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Do immune responses to inhaled skin flakes modulate the expression of allergic disease?

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Summary

We examine the nature of the immune responses to inhaled skin particles and query whether early exposure could play a role in providing protection against the development of allergic disease. Currently, the main hypothesis used to explain environmental modulation of allergic diseases, the 'hygiene hypothesis', is linked exclusively to microbial exposures acting upon the innate immune system. However, many of the exposures sustaining this hypothesis also involve co-exposure to skin flakes from humans or animals. Such skin flakes contain a complex mixture of antigens, glycolipids and small peptides that may induce immune responses. Should these responses prove relevant to the modulation of allergic diseases, it provides new opportunities to better understand the epidemic of allergic disease and to develop new interventions for its prevention.

Keywords allergic disease, asthma, atopy, dander, immunoregulation, innate, skin flakes

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Introduction

The increase in the global prevalence of allergy and allergic diseases, particularly over the past 50 years, has been largely attributed to complex changes in both lifestyle and environment [1]. Despite this global increase, it has been consistently observed that the prevalence of allergic disease is lower in people with two or more older siblings [2, 3], termed the 'sibling effect', and among those raised in close contact with barn animals [4]. Both these observations have been interpreted as evidence of a protective effect of microbial exposures, termed the 'hygiene hypothesis'. The sibling effect has been attributed to either increased childhood infections or exposure to such infections in large families, while increased aerosol exposure to microbial components, such as endotoxin, is associated with confined livestock. However, there are significant doubts on whether differences in infections adequately explain the 'sibling effect' [2, 3, 5]. Similarly, it seems unlikely that the protective effect of exposure to domestic pets can be attributed to increased microbial exposure, as there is no consistent evidence that this occurs. This has led to an alternative hypothesis of high-

dose tolerance to these allergens [6]. This too has been queried [7]. Several other explanations, not linked to microbial exposure (e.g. changes in diet, lack of exercise, increased obesity and *in utero* changes), have also been proposed, see [5], and it is considered likely that additional environmental influences modulating allergic diseases remain to be elucidated [2, 3, 7].

We note that many of the more prominent examples of these protective effects, that is those associated with cats, kids and cows, all involve intensive co-exposure to animal or human dander. The resurgent interest in the immunobiology of human skin, recognized to involve both the innate and adaptive immune systems, provides a basis to question whether bioactive molecules associated with skin may also be inhaled and be immunoregulatory for allergic diseases. Candidate molecules carried by skin flakes include non-self MHC antigens, peptides and glycolipids. We further question whether the changes in personal and domestic hygiene practices would have reduced these exposures and contributed to the increased prevalence of allergic diseases observed globally. As far as we are aware, no such a role has been proposed previously.

Exposure to airborne skin

The particles in the air we breathe are an inordinately complex mixture, with exposure changing with activity, time and location. We, like others [8], have observed that skin flakes constitute a substantial proportion of the recognizable particles present in air samples collected in houses. This is not surprising, given that we shed ~5 g of skin a week, releasing an estimated 10^7 particles/person/day, and that human skin constitutes a major component of house dust. Such dust is constantly re-aerosolized and due to body thermals, the mean concentration in the breathing zone ($44 \pm 3 \mu\text{g}/\text{m}^3$) is more than twice that in the ambient air [9]. The majority of particles circulating in indoor air [10, 11] are small, flaky, proteinaceous particles $<1\text{--}2 \mu\text{m}$ in diameter, while the majority of the skin flakes freshly emitted by humans are larger, with an aerodynamic diameter of $5\text{--}15 \mu\text{m}$ [12]. Although the site of deposition in the airways of particles with the thin, flat shape of skin flakes is difficult to predict using conventional deposition models, it could be expected that the smaller particles would mainly be deposited in the lower respiratory tract and the larger particles in the upper airways. There is strong, albeit indirect evidence that cat dander particles reach the lower airways [13]. The question is not whether we are constantly exposed from birth onwards, but rather what effects this has.

Immune responses to human and animal dander

The front line of the defence system of the outermost layer of the skin, the mammalian stratum corneum (SC), involves a range of glycolipids and antimicrobial peptides. Presumably such molecules remain on skin flakes post-desquamation, along with a variety of antigens, and these, on inhalation, may regulate immune responses within the airways.

