

Development of long term tolerance versus sensitisation to environmental allergens during the perinatal period

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Evidence is steadily accumulating which indicates that the patterns of T cell reactivity against the environmental antigens that determine the allergen responder phenotype in adulthood are, in many cases, established during infancy. The underlying regulatory processes which determine the nature of long-term immunological memory against these antigens appear to involve a combination of classical high zone and low zone tolerance mechanisms. It appears likely that these T cell responses are initiated before birth via the transplacental transfer of low levels of allergen to which mothers are exposed during pregnancy.

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Abbreviations

APC	antigen-presenting cell
BCG	Bacillus Calmette-Guerin
DC	dendritic cell
DTH	delayed type hypersensitivity
GIT	gastrointestinal tract
HDM	house dust mite
PBL	peripheral blood lymphocyte
RLN	regional lymph node
SPT	skin prick test
Th	T helper

Introduction

The prevalence of allergic disease is increasing at an unprecedented rate in many countries, particularly in the First World, and this general trend is most noticeable amongst the pediatric populations. The spiralling costs of treating established atopy [1] provide an urgent imperative for the development of effective preventive measures to limit further increases in disease prevalence. This approach presupposes a detailed knowledge of the aetiology of allergic diseases. Until comparatively recently, the focus of research in this field was, almost exclusively, upon aspects of disease pathogenesis in adults. Recent evidence suggesting that the seeds for allergic disease in the adult are sown during the perinatal life phase, however, has stimulated a resurgence of interest in the regulation of the relevant aspects of T cell immunity in infancy and early childhood, and the recent developments in this area are reviewed below.

Responder phenotype à la Th1/Th2 paradigm

Prior to the landmark studies on the functional heterogeneity within T helper (Th) cell subpopulations in the mouse (reviewed in [2,3]), allergic disease in humans was broadly conceptualised as an aberration of suppressor cell activity which permitted the development of unnecessary immunity against nonpathogenic antigens (allergens) that are ubiquitous in the natural environment. It became evident, however, that this notion was not consistent with a wide body of seroepidemiological data indicating that virtually all adults exhibit serum antibodies directed against common environmental allergens [4,5], with the clinical responder phenotype being determined instead by the isotype of the antibody produced (that is, allergic reactivity being associated specifically with IgE).

This dichotomy was to a large extent resolved via the initial studies from the Kapsenberg [6] and Romagnani [7] groups, and the many follow-up reports from other laboratories, focusing on T cell responses in adults to ubiquitous aeroallergens. In broad terms, these studies demonstrated that allergen-specific CD4⁺ Th clones could be isolated from the peripheral blood lymphocytes (PBLs) of both allergen-responding atopic patients and clinically unresponsive nonatopic controls, the key difference being in the respective cytokine patterns of the clones, that is Th2-like (especially IL-4, IL-5) in atopic patients versus Th1-like (IL-2, IFN- γ) in the normal controls. The Th1/Th2 system was originally defined in the mouse. There is still debate in human clinical immunology circles as to the degree to which it can be applied to man (for example, the distribution of IL-10 is different etc.). Recent studies, however, employing sensitive methods for the assessment of allergen-specific cytokine responses in freshly isolated unmanipulated PBL T cell populations in short term bulk culture have achieved results comparing atopic patients and controls, comparable to those achieved with T cell clones [8,9].

The literature cited above relates exclusively to aeroallergens, which are responsible for the bulk of the allergic disease burden amongst adult populations. The situation with respect to potential allergens in the diet is less clear. In general terms, it is obvious that the prevalence of food allergy remains (in most situations) extremely low, despite the continuous exposure to these agents, inferring that the endogenous control mechanisms which suppress the development of reactivity to food allergens are intrinsically more efficient than those regulating the corresponding responses to airborne allergens. Interestingly, the presence

of food allergen specific antibodies in serum is also virtually universal [4,5], but the persistence of food-specific Th2 cell dependent IgE antibody production in adulthood is rare compared to that seen with respect to airborne allergens.

