

Primary Sensitization to Inhalant Allergens

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Evidence suggests that allergen-specific helper T (Th) cell memory, which underlies expression of atopy in adulthood, is programmed during very early life.

Predisposition to primary allergic sensitization per se may be related to the function of discrete gene(s) that regulate responses to microbial stimuli, particularly during early infancy, whereas a second set of genes may control tissue expression of atopic disease.

ALLERGEN-SPECIFIC T CELL PRIMING

Formal proof that priming of fetal T cells against dietary and inhalant allergens commonly occurs transplacentally has been provided by our laboratory, via the derivation and DNA genotyping of allergen-specific T cell clones from cord blood (1). Analysis of the cytokine profiles of these clones, and of short-term allergen-stimulated bulk cultures of cord-derived peripheral blood mononuclear cells (PBMCs), indicated consistent production of the Th2 cytokines interleukin 4 (IL-4), IL-5, IL-6, IL-9, IL-10, and IL-13 with less frequent and (when detected) comparatively lower levels of interferon γ (IFN- γ) (usually restricted to mRNA [1]). In addition, in a prospective study of a cohort of infants monitored from birth to age 2 yr, it was demonstrated that children who developed atopic symptoms within the study period displayed significantly lower levels of allergen-specific IL-6, IL-10, and IL-13 protein and IL-4 mRNA production in stimulated cord blood cultures, compared with their counterparts who did not develop disease (2). Collectively, these findings are consistent with the hypothesis that allergen-specific Th cell priming is initiated *in utero*, is universally Th2 polarized, and paradoxically is initially more efficient in subjects expressing the nonatopic phenotype.

ALLERGEN-DRIVEN IMMUNE DEVIATION DURING INFANCY

Cross-sectional studies on age-dependent changes in allergen-specific T cell reactivity have demonstrated divergent patterns of lymphoproliferative responses to dietary and inhalant allergens, the former displaying downregulation (consistent with the onset of high zone tolerance) during early postnatal life, in contrast to progressive consolidation of responses to inhalants (3–5). Cytokine responses to typical inhalants such as house dust mite (HDM) partition into adult-equivalent Th2-like and Th1-like patterns by age 5 yr in HDM skin prick test-positive (HDM-SPT⁺) and SPT[−] subjects, respectively, and a similar divergence is seen between atopic family history-positive and negative groups by late infancy, before consistent expression of SPT reactivity in the atopic subjects (5). A clearer picture of these changing patterns of HDM-specific cytokine production has emerged from prospective studies of a cohort of children

bled at 6-mo intervals up to 2 yr of age (2). In this study, PBMCs from the subgroup who developed physician-diagnosed atopy at age 2 yr displayed age-dependent increases in allergen-induced Th2 cytokine production, starting from a low baseline in cord blood; in contrast, Th2 responses in PBMCs from nonatopic children showed the reverse pattern, declining from (higher) fetal levels to low levels by age 2 yr, and this was paralleled by a more rapid postnatal upregulation of allergen-specific IFN- γ responses (2). Thus development of atopy appears to be associated with postnatal consolidation of fetally primed allergen-specific Th2 responses, whereas nonatopic subjects appear to successfully immune deviate these responses toward the Th1 cytokine phenotype.

GENETIC RISK OF ATOPY AND POSTNATAL MATURATION OF T CELL FUNCTION

Healthy human neonates exhibit a range of transient developmental deficiencies in adaptive immune function. In particular, the capacity to express certain Th1-associated responses is low, mirroring the generalized Th2 polarization that is characteristic of the fetal immunological milieu (reviewed in Reference 6). Th1 functions, in particular the capacity to produce IFN- γ , increase progressively during infancy and early childhood. However, it appears that this developmental process is distorted in association with the atopic genotype.

