

Primary Allergic Sensitization to Environmental Antigens: Perinatal T Cell Priming as a Determinant of Responder Phenotype in Adulthood

By Patrick G. Holt

From the Division of Cell Biology, TVW Telethon Institute for Child Health Research, West Perth 6872, Western Australia

The prevalence and severity of allergic diseases, in particular those affecting the respiratory system, are increasing at an alarming rate in the developed countries, and the international research effort into the etiology and pathogenesis of these syndromes is rapidly expanding to meet this challenge. The immunoinflammatory reactions that mediate airway tissue damage in allergic (atopic) subjects stem from aberrant T cell responses to a range of airborne environmental antigens that seemingly are ignored by non-atopic individuals. At the T cell level, allergic reactivity manifests as production of a Th-2-like cytokine profile at each challenge (1, 2), and mounting evidence (reviewed briefly below) suggests that the development of this pattern of T cell sensitization is frequently associated with exposure to high levels of the relevant antigens during early infancy.

This finding contrasts with the traditional experimental literature, in which parenteral antigenic challenge of neonatal animals leads preferentially to tolerance induction, a process most commonly ascribed to T cell anergy and/or deletion (3–5). However in this issue of the journal, Singh et al. (6) present the results of a comprehensive study from a murine model, which focuses on the pattern of underlying antigen-specific T cell cytokine responses during the induction and subsequent expression of classical neonatal tolerance. They demonstrate that neonatal antigen exposure triggers an initially heterogeneous T cell response containing both Th-1- and Th-2-like elements, which results not in eventual T cell deletion/anergy but instead in “priming” for subsequent immune deviation toward a pattern of T cell immunity that is skewed toward Th-2. Their discussion of the relevance of this neonatal tolerance model is couched specifically in terms of autoimmune diseases. However, as argued below, the underlying T cell selection process is equally relevant to the etiology of human allergic diseases.

Protection against Allergic Sensitization: Exclusion versus Regulation

Until comparatively recently, expression of the allergic responder phenotype in humans was held to result from failure of a series of active and passive exclusion mechanisms, operative at mucosal surfaces, which limits contact

between inert environmental antigens such as pollen proteins (potential allergens) and the T cell system. Identification of secretory IgA deficiency as a predisposing factor toward development of allergic disease (7) served to reinforce this general view.

However, parallel findings in the mucosal immunology literature have increasingly cast doubt on this simplistic model. In particular, the results of studies of the phenomenon of oral tolerance (8) and its respiratory tract equivalent (9) argued that protection against the development of immediate and delayed hypersensitivity to ingested and inhaled allergens depended on a series of cognate immunological processes, the triggering of which (by definition) required the passage of sufficient allergen across epithelial barriers to effectively engage the T cell system.

The nature of the regulatory mechanism(s) underlying these latter processes is the subject of ongoing, intense debate. Oral tolerance has been variously ascribed to active suppression mediated by regulatory T cells (10, 11), the leakage into blood of tolerogenic low molecular weight peptides derived from protein antigens digested in the gut (12), or induction of specific T cell anergy (13). This controversy has been resolved partly by the finding that different mechanisms operated at the extremes of the allergen dose response curve, viz., repeated low dose feeding induces regulatory T cells that mediate immune deviation, whereas progressively higher allergen doses induce T cell anergy (14, 15) or eventually T cell deletion (16). However, the mechanism(s) underlying this particular form of immune deviation remains unclear.

Repeated exposure of rodents to aerosols containing low levels of allergen also induces long-lasting tolerance, which is now recognized as immune deviation (17). This results in selective suppression of both IgE and delayed type hypersensitivity (DTH) responses, with concomitant preservation of IgG_{2a/2b} production (9, 18). CD8⁺ regulatory T cells, in particular a subset of apparently antigen-specific CD8⁺ T γ/δ cells (18), play an important role in this process. An additional finding from these studies was that the initial activation of these CD8⁺ regulatory T cells was absolutely dependent on an initial short-lived burst of help from CD4⁺ Th-2 cells responding to the same antigen (17), and the presence of the latter cells in the regional

lymph nodes draining the airway mucosa of exposed animals was associated with a transient phase of specific IgE production (17). Moreover, this finding echoes the original report describing oral tolerance (19), in which guinea pigs placed on a diet supplemented with OVA developed severe but transient (IgE-mediated) food allergy to OVA, before the eventual onset of protective tolerance.