A further notable difference between adult T cell reactivity to dietary antigens versus aeroallergens is obvious from the parallel studies on *in vitro* lymphoproliferation: whereas virtually 100% of adult lymphocytes respond *in vitro* to inhalant allergens, lymphoproliferative responses to common food allergens such as ovalbumin are demonstrable in less than 20% of the population [8•], inferring fundamental differences in the nature of the underlying regulatory mechanisms.

Basic principles defined in animal models

T cell immunity to mucosally delivered allergens

Insight into the nature of the mechanisms regulating T cell responses to inhaled versus ingested allergen has been gained from recent studies in animal models. Our laboratory has a longstanding interest in rat/mouse models involving repeated aerosol exposure to soluble protein antigens (reviewed in [10]). These studies have focused on the nature of the control mechanisms that normally prevent Th2 cell dependent primary IgE responses to inhaled antigens, as the latter usually only occur in animals with underlying deficiencies in either the barrier function in the airway epithelium or in those animals co-exposed to microbial stimuli known to perturb the adaptive immune function. These studies collectively demonstrate that, in immunocompetent adult animals, Th2 cell dependent immunity to inhaled antigens is normally silenced via a form of immune deviation, in which the 'default' IgE response that initially occurs in the respiratory tract regional lymph nodes (RLNs) soon after exposure to antigen is supplanted by the emergence of a later Th1 cell polarised response dominated by IFN- γ [11–13].

The corresponding process in the gastrointestinal tract (GIT), the phenomenon of oral tolerance to dietary antigen, also involves an initial 'default' towards the Th2 cytokine phenotype [14], but IgE (and delayed type hypersensitivity [DTH]) responses are normally rapidly terminated [10]. Studies [15,16] suggest that the nature of the underlying T cell regulatory mechanisms in the GIT are a function of antigen dosage, as high dose feeding leads to T cell anergy and/or deletion via high zone tolerance, whereas responses to low dosages of ingested antigen are regulated via immune deviation.

It is pertinent to note, in the context of further discussion below, that firstly, although these mechanisms (immune deviation, oral tolerance) operate effectively in immunocompetent adult animals, they function poorly during infancy (reviewed in [10]). Secondly, their role appears to be that of shaping long-term immunological memory in naive or weakly primed animals, and they

are markedly less effective in deviating/terminating long-standing responses driven by mature T memory cell populations [10].

Antigen-presenting cells and the phenomenon of neonatal tolerance

Highly relevant to this are the recent studies [17•,18•] on the mechanism of neonatal tolerance to parenterally administered antigens. These studies demonstrate that the failure of newborn animals to develop adult-equivalent T cell memory following parenteral immunisation is due to an intrinsic skewing within the T cell compartment towards the Th2 cytokine phenotype, leading to a failure to prime for Th1 immunity [17•,18•]. This Th2 skew during infancy is a continuation of the pattern characteristic of the fetal Th cell compartment, which is constitutively polarised towards a Th2 phenotype in order to minimise the intrauterine production of Th1 cytokines that are highly toxic towards the placenta [19].

Of further interest is the finding that the cells primarily responsible for the maintenance of the Th2 skew in neonatal mice are the dendritic cells (DCs) [17•], the principal antigen-presenting cells (APCs) involved in stimulating primary immune responses. In this context, we have previously established that DC populations within the airway epithelium of adults represent the principal APC in the respiratory tract [20,21], forming a dense network equivalent to the Langerhans cell network in the epidermis, and hence play a central role in regulating immune responses to inhaled antigen. The rate of postnatal maturation of this DC network is relatively slow, and appears to be the rate limiting factor in the development of local immunocompetence within the respiratory tract during infancy [22,23•]. On the basis of data from the Matzinger group [17•], it may reasonably be predicted that the airway DC population would be inefficient in generating Th1 polarised immunity to inhaled allergens during this life phase.

Development of T cell memory against allergens: where, when and how?