Thus, T cells from subjects at high genetic risk of atopy on the basis of positive family history display a lower capacity to generate both IFN- γ and IL-4 during infancy and early childhood, relative to their family history-negative counterparts; this deficiency is consistently more marked for IFN- γ (1, 6, 7). This relative deficiency in Th1 function in atopic children is associated with a decreased capacity to generate stable immunological memory in response to vaccination with agents such as BCG (8) or the diphtheria/pertussis/tetanus vaccine (9), and with a generalized relative deficiency in CD45RO⁺ (memory) T cells in the peripheral circulation (10).

We speculate that this relative delay in postnatal upregulation of Th1 function in young children with the atopic genotype results in a decreased capacity for immune deviation of their fetally primed allergen-specific Th2 responses toward Th1-polarized immunity, resulting instead in consolidation of the former into Th2-polarized immunological memory (6, 11). As noted above, Th1 function(s) appear depressed in atopic infants relative to age-matched nonatopic infants, and this may reflect the postnatal persistence of regulatory mechanism(s) that normally protect the placental unit against potential damage from toxic Th1 cytokines such as IFN- γ (6, 12). The basis for this persistence in atopic individuals is unknown. In this context it has been widely speculated that respiratory viral infections in early childhood may play an important role in this process via bystander stimulation of Th1 function and, further, that variations in the load and timing of respiratory infections during this life phase may underlie the differences in atopy prevalence that are linked to socioeconomic factors (13–16).

However, this may be an oversimplification. It has been clearly established from a longstanding experimental literature that the normal stimulus for the postnatal upregulation of Th1 function(s) in mammals is contact with microbial signals

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in the extrauterine environment, in particular signals derived from the gastrointestinal microflora (reviewed in References 6 and 16). Conceivably, the socioeconomic class-related differences in respiratory infection history inferred from epidemiological studies (13–15) may be functioning to a significant degree as a surrogate for more biologically important variations in gastrointestinal microbial stimulation during infancy (16). A hint that this may be the case can be seen in a report highlighting differences between gastrointestinal flora in allergy-prone Swedish infants and those from Estonia, where prevalence rates are considerably lower (17). This complex issue merits more detailed investigation.

THE SEARCH FOR ATOPY-ASSOCIATED GENES: INDUCTION VERSUS EXPRESSION OF DISEASE

The current focus of research in this area emphasizes genes encoding molecules known to play important roles in tissue expression of atopic inflammation, such as cytokines and their receptors. However, the findings reviewed here suggest that broadening this focus to include much earlier events in this disease process may be warranted. Thus, we have argued above that the primary genetic defect in the inductive phase of atopy, namely, initial allergic sensitization, lies in the pathway involving control of Th1 function during the perinatal period. Given the importance of environmentally derived microbial signaling in this process, gene(s) associated with recognition and response to stimuli such as bacterial lipopolysaccharide (LPS) must be considered as possible candidates (11). It is of interest to note that one study (18) has linked a polymorphism in the CD14 gene (encoding the high-affinity receptor for bacterial LPS) to severity of atopic disease in a pediatric population, and this may be a forerunner of further interesting developments in this area.

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DISCUSSION

Platts-Mills: The cytokine production by the nonatopic group is very poor; many people would class that as a negative response or a failure of response.

Holt: I have some difficulty with that. If you stimulate a T cell culture and you get measurable cytokine, even though it may be low, it is a positive response. The best interpretation is that there are responsive T cells present in low precursor frequency, rather than being a nonresponse. It may be a trivial response in terms of pathology, but it exists.

Platts-Mills: You used the term "Th1 consolidation" to describe what happens to nonallergic individuals over time. Given the number of people concerned, wouldn't you expect to see some individuals with overt forms of Th1 immunity such as delayed hypersensitivity skin tests or high-titer IgG1 antibodies giving precipitins? We have never found either of these forms of immunity to cat or mite allergens.

Holt: The simplest explanation is that the frequency of allergen-specific Th1 cells is not high enough. Also, we have observed IL-10 production concomitantly with IFN- γ from allergen-stimulated T cells from nonatopics. IL-10 may also provide negative feedback.