Etiology of Allergic Disease in Humans: Significance of Neonatal Allergen Exposure

By far the most common allergies in humans are those induced by airborne antigens, which suggests that the mechanism(s) that normally protects against allergic sensitization at mucosal surfaces operates at a lower level of efficiency in the respiratory tract than, for example, in the gut (20). The fact that these latter mechanisms are predominantly antigen-specific and based on a form (or forms) of immune deviation can be deduced from three lines of evidence: (a) the precursor frequency of T cells reactive to the major inhalant allergens is comparable in the blood of allergic and nonallergic subjects (21); (b) sensitive short-term T cell activation assays demonstrate specific reactivity to inhalant allergens in nearly 100% of blood samples from both allergic and normal subjects (22, 23); and (c) cytokine-secreting T cell clones reactive to inhalant allergens can be readily propagated from bulk cultures of allergen-stimulated blood cells from both normals and atopics (1, 2). Furthermore, consistent with what has been learned from the study of IgE switch regulation in the murine system, the cytokine profiles of allergen-specific T cell clones isolated from atopics are skewed toward a Th-2-like (or in some cases, Th-0-like) profile, compared with a TH-1-like profile in non-atopics (1, 2).

Our understanding of the etiology of allergic disease accordingly turns on the question of how the T cell system is primed and locked into one of these mutually exclusive patterns of reactivity. In the animals studies, it is clear that antigen-driven selection for long-term Th-1-like versus Th-2-like memory against individual inhaled antigens occurs over the course of the first few exposures and, once consolidated, is not readily reversible (9, 20). With respect to exposure of humans to airborne allergens that are ubiquitous in the natural environment, the corresponding period of initial exposure of the immunologically naive immune system generally occurs during late fetal and early postnatal life.

The first indication that antigen-driven immune responses occurring during very early life could influence the expression of the allergic phenotype in adulthood was provided in a series of independent studies, performed with children and young adults, that demonstrated that being born during the pollen season was associated with a significantly increased risk of developing pollen allergy in later life (reviewed in reference 24). In this context, the capacity to develop oral tolerance and its respiratory tract equivalent is poorly developed at birth in experimental animals and does not reach adult-equivalent levels of competence until approximately the time of weaning (25, 26). It is tempting

to speculate that similar developmental deficiencies in immune function in human infants may underlie their heightened susceptibility to allergic sensitization against mucosally presented antigens.

An indirect window into the effects of allergen exposure during this key period on underlying T cell immunity is provided via a series of prospective seroepidemiological studies that have tracked allergen-specific antibody titers in individual subjects from birth through early childhood. These studies have uncovered an intriguing pattern of change in the type and magnitude of allergen-specific antibody responses at different ages. Thus, virtually all children initiate IgG₁ production against both inhalant and food allergens by 3 mo of age (27–29); this production typically (especially in relation to food allergens) peaks in affinity and titer during infancy and wanes thereafter (29), whereas corresponding IgG₄ responses continue to develop (28, 29). The intrinsic biphasic nature of the immune response to environmental allergens in most children is even more marked when individual IgE titers are tracked: after infants are introduced to solid foods, typically they develop varying levels of IgE antibodies against one or more common food antigens, such as egg, cow's milk, etc.; titers usually peak in infants at ~9 mo of age and rapidly wane thereafter (30). Corresponding low-level IgE responses to inhalant allergens develop more slowly, presumably because inhalant antigens produce much lower overall levels of stimulation in comparison with dietary allergens, but nevertheless they are detectable in virtually all children by the age of 2–3 yr (20, 30). However, as is the case with dietary allergens, these responses usually wane, but they do so comparatively slowly and often do not disappear until ~5–6 yr of age (20, 30).

