Sensitization to airborne environmental allergens: unresolved issues

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Despite considerable advances in identifying the environmental agents that trigger allergy and the immunological mechanisms involved, progress in developing effective treatments remains frustratingly slow. Is it time to rethink some of the paradigms guiding this research?

The first modern reports of allergic disease date back to the early nineteenth century and refer to hay fever, which was perceived at the time as a disease of the aristocracy and urban dwellers. The rural working class was considered to have become tolerant through high dose exposure in the fields, an observation very much resonant with contemporary ideas we will discuss here.

Allergic sensitization and attendant diseases such as hay fever and atopic asthma remained relatively infrequent over the next 100 years, but sensitization prevalence began to increase steadily in the 1970s, reaching 40% in many industrialized countries. The degree to which this increase is being carried forward into the twenty-first century is uncertain, but is clear that a variety of social and environmental changes that are ongoing and in some cases are accelerating have the potential to further influence susceptibility to allergic sensitization. The environmental agents responsible for the bulk of allergic disease worldwide are the main indoor allergens, particularly those derived from arthropod (mite and cockroach) and animal (dog and cat) sources and the seasonal pollens. As for human exposure to these agents, pollution and global warming can potentially affect both indoor and outdoor allergens. Increased temperature increases the duration of the pollen season and the abundance of pollen. The increased carbon dioxide associated with global warming has a direct stimulatory effect on pollinosis[1] and thus on the amount of pollen in polluted cities, which paradoxically is often higher than that in rural regions. Climatic changes are also contributing to the geographic spread of allergens, exemplified by the march of ragweed into Europe and Asia[2].

Changes in lifestyle and housing can also affect both exposure to allergens and susceptibility to sensitization. A good example is the infestation of Western-style houses, which are becoming increasingly common in heavily populated tropical regions, with the glycyphagus dust mite *Blomia tropicalis*. It is estimated that 1 billion people are now at risk of sensitization to this allergen, which was considered only a regional curiosity a decade ago. Over the last 25 years during which international allergy prevalence has soared, a series of substantial research advances have provided a broad understanding of underlying disease mechanisms. The principal focus of this research has been characterization of the main environmental allergens and elucidation of the mechanisms underlying induction and expression of allergic reactivity. Despite considerable progress, attempts to prevent disease onset through controlling allergen exposure and to modify the pathogenesis of established allergic disease through antagonizing T helper type 2 (T_H2)–associated effector mechanisms have proven disappointing. This failure suggests that some of the interpretations placed on the central findings from this body of research require re-evaluation. In the discussion here, we focus on three issues that are emerging as increasingly contentious and are challenging existing paradigms relating to atopy pathogenesis: the relationship(s) between the amounts and timing of initial exposure to aeroallergens and the development of sensitization; the relative functions of T_H1 and T_H2 cytokines in expression of allergic disease; and the properties of the allergens that drive allergic responses.

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Determinants of responder phenotype

Initial sensitization to allergens not previously encountered can occur throughout life, as demonstrated by the *de novo* development of allergy in adults to newly encountered occupational antigens, such as allergy to latex, for health workers, and allergy to mouse urinary proteins, for animal house attendants. Adult immigrants also commonly become sensitized to the allergens characteristic of their adopted environment soon after arrival, and many subsequently experience the symptoms of allergic disease *de novo*[3]. It is, however, often difficult to separate the contribution to this process of changes in lifestyle from the simple introduction of new allergens into their environment. The spread of ragweed and birch throughout Italy provides one example of new exposure without attendant cultural changes, with local adults of all ages developing allergies to these pollens[2]. The situation in children, however, may be more complex. In particular, studies of child adoption indicate that the development of sensitization to allergens unique to the new environment is most frequent in children adopted before 2 years of age[4].