Approximately 50% of the lipids of the SC are glycolipids, and include at least nine major ceramide fractions. Within other systems these molecules have been shown to have a range of potent immunomodulatory activities, including signalling cell-fate outcomes and/or apoptosis [14]. Such glycolipids are currently of intense interest, as a marine glycolipid, α -galactosylceramide (αGC), is a potent stimulator of human natural killer T (NKT) cells that play a critical role in modulating allergic diseases, policing both T helper type 1 (Th1) and Th2 cell subsets. These glycolipids are presented via the CD1 family of MHC-Class I-like molecules, and CD1 are found in the SC keratinocytes under some circumstances [15]. The role of αGC in experimental allergic disease models is complex; in some models, it prevents airways inflammation [16], while in others, it induces it [17]. The range of natural ligands that stimulate NKT cells in humans has not been established, and has been suggested to include self-glycolipids; see [16]. Until this is explored further, the potential of the nascent

infant immune system to be regulated by inhaled self or non-self-glycolipids remains unknown.

There is also intense interest in the numerous, multifunctional peptides present in the skin, which include potent effectors of innate immunity, and possess antimicrobial, chemokine, neuropeptide and immunoregulatory activities. A recent review [18] lists over 40 such peptides, with the most prominent being the β -defensins and the cathelicidins [19]. Two recent studies identified roles directly associated with asthma: human β -defensin-1 (HBD-1) is constitutively expressed in the airways and may have a role in the pathogenesis of asthma [20], while variation in haplotypes of the gene for HBD-1 may affect asthma prevalence [21]. Both the β -defensins and the cathelicidins are greatly increased in neonatal, compared with adult skin [22]. Cystatin, another small molecule abundant in sweat, is a potent inhibitor of mite allergen Der p 1 [23]. Again, quantitative studies are required to explore whether these peptides, when inhaled with skin, modulate immune responses.

Human and animal dander will also carry a diverse range of self and 'non-self' antigens. HLA antigens are perhaps the most intriguing of potential candidates as a source of stimulation, as the number of cells responding to alloantigen presentation is much greater than with conventional antigen presentation. A strong stimulatory response to 'non-self' would be consistent with a 'sibling effect'. The extent to which such histocompatibility alloantigens survive on skin flakes requires further exploration. Two studies immunostaining tissue sections with anti-HLA antibodies found that such antigens were almost completely diminished in normal SC [24], although one appeared to show staining in eczematous SC lesions [25]. However, other studies have measured non-self HLA antibodies in the serum, detecting them in 22% and 27% of transfusion-free children tested [26, 27] as well as in adults [28, 29]. The origins of the antigen exposure are speculative; suggestions include 'environmental factors', cross-reacting infections and blood transfusions.

The SC can generate adaptive responses. We [30] and others [31] have shown that normal and atopic subjects have IgE and IgG to a proportion of inhaled particles. In our study, these antigens are likely to include human allergens [32], known to be associated with skin-flakes. Additionally, in a small study using serum from five 18-month-old infants, we found that between ~30% and 50% of skin-like particles in their bed dust eluted antigens that bound their IgG (unpublished observations). Indeed, there is a long history of observations in this area [33–36]. Extracts of normal SC have been demonstrated to contain undescribed T cell antigens of high potency but limited structural diversity [37]; these appear to be analogous in effect to superantigens but require MHC Class II presentation.

Studies in animal models have shown that intratracheal delivery of alloantigen can generate regulatory T cells that

induce antigen-specific tolerance [38], and in an animal model of diabetes, T-regulatory cells were capable of bystander suppression of heterologous immunity [39]. Together, these indicate a mechanism of how inhaled alloantigens may operate to inhibit allergic diseases.

Antibodies against xenoantigens are absent at birth and reach adult levels by the second month of life [40]. It is also notable that the use of feather bedding by children is associated with reduced asthma and dust mite sensitization [41]. While this protection has been attributed to reduced mite allergen exposure, our observations are that aeroallergen exposure from feather bedding is not markedly different from other bedding, but that feather bedding is a potent source of airborne feather fragments (E.R. Tovey, 2007, unpublished observations) and so its use may provide exposure to the complex array of keratin xenoantigens.

Finally, both the potential and complexity of the immunomodulation of allergy by complex biological mixtures that are dander-rich is indicated in a recent study [42], where soluble components of a barn dust extract, not associated with endotoxin, suppressed both IgE and airway hyperresponsiveness responses in a murine model of allergic asthma.

