

Strategic targets for primary prevention of allergic disease in childhood

Holt PG, Macaubas C, Sly PD. Strategic targets for primary prevention of allergic disease in childhood.
Allergy 1998; 53: 72–76. © Munksgaard 1998.

P. G. Holt, C. Macaubas, P. D. Sly

TVW Telethon Institute for Child Health
Research (affiliated with the University of
Western Australia), West Perth, Australia

P. G. Holt
Division of Cell Biology
TVW Telethon Institute for Child Health
Research
PO Box 855
West Perth WA 6872
Australia

The progressive increase in the prevalence of allergic disease and the spiralling costs associated with treatment, particularly of chronic sufferers, provide an urgent imperative for research into new approaches to this problem. Prevention is clearly more desirable and more cost-efficient than cure. But is it practicable? The best case scenario for such an approach would be if the expression of atopy was preceded by a relatively prolonged inductive phase, during which susceptible subjects displayed a readily identifiable “predisease” phenotype, and moreover that the markers defining this phenotype should in turn point to strategic intervention targets. As detailed below, recent developments in our understanding of the basic immunology underlying primary allergic sensitization in man have brought us tantalizingly close to that point, to the extent that several early treatment options can now seriously be considered. The studies which support this proposition are reviewed below.

Priming of the T-cell system against environmental allergens: intrauterine schooling of the developing immune system

Until very recently, T-cell priming against antigens from the external environment was held to commence with the baby's first breath. However, the presence of low-level lymphoproliferative responses in cord blood (CB) against common dietary and inhalant allergens (1–4) has suggested that the small amounts of allergens ingested/inhaled by the mother may be sufficient to prime

the fetal immune system via transplacental leakage, particularly during the last trimester of pregnancy (5). This proposition was widely challenged, and many workers in this field have argued that the responses are due to contamination of CB with low levels of maternal T cells, or, alternatively, represent anti-idiotypic responses induced by maternal antibodies, or cross-reactivity against other more ubiquitous (e.g., microbial) antigens. However, these counterarguments have essentially been refuted in recent studies from our laboratory involving DNA genotyping of fetal allergen-specific T-cell clones in combination with T-cell epitope analysis of individual lymphoproliferative responses (6); additionally, the lack of proliferative responses to tetanus antigen, despite the presence of relevant maternal antibodies, argues against the importance of anti-idiotypic interactions in this context (4, 6).

Analysis of allergen-specific T-cell cytokine profiles in these fetally primed T cells indicates prominent Th2 polarization, evidenced by mRNA responses specific for interleukin (IL)-4, IL-5, IL-6, and IL-9 (6), and cytokine protein (IL-10 and IL-13) detectable by ELISA (6, 7). Interferon-gamma (IFN- γ) responses are, in contrast, relatively infrequent and require the use of very high polymerase chain reaction (PCR) cycle numbers for detection (7).

Postnatal maturation of allergen-specific T-cell responses

Cross-sectional studies employing standard lymphoproliferation techniques indicate postnatal

divergence of T-cell responses to dietary and inhalant allergens. Thus, by the age of 5 years, responses to dietary allergens such as ovalbumin (OVA), which are frequent at birth, have largely disappeared, presumably indicating downregulation by some form of high zone tolerance (4, 8, 9). In contrast, responses to inhalant allergens such as those of house-dust mite (HDM) persist and eventually expand, and, by the age of 5 years, partition into adult-like Th1-polarized profiles (skin-test-negative subjects) as opposed to Th2-polarized profiles in skin-test-reactive atopics (8, 9), the latter pattern being most frequent in children with positive atopic family history.

Selection for Th1- as opposed to Th2-polarized memory against inhalant allergens: what determines the pathway followed?

The pattern of Th1-polarized T-cell cytokine responses which develops postnatally in nonatopics indicates the successful operation of an underlying process of immune deviation; i.e., the divergence of pre-existing (fetally primed) Th2 immunity toward Th1. This process fails in atopics, leading instead to persistence and boosting of fetal Th2 responses (10).

How did the Th2 responses develop initially in virtually all children? The answer to this question is supplied in the recent literature on the immunology of pregnancy. Collectively, this argues that the fetal immunologic milieu is maintained in a Th2-polarized state via the secretion of a range of Th1-antagonistic factors (IL-4, IL-10, prostaglandin (PG)_E₂, progesterone) by the placenta (11, 12), the purpose of which is to dampen Th1 responses (which are toxic to the placenta) at the fetomaternal interface. Accordingly, any immune responses initiated *in utero* are directed toward the Th2 compartment.

