Programming for Responsiveness to Environmental Antigens That Trigger Allergic Respiratory Disease in Adulthood Is Initiated during the Perinatal Period

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Allergy to airborne environmental antigens (allergens) is a major cause of asthma in children and adults. This review argues that the development of allergen-specific immunologic memory of the type that predisposes to allergy development is the end result of a T-cell selection process operative during infancy, which is triggered via encounters between the immature immune system and incoming airborne allergens from the environment. In normal individuals this process leads to the development of allergen-specific T-memory cells that secure the T helper (Th)-1 pattern of cytokines, which actively suppress the growth of their allergy-inducing Th-2 cytokine-secreting counterparts. However, these protective allergen-reactive Th-1 memory cells fail to develop in some individuals, permitting the subsequent proliferation of allergen-specific Th-2 cells that can trigger allergic reactions. Recent evidence suggests that genetic predisposition to allergy may be due in part to hyperactivity of control mechanisms operative *in utero* and which normally protect the fetoplacental unit against the toxic effect of Th-1 cytokines. — *Environ Health Perspect* 106(Suppl 3):795–800 (1998). *http://ehpnet1.niehs.nih.gov/docs/1998/Suppl-3/795-800holt/abstract.html*

Key words: allergy, asthma, environmental allergens, T cells, cytokines

Introduction

There is increasing evidence that the prevalence of allergic respiratory diseases, atopic asthma in particular, is currently rising at an unprecedented rate (*1–4*). It is also becoming clear that this rise is largely restricted to First World countries, to the extent that it is widely believed to be linked to Western lifestyle (*5,6*). A wide range of environmental antigens, or allergens, have been identified as eliciting agents, the most potent of which are found in the indoor environment (*7*). Comparative studies on populations in the western versus eastern (formerly socialist) parts of Germany suggest that this rise in the west commenced in the late 1950s or early 1960s coincident with the surge in postwar economic growth, whereas prevalence rates in the economically stagnant east remained stable (*2*), and similar trends are emerging from studies in Scandinavia and the Baltic States (*2*).

Collectively, these epidemiologic findings argue strongly that environmental factors are primarily responsible for the changes in disease prevalence, and the debate is intensifying internationally as to the precise nature of these factors. The list of candidates are legion and include diet, housing design, population density, and allergen load in the environment. Particular interest is focused on air pollution and respiratory infections and how these factors interact with the components of the immunoinflammatory system that are associated with the manifestations of allergy (*8–10*).

To determine how these environmental factors operate, it is necessary to first gain a broad understanding of the cellular and molecular mechanisms underlying the induction and expression of allergic reactions; the purpose of this review is to highlight a series of recent advances in this rapidly moving field. In particular, given the compelling findings in the current epidemiologic literature, which indicate that these time-related rises in prevalence of allergic respiratory disease manifest initially in young children, the principal focus of this review is the issue of primary allergic sensitization in early life.

Why Allergy?

The disease asthma is clearly a multifactorial process, but there is now an international consensus that an essential feature of the disease is inflammation of the airway mucosa. The latter may be triggered by chemical irritants, virus infection [particularly in infants with small airways (*11*)], or environmental allergens (*12*). All of these factors are potentially interactive; in particular, allergic asthma can be exacerbated by viral infection (*13,14*) or air pollutants (*15*).

However, seroepidemiologic studies on large populations indicate a strong correlation between serum titers of immunoglobulin (Ig)E (the allergic antibody) and the manifestation of asthma in all age groups (*16*), which points to allergy as a major determinant of the asthmatic phenotype. In this context, it is also of interest to note that recent comparative studies on the immunopathology of atopic (allergic) versus intrinsic (of hitherto unknown etiology) asthma have demonstrated virtually identical immunoinflammatory infiltrates in airway biopsy samples from patients with these two forms of the disease (*17*).

Allergy Effector Mechanisms: Induction and Regulation

Allergic reactions are the result of a cellular cascade that occurs over a distinct biphasic time scale. The initial or immediate phase of allergy occurs within seconds to minutes of encountering an antigen (namely, an allergen) to which the subject has previously been sensitized. The allergen becomes bound to a specific IgE antibody attached to the surface of mast cells, which are abundant in tissues at the body's mucosal surfaces. The formation of IgE–allergen complexes on the surface of the mast cells triggers the rapid release of intracellular granules containing edema-inducing

This paper is based on a presentation at the First National Conference on Children's Environmental Health: Research, Practice, Prevention, and Policy held 21–23 February 1997 in Washington, DC. Manuscript received at *EHP* 31 October 1997; accepted 9 February 1998.

