

REVIEW ARTICLE

The multi-faceted role of allergen exposure to the local airway mucosa

K. Golebski^{1,*}, K. I. L. Röschmann^{1,*}, S. Toppila-Salmi², H. Hammad^{3,4}, B. N. Lambrecht^{3,4}, R. Renkonen⁵, W. J. Fokkens¹ & C. M. van Drunen¹

¹Department of Otorhinolaryngology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; ²Helsinki University Central Hospital, Skin and Allergy Hospital & Transplantation Laboratory, Haartman Institute, University of Helsinki, Helsinki, Finland; ³Department of Internal Medicine, University of Ghent, Ghent, Belgium; ⁴Department of Molecular Biology Research, VIB, Wijnjaarde, Belgium; ⁵Transplantation Laboratory, Haartman Institute, University of Helsinki & Helsinki University Central Hospital, HUSLAB, Helsinki, Finland

To cite this article: Golebski K, Röschmann KIL, Toppila-Salmi S, Hammad H, Lambrecht BN, Renkonen R, Fokkens WJ, van Drunen CM. The multi-faceted role of allergen exposure to the local airway mucosa. *Allergy* 2012; DOI: 10.1111/all.12080.

Keywords

allergens; dendritic cells; epithelium; immunology; innate immunity.

Correspondence

Cornelis M. van Drunen, Department of Otorhinolaryngology, Academic Medical Center, University of Amsterdam, Amsterdam, PO Box 22700, The Netherlands.
Tel.: +31 20 566 4721
Fax: +31 20 566 9662
E-mail: c.m.vandrunen@amc.uva.nl

*These authors contributed equally to this work.

Accepted for publication 18 October 2012

DOI:10.1111/all.12080

Edited by: Thomas Bieber

Abstract

Airway epithelial cells are the first to encounter aeroallergens and therefore have recently become an interesting target of many studies investigating their involvement in the modulation of allergic inflammatory responses. Disruption of a passive structural barrier composed of epithelial cells by intrinsic proteolytic activity of allergens may facilitate allergen penetration into local tissues and additionally affect chronic and ongoing inflammatory processes in respiratory tissues. Furthermore, the ability of rhinoviruses to disrupt and interfere with epithelial tight junctions may alter the barrier integrity and enable a passive passage of inhaled allergens through the airway epithelium. On the other hand, epithelial cells are no longer considered to act only as a physical barrier toward inhaled allergens, but also to actively contribute to airway inflammation by detecting and responding to environmental factors. Epithelial cells can produce mediators, which may affect the recruitment and activation of more specialized immune cells to the local tissue and also create a microenvironment in which these activated immune cells may function and propagate the inflammatory processes. This review presents the dual role of epithelium acting as a passive and active barrier when encountering an inhaled allergen and how this double role contributes to the start of local immune responses.

Background

The approach to understanding allergy seems to be changing from a reductionistic view to a more holistic view. This comes with the realization that the cells of the immune system fulfill their roles in the defense against potential threats to the organism in the context of a local tissue that is exposed to the environment. This applies both for the beginning of an immune response where the potential threat is first detected and at the end of an immune response where immune effector cells try to eliminate this threat.

When allergens are able to settle in our mucus, some significant changes may occur. Spores from fungi and pollen from grasses or trees may start to germinate and release their contents, and the proteins that make up the allergenic mixture may get processed by the proteases that are part of the

mucus layer. All these effects may modify the allergenic capacity of allergens well before they come into contact with any cells of the immune system. If we also consider that the allergens would become mixed with other environmental factors like microbial products from resident bacteria or viruses, then the triggers we study in our experimental settings are probably far removed from those we would encounter in real life. Figures 1 and 2 show a representation of the airway mucosa that will lead us through this review.

Uptake of allergens by epithelial cells/transport through the epithelium

The first questions that arise when allergens encounter the airway epithelium are (i) how do they overcome the epithelial barrier to be recognized by immune cells? and (ii) does the

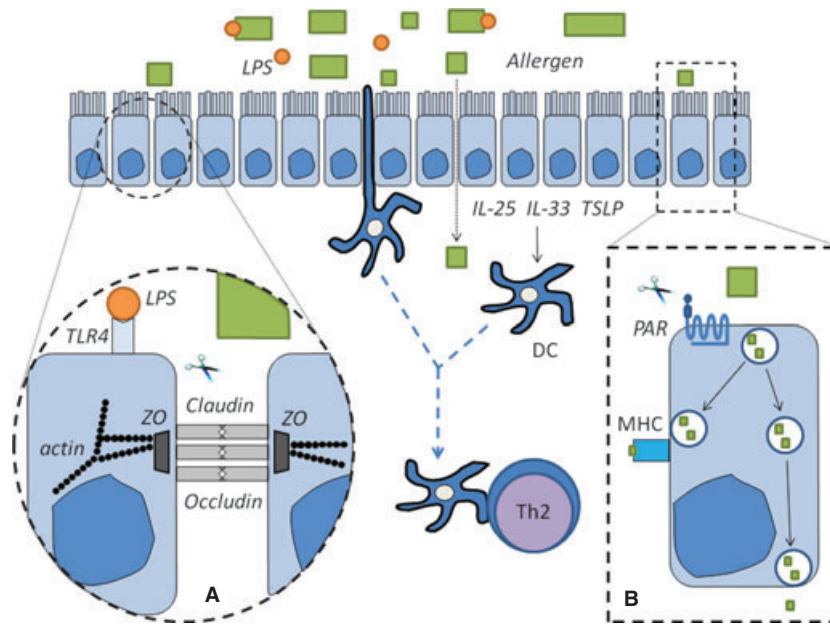


Figure 1 Cross-sectional overview of the airway mucosa tracing the different paths an allergen may travel. In panel A, we see the action of allergen on the proteins of the tight junctions (ZO, Occludin, Claudin) or their contribution to TLR-4 signaling. In panel B, the two routes allergen may follow after epithelial uptake, leading either to their transport to the subepithelial layer or toward

the MHC compartment of the epithelial cells, and in addition, the way protease components of allergen may activate epithelial cells through protease-activated receptors (PAR). In the central part, we see the allergen reaching professional antigen presenting cells that with the help of epithelial mediators may induce a Th2 response.

