

Atopic versus infectious diseases in childhood: a question of balance?

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There is an increasing interest in the concept that respiratory tract infections during early childhood may in some circumstances confer protection against sensitisation to aeroallergens, via “bystander” stimulation of Th-1 associated immune functions in the regional lymph nodes draining the airway mucosa. We hypothesise below that this phenomenon may be but one component of a broader process operative during early postnatal life, in which generalised contact with the microbial environment plays an obligatory role in stimulating the functional maturation of the Th-1 arm of the immune response. We argue further that one of the most potent sources of such stimulation is provided by the normal commensal flora of the gastrointestinal tract, which is established during early infancy.

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The hallmark of atopic disease in adults is the expression of T-cell immunity to airborne environmental allergens that is polarised towards the Th-2 cytokine profile (IL-4/IL-5), as opposed to the Th-1 profile (IL-2/IFN γ) characteristic of non-atopics. Recent evidence (reviewed in 1, 2) suggests that these mutually exclusive forms of immunological memory are most commonly established via antigen-driven T-cell selection during early childhood. The outcome of this selection process is determined by a range of interacting genetic and environmental factors that have been identified epidemiologically (3) but which remain poorly understood at the molecular level.

In this context, interest has recently become focused on the potential role of respiratory infections in childhood, on the basis of epidemiological reports suggesting a protective effect of early infection on the subsequent expression of the atopic phenotype (4). This suggestion is mechanistically plausible, given the known Th-2-inhibitory effects of the cytokines released during host defence responses to respiratory infections, particularly as these infection-related cytokine responses occur predominantly within the same regional lymph nodes in which inhaled allergen-specific Th-memory cell selection is carried out (5). It may also be relevant to recent find-

ings suggesting that changes in public health measures in First World countries targeted particularly at minimising infections during infancy, are associated with increased prevalence of allergic diseases.

Recent conceptual developments stemming from basic research in several areas, in particular relating to the immunology of pregnancy, provide fresh insight into these and related issues, as discussed below.

Polarisation of Th-cell function(s) *in utero*

Recent findings indicate that the cytokine milieu at the fetomaternal interface is constitutively polarised towards the Th-2 profile (6), largely via intrauterine production of high levels of IL-4 and IL-10. This process appears to be an evolutionary adaptation designed to protect the placenta against the toxic effects of Th-1 cytokines such as IFN γ (6–f8), the production of which *in utero* is a major cause of fetal loss. This polarisation is maintained for variable periods postnatally, as evidenced by the selective expansion of Th-2 memory cells in antigen-challenged newborns (9–11) and the attendant low capacity of infant peripheral blood mononuclear cells (PBMC) to produce Th-1 cytokines such as IFN γ following *in vitro* stimulation (12, 13).

Perinatal Th-cell cytokine production and the atopic genotype

Several laboratories have independently reported that the Th-1 cytokine production capacity of cord blood mononuclear cells from newborns with atopic family history is significantly below that of their history-negative counterparts (14–18), suggesting that the “normal” intrauterine Th-2 skew may be exaggerated in individuals expressing the atopic genotype. This provides a plausible explanation for one of the hallmark observations in the allergy literature, notably the enhanced general risk of primary allergic sensitisation (now definable as development of Th-2 skewed immunological memory) which is associated with early postnatal exposure to high doses of allergen, the risk being greatest in children with positive atopic family history (3, 19). The risk may be further exaggerated when initial weak priming of the fetal T-cell system to environmental allergens occurs via transplacental allergen exposure (reviewed in (2)), which again proceeds within a Th-2 context (20), and is dominated by high-level allergen-specific IL-10 production (21).

The apparent Th-2 skewing of cytokine function in atopic family history positive children is maintained for several years postnatally, prompting our earlier suggestion that the kinetics of maturation of Th-1-related functions may be a primary aetiological factor in atopy (22, 23). In particular, sluggish development of this aspect of adaptive immunity may inhibit the postnatal immune deviation of fetal Th-2-skewed responses to allergens towards the Th-1 cytokine phenotype, a process which appears to occur efficiently in non-atopic children over the first 5 years of life, but which fails in their atopic counterparts (24).

