a number of authors have suggested that the nature of the relationship(s) between infection and asthma/atopy in early life may be more complex than previously considered. For example, it has been suggested that RSV-induced wheeze in infancy may be a marker for pre-existing atopic predisposition, as opposed to a stimulant for Th2 differentiation [50]. Recent results from studies relating to host responses to RSV in early life are beginning to clarify this issue, and these are discussed in the conclusion of this review.

Interactions between infection and allergic sensitization in asthma and childhood: dissection of cause/effect relationships

As noted earlier, a key issue that requires further clarification concerns the nature of the short- and long-term sequelae of lower respiratory tract infections, in particular the nature of the cause/effect interrelationships between atopy/asthma.

The scheme mentioned later and detailed in figure 1 illustrates the potential causal pathways in the aetiology of asthma, representing the authors′ current view of how a significant proportion of the contentious literature in this area can be reconciled. The following principal observations underpin the atopy component of this scheme. 1) Allergen-specific Th-cell priming occurs in the perinatal period [8–13], and these early immune responses become compartmentalized into Th1- or Th2-polarized memory during the preschool years [14–18]. 2) The symptomatology associated with the sensitization to inhalants during childhood does not usually progress beyond intermittent wheeze, and severe long-term sequelae appear restricted to a subset of atopics [19] manifesting high levels of Th2-mediated inflammation [56–58] and subsequent airway remodelling [57, 58]. 3) The excessive airway inflammation in this subset implies

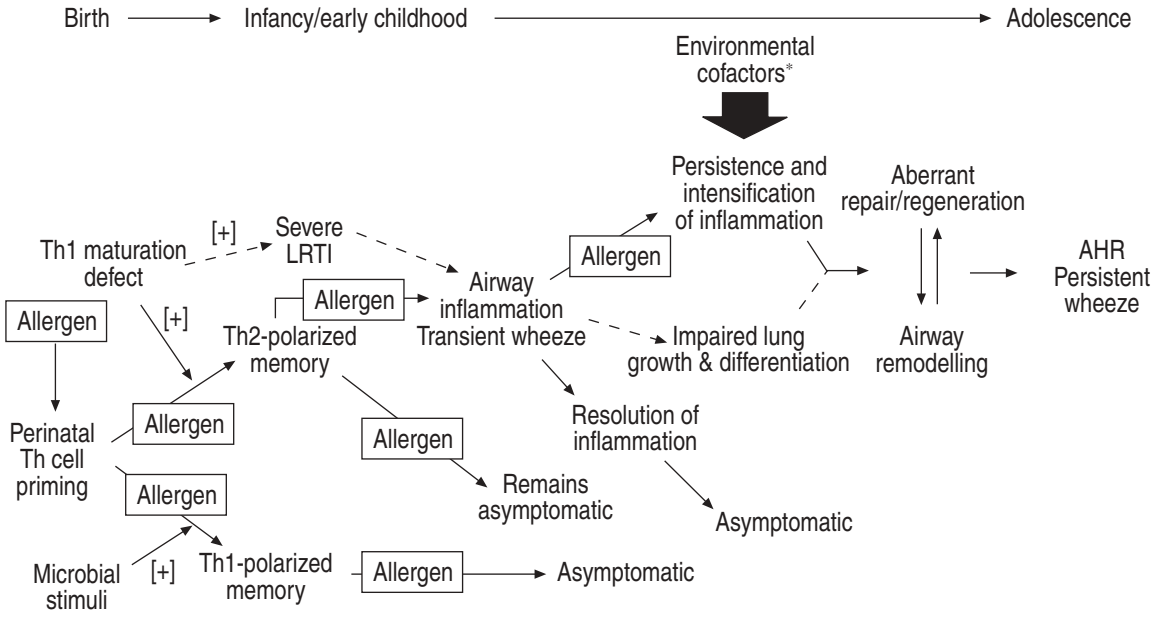

Fig. 1.–A multistage model for interactions between infections and atopy in the aetiology of asthma. LRTI: lower respiratory tract infection; AHR: airway hyperresponsiveness; Th: T-helper cell; [+]: stimulates or predisposes to. *: includes virus infections, environmental irritants, allergen load/type, *etc*. See text for background references relating to the construction of these pathways.

the existence of additional host and environmental cofactors which are "pro-inflammatory"; these may include genetic factors associated with dysregulation of immunological control mechanisms within the airway mucosa, and a series of environmental factors [58]. 4) The earlier that Th2-polarized memory against inhalant allergens develops, the more severe are the long-term consequences in relation to AHR [59, 60], implying that repeated cycles of airway inflammation during rapid lung growth may establish some form of "matrix" for development of subsequent changes associated with airway remodelling. 5) Genetic risk for atopy is associated with sluggish postnatal maturation of adaptive immune function, in particular, Th1 function [20, 27].

With respect to the infection component in the scheme, the relevant recent observations are as follows: 1) genetic risk for atopy has been reported to be associated with increased susceptibility to severe RSV infection and bronchiolitis [61]; atopy in children has also been linked to increased susceptibility to measles-mumps-rubella [62] as well as to frequent upper respiratory tract infections [63]; 2) IFN-γ responses in peripheral blood mononuclear cells are attenuated during acute RSV infection in children who develop bronchiolitis [59–69], suggesting that downregulated Th1 function may be associated with pathogenesis; 3) susceptibility to development of acute RSV bronchiolitis during infancy is associated with decreased levels of IL-12 in cord blood relative to subjects who do not develop bronchiolitis [70], suggesting a pre-existing deficiency in Th1 function in this group; 4) nonatopic wheezing children, in whom the principal stimulus for wheeze is viral infection *per se*, also have manifest reduced IFN-γ responses [71]; and 5) a recent study by the authors' group on a prospective cohort of infants followed to 18 months indicated that the development of T-cell memory to RSV (as a surrogate marker for RSV infection) during the observation period was inversely related to kinetics of postnatal maturation of IFN-γ responses [72].

Collectively, these findings suggest that a comparable genetic defect, associated with delayed postnatal maturation of Th1 function, may underlie susceptibility both to atopic sensitization and the development of severe respiratory viral infections, as well as their spread to the lower airways. Such infections trigger wheeze in the short term, and in a significant proportion of cases they may be of sufficient severity to subtly impair ongoing lung growth and differentiation, thus initiating changes, which subsequently synergize with those resulting from later episodes of airway inflammation, triggered by other stimuli such as allergens.

The present scheme cannot account for all of the potential roles of viral infections in the aetiology of asthma, in particular those associated with adult-onset nonatopic asthma, although the findings of Leech *et al.* [71] may be relevant to this issue. However, the scheme does provide a rational framework for future systematic research into this complex problem.

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