

Primary sensitization to inhalant allergens

Macaubas C, Prescott SL, Venaille TJ, Holt BJ, Smallacombe TB, Sly PD, Holt PG. Primary sensitization to inhalant allergens. *Pediatr Allergy Immunol* 2000; Suppl. 13: 9–11. © Munksgaard 2000.

The neonatal T-cell system is capable of responding to allergens at birth, indicating the occurrence of prenatal sensitization, and the cytokine profile of these responses is skewed towards the Th-2 type. This response is further modified by postnatal exposure to different types of allergens. In relation to inhalant allergen (employed by HDM) the low level fetal Th-2 responses in non-atopics appear to be down-regulated rapidly after birth, parallel to an increase in allergen-specific IFN- γ production. In contrast, atopics appear to consolidate their initial Th-2 responses, and around the age of 6 exhibit a cytokine response profile similar to the adult pattern. A pre-existing deficiency in IFN- γ production may be one of the key factors determining the postnatal persistence of Th-2 responses in atopics.

**Claudia Macaubas,
Susan L. Prescott,
Thierry J. Venaille, Barbara J. Holt,
Troy B. Smallacombe, Peter D. Sly
and Patrick G. Holt**

Division of Cell Biology, TVW Telethon Institute
for Child Health Research, Perth, Australia
(affiliated with The University of Western
Australia)

Key words: allergy; infancy; cytokines;
Th-1/Th-2

Dr Claudia Macaubas, Division of Cell Biology,
TVW Telethon Institute for Child Health
Research, PO Box 855, West Perth WA 6872,
Australia.

e-mail: macaubas@icmr.uwa.edu.au

Accepted 13 December 1999

Prenatal sensitization to environmental allergens

Evidence has been accumulating in the last few years that allergic sensitization may occur very early in life. Cord blood mononuclear cells (CBMC) have been shown to proliferate in response to a range of inhalant and dietary allergens (1, 2). We have detected proliferation to crude house dust mite (HDM) in 46% of cord blood samples, to Ovalbumin (OVA) in 42% and to the major HDM allergen Der p1 in 73% (3). Studies (4) have suggested that fetal T-cell priming can be initiated as early as 22 weeks of gestation. We have recently obtained direct evidence for the fetal origin of the allergen-responder cells in CBMC by genotyping of T cell clones derived from allergen-stimulated bulk cultures (3). These findings are collectively consistent with initial allergen-specific T-cell sensitization occurring before birth, a potential key factor in the development of the adult atopic profile (5).

Analysis of the cytokine responses from cord blood cells in the study cited above (3) has shown that CBMC produce, at low level, a range of Th-2 type cytokines in response to *in vitro* stimulation with allergens. Production of mRNA encoding IL-4, IL-5 and IL-9 in response to HDM and OVA was detected. At the protein level, produc-

tion of IL-6, IL-10 and IL-13 to the same allergens was also observed. The production of cytokines was detected in CBMC from babies with and without familial risk of atopy. In CBMC, IL-10 and IL-13 were produced mainly by CD4+ T cells, while IL-6 came mostly from non-CD4+ T cells. Overall, IFN- γ response to allergens, as seen by RT-PCR, was almost undetectable in cells from most of the neonates.

This low level Th-2 skewed response in neonates may be a consequence of the intrauterine environment. Th-2 type cytokines appear to be required for a successful pregnancy, probably by virtue of their antagonistic effects against production of Th-1 cytokines, which are toxic for the placenta (6). Th-2 cytokines produced by the placenta or other pregnancy-related tissues may influence fetal sensitization (7). Also, the low dose of allergens which are likely to reach the fetus from the maternal circulation may prime the immune system preferentially for a Th-2 response (8).

Allergen response after environmental exposure

Responses to allergens appear to be radically modified following postnatal exposure. While proliferative responses to HDM and OVA are

detected at birth, with age the responses to OVA decrease, while there is an increase in responses to HDM (9). By adulthood, using serum-free medium, proliferation to HDM can be detected in virtually all individuals, independent of atopy (10). Furthermore, in a local cohort of around 2000 children followed from birth, at the age of 6 years, 35% of the children had a positive skin prick test (SPT) to one or more inhalant allergen (HDM, cat dander, mould or rye grass). In contrast, only 2% showed positive SPT to OVA. We also have shown that with age there is a shrinkage in the number of OVA peptides that were recognized at birth (11, 12).

However, this low or absent proliferative response to OVA is accompanied by covert cytokine responses. After *in vitro* restimulation with OVA, we observed production of IL-6 and IL-10 protein (by ELISA) and IL-13 mRNA (by RT-PCR) from PBMC from 6-year-old children who are not atopic to OVA (unpublished).

The distinct patterns of OVA (food) and HDM (inhalant) proliferative responses may be a consequence of dose of allergen, usually high doses for food versus low for inhalant, and route of administration (13). These factors may favour diverse patterns of sensitization and long term

response to different groups of environmental allergens.