It is interesting to note, in this context, the recent prospective study from Shirakawa et al. on Japanese school children (25). This demonstrated that delayed postnatal maturation of capacity to respond positively to BCG vaccination via development of tuberculin hypersensitivity, which is a sensitive index of Th-1-dependent immune competence, was associated with high risk for atopy at the age of 12 years.

Th-cell maturation and postnatal survival: confrontation with the microbial environment

It is clear that protection against bacterial and viral pathogens relies on effective expression of Th-1-immunity (26, 27), and it is accordingly axiomatic that an effective mechanism must exist to rapidly redress the fetal Th-1/Th-2 imbalance following birth via selective stimulation of Th-1 functions.

Is the relevant mechanism “infection” *per se*? On teleological grounds, we argue that it is unlikely that

evolution would select a stimulus which is inherently injurious (and indeed potentially fatal) to the host, as the primary trigger for postnatal maturation of a homeostatic mechanism which is fundamental to the overall survival of the species. Instead, we suggest that the primary signal for functional maturation of the immunoinflammatory system during infancy and early childhood is provided not by pathogens, but by stimulation from commensal microbial flora, the most quantitatively important being that of the gastrointestinal tract (GIT).

Microbial products typified by cell wall-derived lipopolysaccharides (LPS) are recognised as “nature’s Th-1 adjuvants”, and provide activation signals to virtually all bone marrow derived cell types through (*inter alia*) a series of high and low affinity LPS receptors (26). It is conceivable that stimuli derived from commensal flora may exert their effects on immunoinflammatory cells at the periphery, or on their precursors in the bone marrow and possibly the thymus. It is also likely that local stimulation of cells involved in Th-1/Th-2 regulation at sites of bacterial and/or viral infection, particularly in the respiratory tract, would be additive in this process (5).

Support for an important role for GIT commensal flora in the maintenance of immunological homeostasis is provided in the comprehensive germ-free literature over the last 25 years. Gnotobiotic animals of many species maintained postnatally in sterile environments fail to develop normal adaptive immune functions (in particular the capacity to express Th-1-dependent immunity at the periphery (28)) until they are provided with a normal GIT microflora.

Th-1/Th-2 balance in immune responses: a single rate-limiting step?

One of the most intensively studied experimental models in basic immunology is the phenomenon of neonatal tolerance, in which immunisation of fetal or newborn animals with exogenous antigen creates a state of selective unresponsiveness (tolerance) to the same antigen in adulthood. However, virtually all of this literature has relied on the use of Th-1-dependent readout systems for assessment of immunity in adult animals (such as DTH, allograft rejection and IgG_{2a/b}). More recent research has conclusively demonstrated that this traditional choice of assay systems has prevented demonstration of a much more significant reality: that neonatal immunisation covertly primes Th-2 memory cells, the reactivation of which in adulthood by antigen rechallenge inhibits the subsequent expression of specific Th-1 immunity (10, 29).

More importantly, this work has also led to identification of a single cell type that was responsible for selective Th-2 priming in newborns, namely antigen