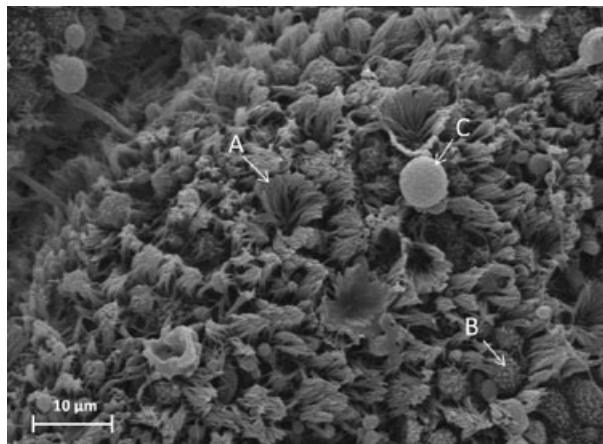


Figure 2 Surface of the inferior turbinate of the nose. In this scanning electron microscope picture, we can identify (A) the ciliated epithelial cells, (B) the microvilli carrying tops of goblet cells, and (C) some undefined (inflammatory) cells attached to the surface.

airway epithelium play a passive or active role in this context?

Assuming a rather passive position of airway epithelium, much attention has been paid to intrinsic features of allergens that would facilitate their passage through the epithelium. Proteolytic activity as a general feature of major allergens has been proposed to be involved in the pathogenesis of

allergies by enabling the passage of allergens through the epithelial barrier. Airborne allergens derived from sources such as house dust mite (HDM), cockroach, fungi, or pollen were identified as cysteine, serine, or aspartic proteases (1, 2). Der-p1, a major allergen from the house dust mite *Dermatophagoides pteronyssinus*, has been shown to be a cysteine protease (3). Furthermore, HDM derived Der-p3 also displays the properties of a cysteine protease, while Der-p6 and Der-p9 are functional serine proteases. Also for Pen-ch13, an allergenic component from *Penicillium chrysogenum*, a serine protease activity could be described (1). Phl-p1, a major allergen from Timothy grass pollen (*Phleum pratense*), shows a high sequence homology to the cysteine proteases papain and Der-p1 (4). While for recombinant Phl-p1 expressed in *Pichia pastoris* a proteolytic activity could be shown (5), natural Phl-p1 failed to display proteolytic abilities both *in vitro* and *ex vivo* (6).

Apart from these intrinsic features of allergens, a disruption of airway epithelium barrier function is also induced by many environmental factors or is a phenomenon in many diseases. Exposure of bronchial epithelial cells to cigarette smoke extract with concentrations comparable to those found in smokers induces the development of paracellular gaps and a decrease in transepithelial resistance. Cigarette smoke exposure induces a more than threefold increase in allergen penetration and the resulting increased subepithelial allergen concentrations provoke a substantial augmentation of histamine release from sensitized basophils (7). Further-

more, an increased alveolar epithelial permeability has been found in cigarette smokers (8) and mice that are exposed to cigarette smoke become more easily sensitized to respiratory allergens (9). Thus, cigarette smoke is a potent factor of reducing the barrier function of the epithelium and may contribute to sensitization and increased allergic inflammation.

It is well established that viral respiratory tract infections are important triggers of acute exacerbations of chronic airway diseases such as asthma, cystic fibrosis, and chronic obstructive pulmonary disease (COPD) (10–13). Furthermore, during infancy, these infections are associated with an increased risk for the development of asthma later in life (14, 15). Airway epithelial cells are the principal host for respiratory viruses. Influenza virus and respiratory syncytial virus (RSV) have been shown to have cytotoxic effects on bronchial epithelium (16); furthermore, respiratory viruses can interfere with the structural integrity of tight junctions, leading to an increase in paracellular permeability. Rhinoviruses are able to disrupt tight junctions (17) as well, and infection of AEC with RSV results in a decreased transepithelial resistance (18). In addition, mediators produced by AEC or infiltrating immune competent cells upon viral infection may influence cell-to-cell contact by interacting with tight junctions and desmosomes. This disruption of the barrier integrity will last even postinfection so may again enable a passive passage of inhaled allergens through the airway epithelium (19).

In some diseases, the epithelial integrity is affected, which would also allow allergens to penetrate our defenses more readily. In this line, recent publications show that loss-of-function mutations within the filaggrin gene are linked to atopic dermatitis (AD), with AD patients having an increased frequency of filaggrin null mutations (20, 21). As filaggrin, among other processes, contributes to the ultrastructural organization of intermediate filaments (22), dysfunctional filaggrin has been suspected to be responsible for the impaired barrier function of the skin, enabling allergen penetration followed by local inflammation. Atopic dermatitis is just one example, as epithelial disruptions are also seen in asthma (23), chronic rhinosinusitis with nasal polyposis (24), and when we extend our view to the digestive tract, also in inflammatory bowel disease (25) and Crohn's disease (26). These disruptions are likely to be the reason for the increased allergen penetration seen in quite some studies (27–29). An excessive mucosal permeability has been reported to exist in atopic individuals (30). However, in a more recent study involving patients with perennial HDM-induced allergic rhinitis, the absorption capacity of the airway mucosa did not differ from that recorded in healthy subjects (31). Others studies showed a rapid and high absorption of ovalbumin through the respiratory epithelium in both the nose and lower airways, with a higher absorption in the lower airways than in nasal mucosa (32).

Several lines of evidence suggest that intrinsic proteolytic activity of allergens may indeed facilitate allergen penetration into local tissues, but on the other hand, the proteolytic activity may also affect chronic inflammation in a more direct fashion. In this context, proteolytic allergens have been shown to down-regulate the antiprotease-based defense

within the mucosa, leading to tissue damage and immune activation, with group one mite allergens degrading airway α 1-antitrypsin inhibitor (33), degrading and inactivating lung surfactant proteins A and D (34), and cleaving elastase-specific inhibitors and secretory leukocyte protease inhibitors (35, 36). The importance of the balance or conversely misbalance between protease and their inhibitor could also underlie the pathogenic mechanism in Netherton's syndrome. This disease with severe disruptions in the skin integrity has been linked to mutations in SPINK5, a gene encoding a protease inhibitor (37).