What determines the phenotype of long-term cytokine responses which are programmed during further development of these responses postnatally? Why do these differ in atopics and non-atopics? A long-standing animal model literature indicates that the principal stimulus for postnatal development of adaptive immune function is contact with the microbial environment, both pathogens and commensals (12–15). It is also clear from various studies that the T-cell system in early postnatal life maintains the fetal Th2 bias for variable periods postnatally, both in experimental animals (16–18) and in man (reviewed in refs. 10, 19). Moreover, the Th1 “deficit” in early life is maintained for up to 5 years (20). Evidence from several independent laboratories also indicates that

the kinetics of postnatal maturation of Th1 function(s) is slower in children at high genetic risk of atopy. This conclusion follows from *in vitro* studies on polyclonal stimulation of IFN- γ production in neonates and infants (3, 21–25), and from *in vivo* studies on the responsiveness of infants and children to both BCG (26) and diphtheria/pertussis/tetanus vaccination (27), all of which are reduced in association with the atopic phenotype. This transient maturational deficit in Th1 function is hypothesized to compromise the efficiency of downregulation (i.e., immune deviation) of fetally primed Th2 responses during early childhood (10, 12).

Th1/Th2 regulation and expression of allergic disease

Large-scale population studies confirm the strong relationship between markers of Th2 reactivity, such as elevated serum IgE, and atopic disease expression (28). However, it is clear that while a very high proportion of schoolchildren with current atopy symptoms (such as atopic asthma) are skin test positive (SPT⁺) to at least one inhalant allergen, only a subset of the SPT⁺ atopic population develop severe disease (29).

Clearly, additional factor(s), genetic and/or environmental, are required for subsequent chronic disease expression. We have argued previously that the target tissue, as exemplified by the airway mucosa in atopic asthma, needs to exceed a “critical threshold” level of local inflammatory damage, before the final development of the airways hyper-reactivity which is the hallmark of chronic disease (30). The most potent of the relevant environmental factors appears to be virus infection (31), and it is likely that these function by pushing the level of airway mucosal inflammation over the threshold referred to above. Additional factors which may be operative here include diet (32) and air pollution (33).

Potential intervention strategies

In principle, this new information can be applied to the rational development of new intervention strategies that aim at interrupting the process described in Fig. 1 at a point prior to the manifestation of chronic inflammatory disease.

We envisage the following five major categories of interventions.

Early use of anti-inflammatories

In the scheme in Fig. 1, progression from expression of Th2-dependent SPT reactivity to one or

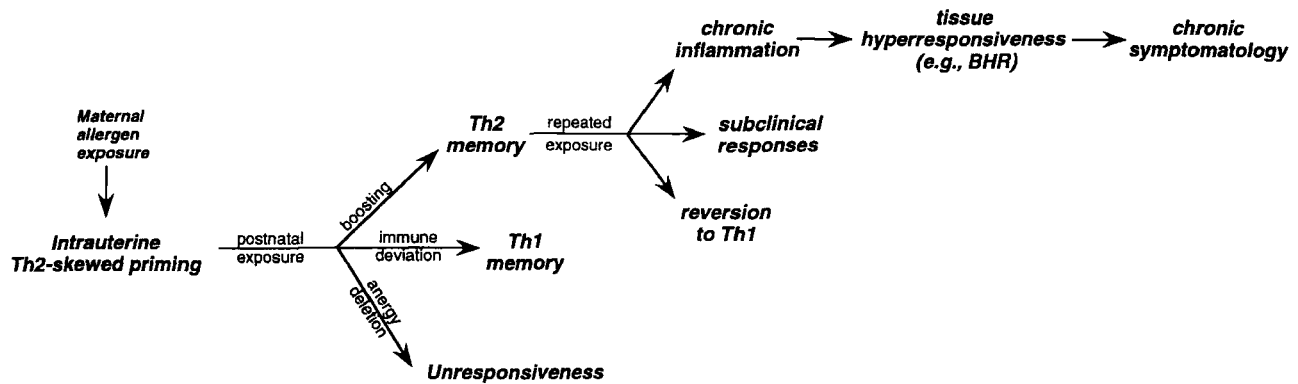


Fig. 1. Induction and expression of allergic disease. BHR: bronchial hyperresponsiveness.

more inhalant allergens through to subsequent full-blown disease expression is dependent upon the accumulation of a suprathreshold level of inflammatory damage in the target tissue. It is also likely that repeated cycles of local Th2-mediated inflammation would provide the key cytokine signals required to drive the further expansion of these Th2 response(s), thus inhibiting mechanism(s) which may otherwise redirect reactivity back toward the Th1 phenotype.