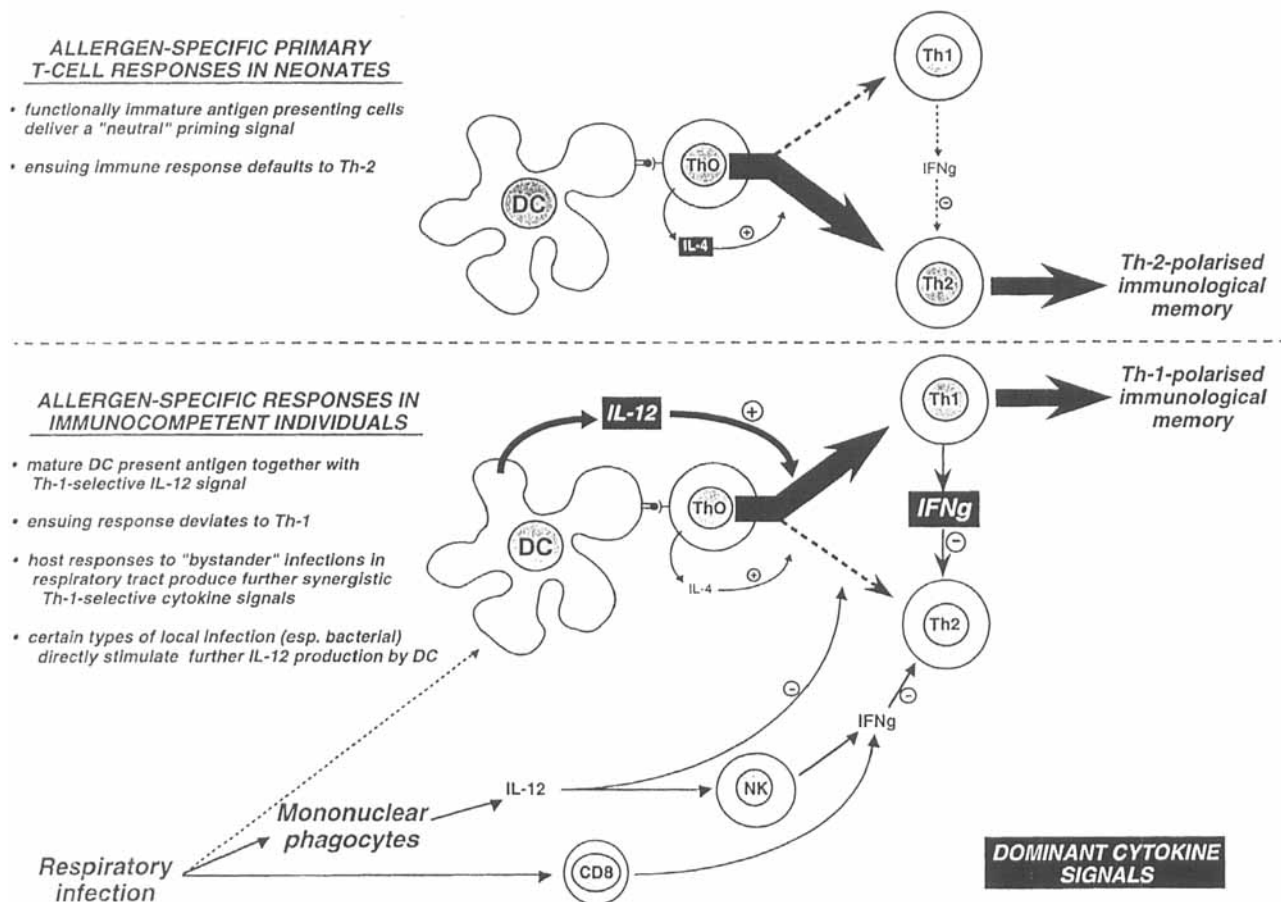


Fig. 1.

presenting Dendritic Cells (DC) (29). In immunoincompetent adult animals, these cells are highly specialised for the acquisition and presentation of exogenous antigen to naive T-lymphocytes, thus initiating Th-memory cell development. In doing so they preferentially select for Th-1-polarised memory via secretion of Th-2-inhibitory cytokines (in particular IL-12) during antigen presentation (30). However, in neonates, one or more key aspects of their Th-1-selective functions fail, and in the absence of this regulatory signal primary immune responses automatically "default" towards Th-2 (29). This default pathway (illustrated in Fig. 1) appears to be a direct consequence of the propensity of naive T-cells to secrete small amounts of Th-2-selective IL-4 during initial stimulation (31).

One of the most potent exogenous stimuli identified for activation of antigen presentation mechanisms and IL-12 production by DC is interaction with microorganisms, in particular LPS from bacterial cell walls (32, 33). We suggest therefore that LPS from gut commensal flora provides one of the major stimuli for postnatal maturation of DC functions. As the transient maturational defect in DC function is common to both peripheral tissue sites

such as the lung (see below) and to central lymphoid compartments (29), it is likely that the principal target for GIT-derived LPS would be relevant stem cells in the bone marrow and/or blood borne DC precursors derived from the latter. It is of interest to note in this context that monocytes, a substantial subpopulation of which share a common precursor with DC, are also known to be functionally deficient during infancy (34).

Dendritic Cells and inhaled allergen-specific Th-memory cell selection

An important role for DC in this process can be deduced from several lines of evidence. The presence of a highly developed network of MHC class II-expressing DC in the epithelium lining the conducting airways has been demonstrated in adult humans and experimental animals (35). These represent the most dynamic peripheral DC population yet described, providing continuous and intensive surveillance of the airway epithelium for inhaled antigens, which are rapidly transported to regional lymph nodes for presentation to the T-cell system (36, 37). These DC are the only professional antigen presenting cells

present in the airway epithelium in the steady state, and hence are highly likely to play a major role in Th-1/Th-2 selection during immune responses to inhaled allergens.