The author's laboratory is supported by the National Health and Medical Research Council of Australia and Glaxo Wellcome, United Kingdom.

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Abbreviations used: APC, antigen-presenting cell; DC, dendritic cell; IFN, interferon; Ig, immunoglobulin; IL, interleukin; PBL, peripheral blood leukocytes; Th, T helper; Th-0, immunologically naive T helper.

vaso-active amines such as histamine, causing local swelling that (if it occurs in the airways) can result in bronchoconstriction. This immediate response is followed by the slower release from mast cells of other classes of bioactive molecules, in particular cytokines and chemokines, which recruit other cell types into the inflamed tissue site. This second wave of cells, in particular T lymphocytes and eosinophils (*18*), steadily accumulates over the next 6 to 12 hr, becomes activated, and releases a different range of inflammatory mediators, provoking a late phase response that can result in more severe and prolonged bronchoconstriction in the airways than is seen in the acute phase.

This inflammatory cascade also involves contributions from platelets, polymorphonuclear leukocytes, basophils, monocytes, macrophages, and dendritic cells, and parallel contributions from acellular inflammatory elements such as the complement system.

However, there is one cellular mechanism that is an absolute prerequisite for the manifestation of allergic reactivity, namely, the development of T-lymphocyte responsiveness to the allergen. These T lymphocytes determine the level of involvement of the most potent cellular mediators of allergic tissue damage, mast cells and eosinophils, in the host response. They regulate the former by secretion of the cytokine interleukin (IL)-4, which controls the synthesis of the IgE antibodies that arm mast cells, and they also regulate eosinophils through production of IL-5, the principal cytokine that controls eosinophil differentiation and activation (*19,20*). Unless T lymphocytes that have become sensitized (i.e., immune) to environmental allergens are present and can thus respond upon subsequent allergen reexposure by secretion of IL-4 and IL-5, allergic reactions cannot be initiated.

Hence, the principal focus of immunologic research in the asthma and allergy area has progressively turned toward the issue of T-cell reactivity to allergens, in particular the question of how sensitivity to different classes of environmental allergens encountered at the body's mucosal surfaces is programmed into long-term immunologic memory.

Regulation of T-Cell Immunity at Mucosal Surfaces: Insight from Experimental Models

The host response to low-level antigen exposure at mucosal surfaces is regulated via the immunologic equivalent of the yin–yang principle, as described in Figure 1 (*19,20*). The response is initiated by specialized antigen-presenting dendritic cells (DCs), which are distributed as a tightly meshed network within the epithelium overlying the body's mucosas, in particular in the respiratory tract (*21–23*). Their function is to sample incoming inhaled antigens and transport them to regional lymph nodes for presentation to T lymphocytes (*23,24*). In the scheme detailed in Figure 1, the initial T lymphocyte stimulated is an immunologically naive precursor (T helper-0 [Th-0]) bearing a receptor that is capable of recognizing DC-bound antigen. This recognition event triggers the Th-0 cell into clonal expansion down one of two optional differentiation pathways, the choice of which is determined by the balance of cytokines present in the surrounding milieu. Th-0 cells automatically secrete low levels of IL-4 following initial stimulation (*25*), and in the absence of countermanding signals, this cytokine promotes the differentiation of Th-0 into T helper (Th)-2 cells, which (in the mouse) secrete IL-4, IL-5, and IL-10. However, if significant levels of other cytokines that can counteract the effects of IL-4 are present (in particular IL-12 from nearby macrophages or activated DCs, or interferon [IFN] from cytotoxic T cells or natural killer cells [Figure 1]), the differentiation of Th-0 cells may be pushed toward the Th-1 phenotype, which all are major producers of IFN-γ. Downstream of these initial events, a further level of competitive cross-regulation occurs between growing clones of Th-1 and Th-2 cells, involving the secretion of Th-2-inhibitory IFN-γ by Th-1 cells and secretion of Th-1-inhibitory IL-10 by Th-2 cells.

Eventually, one of these mutually antagonistic Th-cell phenotypes gains dominance in the developing immune response, progressively locking out its competitors (*26*). This lock is programmed into long-term immunologic memory and is reinforced at each subsequent exposure via the preferential stimulation of the dominant Th-cell phenotype. This overall process is known as immune deviation.

In such a scheme, if initial immune responses to an inhaled environmental allergen led to the selection of Th-2 memory cells, subsequent reexposures would progressively expand the number of these cells at the periphery, stimulating increasingly more vigorous IL-4- and IL-5-dependent IgE, mast cell, and eosinophil responses of the type associated with allergy. Conversely, if the response was initially polarized toward the selection of IFN-γ-secreting Th-1 memory cells, reexposure would stimulate the production of IgA and IgG subclass antibodies, which assist in rapid clearance of allergen from the body, thus minimizing involvement from inflammatory cells.