A series of laboratories have now independently demonstrated that T cells capable of proliferation *in vitro* in response to both the dietary and inhalant classes of allergens are common in cord blood samples (reviewed in [22]). This suggests that initial T cell priming may occur as a result of the transplacental transfer of the relevant allergen, particularly during the last trimester [23•]. There is some indication that this priming process may be strongest in infants with positive atopic family histories [24,25•], and may be enhanced via the transplacental transfer of maternal allergen-specific IgG subclass antibodies [26•].

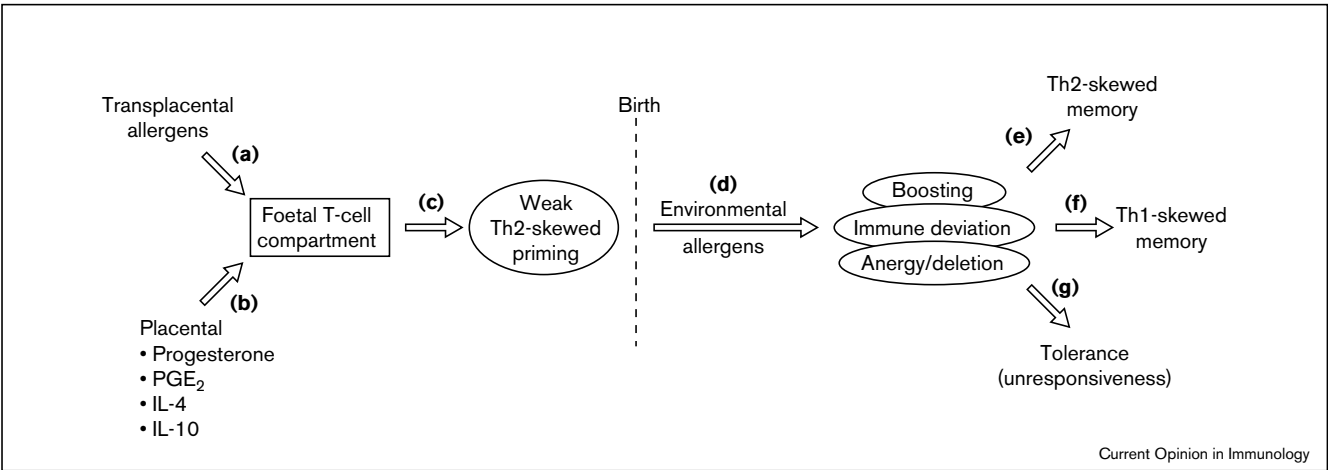
We have recently obtained preliminary evidence that these fetal responses are constitutively polarised towards the Th2 cytokine phenotype in virtually all subjects, and are dominated by the high-level production of the Th1

inhibitory cytokine IL-10 [25••]. Studies in progress in our laboratory indicate that the Th2 cytokines IL-4, IL-5, IL-6, IL-9 and IL-13 are also a consistent feature of these fetal responses, whereas IFN- γ is rarely found (our unpublished data). It has also been suggested that high-level production of Th2 cytokines by allergen-specific cord blood cells at the clonal level may constitute a risk factor for the subsequent development of atopy [27].

During early infancy, these weakly primed Th2 responses are subject to modulation via direct stimulation with incoming environmental allergen. Some indication of the time frame over which this modulation process occurs is provided by the studies on the production of IgG and IgE antibodies against dietary and inhalant allergens, which is initiated in most infants within the first few weeks of life [28,29]. Consistent with that described in animal models [10], IgE responses to the dietary allergens are biphasic in virtually all children and peak early during infancy (within nine months) and with few exceptions (a subset of atopics) are permanently terminated by two to three years of age [29,30]; in contrast, corresponding low level IgG/IgA responses commonly persist into adulthood [4,5].

In contrast, corresponding IgE responses to aeroallergens are slow to develop postnatally and usually do not manifest until two years of age [29,31]. In nonatopic children responses are biphasic but may persist at a low level up to an age of four to seven years before terminating, whereas in atopic children (up to 35% of current five year old cohorts in First World countries) these IgE responses persist and may amplify further with increasing age.

