

P.G. Holt
C. Macaubas
S.L. Prescott
P.D. Sly

Microbial stimulation as an aetiologic factor in atopic disease

Authors' affiliations:

P.G. Holt, C. Macaubas, S.L. Prescott, P.D. Sly,
Division of Cell Biology, TVW Telethon Institute
for Child Health Research, West Perth, Australia

Correspondence to:

Professor P. G. Holt
Division of Cell Biology
TVW Telethon Institute for Child Health
Research
PO Box 855
West Perth WA 6872
Australia
Tel. 61 8 9340 8592
Fax: 61 8 9381 8086
Email: patrick@ichr.uwa.edu.au

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Epidemiologic evidence from various sources suggests that exposure to microbial stimuli during early childhood can influence the induction and expression of atopic diseases, particularly in the respiratory tract. Moreover, these effects may have long-lasting consequences in relation to expression of the atopic phenotype in adulthood. This review discusses key aspects of this evidence in relation to the underlying mechanisms which regulate T-helper (Th)-cell function; in particular, the generation of Th-memory cells responsive to inhalant allergens.

It is now recognized that T-cell immunity in adults to common airborne environmental allergens is virtually universal (1), and that clinical responder phenotype is determined by the underlying cytokine balance within respective allergen-specific Th-memory populations. Thus, nonatopics typically manifest responses dominated by Th-1 cytokines, particularly IFN- γ , while responses in atopics are polarized toward the Th-2 cytokine profile in which IL-4, IL-5, and IL-13 appear to be predominant (2, 3). Clearly, if the mechanism(s) responsible for the disparity in responses between atopics and nonatopics can be elucidated, it should be feasible to develop new therapeutic (or, ideally, prophylactic) strategies to inhibit the disease process.

Atopy is a complex disease process resulting from interactions between genetic and environmental factors, several of which are reviewed in detail elsewhere in this supplement. This discussion will focus upon the potential role of infectious and commensal microorganisms in this disease, particularly during the early postnatal period.

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Host immune responses to allergens and infections: reciprocity resulting in antagonism?

The notion that certain types of infections may actively inhibit the development of allergy derives from the animal models which originally demonstrated the heterogeneous nature of Th-cell activity, notably the existence of cross-regulating Th-1 and Th-2 subsets which compete for dominance in immune responses via the secretion of mutually antagonistic cytokines (4). In these models, IFN- γ -dependent cellular immune responses central to the host response to infections are reciprocally related to IL-4-dependent humoral immune responses. The subsequent elucidation of the Th-1-selective role of IL-12 and IFN- α , which are produced during infection by stimulated NK cells and mononuclear phagocytes, together with the demonstration that high levels of Th-2-antagonistic IFN- γ are also secreted by NK cells and virus-specific CD8⁺ T cells, further supports the potential "bystander" role of infections in promoting the development of Th-1 (as opposed to Th-2) immunity to allergens (3). The latter would appear to be particularly logical with respect to the role of respiratory viral infections in inhalant allergy, as the relevant T-cell responses colocalize in the regional lymph nodes draining the airway mucosa.

However, an important variable in this process is the status of host allergen-specific immunity at the time of infection. Animal studies indicate that once Th-2 responses are fully developed (i.e., a large population of mature Th-2 memory cells becomes established), Th-1 responses are potentially "locked out" by the strength of dominant Th-1-antagonistic cytokine signals (5); i.e., the Th-1 cytokines produced in bystander host antiviral responses may be incapable of reversing the cytokine polarity in ongoing Th-2 responses to allergens. It is thus likely that any protective effects of infection in relation to Th-2-mediated allergy may be restricted to the early phase of allergy development, in which the relevant Th-2 populations are relatively small.

The epidemiologic background

Respiratory infections in asthmatics

Respiratory infections have been recognized for many years as precipitating factors in atopic asthma (reviewed in ref. 6). Recent prospective studies on cohorts of schoolchildren indicate that these effects are highly selective for subjects

with pre-existing allergic respiratory diseases (7), suggesting that airways inflammation induced by the host response to infectious agents (especially viruses) can synergize with that resulting from inhalant allergen exposure, thus precipitating asthma attacks in previously stable subjects. Under certain circumstances, it can be hypothesized that potentially "protective" effects of Th-1 cytokines produced during the host response to the infection are of insufficient intensity to reverse ongoing allergen-specific Th-2 immunity, and instead simply add to the sum of local airway tissue damage. In addition, it is clear that host responses to certain respiratory viral antigens can in some circumstances be intrinsically Th-2 polarized, as in the case of the G protein of RSV (8).

