

Prophylactic Allergen Vaccination: Current and Future Perspectives

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Allergen specific immunotherapy has traditionally been viewed as a treatment for established atopic disease. The disease target of choice has principally been allergic rhinitis, and in contrast there has been widespread reluctance in sectors of the clinical community to extend this approach to treatment of atopic asthma. However as understanding of the underlying pathogenic mechanisms which drive asthma aetiology and pathogenesis increases, the rationales for considering immunotherapy in this treatment context are expanding, particularly in relation to primary and secondary prevention of asthma in childhood. This brief review highlights recent development relating to this issue.

Introduction

It has become clear from a range of prospective studies that the development of asthma which persists to the end of the preschool years, particularly in children who are sensitised to inhalant allergens, is likely to be associated with subsequent progression to more severe forms of the disease during the school years and frequently into adult life. The potentially most effective way to tackle this growing problem is to identify at risk groups very early in this process and institute targeted therapies aimed at prevention of disease progression [1,2]. The design of effective therapeutic strategies to achieve this end requires a thorough understanding of the mechanisms underlying the initiation phase of this complex disease process. Until comparatively recently this aspect of asthma research has been sidelined in the rush to identify pathogenic mechanisms and associated drug targets for treatment of severe chronic disease in adults. The results of this approach to date have been disappointing, and the search for anti-inflammatory drugs which can improve on results obtained with steroids appears as frustrating as ever. In particular, disease modification (as opposed to symptomatic relief) still remains an elusive goal. However, this picture is changing as the pace of research on the aetiology of atopic diseases and of wheezing syndromes in childhood has accelerated, especially over the last decade. In particu-

lar, some important leads have come from studies on the control mechanisms that confer normal resistance against primary allergic sensitisation to inhalants.

Regulation of T cell immunity in the airways: insights from animal model systems

The airway epithelium is in constant contact with non pathogenic inhaled proteins which are ubiquitous in the natural environment, including aeroallergens (which are intrinsically harmless unless they elicit an inappropriate immune response), and also airborne viral and bacterial pathogens. The maintenance of immunological homeostasis in this microenvironment requires that the immune system recognises the difference between these classes of antigens and restricts local T cell activation events to those necessary for elimination of pathogens, while ignoring the much more common non pathogenic antigens/allergens. The key to this balancing act is the presence of cell populations at the interface of the airway epithelium and the external environment which are specialised for antigen sampling, and capable of rapidly transporting capture antigen to the central immune system for elicitation of “appropriate” immune responses. The relevant cells are dense networks of airway mucosal dendritic cells (AMDC) first described by my group in the late 1980s [3,4], and subsequently characterised in detail by numerous investigators (reviewed in [5]).

Two key features of the functional phenotype of these AMDC are their capacity to sample airway luminal antigens/allergens through the intact airway epithelium via extrusion of dendrites through intact tight junctions [6], and maintenance of their functional phenotype while resident in airway tissue to a purely “antigen uptake/processing” mode, not developing capability to present acquired antigens to T cells until they migrate to regional lymph nodes to complete their life cycle. This strict compartmentalisation of functions serves to minimise possibilities of T cell mediated damage of the airway mucosa to inhaled antigen/allergen. Recent experimental model studies have demonstrated that even in sensitised animals, capacity to elicit T cell mediated inflammation to aerosol exposure to allergen requires an obligatory initial “maturation/activation” step on the part of the AMDC, which is normally prevented via a series of local control mechanisms [7,8]. Spontaneous failure of these control mechanisms, and/or triggering of premature maturation/activation of members of the AMDC network by environmental agents, is believed to be central to the expression of inhalant allergy [5].

An additional homeostatic mechanism which plays a central role in the maintenance of immunological homeostasis in the airways in the steady state is the process of inhalation tolerance which is the respiratory tract arm of “mucosal tolerance”. First described in my lab in the early 1980s, this process has been characterised in detail in a variety of experimental settings [9–13]. An important finding has been the fact that the efficiency of the tolerance process is genetically determined; with the threshold allergen exposure levels required to elicit protective tolerance varying over a multi log scale range. Notably, atopy-prone “high-IgE-responder” animal strains require sustained high level exposure to elicit tolerance, while atopy-resistant “low-IgE-responders” are readily tolerised via much lower exposures [14–16].