Regulation of immune responses to environmental allergens encountered at mucosal surfaces in the gastrointestinal tract involves immune deviation, but in situations where high concentrations of food allergens are involved, a second series of control mechanisms becomes operative, which results in the clonal deletion of

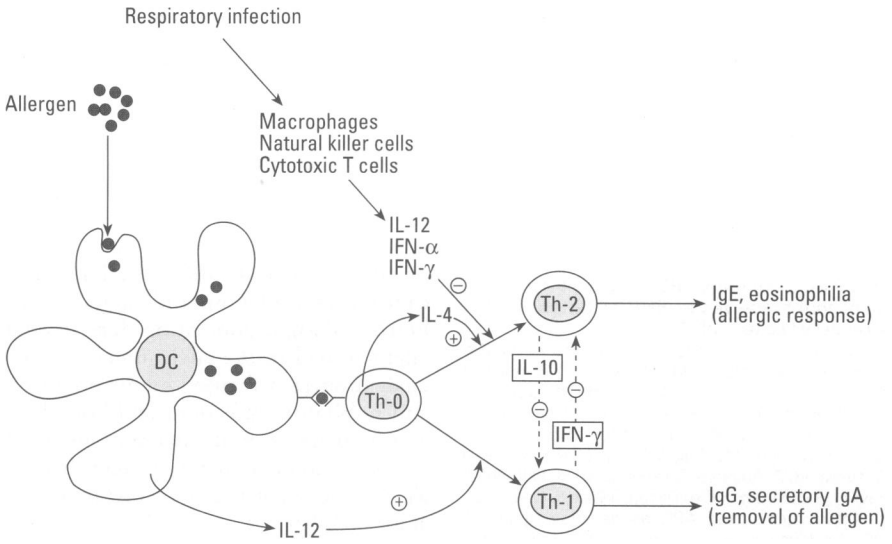

Figure 1. Cytokine-driven selection of alternate forms of allergen-specific immunologic memory via immune deviation.

responder T lymphocytes (*27*). The tandem operation of these two regulatory processes summate to the phenomenon of oral tolerance, which is believed to be the principal mechanism that protects against food allergy and associated immunologic enteropathies.

It is of interest to note that both oral tolerance and its respiratory tract equivalent function poorly in newborn animals, and that these mechanisms do not become fully functional until around the time of weaning (*27*).

Regulation of T-Cell Immunity to Environmental Allergens in Humans: Application of the Murine Th-1 and Th-2 Paradigm

Research over the last 6 to 7 years, in particular in the area of T-cell cloning, has established that it is the nature of an individual's immune responsiveness to environmental allergens, as opposed to the expression of active T-cell immunity per se, that determines clinical responder phenotype. Thus, individuals expressing allergy to antigens such as pollens (e.g., as rhinitis or bronchial asthma) contain circulating pollen-reactive T cells that can be isolated by cloning techniques and that upon *in utero* stimulation with the same allergen will secrete Th-2 cytokines such as IL-4 and IL-5. In contrast, nonallergic individuals contain allergen-reactive T cells that respond via secretion of Th-1 cytokines such as IFN-γ, with little accompanying IL-4 or IL-5 activity (*28,29*). These differences are not necessarily absolute, but instead reflect the nature of equilibrium in respective immune responses, i.e., allergen-specific responses in atopics are dominated by Th-2 cytokines, whereas Th-1 cytokines are dominant in the same responses in normals. This presumably indicates the relative frequency of Th-1 and Th-2 cells in respective Th memory compartments.

Allergen-Specific T-Helper Memory Development in Humans: How and When?

The evidence from animal model systems suggests that the key events determining the nature of T-cell memory to mucosally presented allergens occur at or around the time of the first few exposures. Accordingly, there is increasing interest in the nature of allergen-specific T-cell immunity to these antigens during infancy and early childhood, the period during which the human immune system ostensibly first encounters these agents.

Recent studies reviewed by Holt (*27*) demonstrate that IgG and IgE antibody production against food and inhalant allergens is indeed initiated during infancy, which pinpoints this life period as a crucial phase in the process underlying development of allergic disease. Prospective cohort studies examining both antibody (*29*) and T-cell lymphoproliferative responses (*30*) suggest that repeated exposure to environmental allergens during this period stimulates a variety of different T-cell regulatory mechanisms depending on the levels of allergen exposure. Notably, high-dose exposure to food allergens appears to trigger high-zone tolerance mechanisms, leading in most individuals to the efficient deletion of the bulk of food allergen-specific T cells. In contrast, exposure levels to inhalant allergens are 1 to 2 orders of magnitude lower and instead preferentially trigger immune deviation mechanisms that select for allergen-specific Th-2 memory cells in atopics or Th-1 memory cells in normals (*27,30,31*).