Infections and the *de novo* induction of atopy

The evidence that childhood infections may "protect" against the development of atopy is essentially inferential, and is based on a series of cross-sectional epidemiologic studies, starting with the initial observations of Strachan (9) on paediatric populations in the UK. These and subsequent studies (10) have indicated that the expression of atopy is inversely related to social class, family size, and birth order. Each of these parameters can be taken as a surrogate of likely infection frequency; i.e., low socio-economic status, large family size, and, especially, having a large number of elder siblings, are likely to maximize exposure to respiratory infections during childhood.

Indirect support for this theory is provided by studies which have identified a gradient in atopy prevalence between paediatric populations in Eastern and Western Europe (11, 12), together with evidence of higher frequency/intensity of respiratory infections in the Eastern population (13).

Additional support has come from surveys which have demonstrated an inverse relationship between measles infection in childhood and subsequent sensitization to allergen of house-dust mite (14), and a similar relationship between seropositivity to hepatitis A virus and atopic status (15).

It has further been shown that atopy in 12-year-old Japanese children is inversely related to DHT reactivity to tuberculin, leading to the suggestion that early exposure to *Mycobacterium tuberculosis* protects against the subsequent development of atopy (16). It is important to note that this study found no evidence of active infection.

However, as outlined below, the interpretation of this study is complicated by recent findings indicating that the immune response of children with the atopic genotype is qualitatively and quantitatively different from their non-atopic counterparts.

Primary T-cell sensitization to environmental allergens: the importance of the perinatal period

Independent studies from several laboratories (reviewed in ref. 17) have demonstrated putative allergen-specific T cells in cord blood, suggesting transplacental priming, and formal proof of the foetal origin of these cells has recently been provided by DNA genotyping techniques (18). Moreover, these T cells predominantly express the Th-2 cytokine phenotype in all children, regardless of their atopic family history (18, 19).

Cross-sectional studies on older age groups indicate that by the age of 5 years, most T-cell responses to food allergens are functionally silenced, presumably via high-zone tolerance mechanisms driven by exposure to high levels of allergen in the diet (20). In contrast, individual responses to inhalant allergens segregate into Th-1-like or Th-2-like, the latter subjects expressing specific skin prick test (SPT) reactivity and allergen-specific IgE antibody in serum (20, 21). These findings indicate that the reciprocal patterns of allergen-specific T-cell immunity which distinguish atopic and nonatopic adults are programmed into the Th-cell memory compartment in early childhood.

Thus, the key to understanding the enigma of atopy is elucidation of the mechanism(s) responsible for the early postnatal divergence of these allergen-specific T-cell responses between atopic and nonatopic children; i.e., why are foetally primed Th-2 responses boosted postnatally in the latter, while they are "immune deviated" toward the Th-1 phenotype in nonatopics.

Th-1/Th-2 switch regulation during childhood: a genetically programmed maturational defect in atopics?

It is now clear from many studies that adaptive immune function is deficient (relative to adults) at birth, and does not fully mature until around the (median) age of 5 years (22). During this period, the Th-1/Th-2 balance in all children favours the Th-2 cytokine phenotype, which probably represents a carry over from foetal life in which Th-1 responsiveness at the foetomaternal interface is preferentially suppressed, in order to protect the placenta from the toxic effects of Th-1 cytokines (23).

Recent studies from our laboratory indicate that this transient maturational deficit in Th-1 function is con-

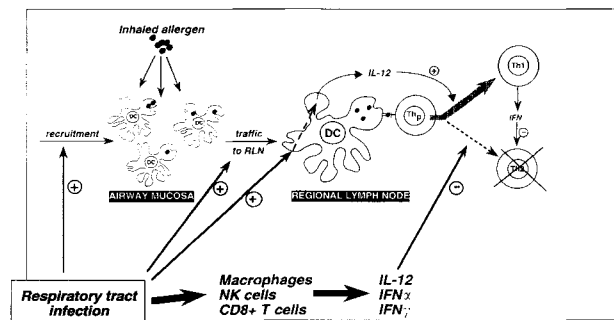


Figure 1. Airway DC-mediated immune deviation of Th2 responses to allergens: "bystander" stimulation of Th1 immunity via local infections.

siderably more marked in children with the atopic genotype, whose PBMC produce less IFN- γ per stimulation event during infancy (24), and in cord blood (reviewed in refs. 17, 22). We have hypothesized that this transient maturational defect in Th-1 cytokine-producing capacity compromises the operation of the immune deviation process which is required to downregulate foetally primed, low-level, allergen-specific Th-2 responses, thus facilitating the generation of Th1-polarized immunologic memory (22, 25).