Platts-Mills: With dust mite you can claim that the dose of antigen was never high enough, but with cat it seems to me that the exposure dose range should be quite sufficient to produce a true Th1-type response.

Holt: There are no direct data available, but we don't see it in the experimental model studies, which looked at the maturation of these responses to inhaled antigens. The most typical finding is that the Th1-dependent subclasses climb progressively with chronic exposure up to a midrange and then stop climbing. They seem to lock in the moderate-range level. If you line these animals up against immunologically naive animals and stimulate the immunologically naive ones with an adjuvant, you get a primary immune response that is 3 to 10 times higher than this permanently locked-in level. If you try and mimic this lock via antibody feedback by taking even quite high levels of antibody and transferring that from animal

to animal, it does not happen. You can transfer it with whole splenocytes. So, whatever the mechanism is, it seems to be intrinsic within the T cell compartment. So, it is some sort of feedback that is coming from the T cell system.

Kauffman: In the lung tissue of healthy individuals the clearance capacity for all kind of particles is quite high. I would like to suggest that the epithelial barrier function in atopics and in asthmatic individuals is abnormal, which will allow antigens to reach the immune system and to induce a Th2-type response. So, the explanation for why we do not see a Th1-type response lies in the normality of the epithelial system in healthy individuals.

Holt: We wait for the publication of proof. It is a great idea.

Platts-Mills: There is actually a paper by Kontou-Karakitsos and colleagues [*J. Allergy Clin. Immunol.* 1975;55:241—Ed.], looking at antigen transfer across allergic and nonallergic noses and looking at PK responses on the back, and they could not find any difference in antigen transfer between allergic and nonallergic individuals.

Björkstén: Dr. Cases published in *Clin. Exp. Allergy* last year on *Fel d 1* complexes. She only found these in allergic individuals. There was very little relationship to whether they were exposed to cats or not. None of the 30 or so nonatopic controls including cat owners had detectable *Fel d 1*.

Von Mutius: In your recent *Lancet* paper the definition of atopy basically is: atopic dermatitis. Could this possibly be a group that is positive to food allergens? Does sensitization to food allergens differ from sensitization to inhalant allergens?

Holt: Few of the children had atopic dermatitis. None in that group were positive to the food panel that was used. We used about six food allergens in the panel for skin prick testing.

Platts-Mills: In your recent *Lancet* paper there are two sets of children. There are children who are judged to be high risk at birth and had atopy at age 2. And those had had low risk at birth and did not have atopy, but the paper does not define how big the overall cohort is, because, clearly, that is not all the children. There are 16 or 18 at one end and 18 at the other end, but how many are there in the middle?

Holt: There is about another third in the middle. What happened was this. The study was pared down from about 55 or so down to those from whom we had full blood samples. We elected finally to look at those who had both family history and symptoms, and those who had neither. The ones in the middle were not looked at simply because they were a heterogeneous group and we were trying to look at the extremes.

Platts-Mills: A lot of atopic children have not declared themselves as atopic at age 2. It is surprising to get this cleanness of your separation when your definition of atopy is something at age 2.

Holt: We were surprised also, but that is the data.

Neijens: Are any of the food-related immunological parameters in infancy indicative of the progression to allergic asthma?

Holt: When you look at the response to, for example, OVA in cord blood you see responses to about seven epitopes. By the age of 6 months it has generally shrunk to two epitopes and by now, rather than about 65 to 75% of the population responding by lymphoproliferation, it is down to about 20%. By age 2 it is almost bottomed out and then by age 5 we see a slow leak back and it is now about back to 20 to 25% of the population, but it is still down to about two epitopes. The pattern is almost the opposite of what we see to inhalants. The cytokine story also is quite different. The only cytokine signals we can get out of those ovalbumin responses at age 9 months are IL-13 message and IL-6 protein, but the latter is entirely derived from the non-T cell compartment, i.e., from monocytes that are using the small amount of antibody and are activated via Fc receptors. In addition, we can often get a little bit of IL-10

that we can measure as protein. So, it is certainly not T cell deletion, but a form of anergy.