On this basis, respiratory allergy (including atopic asthma) can be viewed as a failure of the immune deviation mechanisms that should normally select for Th-1 memory cells during these allergen-driven immune responses in early life. The reason for the disparity between atopics and normals is unknown, but tantalizing hints as to the nature of the underlying Th-cell selection mechanisms have appeared in the very recent literature (*32,33*), in particular that relating to the immunology of pregnancy.

Work from a number of groups [reviewed by Wegmann et al. (*32*) and Mosmann and Sad (*33*)] has now established that the cytokine milieu at the feto-maternal interface is constitutively skewed away from the Th-1 phenotype. This apparently represents an evolutionary adaptation designed to protect the integrity of the feto-placental unit, which is exquisitely sensitive to the toxic effects of Th-1 cytokines such as IFN-γ (*34,35*). One of the major mechanism(s) underlying this process involves the constitutive secretion of Th-2-selective cytokines such as IL-4 and IL-10 by placental cells (*32,33*)—trophoblasts in particular (*36*). This blanket of Th-2 cytokines has the effect of skewing neonatal responses towards selection for Th-2 memory cells.

It is also evident that this Th-2 skew is maintained for varying periods postnatally, as infant mice display preferential expansion of Th-2 memory cells during the preweaning period (*37,38*). Indirect evidence strongly suggests that the same situation applies in humans. It is widely recognized that peripheral blood leukocytes (PBL) from human infants display poor IFN-γ-secreting capacity, which can persist for up to 5 years postnatally (*39*).

The Sensitization Window during Infancy

It is of further interest to note that infant (*40*) and neonatal (*41–43*) PBL from subjects with atopic family history manifest significantly lower IFN-γ-secreting capacity than their family history negative counterparts, suggesting that hyperactivity of the mechanism(s) responsible for maintenance of the Th-2 skew during fetal life may be inherent in the atopic genotype.

This provides an explanation for one of the enigmas of the allergy literature, notably the existence of a transient high-risk window for allergic sensitization during infancy. The latter concept derives from a wide body of epidemiology demonstrating that exposure to relatively high levels of inhalant allergens during the first few months of life (exemplified by birth during the pollen season) is associated with markedly increased risk for expression of allergic respiratory disease in response to exposure to the same allergens in adult life (*2,44*), particularly in infants with positive atopic family history (*45*). These findings are not universally accepted, as some studies suggest that the highest risk is associated with birth 2 to 3 months before the peak of the pollen season (*46*), whereas others have failed to detect such relationships (*47*). However, it has been argued that these contrasting findings may be due to variations in the degree of fluctuation in aeroallergen levels throughout the year in different geographical areas.

In the context of the many studies where birth-month effects have been detected, it can be argued that initial exposure of newborns to high levels of environmental allergens at a time when their immune systems are still partially locked into the Th-2-skewed cytokine phenotype characteristic of fetal life would maximize the likelihood of development of potentially pathogenic Th-2-skewed allergen-specific Th-cell memory. Furthermore, this risk may be highest in infants of the atopic genotype, in whom the perinatal Th-2 skew appears strongest.

Evidence is also emerging that this risk may be further amplified via prenatal allergen exposure. Reports from several independent laboratories suggest that initial priming

of the fetal immune system against environmental allergens can occur *in utero* in many instances (*30,42,48–50*), possibly via transplacental transport of allergen-specific IgG antibody and low levels of native or processed allergen from the maternal circulation (*30*). Moreover, recent work from our laboratory indicates that these early T-cell responses are dominated by Th-2-selective cytokines, in particular IL-10 (*51*).

Cellular Mechanism(s) Underlying the Th-2 Skew during Infancy

A further series of publications during the last year from yet another area of the perinatal immunology literature has provided further insight into this process and may prove to be a major watershed in the allergy and asthma literature. These publications concern the phenomenon of neonatal tolerance, an experimental system that has been studied extensively since the 1960s and has provided much of the theoretical background to our current understanding of discrimination between self and nonself antigens in the immune response.