In contrast to this rather passive role of the epithelium, recently there is an increased interest in the process of direct uptake of allergens by AEC, putting these cells in a more active role (38, 39). Blume et al. have been able to show that there is a difference in allergen uptake between keratinocytes and respiratory epithelium. Although obtained from lung epithelial cell lines, the results suggest a transcytosis of allergens by AEC, whereas in primary human keratinocytes, allergens accumulate in lysosomes, a prerequisite for internal processing, and co-localize with MHC II molecules (40). In contrast to these data, a striking specificity has been observed concerning the uptake of the major birch pollen allergen Bet-v1 by conjunctival epithelial cells of allergic individuals. Already one minute after *in vivo* challenge of birch pollen allergic individuals with the respective allergen, a binding of Bet-v1 to conjunctival epithelial surfaces could have been observed (Fig. 3), whereas in healthy individuals, no binding could be detected. Also the entry of Bet-v1 into the epithelial cells was evident in allergic patients, with a fast distribution, indicating

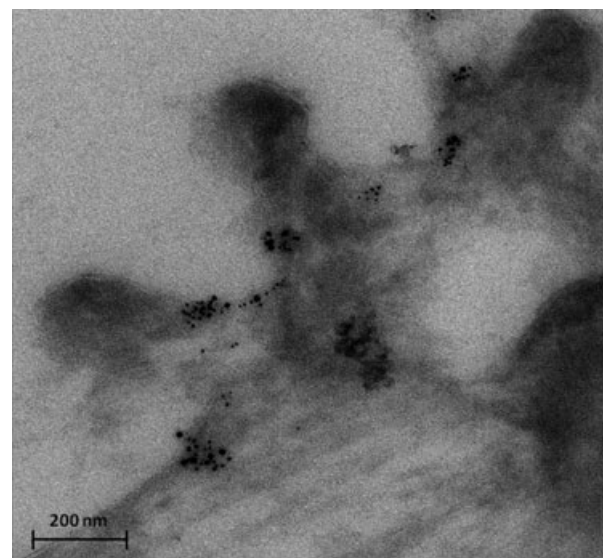


Figure 3 Bet-v1 allergen binding and passing through the conjunctival epithelium obtained from an allergic individual. Double immunogold TEM staining with an anti-Bet-v1 5 nm gold-labeled antibody and an anti-caveolin 2 antibody labeled with 10 nm gold; 27 500 magnification. Bet-v1 is seen to strongly co-localize with a caveolar marker protein caveolin 2.

rapid traffic through the epithelium, while epithelium of healthy subjects did not bind or transport Bet-v1 (41). Although the specific transport mechanism for birch pollen allergens remains to be determined, data indicate an active lipid raft and caveolae-dependent transport (42). In line with this, a major soya bean allergen has been shown to be transported through intestinal epithelial cells via a caveolae-mediated mechanism, with the allergens associating with lipid raft microdomains and an abolishment of endocytosis of the allergen after disruption of caveolae/lipid raft microdomains (43). Interestingly, the transport of birch pollen allergens seems to be allergen-specific, as grass pollen allergens do not bind to epithelial cells from birch pollen allergic individuals and *vice versa*, indicating an antibody-dependent process to be involved.

Antibody-specific recognition c.q. uptake may not be the only mechanism by which airway epithelium may interact with allergens. As many allergens are glycoproteins, carbohydrate-recognizing pattern recognition receptors (PRR) expressed on AEC might also be involved in allergen uptake. Dectin-1 for example recognizes $\beta(1, 3)$ -linked glucans, carbohydrates that are found in the cell walls of plants and fungi (44), but its expression is most prominent in myeloid cells, with a rather weak expression in epithelial cells (45). The surfactant proteins (SP) A and D, which are secreted by type-II pneumocytes, belong to the family of collectins and bind to glycosylated pathogens and allergens, leading to an increased phagocytosis by alveolar macrophages (46). It has been shown that SP-D binds to different carbohydrates that can be found on the surface of subpollen particles (47). Furthermore, SP-D promotes the attachment of allergen particles to bronchial epithelial cells, while the percentage of internalized particles remains unchanged (48), which might indicate that active allergen uptake by AEC might be the rate-limiting step. Interesting data are emerging that suggest a broad role for complex carbohydrates, in particular glucans, in allergen-associated Th2 responses. For example, β -glucan structures present in the peanut allergen Ara-h1 have been shown to display Th2 inducing characteristics (49).

The fate of the allergen

When we consider the paths, which the allergen might follow, we have seen that the allergen might pass between neighboring epithelial cells or might be taken up by epithelial cells and then released again into the subepithelial layer. There, it can be taken up by tissue resident dendritic cells to start the immune response or trigger cross-linking of IgE molecules on the cell surface of mast cells and basophiles in the effector phase (50). In addition, some of the allergens find their way into the bloodstream (28, 32) of which the consequences, up to date, are not clear. To a certain degree, it might not be required for the allergen to pass the epithelium as dendritic cell through cytoplasmic extensions would be able to reach into to the lumen (51, 52) and take up the allergen directly. Whether or not these extensions are presented under all conditions or whether the extensions are triggered by the interaction of allergen with the mucosal layer is unclear (53).

A second more active role of the epithelium is also possible as epithelial cells are known to express MHC class I and II molecules (54, 55), and respiratory epithelium has been reported to process and present antigens in the context of MHC II (56). Given the fact that AEC express surface molecules and growth factors that are associated with intraepithelial lymphocytes (IEL) and antigen presentation, namely IL-7, stem cell factor, ICAM-1, HLA-DR, CD40, CD80, CD86, E-cadherin (the ligand for $\alpha E\beta 7$ integrin on IEL), or the CD2-ligand CD58 (56–60), there might be the possibility that inhaled antigens may be presented rapidly and directly via AEC to IEL within the airway mucosa. Whether or not this interaction leads to a productive immune response remains to be explored as most models seem to point toward an immunosuppressive or anergic response (61, 62).