Neijens: Do you think that these reactions to foods, which are a first try-out of the immune system, may tell us a little bit about the capacity of the immune system to react?

Holt: The lessons from the mucosal immunology literature on experimental animal models are that the nature of T cell regulation seems to be determined to a large extent by the dose-response curve. When you pump in very high levels of antigen at a mucosal surface, you activate mechanisms that lead in some circumstances to T cell deletion. If you come down the dose-response curve, it is T cell anergy. Finally, maybe a couple of logs away, you are now in the part of the dose-response curve where immune deviation seems to be the only mechanism available. For inhaled antigens such as *Fel d 1* and *Der p 1*, because of the low exposure levels the one available mechanism is immune deviation, whereas in the GIT, where intermittent exposure to these antigens will occur over a much broader dose-response curve, there is potentially access to all those forms of regulation. For that reason the immune system has got more backups and so, at the population level, this may be why you see a lot less food allergy than you see inhalant allergy.

Unidentified: Presentation to the nose might be completely different from presentation to the lungs.

Platts-Mills: I would see that completely the other way. The nose is an extremely good system for filtering antigen coming through it and presenting it to the lymphatic system of the nose.

Holt: The main areas where you generate either primary responses or where these regulatory events occur in response to inhalation of antigen in experimental animal models happen to be the lymph nodes that drain the nose or perhaps the pharynx. I suspect that this is much the same in humans. It may well be that the pattern of cytokine production in the lymph nodes that are draining the upper airways, in contrast to the lower conducting airways, is intrinsically different.

Renz: What really is the normal way of the physiological type of immunity after encountering allergens in the airways? Is it really Th1 immunity, as implied by your question, or is it T cell tolerance?

Holt: If you take immunocompetent animals and get them to inhale antigen (or feed the same antigen) the first antibody they make is IgE. If you keep up antigen exposure these immune responses spontaneously immune deviate. We originally thought that it was feedback from the T cell compartment, but a number of experiments in knockout animals show that you still get this deviation in the absence of CD8⁺ cells or T γ - δ cells or IFN- γ , so these mechanisms are probably secondary. We are now looking at the very early phase of the immune response that Dr. Kapsenberg was talking about, where Th1/Th2 selection seems to occur, which is at the level of the APC. In the rat model we purified airway mucosal dendritic cells, pulsed them with antigen, and put them back. It was very clear that they are priming for Th2. There are data from other people coming through soon where exactly the same answer seems to be coming from the gut. To us, this looks like what we are seeing in humans. The reason why we became interested in this whole issue, at least in the pediatric arena, was the study by Hattevig *et al.* They tracked children from birth through to age 13. With low-level IgE assays all of the normal kids also were found to be starting off making IgE responses to foods and many also to inhalants, exactly as in the animal systems. These responses were bi-phasic. So, the answer is: There is nothing abnormal in at least starting off a Th2 response. What seems to be abnormal is if the system does not make a correction at the right stage, and tempers this response by some sort of feedback.

Lamers: If you consolidate the immune response into a certain direction, will it be responding to new incoming antigens in the same way?

Holt: No, we are talking about antigen-specific responses. Each event has its own regulatory tale to tell and it is all about what the microenvironment was at key stages in the development of those immune responses. So, for example, if you look into atopy, the typical pattern you will see is that they are responding to a small number of allergens in this aberrant fashion. They may be making slightly more Th2 cytokines when you look at other immune responses, which can include immune responses to vaccines which are starting to become recognized, but they are not making the very high-level responses that are causing pathology. This seems to be limited to a small number.

