

Allergen interactions with epithelium

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Purpose of review

Allergies are a global health problem with rapidly increasing prevalence but still lacking pathogenetic knowledge or optimal treatment. The objective is to add to the conventional thinking that allergies are caused by overactive, mainly T-cell-mediated, immunological responses and thus to raise the putative role of altered epithelial functions.

Recent findings

Birch pollen allergen was rapidly and actively transported through the respiratory epithelium via caveolar-dependent mechanisms only in patients allergic to birch pollen but not their healthy controls. Transcriptomic analyses showed that whereas healthy individuals raised a strong epithelial response after intranasal allergen challenge, the allergic patients had a reduced response. Thus allergies could also be due to hyporeactive responses on the epithelial level.

Summary

Epithelium has emerged as an active and complex organ with mechanical, biochemical and immunological functions. The increasing awareness that epithelium interacts actively with allergens might provide new targets for the prevention and management of allergy.

Keywords

allergen, barrier, caveolae, pollen, respiratory epithelium

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Introduction

Allergic disease is a symptomatic type I hypersensitivity response caused by allergen cross-linking specifically with anti-IgE molecules on the surface of presensitized mast cells. With the prevalence of up to 20%, allergic disorders affect widely general health and cause remarkable indirect costs worldwide. We would put out two, at least partially, different theories concerning the reason of allergic diseases. The first is that allergy is mainly caused by unbalanced and overactive immunological responses against allergens, primarily driven by Th2 cells. The second is that physical and functional defects on the epithelial barrier result in allergy. Because there is a wealth of literature on the T-cell responses related to allergy [1,2], our objective is to focus on epithelial interactions [3–5,6].

The concept of unified airways suggests that apart from the anatomic continuity, an inflammation in one part influences the homeostasis of other parts [7,8,9]. We focused on birch pollen-caused rhinoconjunctivitis because it is the most common allergy form in North Europe and because specimens are easy to collect from

individuals [10,11,12]. In-vivo challenge with birch pollen allergen, Bet v 1, leads to leukocyte extravasation into conjunctival and nasal mucosa in minutes only in patients allergic to birch pollen but not in healthy individuals. Next, we aimed at observing the contact of Bet v 1 with the respiratory epithelium during the first minutes after the birch pollen challenge.

Allergens bind to and enter in the respiratory epithelium

We demonstrated that in-vivo birch pollen challenge leads to a very rapid binding of Bet v 1 to the pseudo-stratified nasal or stratified conjunctival epithelium. Bet v 1 was localized in small clusters on epithelial surfaces and taken up into epithelium only in patients allergic to birch pollen [10,12]. The great majority of Bet v 1 allergens within the epithelial cells were colocalized with caveolar markers, such as caveolin 2 or flotillin 2. Thus we suggested that this uptake process involves an active lipid raft and caveolar-dependent transport. Thorough immunoelectron microscopy with about 500 merged frames (with 27 000× magnification) revealed that Bet v 1 could travel through the nasal epithelium and reach

mast cells located under the basement membrane. Our first hand evidence gathered by various microscopy techniques suggested that the Bet v 1 allergen bound to and travelled through the nasal and conjunctival epithelium via a lipid raft and caveolar-dependent process [10^{••},12].

After providing proof of this novel hypothesis of active allergen transport, we next wanted to identify the Bet v 1-associated proteins acting as putative receptors and/or participating in intraepithelial transport by using affinity columns, high-pressure liquid chromatography (HPLC), and mass spectrometry. We performed affinity chromatography using nasal epithelial cell lysates collected from both healthy and allergic patients and the covalently matrix-bound Bet v 1 antigen. The Bet v 1-associated proteins were released from the matrix and subjected to 2D HPLC and mass spectrometry MS² analyses and the relevant peptides were identified. By using both database-related and de-novo identifications we generated a shortlist of 16 Bet v 1-binding proteins found solely from the nasal epithelial cells of allergic patients. Six out of these 16 proteins (e.g. 40%) were caveolar proteins: ACTG, PLEC1, STML2, KCNA5, CALM, and ANXA2. Because human proteome consists of less than 1% of caveolar proteins, they were highly enriched only within allergic patients [10^{••}]. This observation on its own clearly supported the hypothesis that Bet v 1 could travel through the epithelium within caveolae.

To provide further evidence that birch pollen allergen is transported via lipid rafts and caveolae, we performed the 3D modelling of Bet v 1 and its possible lipid ligands. Bet v 1 binds to cholesterol and enters the epithelium of allergic patients via cholesterol and glycolipid-rich caveola. We modelled putative lipid ligands for Bet v 1 allergen on the atomic level with computational ligand docking, and showed that the hydrophobic cavity of Bet v 1 has different binding sites for different ligands and also identified several groups of ligand type-specific amino acids. Bet v 1 proteins also bind and transport more complex amphiphilic molecules like ceramides, sphingomyelins and even glycolipids, which are all present on caveolar surfaces. We created a binding mode, in which the hydrophobic tail groups of lipids were located in the Y-shaped cavity within the Bet v 1 molecule, whereas the polar head group remained solvent accessible [13]. This could provide a molecular mechanism for birch pollen entry to the epithelial tissue of allergic patients.