Interestingly, in a recent study, airway DCs were found to build clusters with nerves and proliferating T cells in a model of allergic airway inflammation, but these interactions occurred beneath the smooth muscle layer and not in the epithelium (63). However, further studies have to be performed to investigate the exact T-cell subpopulation involved and the general role of AEC concerning uptake of allergens and antigen presentation within the airways, as through epithelial exosomes would be able to stimulate immune responses at a distance (64).

Allergens affecting cellular function

Disruption of cell–cell contacts not only allows allergens or other environmental factors to pass into local tissues, but it also affects the activity of the cells themselves (65–69). However, to a certain extent, it is difficult to determine whether it is the dissociation *per se* that induces these changes. During the dissociation induced by virus, local tissue cells can be activated through the presence of Toll-like receptor (TLR)-3 that recognizes viral RNA, and during the dissociation induced by allergens, cells can become activated through protease-activated receptors (PAR) that for their stimulation depend directly on the protease activity contained with allergen extracts. So the protease activity of Der-p1 and Pen-ch13, among others, not only is responsible for the disruption of the cell–cell contacts, but also for the activation of epithelial cells through PARs (9, 11). We have some unpublished evidence that disruption *per se* could affect cellular activity directly, where we were able to show that disruption of cell–cell contacts in a nonenzymatic fashion induces mediators release from airway epithelial cells, although in this model the mediator release is much stronger when using an enzymatic approach that would also trigger the PAR receptors.

Recently, it became apparent that the ability of allergens to bind lipids (70–73) may contribute to the uptake and/or the allergenicity of certain allergens. This exciting observation came from the work on the innate pattern recognition receptor TLR-4, where it was shown that Der-p2 was identified to resemble the critical TLR-4 co-factor MD-2 not only structurally but also functionally (74, 75). The role of MD-2 to present the lipid molecule LPS to TLR-4 can be taken over by Der-p2 and as several other members of the MD-2-like

lipid-binding family are major allergens (76), this could point to a more general importance of major allergens being able to bind lipids.

This direct activation of cells by allergen applies both to epithelial cells and to tissue resident dendritic cells, which even if we would disregard the indirect effect of mediators produced by one cell on the activity of the other cell, mounts to a complex interplay between both cell types. A small but important step to tease apart the different contribution comes from the observation in mouse where we showed that the absence of TLR-4 on lung structural cells, while retaining TLR-4 on dendritic cells, abolished allergic airway inflammation caused by the HDM allergen and clearly reduced dendritic cell activation (77).

The local microenvironment

Up to now, we have focused mostly on the direct effect of allergens on epithelial or other tissue resident cells. In this part, we want to highlight some important factors that are secreted by epithelial cells and that will have an impact on immune competent in general and on dendritic cells in particular. This selection is far from exhaustive, but these factors were selected on their reported contribution to the allergic response.

Several reports have demonstrated that allergens can directly activate dendritic cells *in vitro* (49, 78). However, we should not forget that dendritic cells are active within a local tissue and that signals from the environment will reach DCs both directly and indirectly. As a consequence, the regulation of dendritic cell functions is co-dependent on the crosstalk with neighboring epithelial cells (79, 80) and the mediators they produce. The level of these mediators may themselves be affected by environmental factors and will ultimately influence the recruitment of DCs to local tissues, their activity, and even their differentiation. Recent studies have strengthened the idea that aberrant expression of genes within the epithelium might be a key driver of the inflammatory response, confirming the important role of structural cells like AEC within the initiation or treatment of complex disorders like allergy (81–89). Furthermore, secretion of CCL2 and CCL20 chemokines by *in vitro* HDM-stimulated epithelial cells was demonstrated in context of environment creation for the immature DCs chemotaxis (90). This was also seen in a model of lung epithelium exposed to the purified major allergen Phl-p1 (91). Interestingly, CCL20 release is not directly dependent on TLR2/4 triggering or PARs activation, but relies on β -glucan moieties within the HDM (92).

Airway epithelium of diseased (93) and also healthy individuals produces a variety of mediators in response to inhaled allergens that may affect the immune cells differentiation toward the allergic Th2 response. Increased baseline levels of IL-6, IL-8, GM-CSF, and TNF- α were detected in epithelial cells obtained from rhinitic individuals (94) and additionally IL-6, IL-8, IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) from healthy epithelium in response to allergens (77, 95). Expression of TSLP, a cytokine that

affects DCs, B cells, and Th2 cells is associated with asthma and allergic inflammatory states. One of the effects of TSLP is the induction of Th2 responses via up-regulation of OX40L on DCs, as OX40/OX40L interaction triggers and maintains the TSLP-mediated allergic reaction (96). However, another mouse model suggests that allergen-induced TSLP production by nasal epithelial cells requires the additional involvement of mast cell, possibly via Fc ϵ RI, as allergen alone was not sufficient for the induction of TSLP production (97). The relative importance of TSLP was shown in a model where lung epithelium specific-TSLP overexpression induced the development of an inflammatory state defined by the influx of Th2 cells and eosinophils (98). Airways inflammation triggered by DCs responding to overexpressed TSLP also led to an induction of airways hyper-responsiveness after exposure to metacholine. Moreover, it was demonstrated that TSLP may directly interact with naïve CD4⁺ T cells promoting their type helper two conversion (99).

Another cytokine produced by, among others, structural cells relevant for Th2 skewing of immune responses is IL-25. Similarly to TSLP, IL-25 overexpression in mouse lung epithelium leads to mucus production and airway infiltration of eosinophils and macrophages (100), typical features of the Th2 allergic inflammation. Moreover, allergen provocation in asthmatic individuals resulted in an increased production of IL-25 and its receptor in bronchial mucosa and indicated T cells, mast cells, eosinophils as potential targets and sources of this cytokines with respect to the allergic inflammation (101). Takahashi and colleagues demonstrated that IL-22 attenuates IL-25 expression in lung epithelium leading to an inhibition of allergen triggered airways inflammation, which suggests a strong and significant IL-25 involvement in allergic reactions (102).