The precise mechanisms underlying tolerance induction have yet to be determined. However the AMDC are central to the process, and it has been suggested that a specific plasmacytoid subset within this population may be responsible [17].

The role of AMDC in host responses to microbial challenge in the airways

The immune surveillance role of the AMDC population is principally that of alerting the immune system to incoming airborne pathogens to elicit primary immune responses, and where required to stimulate secondary immunity in the airway mucosa during subsequent cycles of infection. The former is achieved via the antigen capture/transport role of AMDC, while the latter requires functional up-regulation of AMDC *in situ*. Precisely how this is achieved is unknown, but significant insight is available from bacterial and viral exposure models in rats and mice. In particular, it is clear that airway exposure to microbial breakdown products including LPS [18,19] markedly accelerates the dynamics of antigen sampling in the epithelium/mucosa. Moreover it has been shown that a permissive LPS-equivalent signal is an obligatory requirement to stimulate sufficient functional maturation/activation of AMDC to enable them to “recognise” incoming non-pathogenic antigen/allergen [5].

Of particular relevance to recent human studies are reports demonstrating the profound effects of respiratory viral infections on the AMDC network in rats and mice. In particular, Sendai virus infection rapidly expands the AMDC network in rats [20,21], and in contrast to observations in airway challenge models involving non-living microbial stimuli or allergens where network activity returns rapidly to baseline after withdrawal of the agents [18,19], the AMDC network remains markedly expanded for >40 days post viral clearance from the lungs [20,21]. Of additional note, a recent report has demonstrated a novel pathway via which Type 1 IFN produced in airway tissues during a respiratory virus infection in mice stimulates large scale up-regulation of FcεR1a expression on lung DC [22]. Earlier studies with Langerhans cells demonstrate that arming with FcεR1a strongly enhances allergen uptake, processing and subsequent presentation to T cells. Collectively, these observations provide a plausible explanation for recent human epidemiological findings relating to the respective roles of atopy and viral infection in asthma pathogenesis, which is discussed below.

Aetiology of asthma in early childhood: insight from birth cohort studies

A series of prospective birth cohorts which have tracked populations of children through early childhood and into their teen years (reviewed in [2]) have provided a broad framework for our understanding of how the persistent asthma phenotype develops in early life. These findings have been incorporated into a working model of asthma aetiology in childhood, which also serves as a template for design of early intervention strategies [23]. The key feature of this model and of the underlying data sets from which it is derived is the consistent finding that while early atopic sensitisation to inhalant allergens and severe respiratory viral infections triggering wheeze during infancy can function as independent risk factors for asthma development,

this risk is maximal in children experiencing *both* insults [2,23]. This suggests underlying synergistic interactions between inflammatory mechanisms triggered in the airway mucosa via viruses and allergens. As noted above there is now evidence for sustained up-regulation of AMDC networks during and following respiratory viral infection, and this provides a potential mechanism for intensification of allergen driven airways inflammation in sensitised children; and this is nominated in Figure 1 in Reference [2] as a potential interaction mechanism linking anti-viral and atopic inflammatory mechanisms, in the overall asthma causal pathway. It is also pertinent to note in this context earlier publications indicating that the kinetics of postnatal maturation of the AMDC network in rodents is fastest in the nasal turbinates which is the site of maximal contact in the airways with incoming microbial stimuli [24], and findings in *post mortem* airway tissue samples from human infants with histories of severe airways infection demonstrating expanded AMDC populations [25].

The downstream effects of these interactions in relation to initiation of asthma development during early childhood are incompletely understood, but are postulated to involve inflammation-mediated changes to lung growth and differentiation. This life phase is a period of intensive growth and remodelling of lung and airway tissues, and disturbance of this process may perturb the orderly establishment of complex structure/function relationships that are central to development of normal respiratory function in later childhood (reviewed in [23]). It is pertinent to note in this context the evidence indicating that lung function in individual children tends to “track” over time in a stable fashion within the population centile describing their functional capacity during infancy, and further that any events triggering a drop to a lower centile during this critical period has the potential to carry forward into later life [23,26,27]. Thus, either short term bursts of intense airways inflammation or sustained periods of moderate inflammation may have long term deleterious consequences in relation to subsequent lung function.

