

Prenatal sensitisation

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There has been an increase in the prevalence and severity of allergic disorders and, in particular, asthma in children, though clear-cut epidemiological evidence to support this has been slow to accumulate, mainly because of lack of an agreed definition of asthma suitable for population surveys. One study from South Wales, using identical ascertainment among 12-year-olds, showed a change in point prevalence between 1973 and 1988 from 4 to 9% for asthma, 5 to 16% for eczema and 9 to 15% for hay-fever (1). This increase has occurred in many countries and has been demonstrated in Finnish military conscripts (2), in Taiwanese schoolchildren (3), Australian children (4) and adolescent Maories in New Zealand (5). While diagnostic transfer might account for the increase in some of the studies, it is clearly not the case for the majority (6). Furthermore, there have been enormous increases in hospital admissions for childhood asthma over the last 8–10 years with no reduction in severity on admission, no increase in readmission ratio and no evidence of diagnostic transfer (7). A relatively small shift in population susceptibility due to changes in the environment could account for the increase in frequency of disease and the even greater increase in numbers experiencing severe disease.

Whilst heritability forms the basis of the definition of atopy, it is clear that the environment must have had a major influence on phenotype (8). If this population shift is to be reversed, then detailed study of the environmental causes is essential. Longitudinal cohort studies will be required to establish which of the many possible factors are critical to the development of allergy and subsequent asthma. The ultimate aim of such studies must be to identify avoidable factors which might lead to the establishment of protocols of prevention. Many of the assertions about prevention at present are based on rather limited, usually retrospective evidence, and while

early results from prospective studies look encouraging (9), longer term follow-up has revealed little or no effect (10, 11).

Fetal and neonatal immune responses

It is known that mature circulating T cells can be detected in the fetus by 18 weeks of gestation (12) and therefore antigen exposure via the mother could initiate sensitisation and immune memory from this early stage (previous studies have already shown that food allergen avoidance in the 3rd trimester of pregnancy is ineffective, possibly being too late (11, 13)). Our detection of specific cord blood mononuclear cell responses to allergen implies antenatal immune activation (14, 15). We also have evidence that maternal exposure to house dust mite allergen during the third trimester of pregnancy results in increased neonatal T cell proliferation to house mite allergen (16) and that maternal exposure to birch pollen after 22 weeks of pregnancy is associated with raised infant T cell responses to birch pollen at birth. Furthermore, house dust mite exposure in the first month, but not the third month after birth, strongly influences the development of house dust mite skin prick test sensitivity at 1 year of age (16) and wheeze at 3 years of age. From previous studies this is likely to translate into a strong association between allergen exposure during pregnancy and the first month of life with the subsequent development of atopic asthma (17). Studies of T cell activation, specific proliferative responses to allergens and the cytokine gene expression and cytokine release of triggered cells from terminated fetuses and premature babies are underway to determine whether commencing allergen avoidance during the second trimester of pregnancy result in the prevention of these raised responses.

T cells and cytokines

Based initially on studies in mice, allergen responsive helper T cell clones from allergic individuals have been shown to selectively produce cytokines of the IL-4 gene cluster encoded on chromosome 5_q31 namely, IL-4, IL-5, IL-6, IL-10 and IL-13 (Th-2 like). Non-atopic T cell clones produce IL-2, IFN- γ and TNF β (Th-1 like) (18), whilst subtypes produce IL-3 and GM-CSF (19). These cytokines have a wide range of effects, including the activation and recruitment of accessory cells in the allergic response, specifically mast cells, eosinophils and basophils. Both in murine models of atopy and in human allergy, it has been shown that opposing signals from Th-1 cells producing IFN- γ and Th-2 cells producing IL-4 and IL-13 influence CD40-dependent IgE synthesis by B cells (19–23), whilst IFN- γ inhibits transformation to this isotype (24). Of particular relevance is that when compared to adult T cells, those from neonates have a reduced IFN- γ production after polyclonal stimulation (25–27), and the same has been shown for IL-4 production (28). Holt et al (29) have demonstrated that although the IFN- γ -producing capacity by CD4⁺ T cell clones from babies was reduced relative to adults, it was significantly lower still in babies born to allergic families. It has also been demonstrated that IFN- γ production by cord blood mononuclear cells is reduced in those newborns with a family history of atopic disease (30). We have found raised cord blood T cell proliferative responses and defective IFN- γ production to stimulation with specific allergens in babies who have subsequently developed allergic problems in relation to those allergens (14), and these observations have been partly confirmed by others (31). We have also found that babies born to atopic asthmatic parents who have developed allergic manifestations by 1 year of age, have significantly lower CD4⁺/CD45RO⁺ (memory) and CD4⁺/CD25⁺ (activated) T cells in the cord blood, compared with babies who were born in a similar situation but have not developed allergic disease or in babies born to non-allergic parents (15). We have demonstrated that by 6 months of age, these differences are lost (32), presumably because the relevant T cell populations have moved into the target organs including the lungs, under the influence of post-natal allergen exposure. This trafficking of T cells has not been properly documented in humans.