IL-33 (or IL-1-F11 or nuclear factor from high endothelial venules) is a cytokine released by damaged epithelium and it triggers IL-1 receptor-related protein ST2 to promote type two inflammation via NF- κ B/MAPK pathways (103). Little is known about IL-33, probably due to the fact that airway epithelial cells store this cytokine in nuclei, potentially as a nuclear factor (80, 104), and the mechanism of its secretion to our knowledge has not been elucidated. However, Prefontaine et al. (105) suggest that IL-33 levels in bronchoalveolar lavage fluid may be used as a relevant biomarker for the determination of the severity of asthma. There are other studies postulating that IL-33 is sufficient and required for a severe allergic inflammation in the lung through an activation of the expression of other pro-inflammatory cytokines (106). In this recent publication (107), IL-33 rapid production in mouse lung model was demonstrated to be driven by trefoil factor two (TFF2). TFF2 mediator is produced among others in structural cells within hours of injury or allergen challenge (108) to enhance cell repair and it was shown to increase severity of allergic responses in lung triggered by HDM (107). Not only is the TFF2 involved in tissue repair mechanisms, but also seems to play an important role in the initiation and progression of mucosal Th2 immunological responses.

Disease affecting function

To some degree, potential differences in responsiveness of cells between the diseased and healthy state could be considered a chicken or egg problem. However, even if those differences would not be the cause of the disease, they could still contribute to the expression of symptoms, their severity, or their chronicity. The first examples of differential responses that we already encountered were in the uptake of allergens by the epithelium. Not only was the allergen Bet-v1 taken up by Birch allergic individuals and not by healthy individuals, but Bet-v1 was also not taken up by individuals who were allergic to grass rather than birch pollen (R. Renkonen and S. Toppila-Salmi, unpublished observations). Even within one disease, there could be different exponents as concluded from our clinical observations in grass allergic individuals where patients with an isolated pollen allergy would respond more strongly to a nasal provocation with pollen than those with a concomitant house dust mite sensitization (109).

At the epithelial and the *in vivo* level, we are spending a considerable effort studying the impact of HDM (82, 110–112), grass pollen allergens (6, 91), and Bet-v1 (113) using a biosystematical approach to further elucidate allergen-induced responses in health and disease. A number of important observations were made. The first is that (nasal) epithelium from healthy individuals respond so strongly to allergenic triggers. From a molecular perspective, this does not need to surprise us given the many mechanisms by which epithelium can detect and respond to these allergens.

However, it seems contradictory to our clinical concept where only the allergic individual responds to an allergen trigger and the healthy individual would not. This now entices the interesting questions why and where the 'allergic response' peters out in a healthy individual, so that the healthy individual does not become sensitized to the allergens it is constantly exposed to. The second relevant observation indicates that some of the inflammation-mediating genes in the allergic individuals are constantly activated, which would suggest that it is the failure to down-regulate these genes (rather than their initial activation) that would contribute to the allergic disease. A final example of how a disease state could contribute to the allergic response (or even the induction of new sensitization) comes from a mouse model where chronic inflammation is induced in the lung by instilling them

with *ex vivo* activated dendritic cells (114). Long after these original dendritic cells have gone and the lung eosinophilia has perished, the mouse own lung resident dendritic cells show an activated state with increased levels of co-stimulatory molecules that are more efficient at responding to neo-allergens.

Concluding remarks

Although we have come some way in understanding the complex interplay that occurs within local tissues, we are far removed from painting the complete picture. First of all, we have addressed only the epithelium and the dendritic cell, assuming that all epithelial cells are the same (which could be true or not) and that all dendritic cells are same (which is definitely not true). We have completely disregarded the contribution of fibroblasts and smooth muscle cells that seem so important in local remodeling steps seen in upper and lower airways (115, 116). Nor have we considered the role of the newly discovered innate T cells (117, 118) that are so prominently present in nasal polyps (119). These are just a few examples of factors that we have not considered at the initiation of immune responses: a situation that could be simpler than the situation during the effector phase of the immune response. Not in the last place because in the effector phase there are even more players to consider. Even in the simple situation that we have discussed here, we would need to study how our outcomes from *in vivo* models resemble or differ from our *in vitro* or *ex vivo* models before we can start understanding what happens in real life. The scariest realization could be that we are all individuals and that each and every one of us may respond differently, even when conditions are identical.

Conflict of interest

KG, KL, ST-S, HH, BL and RR have no conflict of interest. WF and CvD have received private sector support for research and/or clinical trials related to treatment of allergic and non-allergic rhinitis by Allergopharma, ALK-Abello, GlaxoSmithKline, HAL Allergy, MSD, Optinose UK, and public sector research support by InterUniversity Attraction Poles (Belgium), ZonMW (The Netherlands), and Global Allergy and Asthma European Network (EU).

References

- Chiu LL, Perng DW, Yu CH, Su SN, Chow LP. Mold allergen, pen C 13, induces IL-8 expression in human airway epithelial cells by activating protease-activated receptor 1 and 2. *J Immunol* 2007;**178**:5237–5244.
- Gunawan H, Takai T, Ikeda S, Okumura K, Ogawa H. Protease activity of allergenic pollen of cedar, cypress, juniper, birch and ragweed. *Allergol Int* 2008;**57**:83–91.
- Adam E, Hansen KK, Astudillo FO, Coulon L, Bex F, Duhant X et al. The house dust mite allergen Der p 1, unlike Der p 3, stimulates the expression of interleukin-8 in human airway epithelial cells via a proteinase-activated receptor-2-independent mechanism. *J Biol Chem* 2006;**281**:6910–6923.
- Petersen A, Grobe K, Schramm G, Vieths S, Altmann F, Schlaak M et al. Implications of the grass group I allergens on the sensitization and provocation process. *Int Arch Allergy Immunol* 1999;**118**:411–413.
- Grobe K, Becker WM, Schlaak M, Petersen A. Grass group I allergens (beta-expansins) are novel, papain-related proteinases. *Eur J Biochem* 1999;**263**:33–40.
- Roschmann K, Farhat K, Konig P, Suck R, Ulmer AJ, Petersen A. Timothy grass pollen major allergen Phl p 1 activates respiratory epithelial cells by a non-protease mechanism. *Clin Exp Allergy* 2009;**39**:1358–1369.
- Gangl K, Reininger R, Bernhard D, Campana R, Pree I, Reisinger J et al. Cigarette smoke facilitates allergen penetration across respiratory epithelium. *Allergy* 2009;**64**:398–405.
- Jones JG, Minty BD, Lawler P, Hulands G, Crawley JC, Veall N. Increased alveolar epithelial permeability in cigarette smokers. *Lancet* 1980;**1**:66–68.