On theoretic grounds, the appropriate use of anti-inflammatories (steroidal or nonsteroidal), at a stage after objective evidence of Th2 reactivity is confirmed, may interrupt this potentially self-sustaining process, and permit either partial or complete re-establishment of local immunologic homeostasis through immune deviation mechanisms.

It is pertinent to note that the key cell population which is likely to drive this immune deviation process, airway mucosal dendritic cells (DC), is sensitive to the effects of inhaled steroids (34–37). However, it is not known to what degree steroids influence the immune deviating properties of these DC; i.e., whether they affect the balance between Th1 and Th2 stimulation. It is also relevant to note in this context the results of recent studies which suggest that the use of β_2 -agonists may in some circumstances prevent Th1 development by selective inhibition of IL-12 production by antigen-presenting cells (APC) (38).

Environmental control

It is generally acknowledged that the levels of specific allergen in the immediate environment of individual atopics are positively correlated with intensity of disease expression (39); therefore, on first principles, it can be argued that reduction of allergen levels in the homes of atopic children is desirable. It is not clear, however, to what degree these measures are practicable over the time period

required to influence the progress of disease in subjects already fully sensitized.

However, epidemiologic evidence from various sources indicates that exposure to high levels of inhalant allergens during infancy constitutes a risk factor for allergic disease expression in later life. Our recent findings, indicating that weak Th2 sensitization to inhalant allergens is common at birth in both (future) atopics and nonatopics, coupled with earlier reports from our group and several other laboratories indicating that capacity to generate countermanding Th1 responses is blunted in atopics during infancy, collectively provide a plausible explanation of the earlier epidemiologic findings. Thus, early postnatal exposure of atopics with primed Th2 cells plus poor Th1 function is likely to favor the boosting of their Th2 immunity, rather than stimulating immune deviation toward Th1.

Accordingly, allergen reduction measures may be particularly effective if employed during early infancy.

Immunoprophylactic vaccines

Given the plasticity of immune responses to allergens during early childhood relative to the mature memory responses encountered in adulthood, modified immunotherapeutic strategies, similar to those currently applied in established atopy to immune deviate Th2 responses to Th1, may be effective in early childhood. The key feature of this approach is the fact that the target group have not yet developed relevant mature Th2-memory populations; i.e., they are not yet fully sensitized, and hence the allergen preparations which would be employed would ideally function as immunoprophylactic “vaccines” to preferentially stimulate immune deviation toward Th1 (40).

Several important issues relating to such an approach are yet to be resolved; in particular, the nature of the allergen formulations and the mode of administration. On theoretic grounds, mucosal

exposure (in particular, the intranasal route) would appear to be a logical starting point. A comprehensive animal model literature indicates that this route leads to preferential downregulation of Th2 responses (41), and intranasal allergen drops have been employed in a small number of human clinical trials without detrimental effects. However, the approach is yet to be tested in young children.

In the future, it may be possible to enhance the Th1-stimulatory effects of such preparations via the inclusion of appropriate adjuvants, specifically designed for effectiveness at mucosal surfaces (42).

Immunostimulatory vaccines

As argued above, a temporal delay in the switch from Th2-biased (fetal) to Th1-biased (adult-like) immune function during childhood may be a key factor underlying the predisposition of atopics to generation of long-term, allergen-specific Th2-polarized memory. Hence, measures to hasten this maturation process may prove efficacious in limiting allergic sensitization.

Stimulation of the natural microbial environment, including, in particular, the gut microflora (12–15), is one of the principal stimuli for this process. Consequently, it may be possible to accelerate Th1 “maturation” in atopics via provision of additional microbial-based stimuli, or defined molecules which mimic their effects.

Several approaches may be feasible:

- 1) modulation of gut flora via dietary manipulation
- 2) the use of oral microbial extracts such as those in current use in adults as nonspecific mucosal immunostimulants (43, 44)
- 3) parenteral vaccination with microbial stimulants such as BCG (45, 46)
- 4) the use of orally available small-molecule “adjuvants” which selectively enhance systemic Th1 function (47).

Oral tolerance

As argued earlier (13), the available epidemiologic and immunologic evidence indicates that selective downregulation of Th2-dependent IgE responses to dietary antigens operates at a very high rate of efficiency, even in atopics, as evidenced by the very low prevalence of persistent food allergy in the adult human population.