This early pattern of transient Th-2-dependent antibody production against nonpathogenic environmental antigens appears common to all children and may represent the default response to mucosal challenge; this finding is also consistent with the result of *in vitro* studies demonstrating that naive neonatal CD4⁺ human T cells produce IL-4 at priming (31). Moreover, as noted above, it mirrors the initial Th-2 skewing of the response of immunologically naive experimental animals to “new” ingested or inhaled antigens, during the early phase of tolerance induction.

In the case of responses to dietary allergens, the regulatory process leading to the termination of antigen-specific IgE production is clearly very effective, given the extremely low prevalence of food allergy in the adult population. However, a steadily increasing percentage of the population is displaying hypersensitivity to one or more airborne environmental allergens, which manifest as IgE responses that fail to terminate during childhood and instead persist into adulthood (failure to develop tolerance?) or reappear in adulthood (loss of tolerance?).

Development of T Cell Immunity to Environmental Allergens in Humans: The Emerging Picture

In contrast to the relatively broad serological literature on the postnatal development of immunity to environmen-

tal allergens in humans, direct studies on the nature of the underlying T cell responses have been begun only recently.

The most productive approach has involved prospective studies on peripheral blood T cell reactivity to panels of allergens in cohorts of infants, bled at strategic intervals throughout early childhood. Analyses of the relevant response patterns have thus far relied exclusively on lymphoproliferation assays, because the low precursor frequency of allergen-specific T cells in these early responses militates against the use of conventional cytokine assays.

It is becoming clear from these studies that major differences exist between the patterns of T cell reactivity to food and to inhalant allergens, as predicted by the available data on respective antibody titers during childhood (24). Notably, lymphoproliferative responses to the major inhalant allergens increase markedly in frequency and intensity between infancy and adulthood, in contrast to parallel responses to food allergens, which peak in early infancy and decline thereafter (22, 32). These disparate patterns have been suggested to reflect the relative importance of immune deviation versus T cell deletion mechanisms at the two challenge sites, triggered respectively by repeated exposure to nanogram levels of inhaled versus microgram-to-gram levels of ingested allergens (20, 32).

Elucidation of relevant T-cell cytokine response patterns, particularly during the perinatal period, represents the major priority for future research in this area. The results of preliminary studies on cytokine levels in the blood of infants have detected a transient rise in IL-4 concentrations during early infancy (33, 34), which coincides with the transient peak of food allergen-specific IgE titers that commonly occurs during the first year of life (30). However, the key information still lacking relates to the cytokine phenotype of allergen-specific T helper cells during the initial phase of these immune responses; studies employing semi-quantitative reverse transcriptase-PCR technology to address this important question are in progress at several centers.

When and How Is T Cell Priming to Environmental Allergens Initiated? These questions have not been answered definitively, but some tantalizing clues are available. It is evident that in many cases, initial priming of the T cell system is initiated in utero. This conclusion follows from the rash of recent reports (e.g., references 32, 35–37) demonstrating that lymphoproliferative responses of cord blood cells to food and inhalant allergens are common in infants both with/without atopic family history.

However, whereas responses to ubiquitous environmental allergens are common in cord blood, parallel responses to vaccine antigens such as tetanus toxoid, to which pregnant women are not generally exposed are not found (32). This suggests that transplacentally transferred allergen or processed peptides derived from the latter, perhaps in conjunction with maternally derived IgG-subclass antibodies which are present in virtually all neonatal blood samples (27), may provide the initial trigger(s). In this context, there are several precedents in the literature for induction of tolerance/immune deviation in neonatal animals associ-

ated with transplacental transfer of either specific antibodies (38) or dietary antigens (39).

Does Perinatal Priming to Environmental Allergens Contribute to the Maintenance of Immunological Homeostasis or Does It Pre-dispose to the Development of Allergic Disease? As argued above, it seems unlikely that perinatal priming per se constitutes a risk factor, given that it appears universal. Instead, it may be part of a normal education process in which the immature immune system is primed for the subsequent selection of host protective immunity (in this case, Th-1-like), under drive from environmental allergens that are encountered during subsequent childhood.