Disease progression: chronicity and intensity

The association between atopy and risk for progression of asthma continues beyond the preschool years [2], and the strength of the association between these two diseases appears strongest during the teen years [28]. We have recently completed a detailed respiratory follow-up on a birth cohort of 1380 14year olds and 82% of all current asthma cases were atopic (to be published), which is consistent with this view and with findings from other birth cohorts. Moreover, intensity of atopic sensitisation associated strongly with asthma severity in this group.

Additional evidence in support of the continuing importance of atopy in driving asthma pathogenesis in this age group has come recently from intervention studies aimed at prevention of progression from allergic rhinitis to asthma in pollen allergic but non asthmatic school children. These studies have compared groups of atopic children given allergen-specific immunotherapy versus standard best-practise management not including immunotherapy, and tracking them over two consecutive 5-year periods. The group given immunotherapy displayed significantly reduced rates of asthma development over the observation period [29,30].

An additional series of observations on school children from several independent groups are of particular relevance to this discussion. Notably, acute asthma exacerbations of sufficient severity to require hospitalisation are now recognised to be restricted principally to atopics [31–34]. Moreover the degree of risk for exacerbation is reportedly related to intensity of sensitisation as opposed to sensitisation *per se* [31]. In this context we are in the process of investigations on a cross sectional cohort of 170 asthmatic children recruited following hospitalisation for acute severe exacerbations, and 96% of these children were atopic and moreover were within the upper quartile of atopy intensity relative to the overall age-matched atopic population. These observations are consistent with those reported earlier [31–34] and argue strongly that interactions between atopic and anti-viral inflammatory mechanisms continue to play a central role in driving asthma pathogenesis throughout (at least) the school years.

Conclusions

Accumulating evidence from a variety of experimental and clinical settings is providing an increasingly strong argument for a role for both viral infections and atopy in the aetiology and pathogenesis of asthma. In particular, the presence of underlying atopic sensitisation appears to provide an obligatory “background” which creates susceptibility to the asthma promoting effects of viral infection. This appears to hold for the initiation of asthma development in early postnatal life, and also for subsequent progression to chronicity. Of particular interest is the observation that inhalant allergy also appears to create susceptibility to the acute exacerbation-triggering effects of viral infections.

The most likely candidate mechanism for these interactions involves effects of viral-induced effects on the AMDC which are responsible for triggering Th2 responses associated with the asthma late phase reaction, and the design of drugs to target this interaction appears a logical future step. In the interim the most direct approach, for both primary and secondary prevention of atopic asthma, would appear to be allergen specific immunotherapy. In this regard we are at the early stages of a clinical trial funded by the NIH ITN network, aimed at enhancing the normal development of mucosal tolerance in high risk non-sensitised infants via administration of a mixture of aeroallergens using SLIT methodology. Initial safety/immunogenicity studies on a subcohort of 50 infants treated for one year without significant side effects has been completed, and a stop/go decision related to extension to an overall study size of 200 children is expected soon.

Regardless of the progress of this primary prevention trial strategy, the recent success in reduction of progression from rhinitis to asthma in 6–12year old atopics indicates that this secondary prevention approach merits further and detailed follow-up. However, it may be timely to consider widening of the criteria for trial design in this area to include more severe categories of asthma risk.

In this regard, the increasing strength of the epidemiological data linking underlying atopy with susceptibility to viral induced severe exacerbations, and the accompanying increase in understanding of likely underlying mechanisms, suggests that atopic

children with a history of severe asthma exacerbations may be the group in most need of effective immunotherapy to control their inhalant allergies. This is the group that currently is to a large extent sidelined in the debate concerning potential benefits of immunotherapy, and safety considerations are usually given as the reason. This group of patients are widely considered to be potentially at high risk of anaphylaxis-associated side effects from immunotherapy, but it is unclear whether the data supporting that proposition is valid. In my view it is time to re-examine this issue in detail in light of more recent data, because inadvertent denial of the potential benefits of this type of therapy to this subgroup of patients may be doing them a disservice.