We interpret the findings on atopy and tuberculin reactivity in 12-year-olds (14) cited above as evidence in support of this thesis. In this study, delayed hypersensitivity to tuberculin (as DHT) in 12-year-olds who had been BCG immunized at 3 months and 6 years, was inversely correlated with current atopy. We suggest that the failure of the atopics to develop long-term Th-1-dependent memory (as DHT) is indicative of their delayed maturation of Th-1 competence during infancy and early childhood.

Further supporting evidence of this thesis is contained in a recent prospective cohort study demonstrating an inverse relationship between total serum IgE levels at the age of 2 years and *in vitro* PBMC proliferative responses to antigens in the standard diphtheria/pertussis/tetanus vaccine (26). It is also noteworthy that overall numbers of T-memory cells in PBMC from atopic children are reportedly lower than in their nonatopic counterparts (27, 28).

Maturation of Th-1 competence during early childhood: the importance of microbial stimulation

As noted above, the polarization of the foetal immune system toward the Th-2 cytokine phenotype is an adaptive survival mechanism to protect the integrity of the placenta.

In this sequestered environment, the foetus is not reliant upon functional Th-1 immunity for protection against infections, but this situation changes after birth with the progressive dilution of maternal protective factors.

What alerts the foetal immune system to the potential dangers of the external environment, and triggers Th-1 maturation? The available evidence, based particularly on studies performed in germ-free animals, strongly suggests that the inductive signal is provided by contact with the microbial world, both pathogens and (perhaps more importantly) commensal organisms (reviewed in refs. 25, 29; see also ref. 30).

Consequently, factors which influence quantitative and qualitative aspects of microbial stimulation via the respiratory and gastrointestinal mucosae during early childhood may have a major impact on the kinetics of postnatal maturation of Th-1 competence at a number of different levels. Theoretically, these include effects upon precursor populations within the mononuclear cell compartment in the bone marrow, thymus, etc., where signalling via microbial "signature" molecules such as bacterial lipopolysaccharide, from commensal organisms, interacting with specific receptors such as CD14 may influence various aspects of functional maturation; e.g., upregulation of the IL-12 pathway in immature antigen-presenting cells (APC), before their migration into peripheral tissues. These effects may synergize with those resulting from intercurrent mucosal infections, particularly at the respiratory mucosal sites which represent the principal points of contact with inhaled allergens. One likely scenario in this context (Fig. 1) is infection-induced upregulation of the principal APC network responsible for surveillance of inhaled allergen, the airway intraepithelial dendritic cell (DC) network (31), which is functionally immature in neonates and infants (32, 33). These cells play a vital role in Th-1/Th-2 regulation in immune responses to inhaled allergen, and infection-mediated upregulation of functions such as IL-12 production, and costimulator expression may assist in

deviation of these responses toward the Th-1 cytokine phenotype. It is of interest to note in this context that DC have recently been identified as the principal (*in vivo*) source of IL-12 in experimental animals subjected to microbial stimulation (34).

Vaccination during infancy as a potential modulator of immune responses to allergens

The possible role of vaccination during infancy in this process is the subject of increasingly intense debate. On the one hand, it has been proposed that prevention of certain infections through successful vaccination may in some circumstances indirectly promote atopic sensitization (14) by removal of the microbial stimulation which upregulates Th-1-like immunity. Alternatively, early vaccination with Th-1-stimulatory agents such as BCG is being promoted as a potential preventive measure, based on the epidemiologic findings relating to the inverse relationship between DHT responsiveness and atopy in Japanese children (16) and the recent description of the Th-2-inhibitory effects of BCG in mice (35).

However, it is our firm view that this question must be approached with extreme caution, given the inherent complexities associated with the underlying cellular mechanisms. In particular, it should be stressed that interpretation of the epidemiologic findings relating to the effects of infections and vaccines in atopic children is complicated by the inherent deficiency in their Th-1 competence during the early postnatal period. Until more detailed information is available on the precise nature of this deficiency, it would be premature to contemplate preventive strategies which involve changes to current infant vaccination programmes. For these reasons, comparative studies on antivaccine responses in atopic and nonatopic children represent a high priority within our current research programme.

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