The accompanying paper by Singh et al. (6) illustrates the other side of this coin, viz., how neonatal T cell priming that elicits an initially heterogeneous response can conversely set triggers for later immune deviation toward Th-2-like immunity. Their discussion emphasizes the potential importance of such a mechanism in silencing autoaggressive T cell responses. Analogous to the situation outlined above for allergen reactive T cells, human cord blood also contains surprisingly high numbers of T cells responsive to such autoantigens as acetylcholine receptor and myelin basic protein, which moreover display a mixed Th-1/Th-2 cytokine pattern (40).

Why Should Perinatal T Cell Priming Preferentially Lead to Immune Deviation toward Th-1 Responses against One Class of Antigen (Environmental Allergens) and to Th-2 Responses against Another (Self Antigens)? This question remains to be determined, but the answer is likely to center around differences in the respective modes of antigen presentation to the immune system, both at initial priming and during the ensuing antigen-driven T cell selection process.

In relation to responses to airborne environmental allergens, recent studies using adult experimental animals have demonstrated the central role of a network of intraepithelial dendritic cells (DC), the majority of which express high levels of class II major histocompatibility complex (MHC) in the delivery of inhaled antigens from the airway mucosa to the T cell system (41). However, in neonatal animals, this DC population is predominantly MHC class II low or negative, and they do not develop adult-equivalent patterns of MHC class II expression, or reach comparable densities with the epithelium, until the time of weaning (42). The nature of antigenic signaling to the T cell system in neonatal animals via the airway epithelial DC network is therefore likely to differ both qualitatively and quantitatively from that in adults. It has not yet been formally established that a comparable DC developmental pattern occurs in the airway epithelium of humans during infancy, but the close similarities between respective networks in adult humans and mature experimental animals from several different species (references 41 and 43) suggest that the latter is likely. If so, we can speculate that the kinetics of postnatal maturation of the airway DC network may be one of the key factors in the immune deviation processes that determine the nature of the long-term T cell memory for environmental allergens.

The author's laboratory is supported by the National Health and Medical Research Council of Australia and Glaxo Wellcome.

Address correspondence to P.G. Holt, Division of Cell Biology, TVW Telethon Institute for Child Health Research, P.O. Box 855, West Perth, Western Australia 6872.

Received for publication 11 March 1996.