IL-10 is a product of various cell types, including monocytes, B cells and T cells, and is associated with the differentiation of Th-2 cells. IL-10 inhibits cytokine production, particularly of IFN- γ , by T cells and natural killer cells (33). It has been suggested that IL-10 prevents the production of the IFN- γ inducing cytokine, IL-12 by accessory cells.

One possible mechanism for our findings of reduced IFN- γ production by neonatal T cells is an imbalance of IL-10 and IL-12 production.

Maternal, placental and fetal immunological interactions

We believe that there is a powerful maternal and placental influence on the fetal immune response, which promotes or suppresses Th-2-like activity, but the nature of this influence is not known. Evidence is growing that during pregnancy the maternal immune response becomes heavily biased towards Th-2-phenotype, with increasing IL-4 and IL-10 during gestation being detected in the murine system. Clinically, a picture of reduced cell-mediated immunity has been described in pregnancy (34). The benefit of this loss of cell-mediated immunity may be reduction in NK cell activity. These cells have been shown to have a role in spontaneous abortion and may attack the trophoblast (35). Additionally, IFN- γ is an abortifacient whose effect may be mediated through the activation of NK cells by this cytokine (36). Clearly Th-1-type cell-mediated immune responses are undesirable for maintenance of pregnancy.

To date, most evidence of the role of cytokines in pregnancy has been derived from the murine system. Dudley et al showed increasing IL-4 and decreasing IL-2 production by murine maternal splenocytes during gestation. However, very low levels of these lymphokines were detected in decidual lymphocyte cultures (37). Delassus likewise saw increases in IL-4 and also IL-10 and some increase in IFN- γ in peripheral blood mononuclear cell cultures from maternal blood. In the placenta, increases were seen in IL-4 but IFN- γ did not increase and IL-2 remained low (38).

Very little work has been carried out on human cytokine production in pregnancy. We have recently performed a study investigating T cell proliferative responses and cytokine production from 16 fetuses in the second trimester (15–24 weeks gestation) and 35 babies in the third trimester (26–34 weeks gestation). FACS analysis of fetal T cells showed that whilst CD25⁺ T cells are virtually undetectable at 15 weeks gestation, at 20 weeks they are approximately 50% of the levels at full term. Proliferative responses to PHA were significantly higher in the third trimester than the second trimester ($p < 0.0002$) and increased with progression through gestation. Even more importantly, we have demonstrated the same increase in proliferative response to specific inhalant and ingestant allergens, further supporting the theory of intrauterine priming (39). In addition to the difference seen in proliferative responses, there was also a difference in IFN- γ production during the second and third trimester. IFN- γ production by un-

stimulated cells was higher in the third than second trimester. However, the cells of some babies did not produce measurable IFN- γ whatever the stimulant. We hypothesise that the role of non-specific fetal PBMC IFN- γ production is to counteract the effects of IL-4 produced by the placenta and/or mother. A mechanism such as this would be essential to prevent an allergic (Th-2) phenotype in all newborns. Failure of this mechanism may underlie the development of atopy. We are currently investigating the materno-placental-fetal relationship on cytokine production during pregnancy with particular reference to the development of atopy.

Conclusions

The prevalence of asthma and related allergic disorders has increased considerably over the last 25 years. As genetic stock has not changed, environmental factors must have influenced the phenotype. We have shown that peripheral blood mononuclear cell (PBMC) proliferative responses and cytokine profiles to specific allergens are already altered at birth in babies who subsequently develop allergic disease compared to those with no disease. We have also shown that positive PBMC proliferative responses to specific allergens can be detected from 22 weeks gestation and that these responses were higher in babies whose mothers were exposed to increased allergen concentrations after 22 weeks of pregnancy. Therefore in genetically predisposed individuals, antenatal factors are critical for the development of asthma, and these events include maternal and thereby fetal exposure to allergens and tobacco smoke and materno-placental-fetal immunological interactions.

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