If it were possible to harness successfully these same oral tolerance mechanism(s) to downregulate Th2 responses to inhalant allergens during childhood, a log-scale reduction in the prevalence of respiratory allergies would not be an unrealistic goal. However, the factor limiting this approach is

the availability of the large quantities of inhalant allergens which would be required, given that the allergen exposure range over which oral tolerance normally operates is one-to-several logs above that relevant to inhalant allergens. This logistical problem may be eventually solvable via the use of molecular biologic techniques; e.g., the cloning of genes encoding inhalant allergen epitopes into foodstuffs such as cereals, which could then be used as dietary supplements during early childhood.

Conclusions

As recently as 10 years ago, the approaches outlined above for the potential control of allergic disease would have been considered heretical. However, the realization that the prevalence and severity of these diseases, and their attendant socioeconomic impact, are continuing to rise, despite the best efforts of the pharmaceutical industry and the medical research community, is stimulating increasing interest in new and radical approaches to this problem. Primary prevention strategies of the type outlined above represent one such initiative.

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;67:1003-7.
2. Piccinni M-P, Mecacci F, Sampognaro S, et al. Aeroallergen sensitization can occur during fetal life. *Int Arch Allergy Immunol* 1993;102:301-3.
3. Piastra M, Stabile A, Fioravanti G, Castagnola M, Pani G, Ria F. Cord blood mononuclear cell responsiveness to beta-lactoglobulin: T-cell activity in “atopy-prone” and “non-atopy-prone” newborns. *Int Arch Allergy Immunol* 1994;104:358-65.
4. Holt PG, O’Keeffe 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. *Pediatr Allergy Immunol* 1995;6: 85-90.
5. Jones AC, Miles EA, Warner JO, Colwell BM, Bryant TN, Warner JA. Fetal peripheral blood mononuclear cell proliferative responses to mitogenic and allergenic stimuli during gestation. *Pediatr Allergy Immunol* 1996;7:109-16.
6. 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 (in press).
7. Prescott SL, Macaubas C, Yabuhara A, et al. Developing patterns of T cell memory to environmental allergens in the first two years of life. *Int Arch Allergy Immunol* 1997;113:75-9.
8. 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;27:1261-9.
9. Macaubas C, Sly PD, Burton P, et al. Differential regulation