References

1. Wierenga, E.A., M. Snoek, C. de Groot, I. Chrétien, J.D. Bos, J.M. Jansen, and M.L. Kapsenberg. 1990. Evidence for compartmentalization of functional subsets of CD2⁺ T lymphocytes in atopic patients. *J. Immunol.* 144:4651–4656.
2. Romagnani, S. 1990. Regulation and deregulation of human IgE synthesis. *Immunol. Today.* 11:316–321.
3. Nossal, G.J. 1983. Cellular mechanisms of immunologic tolerance. *Annu. Rev. Immunol.* 1:33–62.
4. Young, C.R., and M.Z. Atassi. 1983. T-lymphocyte recognition of sperm-whale myoglobin. Specificity of T-cell recognition following neonatal tolerance with either myoglobin or synthetic peptides of an antigenic site. *J. Immunogenet.* 10: 161–169.
5. Gammon, G., K. Dunn, N. Shastri, A. Oki, S. Wilbur, and E. Sercarz. 1986. Neonatal tolerance induced to minimal immunogenic peptides is caused by clonal inactivation. *Nature (Lond.)*. 319:413–415.
6. Singh, R.R., B.H. Hahn, and E.E. Sercarz. 1996. 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.* 183:1613–1621.
7. Taylor, B., A.P. Norman, H.A. Orgel, C.R. Stokes, M.W. Turner, and J.F. Soothill. 1973. Transient IgA deficiency and pathogenesis of infantile atopy. *Lancet.* ii:7821–7823.
8. Mowat, A.M. 1987. The regulation of immune responses to dietary protein antigens. *Immunol. Today.* 8:93–98.
9. Holt, P.G., and J.D. Sedgwick. 1987. Suppression of IgE responses following antigen inhalation: a natural homeostatic mechanism which limits sensitization to aeroallergens. *Immunol. Today.* 8:14–15.
10. Zhang, Z., and J.G. Michael. 1990. Orally inducible immune unresponsiveness is abrogated by IFN- γ treatment. *J. Immunol.* 144:4163–4165.
11. Miller, A., O. Lider, A.B. Roberts, and M.B. Sporn. 1992. Suppressor T cells generated by oral tolerization to myelin basic protein suppress both in vitro and in vivo immune responses by the release of TGF- β following antigenic specific triggering. *Proc. Natl. Acad. Sci. USA.* 89:421–425.
12. Lamont, A.G., M. Gordon, and A. Ferguson. 1987. Oral tolerance in protein-deprived mice. II. Evidence of normal 'gut processing' of ovalbumin, but suppressor cell deficiency, in deprived mice. *Immunology.* 61:339–343.
13. Whitacre, C.C., I.E. Gienapp, C.G. Orosz, and D.M. Bitar. 1991. Oral tolerance in experimental autoimmune encephalomyelitis. III. Evidence for clonal anergy. *J. Immunol.* 147: 2155–2163.
14. Gregerson, D.S., W.F. Obritsch, and L.A. Donoso. 1993. Oral tolerance in experimental autoimmune uveoretinitis. *J. Immunol.* 151:5751–5761.
15. Friedman, A., and H.L. Weiner. 1994. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. *Immunology.* 91:6688–6692.
16. Chen, Y., J.-I. Inobe, R. Marks, P. Gonnella, V.K. Kuchroo, and H.L. Weiner. 1995. Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature (Lond.)*. 376:177–180.
17. McMenamin, C., and P.G. Holt. 1993. The natural immune response to inhaled soluble protein antigens involves major histocompatibility complex (MHC) class I-restricted CD8⁺ T cell-mediated but MHC class II-restricted CD4⁺ T cell-dependent immune deviation resulting in selective suppression of IgE production. *J. Exp. Med.* 178:889–899.
18. McMenamin, C., C. Pimm, M. McKersey, and P.G. Holt. 1994. Regulation of CD4⁺-TH-2-dependent IgE responses to inhaled antigen in mice by antigen-specific γ/δ T-cells. *Science (Wash. DC)*. 265:1859–1861.
19. Wells, H.G., and T.B. Osborne. 1911. The biological reactions of the vegetable proteins. I. Anaphylaxis. *J. Infect. Dis.* 8: 66–124.
20. Holt, P.G. 1994. Immunoprophylaxis of atopy: light at the end of the tunnel? *Immunol. Today.* 15:484–489.
21. Halvorsen, R., V. Bosnes, and E. Thorsby. 1986. T cell responses to a *Dermatophagoides farinae* allergen preparation in allergic and healthy controls. *Int. Arch. Allergy Immunol.* 80: 62–69.
22. Upham, J.W., B.J. Holt, M.J. Baron-Hay, A. Yabuhara, B.J. Hales, W.R. Thomas, R.K.S. Loh, P. O'Keeffe, L. Palmer, P. Le Souef, P.D. Sly, P.R. Burton, B.W.S. Robinson, and P.G. Holt. 1995. 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.* 25:634–642.
23. Imada, M., F. Estelle, R. Simons, F.T. Jay, and K.T. Hayglass. 1995. Allergen-stimulated interleukin-4 and interferon-gamma production in primary culture: responses of subjects with allergic rhinitis and normal controls. *Immunology.* 85: 373–380.
24. Holt, P.G., C. McMenamin, and D. Nelson. 1990. Primary sensitization to inhalant allergens during infancy. *Ped. Allergy Immunol.* 1:3–13.
25. Strobel, S., and A. Ferguson. 1984. Immune responses to fed protein antigens in mice. 3. Systemic tolerance or priming is related to age at which antigen is first encountered. *Pediatr. Res.* 18:588–594.
26. Holt, P.G., J. Vines, and D. Britten. 1988. Suppression of IgE responses by antigen inhalation: failure of tolerance mechanism(s) in newborn rats. *Immunology.* 63:591–593.
27. Mariani, F., J.F. Price, and D.M. Kemeny. 1992. The IgG subclass antibody response to an inhalant antigen (*Dermatophagoides pteronyssinus*) during the first year of life: evidence for early stimulation of immune system following natural exposure. *Clin. Exp. Allergy.* 22:29–33.