Lamers: But the studies that have been looking at exposure to mycobacteria suggest that exposure might protect.

Holt: I totally disagree with you. Shirakawa *et al.* selected 900 children for whom the BCG vaccination records were available all the way back to the first BCG vaccination they got at 3 months. The kids who failed to respond at 3 months were then revaccinated before they got to school at age 6. They were then recruited into the study at age 12. The children were then Mantoux tested again. So, you now have a readout as to whether the Th1-dependent memory that results from one or two of their previous BCG vaccinations successfully survived that 6-year period. You can also look back at what has happened to the children who did make that proper Th1 response at 3 months or did not until they were age 6. And the answer is extremely clear. The children that responded to their first or second BCG vaccination and maintained their Mantoux positivity were the children that had a very low level of atopic disease. The ones who failed and were still Mantoux negative when they were retested at age 12, had 4- or 5-fold the levels of atopic disease. Now, some people have chosen to interpret that data on the basis of exposure to wild-type mycobacteria. But the only mycobacterial exposure that we know about in that study was active vaccination. So it is a total surmise that this has got anything to do with environmental exposure. Studies from Scandinavia also did not show an association between early BCG vaccination and the subsequent expression of atopic disease.

Lamers: So this Th1/Th2 consolidation is antigen specific?

Holt: Yes, each immune response has its own tale to tell.

Postma: You showed in your *Lancet* paper that there were differences in atopic and nonatopic children at age 2 with respect to cord blood cytokine profiles. Atopy is reflected by eczema, hay fever, wheeze, or positive RAST/skin test. Were you able to assess whether there is a difference in total IgE levels between the two patient groups? I am convinced that there is the same difference between total IgE as there is with specific IgE.

Holt: At age 2 most children still have low total IgE. At this stage the relationship between total IgE and symptoms is not strong, but becomes progressively stronger with age.

Aalberse: Your cord blood T cell clones would enable you to establish whether the antenatal sensitization takes care of precursor cells that expand later in life. It would in principle be possible to see whether at age 2, or perhaps even at age 6, the same clones are still around, looking at the TCR. Do you think the same T cells will still be present and do they actually contribute to the IgE response that is discriminating between atopy and nonatopy?

Holt: We don't know, but it would be theoretically possible to do that. On the basis of the ovalbumin data I would have my doubts, I must admit, because we see in terms of T cell epitopes a very different spread to what we see around 6 months later. Dr. Thomas is doing what we did with OVA, with *Der p 1*, mapping the epitopes in the cord. The story is not in yet, but it looks like the epitopes that are being recognized in the cord are much more representative of the ones that he sees in adults than we do with ovalbumin, so again this may be a difference between ovalbumin and inhalants.

Aalberse: Do you think that antenatal sensitization is relevant at all for sensitization later in life?

Holt: Yes, I think that in many cases the ensuing immune response develops via modulation of the primary response that starts in early childhood. All of the ensuing immune responses will be influenced by the microenvironment at the point of antigenic challenge. By changing the cytokine pattern at the point of antigen presentation early T cells can influence the immune responses, but these are not necessarily the same clones. In a sense it may be like affinity maturation in the B cell compartment. Obviously, the process is rather different, but the concept is not hugely different.

Kauffman: Is there a role for the alveolar macrophage and specific IgA and the capacity to clear antigen from the airways, preventing the allergic response?

Holt: In young children there are no clear data. In adults, macrophages of atopy are reported to be less capable to clear antigen. There is also very little data available in young experimental animals. We have one Ph.D. program at the moment looking at a range of these issues. There are developmental differences in the capacity of these macrophage populations in the lung that make both IL-10 and IL-12. So, there are differences, but it is not clear how these relate to what is going on.

Kauffman: Is it reasonable to expect that certain responses in the population will go down in a young child by its increasing capacity to remove antigens from the airways?

Holt: No, I don't think we have reason to suspect that yet. It is an interesting idea, but: no data.