The initial observations linking allergen exposure in early childhood with sensitization risk were related to allergy to seasonal allergens

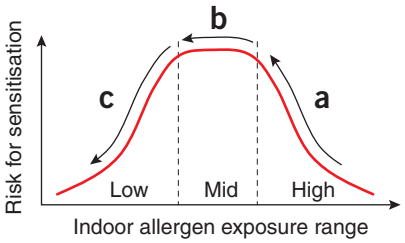


Figure 1 As a consequence of the shape of the exposure–risk curve, reducing exposure in the high exposure range (a; arrow indicates direction) increases the risk for sensitization. In contrast, reducing exposure in the plateau region (b) is without effect. In the low range (c), reduction has the potential to lower sensitization risk.

in northern Europe. Notably, sensitization to birch was associated with birth during the 'pollen season'[5], and subsequent reports confirmed that relationship for a range of other seasonal allergens. Those findings, together with the migration studies, have given rise to two general ideas that have assumed canonical status in this field: early childhood represents the life phase during which risk of allergic sensitization is highest, and the seemingly logical extension that risk increases with exposure intensity over this period.

The idea that infancy represents the 'prime time' for initial sensitization is increasingly supported by a growing pediatric immunology literature. The principal determinant of risk seems to be the maturational status of the immune system in relation to T_H1-T_H2 balance at the time aeroallergens are initially encountered[6], stemming from the generalized T_H2 skewing of immune function during fetal and neonatal life. Many reports have demonstrated the presence of aeroallergen-reactive T cells in cord blood[6], which has been widely interpreted as evidence for transplacental priming. There is credible evidence available on the development of long-lived memory T cells *in utero* against pathogens in situations of high-dose antigenic stimulation. However, the findings relating to putative fetal priming against aeroallergens have been more difficult to reconcile with known dose-response relationships, given the very low cumulative maternal exposure to individual allergens over the duration of a pregnancy. A possible solution to this enigma has been provided by studies demonstrating that allergen-responsive T cells in neonates are recent thymic emigrants, which are $CD4^+CD45RA^+$ and thus are immunologically naive[7]. These cells lack the fine specificity of functionally mature $CD4^+CD45RA^+$ T cells as a consequence of shortened CDR3 length and respond promiscuously to a broad spectrum of antigens[7].

A hallmark of the response phenotype of these promiscuous neonatal T cells is a rapid bust of proliferation and accompanying cytokine production, followed by apoptosis, unless they are 'rescued' by common γ-chain cytokines such as interleukin 7. The functional importance of these responses remains to be defined, but may include (as suggested for comparably promiscuous neonatal $CD8^+$ T cell responses[8]) involvement in short-lived 'natural immunity' during early life, when sources of cytokines required for the amplification of antibody-mediated defense against pathogens are limited because of a lack of specific memory T cells[9]. The predominance of B1 cells at this time provides an equivalent source of low-affinity, multireactive natural antibodies.

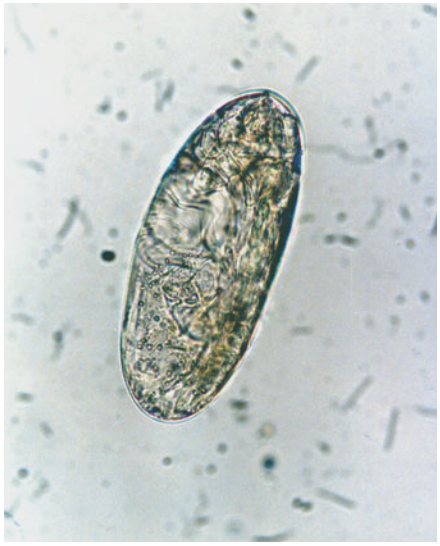
It thus seems that effective priming of aeroallergen-specific memory T cells rarely occurs before birth and instead is initiated during infancy and is consolidated by the end of the preschool years into patterns equivalent to those in adults, such as mixed T_H1-T_H2 responses in atopic people versus a low T_H1-like cytokine response phenotype in nonatopic people[6]. Genetic risk for the consolidation of memory into a T_H2-polarized pattern in childhood has been linked to delayed postnatal maturation of immune competence and in particular to sluggish upregulation of T_H1 activity. This can be partly attributed to a deficit in $CD4^+$ T cell function during infancy and can be demonstrated at the clonal level[6] but also involves delayed functional maturation of associated populations, including antigen-presenting cells and regulatory T cells. Children in the high-risk group also manifest deficiencies in resistance to infections and responsiveness to vaccines, which also seem attributable to attenuated T_H1-associated function.