- of T-cell responses to inhalant versus dietary allergens during early childhood. *Am J Respir Cell Mol Biol* (submitted).
10. Holt PG, Macaubas C. Development of long term tolerance versus sensitisation to environmental allergens during the perinatal period. *Curr Opin Immunol* 1997;9:782-7.
 11. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunol Today* 1993;14:353-6.
 12. Holt PG, Sly PD, Björkstén B. Atopic versus infectious diseases in childhood: a question of balance? *Pediatr Allergy Immunol* 1997;8:53-8.
 13. Holt PG. Environmental factors and primary T-cell sensitisation to inhalant allergens in infancy: reappraisal of the role of infections and air pollution. *Pediatr Allergy Immunol* 1995;6:1-10.
 14. Inagaki H, Suzuki T, Nomoto K, Yoskikai Y. Increased susceptibility to primary infection with *Listeria monocytogenes* in germfree mice may be due to lack of accumulation of L-selectin⁺ CD44⁺ T cells in sites of inflammation. *Infect Immun* 1996;64:3280-7.
 15. Sudo N, Sawamura S-A, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* 1997;159:1739-45.
 16. Ridge JP, Fuchs EJ, Matzinger P. Neonatal tolerance revisited: turning on newborn T cells with dendritic cells. *Science* 1996;271:1723-6.
 17. 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;183:1613-22.
 18. Chen N, Gao Q, Field EH. Expansion of memory Th2 cells over Th1 cells in neonatal primed mice. *Transplantation* 1995;60:1187-93.
 19. 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.
 20. Holt PG, McMenamin C, Nelson D. Primary sensitisation to inhalant allergens during infancy. *Pediatr Allergy Immunol* 1990;1:3-13.
 21. 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;22:1093-9.
 22. 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;4:60-4.
 23. Tang MLK, Kemp AS, Thorburn J, Hill DJ. Reduced interferon- γ secretion in neonates and subsequent atopy. *Lancet* 1994;344:983-6.
 24. Martinez FD, Stern DA, Wright AL, Holberg CJ, Taussig LM, Halonen M. Association of interleukin-2 and interferon- γ production by blood mononuclear cells in infancy with parental allergy skin tests and with subsequent development of atopy. *J Allergy Clin Immunol* 1995;96:652-60.
 25. Liao SY, Liao TN, Chiang BL, et al. Decreased production of IFN- γ and increased production of IL-6 by cord blood mononuclear cells of newborns with a high risk of allergy. *Clin Exp Allergy* 1996;26:397-405.
 26. Shirakawa T, Enomoto T, Shimazu S, Hopkin JM. Inverse association between tuberculin responses and atopic disorder. *Science* 1997;275:77-9.
 27. Prescott SL, Sly PD, Holt PG. Reduced responsiveness to DPT vaccination during infancy is associated with elevated serum IgE. *Lancet* 1998 (in press).
 28. Burrows B, Martinez F, Halonen M, Barbee R, Cline M. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med* 1989;320:271-7.
 29. Woolcock AJ, Peat JK, Trevillion LM. Is the increase in asthma prevalence linked to increase in allergen load? *Allergy* 1995;50:935-40.
 30. Holt PG, Sly PD. Allergic respiratory disease: strategic targets for primary prevention during childhood. *Thorax* 1997;52:1-4.
 31. Johnston SL, Pattemore PK, Sanderson G, et al. The relationship between upper respiratory infections and hospital admissions for asthma: a time-trend analysis. *Am J Respir Crit Care Med* 1996;154:654-60.
 32. Weiss ST. Diet as a risk factor for asthma. *Ciba Found Symp* 1997;206:244-57.
 33. Björkstén B. Risk factors in early childhood for the development of atopic diseases. *Allergy* 1994;49:400-7.
 34. Nelson DJ, McWilliam AS, Haining S, Holt PG. Modulation of airway intraepithelial dendritic cells following exposure to steroids. *Am J Respir Crit Care Med* 1995;151:475-81.
 35. Holt PG, Thomas JA. Steroids inhibit uptake and/or processing but not presentation of antigen by airway dendritic cells. *Immunology* 1997;91:145-50.
 36. Möller GM, Overbeek SE, van Helden-Meeuwsen CG, et al. Increased numbers of dendritic cells in the bronchial mucosa of atopic asthmatic patients: downregulation by inhaled corticosteroids. *Clin Exp Allergy* 1996;26:517-24.
 37. Tunon-de-Lara JM, Redington AE, Bradding P, et al. Dendritic cells in normal and asthmatic airways: expression of the α subunit of the high affinity immunoglobulin E receptor (Fc ϵ RI- α). *Clin Exp Allergy* 1996;26:648-55.
 38. Panina-Bordignon P, Mazzeo D, Lucia PD, et al. Beta γ -antagonists prevent Th1 development by selective inhibition of interleukin-12. *J Clin Invest* 1997;15:1513-19.
 39. Peat JK, Gray EJ, Mellis CM, Leeder SR, Woolcock AJ. Differences in airway responsiveness between children and adults living in the same environment: an epidemiological study in two regions of New South Wales. *Eur Respir J* 1994;7:1805-13.
 40. Holt PG. A potential vaccine strategy for asthma and allied atopic diseases in early childhood. *Lancet* 1994;344:456-8.
 41. Holt PG. Immunoprophylaxis of atopy: light at the end of the tunnel? *Immunol Today* 1994;15:484-9.
 42. Tomasi M, Dertzbaugh MT, Hearn T, Hunter RL. Strong mucosal adjuvanticity of cholera toxin within lipid particles of a new multiple emulsion delivery system for oral immunization. *Eur J Immunol* 1997;27:2720-5.
 43. Collett JP, Shapiro S, Ernst P, et al. Effects of an immunostimulating agent on acute exacerbations and hospitalizations in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1997;156:1719-24.
 44. Lehmann D, Coakley KJ, Coakley CA, et al. Reduction in the incidence of acute bronchitis by an oral *Haemophilus influenzae* vaccine in patients with chronic bronchitis in the highlands of Papua New Guinea. *Am Rev Respir Dis* 1991;144:324-30.
 45. Alm JS, Lilja G, Pershagen G, Scheynius A. Early BCG vaccination and development of atopy. *Lancet* 1997;350:400-3.
 46. Strannegård I-L, Larsson L-O, Wennergren G, Strannegård Ö. Prevalence of allergy in children in relation to prior BCG vaccination and infection with atypical mycobacteria. *Allergy* 1998;53:249-54.
 47. Rhodes J. Covalent chemical events in immune induction: fundamental and therapeutic aspects. *Immunol Today* 1996;17:436-41.