Cytokine responses to HDM in atopic and non-atopic children

As mentioned previously, low level Th-2 skewed responses to HDM can be observed at birth in most newborns, and proliferative responses increase with age. At the protein level, our studies on 6-year-old children showed that after *in vitro* stimulation with HDM, PBMC from atopic children produce several Th-2 type cytokines, concurrently with IFN- γ (11). Non-atopic children showed low or undetectable production of Th-2 type cytokines, accompanied by IL-6, IL-10 and IFN- γ responses (14) (Fig. 1).

What happens during the period between birth to the age of 6, where cytokine profiles resemble that of the adult, albeit at a lower level? Our earlier cross sectional study (15) and more recent longitudinal data (12), suggest that atopic children manifest a delayed "maturation" of their cytokine responses. Analysis of HDM-induced cytokine responses by CBMC from children who exhibit atopic symptoms at the age of 2 years

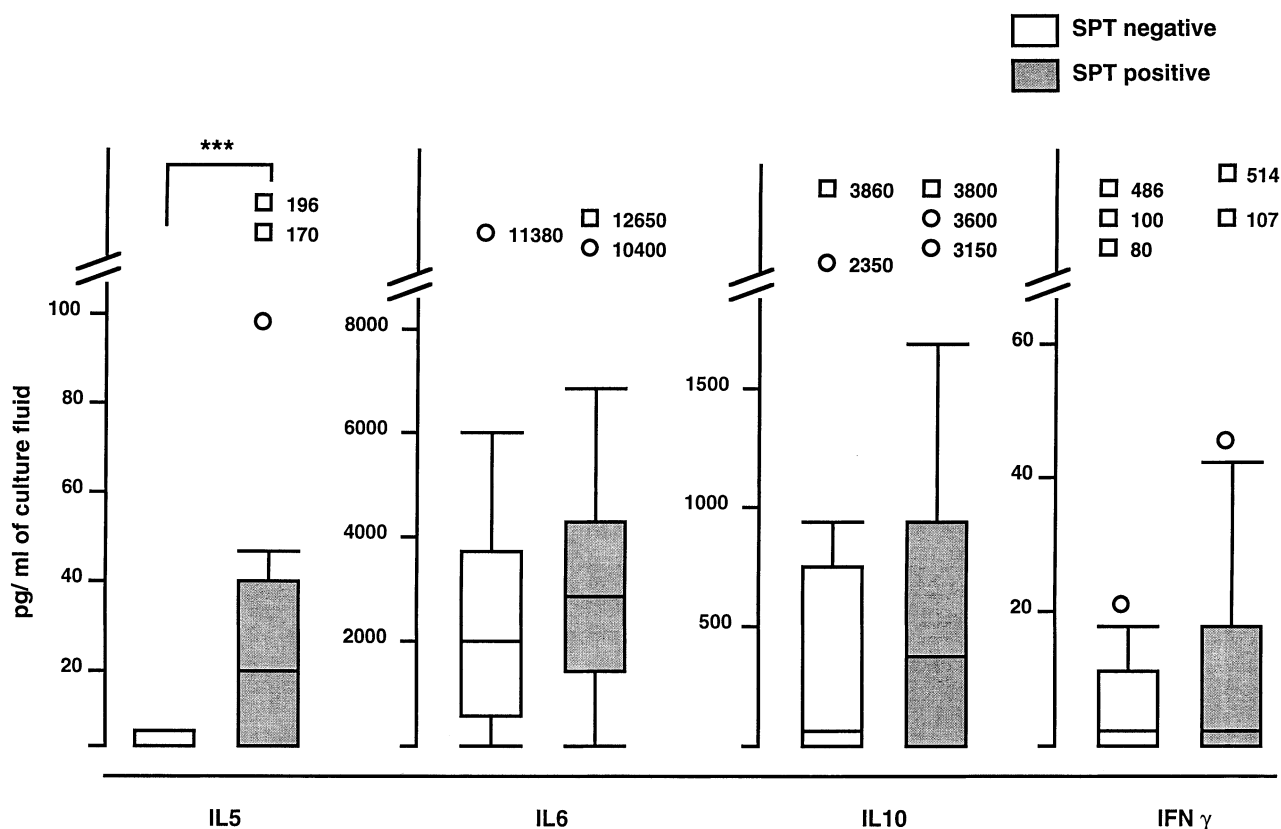


Fig. 1. HDM-induced cytokine protein production by HDM-SPT positive ($n=30$) and HDM-SPT negative ($n=29$) groups at 6 years of age. The 25th and 75th percentiles are indicated. Lines extending from the boxes indicate the highest and lowest values, excluding outliers (circles) and extreme cases (squares). The line across the box represents the median value. *** $P<0.0001$.

showed lower initial cytokine production in comparison to children without symptoms. This was evident for IL-4 (by RT-PCR) and IL-6, IL-10 and IL-13 (by ELISA) (12).

Our group have shown previously that T cell clones derived from infants and young children at risk of atopy produce less IFN- γ and IL-4 than those not at risk (15). Similar findings have subsequently been reported for polyclonally stimulated CBMC from neonates at risk of atopy (16), and for allergen stimulated CBMC from subjects who develop atopy in early childhood (17). We observed a slower postnatal up-regulation of IFN- γ responses to HDM in children who develop atopic symptoms at age 2 years (12). At least part of this deficit in IFN- γ production is due to the CD4⁺ T cells themselves (12).