9. Moerlose KB, Robays LJ, Maes T, Brusselle GG, Tournoy KG, Joos GF. Cigarette smoke exposure facilitates allergic sensitization in mice. *Respir Res* 2006;**7**:49.
10. Caramori G, Ito K, Contoli M, Di SA, Johnston SL, Adcock IM et al. Molecular mechanisms of respiratory virus-induced asthma and COPD exacerbations and pneumonia. *Curr Med Chem* 2006;**13**:2267–2290.
11. De Serres G, Lampron N, La Forge J, Rouleau I, Bourbeau J, Weiss K et al. Importance of viral and bacterial infections in chronic obstructive pulmonary disease exacerbations. *J Clin Virol* 2009;**46**:129–133.
12. Papadopoulos NG, Xepapadaki P, Mallia P, Brusselle G, Watelet JB, Katzipsalti M et al. Mechanisms of virus-induced asthma exacerbations: state-of-the-art. A GA2LEN and Inter-Airways document. *Allergy* 2007;**62**:457–470.
13. Wat D, Gelder C, Hibbitts S, Cafferty F, Bowler I, Pierrepont M et al. The role of respiratory viruses in cystic fibrosis. *J Cyst Fibros* 2008;**7**:320–328.
14. Gern JE. Rhinovirus and the initiation of asthma. *Curr Opin Allergy Clin Immunol* 2009;**9**:73–78.
15. Xepapadaki P, Papadopoulos NG. Viral infections and allergies. *Immunobiology* 2007;**212**:453–459.
16. Folkerts G, Busse WW, Nijkamp FP, Sorkness R, Gern JE. Virus-induced airway hyperresponsiveness and asthma. *Am J Respir Crit Care Med* 1998;**157**:1708–1720.
17. Sajjan U, Wang Q, Zhao Y, Gruenert DC, Hershenson MB. Rhinovirus disrupts the barrier function of polarized airway epithelial cells. *Am J Respir Crit Care Med* 2008;**178**:1271–1281.
18. Singh D, McCann KL, Imani F. MAPK and heat shock protein 27 activation are associated with respiratory syncytial virus induction of human bronchial epithelial monolayer disruption. *Am J Physiol Lung Cell Mol Physiol* 2007;**293**:L436–L445.
19. Kampf C, Relova AJ, Sandler S, Roomans GM. Effects of TNF- α , IFN- γ and IL- β on normal human bronchial epithelial cells. *Eur Respir J* 1999;**14**:84–91.
20. Aslam A, Lloyd-Lavery A, Warrell DA, Misbah S, Ogg GS. Common filaggrin null alleles are not associated with hymenoptera venom allergy in Europeans. *Int Arch Allergy Immunol* 2011;**154**:353–355.
21. Brown SJ, Asai Y, Cordell HJ, Campbell LE, Zhao Y, Liao H et al. Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. *J Allergy Clin Immunol* 2011;**127**:661–667.
22. Brown SJ, McLean WH. One remarkable molecule: filaggrin. *J Invest Dermatol* 2012;**132**:751–762.
23. Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I et al. Defective epithelial barrier function in asthma. *J Allergy Clin Immunol* 2011;**128**:549–556.
24. Saitoh T, Kusunoki T, Yao T, Kawano K, Kojima Y, Miyahara K et al. Relationship between epithelial damage or basement membrane thickness and eosinophilic infiltration in nasal polyps with chronic rhinosinusitis. *Rhinology* 2009;**47**:275–279.
25. Salim SY, Soderholm JD. Importance of disrupted intestinal barrier in inflammatory bowel diseases. *Inflamm Bowel Dis* 2011;**17**:362–381.
26. Zeissig S, Burgel N, Gunzel D, Richter J, Mankertz J, Wahnschaffe U et al. Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. *Gut* 2007;**56**:61–72.
27. Greiff L, Persson CG. On the physiology of airway absorption of allergens. *Allergy* 1999;**54** (Suppl 57):31–36.
28. Passalacqua G, Altrineti V, Mariani G, Falagiani P, Mistrello G, Brizzolaro R et al. Pharmacokinetics of radiolabelled Par j 1 administered intranasally to allergic and healthy subjects. *Clin Exp Allergy* 2005;**35**:880–883.
29. Reisinger J, Triendl A, Kuchler E, Bohle B, Krauth MT, Rauter I et al. IFN- γ -enhanced allergen penetration across respiratory epithelium augments allergic inflammation. *J Allergy Clin Immunol* 2005;**115**:973–981.
30. Salvaggio JE, Cavanaugh JJ, Lowell FC. A comparison of the immunologic responses of normal and atopic individuals to intranasally administered antigen. *J Allergy Clin Immunol* 1964;**35**:62–69.
31. Greiff L, Andersson M, Svensson J, Wollmer P, Lundin S, Persson CG. Absorption across the nasal airway mucosa in house dust mite perennial allergic rhinitis. *Clin Physiol Funct Imaging* 2002;**22**:55–57.