During infancy in rodents, DC are present in low numbers and are functionally defective. The postnatal development of the capacity of young animals to express normal (viz. Th-1-skewed) immunity to inhaled antigens is coincident with the establishment of an adult-equivalent intraepithelial network of DC in the airway walls (38, 39).

The ontogeny of corresponding DC populations in humans has not yet been mapped; however, the low frequency of MHC class II-expressing cells in human neonatal airway tissues (40) in contrast to the high numbers in adults (41), infers that overall developmental patterns will be similar to those in experimental animals. It is also pertinent to note that the reduced capacity of infant PMBC for Th-1 cytokine production in response to *in vitro* polyclonal stimulation can be boosted to levels approaching those of adults, provided that endogenous accessory cells (viz. monocytes and blood borne DC) are replaced by equivalent cells from adults (12, 13). It should also be noted that there is currently only indirect evidence available in relation to the positive effects of microbial stimulation on the postnatal maturation of the airway DC network (38). However, it should be emphasised that LPS provides potent functional maturation signals to human DC *in vitro* which are analogous to the effects shown with murine cells, a feature of which is stimulation of secretion by the DC of the Th-1-selective cytokine IL-12 (32).

Conclusions

Our general hypothesis in relation to the sensitisation phase of atopic disease may thus be summarised as follows:

- (i) initial priming of the T-cell system against environmental allergens is initiated *in utero* or soon after birth, and occurs within an immunological milieu already polarised towards the atopy-inducing Th-2 cytokine phenotype;
- (ii) Th-cell memory development occurs via allergen-driven Th-1/Th-2 selection, when the naive or weakly primed T-cell system is confronted by repeated environmental allergen exposure;
- (iii) the risk of selection for potentially pathogenic allergen-specific Th-2 memory is highest in children with the atopic genotype, as the degree of polarisation of their Th-cell function towards Th-2 is greatest;
- (iv) the Th-1/Th-2 imbalance characteristic of the fetal Th-cell compartment is normally redressed postnatally by preferential enhance-

ment of Th-1 immunity through microbial stimulation, which includes not only intercurrent infections but also the normal GIT microflora. A key focus of this microbial stimulation is the Th-1-selective function(s) of the antigen presenting Dendritic Cells which regulate the inductive phase of primary immune responses.

A logical extension of this argument is that at the population level, quantitative and/or qualitative differences in gastrointestinal microbial colonisation between First World and Third World infants (e.g. (42, 43)) may contribute to accompanying variations in prevalence rates of allergic disease. In addition, the effective application in First World countries of measures that modulate quantitative and qualitative aspects of microbial stimulation during infancy and early childhood may have unforeseen sequelae, which could conceivably include prolongation of the window period during which Th-cell function maintains the Th-2 skew which is characteristic of fetal life. In the context of postnatal allergen-specific Th-1/Th-2 selection, such measures may accordingly help to tip the balance towards Th-2 selection in those in which the Th-2-skew is most exaggerated at birth; viz, infants with positive atopic family history.

The measures in question, which have come to represent the norm in first world countries over the last 20-30 years, include the introduction of effective public health and hygiene programs targeted at water supplies and foodstuffs; vaccination programs which limit the impact of infectious diseases during childhood; widespread use of antibiotics in paediatrics; increasingly stringent infant feeding (and associated hygiene) practices, and reduction in family and school class sizes which limit sharing of infections. Collectively, these measures have markedly reduced mortality and morbidity during early childhood, but (we speculate) at the expense of efficient early education of the immune system, resulting in increasingly frequent "errors" in T-cell memory development against non-pathogenic environmental antigens which can be translated in later life into atopic disease.

The solution to this problem clearly does *not* involve abandoning current public health practices that are demonstrably so effective in protecting the health of our children, but instead lies in gaining a fuller understanding, at the cellular and molecular level, of the mechanisms by which stimuli from the microbial environment promote the normal postnatal maturation of the immune system. The fact that >40% of the current cohort of preschool children in many first world countries express skin prick test reactivity to 1 or more inhalant allergens, provides an urgent imperative for solving this fascinating puzzle.

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