The basis for this phenomenon was believed to be a general hyporesponsiveness of the immature immune system in neonates to parenteral immunization with foreign antigen. This maturational defect is not absolute, as demonstrated by the positive antibody responses to infant vaccination observed in both experimental animals and humans. However, recent studies indicate significant qualitative differences between the same responses in infants and adults. Indeed, in a variety of experimental systems, neonatal experimental animals thus immunized develop tolerance to the antigen, characterizeed by an apparent failure to recognize the antigen if it is presented to them subsequently during adulthood. It was generally believed that some malfunction in the immature neonatal immune system led to the clonal deletion of the T cells that responded to the antigen at the time of initial immunization. However, the assay systems that have been used to test responses in ostensibly tolerant mice were almost exclusively Th-1-dependent, e.g., IgG production, delayed-type hypersensitivity response, graft versus host response, etc. What in fact occurs is that neonatal priming triggers low-level Th-2 memory cell development because of the covert operation of the fetal Th-2-skewing process described above. Upon subsequent rechallenge with the same antigen in adulthood, the neonatally primed Th-2 memory cells are activated and lock out Th-1 responses via immune deviation (*52,53*).

Importantly, the step in the immune response in infant mice at which this lock out occurs has been narrowed down to a singular cellular locus involving the activity of the antigen-presenting cells (APC) that initiate T-cell activation (*52*). The principal APC population responsible for stimulating primary immune responses involving Th-0 cells is the DC. In immunocompetent adults, DCs preferentially stimulate Th-1 memory cell development, but in infants they lack this capacity and instead promote Th-2 responses. If their activity is bypassed by adoptive immunization of newborns with antigen-pulsed syngeneic adult DCs, the ensuing immune response leads to adult-equivalent Th-1 memory development (*52*).

The precise nature of the functional differences between adult and neonatal DC remains to be established. A likely candidate is capacity for production of IL-12, the principal cytokine employed by adult DCs for positive selection of Th-1 memory cells [(*54,55*); Figure 1], and our laboratory is currently focusing on this question in relation to human neonatal DCs.

It is also interesting to note in this context that the reduced capacity of human infant T cells to secrete IFN-γ in response to polyclonal stimulation can be partially corrected by supplementation of the cultures with maternal peripheral blood accessory cells (*56,57*), which again suggests that the general findings in the mouse model are likely to be broadly applicable to humans.

In relation to infant immune responses to the airborne environmental allergens associated with atopic asthma, the relevant APC population involved in Th memory development is the airway intraepithelial DC described above (*21–23*). This network is functionally deficient in rats during infancy and develops functional competence at a slower rate than DCs in other mucosal or lymphoid compartments, hinting at an important role for local tissue factors in the maturation process (*39,57*). Local tissue inflammation appears to be one factor that hastens postnatal maturation of these cells (*56*).

Conclusions

It is becoming increasingly clear that in many individuals the expression of allergic diseases such as atopic asthma is attributable to immunologic errors occurring during infancy, which result in inappropriate selection for allergen-specific Th-2 memory cells. It is also likely that a common cause of such errors may be the sluggish postnatal maturation of the Th-1-selective functions of APC, in particular airway mucosal DC populations.

The prime function of DCs is believed to be generation of protective Th-1 immunity against microbial pathogens presented at mucosal surfaces (*52,58*). Breakdown products of the pathogens themselves (especially bacterial lipopolysaccharides) provide one of the major stimuli for DCs to secrete the potent Th-1-selective cytokine IL-12.

The second major stimulus for IL-12 production by DCs is interaction with CD40 ligand on the surface of activated T cells. However, neonatal and infant T cells are deficient in CD40 ligand expression (*59*), and hence in the absence of strong signaling via CD40 ligand, IL-12 production by DCs in early life may be more dependent on microbial stimulation than is the case in adulthood. This may account in part for the apparent protective effects of respiratory infections in early life in relation to subsequent allergy development, and in turn for some of the differences in prevalence of asthma and atopy related to geography and social class (*2,3,8–10*).

To what extent can these new findings be usefully applied to primary prevention of allergic respiratory diseases? At present this question remains within the realms of research, but a variety of novel strategies based on these concepts are currently under development in several centers.

These strategies include: inhalant allergen avoidance measures aimed at limiting allergen exposure during the period of early infancy in which the fetal Th-2 skew is most marked; development of pharmacologic adjuvant strategies that aim to mimic the DC-stimulatory effects of microbial agents without the attendant tissue damage caused by infections; and immunotherapeutic intervention in allergen-specific immune responses during infancy to directly stimulate Th-1 memory development via a vaccinelike approach (*60*).

The alarming upward trend in the prevalence of atopic respiratory disease in our communities, and the spiraling costs of associated health care, provide urgent imperatives for the vigorous pursuit of these and related strategies for primary disease prevention.

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