Within our proposal, it would be difficult to discount a possible role for commensural micro-organisms carried on normal skin flakes [43]. These too differ between individuals [44] and different animals and would also be affected by personal hygiene. However, on the basis of their respective mass, it might be expected that the contribution of human-derived molecules would greatly exceed that from the microbial sources, and studies [11] indicate that the ratio of skin particles to viable bacteria is greater than 100 : 1.

Implications for studies of allergic diseases

Probably the most common inhaled biological material that the human airways are exposed to is the aerosolized skin of themselves and other people. Despite this, such material has not been considered in the context of its potential to modulate allergic airways disease through both innate and adaptive stimulation. As a result, there has been little interest in the molecules associated with skin that are shed by individuals, or animals, how the associated effector molecules survive over time after the skin is shed and how they differ with the age and identity of the source. Little is also known about how domestic aerosol exposure to these may have changed over the last 50 years, although it is accepted that there have been considerable changes in housing size, furnishings, ventilation, occupant density etc. Some information on hygiene and allergic disease prevalence has been reviewed recently [5]. While the interpretation of data on the use of cleaning products is complex, it has been estimated that their use increased 50–75% in Europe during the period from 1969–1994 [45],

suggesting that our bodies and clothes continued to get even 'cleaner' over the period that the prevalence of asthma and allergic diseases was observed to increase. By the mid-1990s a study of 15-month-old children found that 60% were given a bath or showered once or more a day [46]. Certainly washing with soap will remove cutaneous lipids [47] and other small molecules, and in our view, such changes in personal and domestic hygiene practices could have greatly reduced individuals exposure to these molecules over the past 50 years.

Several studies contain hitherto unexplained observations that would lend support to this concept. The 'sibling effect' is stronger if exposure is to brothers rather than sisters [48, 49], possibly as exposure would involve additional H-Y minor histocompatibility antigens found on male dander. Paradoxically, two recent dust mite allergen avoidance trials resulted in more eczema [50] and sensitization [51] not less, in the intervention groups. Although these interventions reduced dust mite allergen, they almost certainly reduced skin particles as well. Increased infant hygiene at 15 months has been associated with more eczema, asthma and wheeze at 42 months [52]. Additional observations cited as supportive of the hygiene hypothesis can also be interpreted to support this hypothesis, e.g. bedroom sharing [48], greater social crowding and early attendance of childcare, see [2, 3], are all protective for atopy, at least in some studies.

Testing the concept

This concept provides new directions for research. Direct measurement of the spectrum of glycolipids, active peptides and antigens (particularly histocompatibility antigens) associated with aerosolized SC/keratin particles is required. This would include determining the spectrum of such particles recognized by antibody subtypes from atopic and non-atopic infants over time. Further definition of T cell stimulation by dander derived from humans and animals with particular reference to presentation by CD1 family members and NKT cell activation should be performed, as should a re-examination of Th1 and Th2 responses of animals *in vivo*, and humans *in vitro*, to allergens presented in conjunction with skin flakes. The influence of genetic disparity within co-habiting humans on allergic outcomes could be re-examined using existing datasets from transplantation screens and gene–disease interaction studies. Studies on the effect of personal hygiene on a range of lipid, peptide and antigens and their dispersion with skin are also required.

Relevance

So far, the majority of trials testing allergen avoidance and/or dietary modification interventions for the primary prevention of allergic diseases have not produced

encouraging outcomes [53]. Additionally, no interventions based on the 'hygiene hypothesis' have been consistently demonstrated to be both feasible and effective for preventing allergic disease, although a number of promising avenues are being explored [54]. Should early exposure to aerosol skin be relevant to the modulation of allergic diseases, it provides opportunities to develop novel interventions for prevention. These would be directed towards increasing early and varied exposure to epidermal particles derived from human, mammalian and avian sources while research would be directed at the identification and isolation of the molecules responsible. Personal hygiene would be targeted, so that hand washing to reduce infection risk would be encouraged but excessive bathing of young children would be discouraged to increase the shedding of skin carrying effector molecules capable of maximizing immune regulation. Such lifestyle and environmental modifications may prove more achievable and beneficial than current approaches.

A recent review noted that 'the mammalian SC is a remarkable structure that appears lifeless and trivial to the histologist, but in reality has unbelievable complexity, subtleties and importance' [55]. Its effects may be even greater than imagined.

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