Figure 1



Perinatal T-cell priming against environmental allergens – alternate pathways for modulation of responses via direct allergen exposure during infancy. (a) Initial priming of the fetal immune system normally occurs in response to low levels of environmental allergens which cross the placenta. (b) This priming occurs in a milieu (progesterone, prostaglandin E₂ [PGE₂], IL-4 and IL-10) which is strongly inhibitory to Th1-associated functions, resulting in (c) the initial generation of low level Th2 polarised immunological memory. Following birth, (d) the weakly (Th2) primed immune system is directly stimulated by much higher levels of the same environmental allergens, which drive Th cell selection down three alternative pathways: (e) towards boosting of Th2-polarised immunity; (f) towards deviation from the weak Th2-responses towards the Th1 phenotype; and (g) towards induction of T cell anergy or T cell deletion in response to sustained high level exposure. The available evidence suggests that pathways (e) and (f) appear to be important in regulation of responses to aeroallergens, whereas (g) represents the major pathway relevant to food allergens.

These findings are consistent with a general model for postnatal control of allergen-specific T cell responses in which (as for the animal models) the nature of the regulatory mechanisms employed is dictated by the respective allergen exposure levels: high zone tolerance to food antigens which are encountered at high concentrations, leading to silencing of the bulk of reactive T cells via anergy/deletion, versus immune deviation (low zone tolerance) of weakly primed Th2 responses against inhalants towards a Th1 phenotype.

Immune deviation of Th2 responses during infancy: risk factors for failure

As noted above, the immune system of newborn animals is intrinsically Th2-skewed at birth, and the available evidence from experimental animals suggests that this polarisation is maintained for a significant period during infancy [35,36]. The data relating to humans is less clear cut, but it is evident that the capacity to produce the principal Th1 cytokine IFN- γ is generally low during infancy, as a result of developmental deficiencies in both the APC and T cell compartments [37–40,41•], the latter including hyporesponsiveness to IL-12 [42].

There is also evidence from several laboratories that at birth IFN- γ producing capacity is lowest in children with a genetic background of atopy, and remains so throughout infancy [43–46,47•], leading to the suggestion that slow postnatal development of Th1-associated functions is a primary risk factor for atopy [46]. Consistent with this view, a recent report has demonstrated that failure to develop Th1-dependent Delayed Hypersensitivity to Tuberculin in response to Bacillus Calmette-Guerin (BCG) vaccination during infancy and early childhood is much more common amongst children who subsequently develop atopic disease, as compared with their normal counterparts [48].

This proposed developmental defect in Th1-associated adaptive immune function in atopics may simply be a subtle exaggeration (or prolongation) of the normal Th2 skew which is a hallmark of the fetal immune system. In the latter situation, the production of Th1 cytokines *in utero* is tightly downregulated via the combined effects of the placental production of IL-10 and IL-4 [49•], prostaglandin E₂ [50–52], and progesterone [53–54,55•]. Following birth, the influence of these agents wanes, and Th1-associated functions mature, a process that is likely to be accelerated by direct stimulation of the newborn immunoinflammatory system via the microbial environment. In this context, there is growing interest in the role both of infections [56–58,59•] and commensal microbial flora [58,59•,60••] in the stimulation of postnatal immune function maturation during infancy, and in the downstream effects thereof on the aetiology of atopic disease.

Conclusions

Figure 1 encapsulates the data discussed in this review into a broad working hypothesis for the regulation of T cell immunity to environmental allergens. T cell priming *in utero*, in response to low levels of transplacental environmental allergens occurs in the presence of a range of placental Th1-inhibitory factors, resulting in weakly primed Th2-skewed T cell populations. After birth, these cell populations can be driven along the following alternative pathways: Th2-boosting; Th2→Th1 immune deviation; or induction of tolerance. The first two pathways appear to be important for the regulation of immune responses to aeroallergens, whereas the third pathway is important for food allergen responses.

Resistance or sensitivity to allergic disease appears to ultimately be determined by the balance achieved between Th2-boosting and immune deviation during early life. The reasons underlying the apparent failure of immune deviation in atopics remain to be defined, but are likely to involve developmental defects during the process in which the imbalance in fetal Th cell function (*viz.* the Th2 skew) is redressed during infancy.

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