It is noteworthy that this maturation process is itself driven by environmental stimuli, particularly by microbe-associated motifs that engage pattern-recognition receptors such as CD14 and Toll-like receptors. Studies demonstrating extraordinarily low allergy prevalence in farming children exposed to very high microbial loads during infancy and the strong influence of genetic variation in Toll-like receptor function on allergy resistance in these children[9] illustrate the complexity of the underlying gene-environment interactions governing this process.

Is more aeroallergen exposure worse?

Although the findings discussed above provide a plausible explanation for why early life constitutes a time of increased risk for sensitization, they do not shed light on associated dose-response relationships. Nevertheless, it has been widely extrapolated that reducing allergen exposure during this period will lower sensitization risk, and this premise has been tested in a range of 'allergen avoidance' trials involving infants and young children focusing on environmental control measures targeting a reduction in indoor allergen concentrations. Although this approach has benefits in relation to reducing symptoms in sensitized children, with the exception of a single study demonstrating a modest reduction in responses to house dust mites[10], there is no convincing evidence that sensitization itself is reduced, and in some circumstances it may actually be increased. A notable example is an intervention study[11] that achieved a reduction in house dust mite concentrations from 30 μg mites/g dust to 16.6 μg/g. Although children living in the houses modified to reduce the house dust mite concentrations demonstrated improved lung function by 3 years of age, their sensitization rates to house dust mites were double those found in the control children[11].

That finding seems counterintuitive, until additional research is considered. There is a well established experimental literature[12,13] demonstrating that T_H2 priming is preferentially favored by low-dose antigen exposure, whereas higher doses favor T_H1 priming. Moreover, a series of epidemiological studies focusing on intensity of exposure to indoor allergens and subsequent sensitization in children has demonstrated apparent dose-response relationships that fit a biphasic pattern in which, notably, sensitization risk increases with exposure concentration until a plateau is reached, above which risk decreases



Trouble hatching: egg with mite larvae about to hatch. Photo by Nat Malainual (Department of Parasitology, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand).

Pretty but sneezy: Cats and grass are chief sources of allergens. From morguefile.com.

with further increases in exposure (**Fig. 1**). Findings consistent with this relationship were initially reported for cat allergen[14], which attains considerably higher effective exposure concentrations than other indoor allergens, but it has also been demonstrated in relation to house dust mites[15].

An additional immunological mechanism is also likely to contribute to this dose-response relationship. Notably, studies in experimental models[16] have demonstrated that protection from sensitization to aeroallergens normally occurs through a mechanism equivalent to the oral tolerance process. A hallmark of animals 'tolerized' by repeated aerosolized allergen exposure is the inability to subsequently produce immunoglobulin E (IgE) against the inhaled allergen, accompanied by ongoing production of specific IgG antibodies. This response is precisely as described for a substantial subset of nonatopic humans who produce cat-specific IgG4 in the absence of IgE, a phenotype called 'modified T_H2'[14].

So should allergen avoidance be abandoned as a preventative strategy? Based on the curve in **Figure 1**, the answer may depend on the baseline amount of allergen in a given target environment. Thus, reducing allergen load in the high range (**Fig. 1**, a) would increase risk for sensitization, possibly analogous to that in a study[11] in which house dust mites were reduced from 30.3 μg/g to 18.9 μg/g. In contrast, reducing allergen load in the plateau region (**Fig. 1**, b) would predictably be without effect, but if baseline exposure in the target environment were initially in the low range (**Fig. 1**, c), avoidance would have the potential to lower exposure concentrations to below the stimulation threshold required for triggering T_H2 priming, thus reducing sensitization. The last scenario may account for the moderate success of a trial in which a reduction in house dust mites from 16.6 μg/g to 5.3 μg/g resulted in a 28% decrease in sensitization[10].