The deficiency in IFN- γ production in children at risk of atopy may play a key role in the maintenance of dominant Th-2 type responses during early postnatal life. It has been shown that IFN- γ is able directly to inhibit Th-2 responses (18), and IFN- γ deficiency early in life may thus contribute to a favourable environment for persistence of fetal Th-2 type responses to allergens encountered during this period. It is of interest to note in this context that neonates at risk of atopy also show deficient production of IFN- γ in response to bacterial products (19). Work from our group has also found that children with high serum IgE levels have a deficient response to DPT vaccination (20). These observations indicate that differences in immune function, already present at birth, may determine the nature of the interactions between the growing child and environmental and infectious agents.

References

1. KONDO N, KOBAYASHI Y, SHINODA S, et al. Cord blood lymphocyte responses to food antigens for the prediction of allergic disorders. *Arch Dis Child* 1992; 67: 1003–1007.
2. PICCINI M-P, MECACCI F, SAMPOGNARO S, et al. Aero-allergen sensitization can occur during fetal life. *Int Arch Allergy Immunol* 1993; 102: 301–303.
3. PRESCOTT SL, MACAUBAS C, HOLT BJ, et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T-cell responses towards the Th-2 cytokine profile. *J Immunol* 1998; 160: 4730–4737.
4. JONES CA, MILES EA, WARNER JO, et al. Fetal peripheral blood mononuclear cell proliferative responses to mitogenic and allergenic stimuli during gestation. *Pediatr Allergy Immunol* 1996; 7: 109–116.
5. HOLT PG. Primary allergic sensitisation to environmental antigens: perinatal T-cell priming as a determinant of responder phenotype in adulthood. *J Exp Med* 1996; 183: 1297–1301.
6. RAGHUPATHY R. Th1-type immunity is incompatible with successful pregnancy. *Immunol Today* 1997; 18: 478–482.
7. JONES C, KILBURN S, WARNER J, et al. Intrauterine environment and fetal allergic sensitization. *Clin Exp Allergy* 1998; 28: 655–659.
8. SINGH RR, HAHN BH, SERCARZ EE. Neonatal peptide exposure can prime T cells, and upon subsequent immunization induce their immune deviation: implications for antibody vs. T cell-mediated autoimmunity. *J Exp Med* 1996; 183: 1613–1622.
9. HOLT PG, O'KEEFE PO, HOLT BJ, et al. T-cell "priming" against environmental allergens in human neonates: sequential deletion of food antigen specificities during infancy with concomitant expansion of responses to ubiquitous inhalant allergens. *Ped Allergy Immunol* 1995; 6: 85–90.
10. UPHAM JW, HOLT BJ, BARON-HAY MJ, et al. Inhalant allergen-specific T-cell reactivity is detectable in close to 100% of atopic and normal individuals: covert responses are unmasked by serum-free medium. *Clin Exp Allergy* 1995; 25: 634–642.
11. YABUHARA A, MACAUBAS C, PRESCOTT SL, et al. Th-2-polarised immunological memory to inhalant allergens in atopics is established during infancy and early childhood. *Clin Exp Allergy* 1997; 27: 1261–1269.
12. PRESCOTT SL, MACAUBAS C, SMALLACOMBE T, et al. Development of allergen-specific T-cell memory in atopic and normal children. *Lancet* 1999; 353: 196–200.
13. WEINER HL. Oral tolerance. *Proc Natl Acad Sci USA* 1994; 91: 10762–10765.
14. MACAUBAS C, SLY PD, BURTON P, et al. Regulation of Th-cell responses to inhalant allergen during early childhood. *Clin Exp Allergy* 1999; 29: 1223–1231.
15. HOLT PG, CLOUGH JB, HOLT BJ, et al. Genetic "risk" for atopy is associated with delayed postnatal maturation of T-cell competence. *Clin Exp Allergy* 1992; 22: 1093–1099.
16. RINAS U, HORNEFF G, WAHN V. Interferon- γ production by cord-blood mononuclear cells is reduced in newborns with a family history of atopic disease and is independent from cord blood IgE-levels. *Pediatr Allergy Immunol* 1993; 4: 60–64.
17. WARNER JA, MILES EA, JONES AC, et al. Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? *Clin Exp Allergy* 1994; 24: 423–430.
18. NOBLE A, STAYNOV D, KEMENY D. Generation of rat Th2-like cells *in vitro* is interleukin-4-dependent and inhibited by interferon-gamma. *Immunology* 1993; 79: 562–567.
19. POHL D, BOCKELMANN C, FORSTER K, et al. Neonates at risk of atopy show impaired production of interferon-gamma after stimulation with bacterial products (LPS and SEE). *Allergy* 1997; 52: 732–738.
20. PRESCOTT SL, SLY PD, HOLT PG. Raised serum IgE associated with reduced responsiveness to DPT vaccination during infancy. *Lancet* 1998; 351: 1489.