28. Kemeny, D., R. Urbanek, and P. Ewan. 1989. The subclass of IgG antibody in allergic disease. *Clin. Exp. Allergy*. 19:545–549.
29. Devey, M.E., S. Beckman, and D.M. Kemeny. 1993. The functional affinities of antibodies of different IgG subclass to dietary antigens in mothers and their babies. *Clin. Exp. Immunol.* 94:117–121.
30. Hattevig, G., B. Kjellman, and B. Björkstén. 1993. Appearance IgE antibodies to ingested and inhaled allergens during the first 12 years of life in atopic and non-atopic children. *Ped. Allergy Immunol.* 4:182–186.
31. Demeure, C.E., L.-P. Yang, D.G. Byun, H. Ishihara, N. Vezzio, and G. Delespesse. 1995. Human naive CD4 T cells produce interleukin-4 at priming and acquire a Th2 phenotype upon repetitive stimulations in neutral conditions. *Eur. J. Immunol.* 25:2722–2725.
32. Holt, P.G., P.O. O’Keeffe, B.J. Holt, J.W. Upham, M.J. Baron-Hay, C. Suphioglu, B. Knox, G.A. Stewart, W.R. Thomas, and P.D. Sly. 1995. 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.* 6:1–5.
33. Kim, K.-M., and H.M. Mayumi. 1992. IgE, IL-4, and soluble Fc_γRII in the serum of atopic and nonatopic children. *Pediatr. Allergy Immunol.* 3:11–15.
34. Borres, M.P., R. Einarsson, and B. Björkstén. 1995. Serum levels of interleukin-4, soluble CD23 and IFN- γ in relation to the development of allergic disease during the first 18 months of life. *Clin. Exp. Allergy*. 25:543–548.
35. Kondo, N., Y. Kobayashi, S. Shinoda, K. Kasahara, T. Kameyama, S. Iwasa, and T. Orii. 1992. Cord blood lymphocyte responses to food antigens for the prediction of allergic disorders. *Arch. Dis. Child.* 67:1003–1007.
36. Piccini, M.-P., F. Mecacci, S. Sampognaro, R. Manetti, P., Parronchi, E. Maggi, and S. Romagnani. 1993. Aeroallergen sensitization can occur during fetal life. *Int. Arch. Allergy Immunol.* 102:301–303.
37. Warner, J.A., E.A. Miles, A.C. Jones, D.J. Quint, B.M. Colwell, and J.O. Warner. 1994. Is deficiency of interferon gamma production by allergen triggered cord blood cells a predictor of atopic eczema? *Clin. Exp. Allergy*. 24:423–430.
38. Jarrett, E.E.E., and E. Hall. 1983. IgE suppression by maternal IgG. *Immunology*. 48:49–58.
39. Telemo, E., I. Jakobsson, B.R. Weström, and H. Folkesson. 1987. Maternal dietary antigens and the immune response in the offspring of the guinea-pig. *Immunology*. 62:35–38.
40. Yu, M., S. Fredrikson, J. Link, and H. Link. 1995. High numbers of autoantigen-reactive mononuclear cells expressing interferon-gamma (IFN- γ), IL-4 and transforming growth factor-beta (TGF- β) are present in cord blood. *Clin. Exp. Immunol.* 101:190–196.
41. Schon-Hegrad, M.A., J. Oliver, P.G. McMenamin, and P.G. Holt. 1991. Studies on the density, distribution, and surface phenotype of intraepithelial class II MHC antigen (Ia)-bearing dendritic cells (DC) in the conducting airways. *J. Exp. Med.* 173:1345–1356.
42. Nelson, D., C. McMenamin, A.S. McWilliam, M. Brenan, and P.G. Holt. 1994. Development of the airway intraepithelial dendritic cell network in the rat from class II MHC (Ia) negative precursors: differential regulation of Ia expression at different levels of the respiratory tract. *J. Exp. Med.* 179:203–212.
43. Holt, P.G., M.A. Schon-Hegrad, M.J. Phillips, and P.G. McMenamin. 1989. Ia-positive dendritic cells form a tightly meshed network within the human airway epithelium. *Clin. Exp. Allergy*. 19:597–601.