References

1. Holt PG, Sly PD, Martinez FD, Weiss ST, Björkstén B, von Mutius E, Wahn U. Drug development strategies for asthma: in search of a new paradigm. *Nature Immunol* 2004; 5: 695–698.
2. Sly PD, Boner AL, Björkstén B, Bush A, Custovic A, Eigenmann PA, Gern JE, Gerritsen J, Hamelmann E, Helms PJ, et al. Early identification of atopy in the prediction of the development of persistent asthma in children. *Lancet* 2008; 372: 1100–1106.
3. Holt PG, Schon-Hegrad MA, Phillips MJ, McMenamin PG. Ia-positive dendritic cells form a tightly meshed network within the human airway epithelium. *Clin Exp Allergy* 1989; 19: 597–601.
4. Holt PG, Schon-Hegrad MA, Oliver J, Holt BJ, McMenamin PG. A contiguous network of dendritic antigen-presenting cells within the respiratory epithelium. *Int Arch Allergy Appl Immunol* 1990; 91: 155–159.
5. Holt PG, Strickland DH, Wikstrom ME, Jahnsen FL. Regulation of immunological homeostasis in the respiratory tract. *Nat Rev Immunol* 2008; 8: 142–152.
6. Jahnsen FL, Strickland DH, Thomas JA, Tobagus IT, Napoli S, Zosky GR, Turner DJ, Sly PD, Stumbles PA, Holt PG. Accelerated Antigen Sampling and Transport by Airway Mucosal Dendritic Cells following Inhalation of a Bacterial Stimulus. *J Immunol* 2006; 177: 5861–5867.
7. Strickland DH, Stumbles PA, Zosky GR, Subrata LS, Thomas JA, Turner DJ, Sly PD, Holt PG. Reversal of airways hyperresponsiveness by induction of airway mucosal CD4⁺CD25⁺ regulatory T cells. *J Exp Med* 2006; 203: 2649–2660.
8. Huh JC, Strickland DH, Jahnsen FL, Turner DJ, Thomas JA, Napoli S, Tobagus I, Stumbles PA, Sly PD, Holt PG. Bidirectional interactions between antigen-bearing respiratory tract dendritic cells (DCs) and T cells precede the late phase reaction in experimental asthma: DC activation occurs in the airway mucosa but not in the lung parenchyma. *J Exp Med* 2003; 198: 19–30.
9. Umetsu DT, DeKruyff RH. The regulation of allergy and asthma. *Immunol Rev* 2006; 212: 238–255.
10. Hintzen G, Ohl L, del Rio M-L, Rodriguez-Barbosa J-I, Pabst O, Kocks JR, Klege J, Hardtke S, Forster R. Induction of Tolerance to Innocuous Inhaled Antigen Relies on a CCR7-Dependent Dendritic Cell-Mediated Antigen Transport to the Bronchial Lymph Node. *J Immunol* 2006; 177: 7346–7354.
11. McMenamin C, Holt PG. 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* 1993; 178: 889–899.
12. McMenamin C, Pimm C, McKersey M, Holt PG. Regulation of IgE responses to inhaled antigen in mice by antigen-specific gamma delta T cells. *Science* 1994; 265: 1869–1871.
13. Holt PG, Batty JE, Turner KJ. Inhibition of specific IgE responses in mice by pre-exposure to inhaled antigen. *Immunology* 1981; 42: 409–417.
14. Holt PG, Britten D, Sedgwick JD. Suppression of IgE responses by antigen inhalation: studies on the role of genetic and environmental factors. *Immunology* 1987; 60: 97–102.
15. Holt PG, Sly PD. Interactions between respiratory tract infections and atopy in the aetiology of asthma. *Eur Respir J* 2002; 19: 538–545.
16. Holt PG, McMenamin C. Defence against allergic sensitization in the healthy lung: the role of inhalation tolerance. *Clin Exp Allergy* 1989; 19: 255–262.
17. de Heer HJ, Hammad H, Soullie T, Hijdra D, Vos N, Willart MA, Hoogsteden HC, Lambrecht BN. Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. *J Exp Med* 2004; 200: 89–98.