T_H1 and T_H2 cytokines in allergy

The mouse T_H1-T_H2 paradigm has provided a useful framework for research into the pathogenic mechanisms underlying human atopic diseases, but the degree to which interspecies extrapolation can be extended has been questioned. An issue frequently raised is why putative T_H1-polarized memory in nonatopic animals against aeroallergens that are present continuously in the environment apparently never manifests as delayed-type hypersensitivity. The answer may lie in the studies cited above in relation to 'modified T_H2 immunity'. As noted, animals chronically exposed to aeroallergen progressively shut down their IgE response capacity while maintaining parallel IgG antibody production. However, rechallenge studies have indicated that the capacity to generate delayed-type hypersensitivity to the same antigen[17] or to further 'boost' specific IgG antibody production is also covertly suppressed in these animals; that is, this T_H1-like response is internally regulated and cannot be 'boosted' to pathonomonic levels. By analogy to the nomenclature relating to cat-specific IgG4 production in the absence of IgE, this could be described as 'modified T_H1 immunity'.

The situation in atopic animals, however, may be even more complex. As discussed above, children at high risk of atopy start life at the lower extreme of the T_H1 response spectrum, and deficient T_H1 cross-regulation during the early stages of priming may assist in driving their allergen-specific T helper–memory cell development down the T_H2 pathway. However, this developmental deficiency is often transient; for example, along with increased T_H2 responses, atopic children eventually develop higher T_H1 responses to environmental allergens than their nonatopic counterparts[18].

Could hyperproduction of T_H1 cytokines in immune responses to allergens or responses to other agents (such as viruses) encountered at sites of chronic allergen-induced tissue inflammation contribute to the pathogenesis of atopic diseases? An increasing body of indirect evidence indicates that this scenario merits serious consideration. For example, in a study of 11-year-old children, the *in vitro* allergen-specific and polyclonal interferon-γ (IFN-γ) responsiveness of peripheral blood mononuclear cells were independently associated with responsiveness to dermal and/or airway challenge[18]. In older atopic asthmatic children and adults, increased numbers of IFN-γ-secreting T cells have been reported in blood and/or bronchoalveolar lavage fluid, and increased IFN-γ itself has been reported in both compartments in asthmatic adults[18]. It is also noteworthy that in a variety of settings, the associations of IFN-γ with atopic disease severity are linked to $CD8^+$ T cell function. The chief example is fatal asthma[19], a hallmark of which is increased $CD3^+CD8^+$ IFN-γ^+ cells in the bronchial mucosa. Similar observations have been made in relation to $CD8^+$ IFN-γ^+ T cells in blood and sensitization in high-risk children[20]. House dust mite–reactive $CD8^+$ T cells are also increased substantially in blood from house dust mite–sensitized subjects with atopic dermatitis[21], and the high frequency of IFN-γ-secreting cells at lesional sites in this disease suggests that they may have an active function in pathogenesis[22].

Collectively these findings suggest that the potential function of T_H1 cytokines as pathogenic factors in allergen-induced airway disease merits more detailed investigation. In particular, they suggest that the development of therapeutic strategies for controlling chronic T_H2-associated diseases (such as atopic asthma) by deliberate upregulation of T_H1 immunity should be approached with caution.

Immunodominant allergens

From a global perspective, the most important sources of outdoor allergens are pollens of grasses, weeds, olive, birch and conifers. Emanations from mites, cockroaches and cats constitute the most common indoor allergens. The allergens from most of these sources have now been identified. The number of IgE-binding proteins identified for each allergen seems formidable, exemplified by the 20 different allergenic proteins that have been described for both grass pollen and mites. Most sources, however, have immunodominant allergens. Thus, nearly all patients with birch allergy have 80% of their IgE directed to the lipid transport protein Bet v 1 (ref. 23), most of the IgE binding to grass pollen is specific for group 1 and 5 allergens[24] and about 50% of IgE directed to pyroglyphid mites is to the group 1 and 2 allergens[25]. Similar percentages of IgE bind the uteroglobin Fel d 1 in cat allergy and the protein Amb a 1 in ragweed allergy. Cockroach allergens seem to be the exception, with the most potent allergen reacting substantially in only 30–50% of allergic people. Some caution must be taken in directly equating IgE binding with potency. For example, the group 1 grass allergens bind IgE at high titer but are very poor at eliciting allergic responses in the nose and skin. The group 2 grass allergens that only account for 10% of the IgE binding are far more allergenic *in vivo*.