Finally we collected nasal epithelial cell swabs for transcriptomic analyses from birch pollen allergic and healthy individuals during the nonsymptomatic winter season and during the symptomatic spring season at weekly intervals starting at the onset of the birch pollen season. Every individual participating in this study was symptom-free during winter. During the course of this exper-

iment the healthy individuals remained asymptomatic, whereas the allergic patients gradually began to develop clinical symptoms [10^{••},11[•]]. During the asymptomatic winter period, the nasal epithelial transcriptome had about 100 differentially expressed genes between the allergic and healthy individuals. Thus there was a significant difference in epithelial gene expression profiles during wintertime even though neither group had any symptoms. The time series analyses generated the next surprise. The epithelium gathered from healthy individuals displayed 40 significantly altered transcripts during the spring season when birch pollen was in the air compared with the winter season. Out of these 40 transcripts, 20 were unique for this group and the rest were modified in the allergic patients. Of these uniquely regulated genes a significant proportion (up to 40%) belonged to the Gene Ontology category Immune response (GO:0006955). The members of the Immune response category modified only in healthy individuals were: CXL10, CXCL6, CXCL9, CXCR2, IL1 and IL8, NKG7, PGSG and FPR1. This Immune response category has altogether 550 genes and thus represents only a few percentage of all annotated proteins within the human proteome (amigo.geneontology.org). Therefore birch pollen allergen induced a strong epithelial immune response within healthy individuals, whereas this same response was not detected within allergic patients [11[•]]. Also other groups have indicated the altered cellular state of epithelial cells in allergic versus healthy controls. The hyporesponsiveness of the allergic epithelial cells was observed in in-vitro cultivations after the allergen perturbation [14]. Our allergic patients showed 100 uniquely regulated transcripts (converted for further analyses to proteins) in nasal epithelial specimens during increasing symptoms. Dyneins were among the most enriched Gene Ontology categories in these proteins. The whole human proteome has only 19 dynein proteins (0.1%), whereas we observed six of them among the 100 modified epithelial proteins (6%) in the allergic epithelium. Dyneins provide molecular motors for epithelial ciliary movements and intracellular caveolar cargo traffic along the microtubuli.

Is allergy a disease of the epithelium or of the immune system?

Currently it seems that both answers are correct. There are several immunological studies indicating an altered Th2 response, IgE production and other hyperimmune responses, which are not covered further in this review [1,2]. The role of the epithelial barrier function in allergies has recently also been identified [3[•]–5[•]]. In atopic dermatitis, filaggrin mutations have been identified constantly which provokes increasing interest in barrier dysfunction genes [15[•]]. Interestingly, mutations in filaggrin not only cause atopic dermatitis but also confer a

significant risk for other allergic diseases and asthma in context of eczema [16]. The impaired airway epithelial barrier functions with disrupted tight junctions, reduced antioxidant activity and innate immunity are seen also in asthma patients. Such alterations in the barrier could explain the significant susceptibility of asthma patients to respiratory viruses [3[•],4[•],17[•]]. The genome-wide association (GWA) studies have provided a number of hypothesis-free candidate genes linked to epithelial barrier functions for further analyses in asthma and other allergic diseases [17[•]].

The precise transport mechanism of allergens through the respiratory epithelium is still unknown. We know now that lipid rafts and caveolae provide means for transporting [10^{••},11[•],12]. Much larger cargo, such as full *Pseudomonas* bacteria can also actively invade lung epithelial cells via lipid raft and caveolar mechanisms [18]. The food allergens are protected from lysosomal degradation when they are transported across the intestinal epithelium by their binding to cell surface IgE/CD23 (FcεRII). The presence of IgE/CD23 in the intestine opens a gate for intact allergens to transcytose across epithelial cells, which in turn can lead to mast cell-dependent type I anaphylactic responses [19]. This direct interaction with IgE and its low-affinity receptor CD23 has so far been detected in the intestinal but not in the respiratory epithelium. On the contrary, as pollen allergen transport seems to be specific, for example our patients allergic to birch pollen do not transport timothy grass allergen and *vice versa*, respiratory epithelial transport is most probably antibody-dependent also. There is preliminary evidence that ovalbumin allergen (OVA) could be transported rapidly via the respiratory epithelium in both the nose and lower airways in a rodent model [20]. Recently, the internalization of grass pollen allergen into human respiratory epithelial cells has been shown [21[•],22].

Conclusion

Taken together new data from our laboratory and others on the role of epithelial barrier and the active transport of allergens through it might be incorporated to the pathomechanisms of allergy. Also studies suggest that allergen exposure leading to a reduced epithelial response in allergic patients compared with healthy individuals could challenge the concept of hypersensitivity. In fact, if allergy turns to be at least in part a result of epithelial hyposensitivity, it questions the accuracy of the current terminology – and putatively also the future management targets.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 66).

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