32. Hens G, Bobic S, Reekmans K, Ceuppens JL, Hellings PW. Rapid systemic uptake of allergens through the respiratory mucosa. *J Allergy Clin Immunol* 2007;**120**:472–474.
33. Kalsheker NA, Deam S, Chambers L, Sreedharan S, Brocklehurst K, Lomas DA. The house dust mite allergen Der p1 catalytically inactivates α 1-antitrypsin by specific reactive centre loop cleavage: a mechanism that promotes airway inflammation and asthma. *Biochem Biophys Res Commun* 1996;**221**:59–61.
34. Deb R, Shakib F, Reid K, Clark H. Major house dust mite allergens *Dermatophagoides pteronyssinus* 1 and *Dermatophagoides farinae* 1 degrade and inactivate lung surfactant proteins A and D. *J Biol Chem* 2007;**282**:36808–36819.
35. Brown A, Farmer K, MacDonald L, Kalsheker N, Pritchard D, Haslett C et al. House dust mite Der p1 downregulates defenses of the lung by inactivating elastase inhibitors. *Am J Respir Cell Mol Biol* 2003;**29**:381–389.
36. Hughes VS, Page K. German cockroach frass proteases cleave pro-matrix metalloproteinase-9. *Exp Lung Res* 2007;**33**:135–150.
37. Chavanas S, Bodemer C, Rochat A, Hamel-Tellac D, Ali M, Irvine AD et al. Mutations in SPINK5, encoding a serine protease inhibitor, cause Netherton syndrome. *Nat Genet* 2000;**25**:141–142.
38. Renkonen R, Renkonen J, Joenvaara S, Mattila P, Parviainen V, Toppila-Salmi S. Allergens are transported through the respiratory epithelium. *Expert Rev Clin Immunol* 2010;**6**:55–59.
39. Toppila-Salmi S, Renkonen J, Joenvaara S, Mattila P, Renkonen R. Allergen interactions with epithelium. *Curr Opin Allergy Clin Immunol* 2011;**11**:29–32.
40. Blume C, Foerster S, Gilles S, Becker WM, Ring J, Behrendt H et al. Human epithelial cells of the respiratory tract and the skin differentially internalize grass pollen allergens. *J Invest Dermatol* 2009;**129**:1935–1944.
41. Renkonen J, Mattila P, Lehti S, Makinen J, Sormunen R, Tervo T et al. Birch pollen allergen Bet v 1 binds to and is transported through conjunctival epithelium in allergic patients. *Allergy* 2009;**64**:868–875.
42. Joenvaara S, Mattila P, Renkonen J, Makitie A, Toppila-Salmi S, Lehtonen M et al. Caveolar transport through nasal epithelium of birch pollen allergen Bet v 1 in allergic patients. *J Allergy Clin Immunol* 2009;**124**:135–142.
43. Sewekow E, Bimczok D, Kahne T, Faber-Zuschratter H, Kessler LC, Seidel-Morgenstern A et al. The major soyabean allergen P34 resists proteolysis in vitro and is transported through intestinal epithelial cells by a caveolae-mediated mechanism. *Br J Nutr* 2012;**16**:1–9.
44. Reid DM, Gow NA, Brown GD. Pattern recognition: recent insights from Dectin-1. *Curr Opin Immunol* 2009;**21**:30–37.
45. de Vries HS, Plantinga TS, Van Krieken JH, Stienstra R, van Bodegraven AA, Festen EA et al. Genetic association analysis of the functional c.714T>G polymorphism and mucosal expression of dectin-1 in inflammatory bowel disease. *PLoS ONE* 2009;**4**:e7818.
46. Erpenbeck VJ, Malherbe DC, Sommer S, Schmiel A, Steinhilber W, Ghio AJ et al. Surfactant protein D increases phagocytosis and aggregation of pollen-allergen starch granules. *Am J Physiol Lung Cell Mol Physiol* 2005;**288**:L692–L698.
47. Petersen A, Becker WM, Moll H, Blumke M, Schlaak M. Studies on the carbohydrate moieties of the timothy grass pollen allergen Phl p 1. *Electrophoresis* 1995;**16**:869–875.
48. Schleh C, Erpenbeck VJ, Winkler C, Lauenstein HD, Nassimi M, Braun A et al. Allergen particle binding by human primary bronchial epithelial cells is modulated by surfactant protein D. *Respir Res* 2010;**11**:83.
49. Shreffler WG, Castro RR, Kucuk ZY, Charlop-Powers Z, Grishina G, Yoo S et al. The major glycoprotein allergen from *Arachis hypogaea*, Ara h 1, is a ligand of dendritic cell-specific ICAM-grabbing nonintegrin and acts as a Th2 adjuvant in vitro. *J Immunol* 2006;**177**:3677–3685.
50. Falcone FH, Knol EF, Gibbs BF. The role of basophils in the pathogenesis of allergic disease. *Clin Exp Allergy* 2011;**41**:939–947.
51. Jahnsen FL, Strickland DH, Thomas JA, Tobagus IT, Napoli S, Zosky GR et al. Accelerated antigen sampling and transport by airway mucosal dendritic cells following inhalation of