18. McWilliam AS, Nelson D, Thomas JA, Holt PG. Rapid Dendritic Cell recruitment is a hallmark of the acute inflammatory response at mucosal surfaces. *J Exp Med* 1994; 179: 1331–1336.
19. Schon-Hegrad MA, Oliver J, McMenamin PG, Holt PG. Studies on the density, distribution, and surface phenotype of intraepithelial class II major histocompatibility complex antigen (Ia)-bearing dendritic cells (DC) in the conducting airways. *J Exp Med* 1991; 173: 1345–1356.
20. McWilliam AS, Marsh AM, Holt PG. Inflammatory infiltration of the upper airway epithelium during Sendai virus infection: involvement of epithelial Dendritic Cells. *J Virology* 1997; 71: 226–236.
21. McWilliam AS, Napoli S, Marsh AM, Pemper FL, Nelson DJ, Pimm CL, Stumbles PA, Wells TNC, Holt PG. Dendritic Cells are recruited into the airway epithelium during the inflammatory response to a broad spectrum of stimuli. *J Exp Med* 1996; 184: 2429–2432.
22. Grayson MH, Cheung D, Rohlfing MM, Kitchens R, Spiegel DE, Tucker J, Battaile JT, Alevy Y, Yan L, Agapov E, et al. Induction of high-affinity IgE receptor on lung dendritic cells during viral infection leads to mucous cell metaplasia. *J Exp Med* 2007; 204: 2759–2769.
23. Holt PG, Upham JW, Sly PD. Contemporaneous maturation of immunological and respiratory functions during early childhood: Implications for development of asthma prevention strategies. *J Allergy Clin Immunol* 2005; 116: 16–24.
24. Nelson DJ, McMenamin C, McWilliam AS, Brenan M, Holt PG. 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* 1994; 179: 203–212.
25. Tschernig T, de Vries VC, Debertin AS, Braun A, Walles T, Traub F, Pabst R. Density of dendritic cells in the human tracheal mucosa is age dependent and site specific. *Thorax* 2006; 61: 986–991.
26. Hibbert ME, Hudson IL, Lanigan A, Landau LI, Phelan PD. Tracking of lung function in healthy children and adolescents. *Pediatr Pulmonol* 1990; 8: 172–177.
27. Hoo A, Matthias H, Dezateux C, Costeloe K, Stocks J. Respiratory function among preterm infants whose mothers smoked during pregnancy. *Am J Respir Crit Care Med* 1998; 158: 700–705.
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: 271–277.
29. Niggemann B, Jacobsen L, Dreborg S, Ferdousi HA, Halken S, Host A, Koivikko A, Koller D, Norberg LA, Urbanek R, et al. Five-year follow-up on the PAT study: specific immunotherapy and long-term prevention of asthma in children. *Allergy* 2006; 61: 855–859.
30. Jacobsen L, Niggemann B, Dreborg S, Ferdousi HA, Halken S, Host A, Koivikko A, Norberg LA, Valovirta E, Wahn U, et al. Specific immunotherapy has long-term preventive effect of seasonal and perennial asthma: 10-year follow-up on the PAT study. *Allergy* 2007; 62: 943–948.
31. Xatzipsalti M, Kyrana S, Tsolia M, Psarras S, Bossios A, Laza-Stanca V, Johnston SL, Papadopoulos NG. Rhinovirus viremia in children with respiratory infections. *Am J Respir Crit Care Med* 2005; 172: 1037–1040.
32. Parry DE, Busse WW, Sukow KA, Dick CR, Swenson C, Gern JE. Rhinovirus-induced PBMC responses and outcome of experimental infection in allergic subjects. *J Allergy Clin Immunol* 2000; 105: 692–698.
33. Murray CS, Poletti G, Kebadze T, Morris J, Woodcock A, Johnston SL, Custovic A. Study of modifiable risk factors for asthma exacerbations: virus infection and allergen exposure increase the risk of asthma hospital admissions in children. *Thorax* 2006; 61: 376–382.
34. Johnston SL, Pattemore PK, Sanderson G, Smith S, Campbell MJ, Josephs LK, Cunningham A, Robinson BS, Myint SH, Ward ME, 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: 654–660.

Discussion

van Ree: So you look at the change in the number of dendritic cells expressing the high affinity receptor in these children with exacerbations? But have you looked in children that have the same viral infections but no asthma? Is it lower than this?

Holt: No, we are trying to do that at the moment and that is the obvious next step. So what we want to do is see if we can grave this through the various sorts of virus infections, for sure.

Chapman: Pat, have you considered, or is it been looked at, the potential confounding effects of allergen exposure in some of these studies?

Holt: Absolutely. In fact, this model totally relies on the IgE that is bound to the high-affinity receptors being cross-linked. And so it only works with IgE that is against ubiquitous allergens that have to be around at the time of the exacerbations. So I guess the indoor allergens are likely to be the most important ones and, of course, how you could quantitatively account for that is virtually impossible. You know we have to do it different ways, but yes, it will be an important confounder.