The importance of the immunodominant allergens in the etiology of allergy lies in the possibility that responses to these allergens drive sensitization to other antigens. Targeting

immunotherapy selectively to immunodominant allergens thus provides a potential avenue for the development of new, molecularly defined desensitization strategies, and indeed studies involving single-allergen desensitization for ragweed, cat and birch pollen allergy all have shown promise in this context.

Whether the immunodominant allergens have intrinsic adjuvant properties that contribute to their potency remains unknown. It is thus unclear whether removing them or neutralizing host responses to them will 'silence' allergy or redirect it toward different specificities. As shown by IgG binding, different allergens can induce very different immune responses. For example, cat allergens[26] and most cockroach allergens[27] induce IgG in subjects with and without IgE to these specificities, whereas the immunodominant pyroglyphid mite and pollen allergens generally induce only IgG in allergic subjects[28]. Some highly antigenic proteins from complex allergens are not even allergenic, demonstrating that even within an allergen source, antigenicity is not a sufficient property for allergenicity. An example is the nonallergenic pyroglyphid mite ferritin, which induces high T cell proliferation and similar cytokine responses from the T cells of both allergic and nonallergic subjects[29].

From a functional standpoint, the main allergens represent a diverse group of proteins[30]. The group 1 and 2 pyroglyphid mite allergens are a cysteine protease and a lipid-binding protein similar to the myeloid differentiation 1 protein that associates with Toll-like receptor 4. Bet v 1 is one of the pathogenesis-related type 10 proteins that function to transport small lipids. Fel d 1 is a lipid-binding uteroglobin that regulates responses to lipid mediator and the group 1 grass allergens are β-expansins that disrupt noncovalent bonds for the formation of the pollen tube. The cockroach allergens with the highest IgE binding are the aspartate protease–like allergens Bla g 2 and glutathione *S*-transferase Bla g 5 (ref. 27). The only biochemical similarities are in ragweed and conifer allergens, which contain pectate lyases. There is thus little to suggest from this broad perspective that functions of the allergens direct their T_H1 or T_H2 characteristics.

Much attention has been paid to the possible adjuvant function of the cysteine protease activity of pyroglyphid mite allergen, but it now seems that lipid binding is a more frequent property of immunodominant allergens[30]. Lipid binding is also found for the mammalian lipocalin allergens and the lipid-transfer proteins from the pollen of parietaria, olive and mugwort. It could be possible that lipids may facilitate antigen presentation to natural killer T cells via CD1, but although natural killer T cell activity has been shown to be important in animal allergy models, its relevance to human allergy remains unknown. Molecules presented together with allergens can also be important, and attention has been drawn to the abundance of T_H2-inducing phytoprostanes in pollens. Thus, although there are multifaceted relationships between the degree of allergen exposure and the pattern of the allergic response and disease expression, these relationships may differ for different allergens, even within the one source.

Conclusions

Relatively recent changes in lifestyle and in the environment in the industrialized West have conspired to set the scene for an epidemic of allergic diseases such as hay fever and asthma, which were comparative rarities a generation ago. It is clear from the emerging epidemiological literature that history is repeating itself in the developing countries as the pace of industrialization quickens. It is also evident that despite the seemingly straightforward nature of allergy, in which the causative agents (environmental allergens) and the inflammatory pathways they trigger (T_H2-mediated hypersensitivity cascades) are both well characterized, the translation of this knowledge into improved means of prevention and treatment remains frustratingly slow. The hidden complexities highlighted here, which are likely to represent only the tip of a larger iceberg, may in part explain the slow pace of this process.

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