- a bacterial stimulus. *J Immunol* 2006;**177**:5861–5867.
52. Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R et al. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2001;**2**:361–367.
 53. Chieppa M, Rescigno M, Huang AY, Germain RN. Dynamic imaging of dendritic cell extension into the small bowel lumen in response to epithelial cell TLR engagement. *J Exp Med* 2006;**203**:2841–2852.
 54. Londei M, Lamb JR, Bottazzo GF, Feldmann M. Epithelial cells expressing aberrant MHC class II determinants can present antigen to cloned human T cells. *Nature* 1984;**312**:639–641.
 55. Vignola AM, Campbell AM, Chanez P, Bousquet J, Paul-Lacoste P, Michel FB et al. HLA-DR and ICAM-1 expression on bronchial epithelial cells in asthma and chronic bronchitis. *Am Rev Respir Dis* 1993;**148**:689–694.
 56. Oei E, Kalb T, Beuria P, Allez M, Nakazawa A, Azuma M et al. Accessory cell function of airway epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 2004;**287**:L318–L331.
 57. Fournier M, Lebargy F, Le Roy LF, Lenormand E, Pariente R. Intraepithelial T-lymphocyte subsets in the airways of normal subjects and of patients with chronic bronchitis. *Am Rev Respir Dis* 1989;**140**:737–742.
 58. Goto E, Kohrogi H, Hirata N, Tsumori K, Hirotsako S, Hamamoto J et al. Human bronchial intraepithelial T lymphocytes as a distinct T-cell subset: their long-term survival in SCID-Hu chimeras. *Am J Respir Cell Mol Biol* 2000;**22**:405–411.
 59. Kalb TH, Chuang MT, Marom Z, Mayer L. Evidence for accessory cell function by class II MHC antigen-expressing airway epithelial cells. *Am J Respir Cell Mol Biol* 1991;**4**:320–329.
 60. Salik E, Tyorkin M, Mohan S, George I, Becker K, Oei E et al. Antigen trafficking and accessory cell function in respiratory epithelial cells. *Am J Respir Cell Mol Biol* 1999;**21**:365–379.
 61. Westendorf AM, Bruder D, Hansen W, Buer J. Intestinal epithelial antigen induces CD4⁺ T cells with regulatory phenotype in a transgenic autoimmune mouse model. *Ann N Y Acad Sci* 2006;**1072**:401–406.
 62. Westendorf AM, Fleissner D, Groebe L, Jung S, Gruber AD, Hansen W et al. CD4⁺Foxp3⁺ regulatory T cell expansion induced by antigen-driven interaction with intestinal epithelial cells independent of local dendritic cells. *Gut* 2009;**58**:211–219.
 63. Veres TZ, Shevchenko M, Krasteva G, Spies E, Prenzler F, Rochlitz S et al. Dendritic cell-nerve clusters are sites of T cell proliferation in allergic airway inflammation. *Am J Pathol* 2009;**174**:808–817.
 64. Hunderfoan G, Zimmer KP, Strobel S, Gebert A, Ludwig D, Buning J. Luminal antigens access late endosomes of intestinal epithelial cells enriched in MHC I and MHC II molecules: in vivo study in Crohn's ileitis. *Am J Physiol Gastrointest Liver Physiol* 2007;**293**:G798–G808.
 65. Beisswenger C, Platz J, Seifart C, Vogelmeier C, Bals R. Exposure of differentiated airway epithelial cells to volatile smoke in vitro. *Respiration* 2004;**71**:402–409.
 66. Hastie AT, Everts KB, Cho SK, Zangrilli J, Shaver JR, Pollice MB et al. IL-1 beta release from cultured bronchial epithelial cells and bronchoalveolar lavage cells from allergic and normal humans following segmental challenge with ragweed. *Cytokine* 1996;**8**:730–738.
 67. King C, Brennan S, Thompson PJ, Stewart GA. Dust mite proteolytic allergens induce cytokine release from cultured airway epithelium. *J Immunol* 1998;**161**:3645–3651.
 68. Spurrell JC, Wiehler S, Zaheer RS, Sanders SP, Proud D. Human airway epithelial cells produce IP-10 (CXCL10) in vitro and in vivo upon rhinovirus infection. *Am J Physiol Lung Cell Mol Physiol* 2005;**289**:L85–L95.
 69. Taima K, Imaizumi T, Yamashita K, Ishikawa A, Fujita T, Yoshida H et al. Expression of IP-10/CXCL10 is upregulated by double-stranded RNA in BEAS-2B bronchial epithelial cells. *Respiration* 2006;**73**:360–364.
 70. Gonzalez-Rioja R, Asturias JA, Martinez A, Goni FM, Viguera AR. Par j 1 and Par j 2, the two major allergens in *Parietaria judaica*, bind preferentially to monoacylated negative lipids. *FEBS J* 2009;**276**:1762–1775.
 71. Mattila K, Renkonen R. Modelling of Bet v 1 binding to lipids. *Scand J Immunol* 2009;**70**:116–124.
 72. Meenan NA, Ball G, Bromek K, Uhrin D, Cooper A, Kennedy MW et al. Solution structure of a repeated unit of the ABA-1 nematode polyprotein allergen of *Ascaris* reveals a novel fold and two discrete lipid-binding sites. *PLoS Negl Trop Dis* 2011;**5**:e1040–e1044.
 73. Mueller GA, Edwards LL, Aloor JJ, Fessler MB, Glesner J, Pomes A et al. The structure of the dust mite allergen Der p 7 reveals similarities to innate immune proteins. *J Allergy Clin Immunol* 2010;**125**:909–917.
 74. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. *Nat Immunol* 2004;**5**:987–995.
 75. Trompette A, Divanovic S, Visintin A, Blanchard C, Hegde RS, Madan R et al. Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature* 2009;**457**:585–588.
 76. Thomas WR, Hales BJ, Smith WA. Structural biology of allergens. *Curr Allergy Asthma Rep* 2005;**5**:388–393.
 77. Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med* 2009;**15**:410–416.
 78. Huang HJ, Lin YL, Liu CF, Kao HF, Wang JY. Mite allergen decreases DC-SIGN expression and modulates human dendritic cell differentiation and function in allergic asthma. *Mucosal Immunol* 2011;**4**:519–527.
 79. Pawankar R. Epithelial cells as immunoregulators in allergic airway diseases. *Curr Opin Allergy Clin Immunol* 2002;**2**:1–5.
 80. Willart M, Hammad H. Lung dendritic cell-epithelial cell crosstalk in Th2 responses to allergens. *Curr Opin Immunol* 2011;**23**:772–777.
 81. Perros F, Lambrecht BN, Hammad H. TLR4 signalling in pulmonary stromal cells is critical for inflammation and immunity in the airways. *Respir Res* 2011;**12**:125.
 82. Vroeling AB, Jonker MJ, Luiten S, Breit TM, Fokkens WJ, van Drunen CM. Primary nasal epithelium exposed to house dust mite extract shows activated expression in allergic individuals. *Am J Respir Cell Mol Biol* 2008;**38**:293–299.
 83. de Gabory L, Bareille R, Daculsi R, Daculsi R, L Azou BJ, Flahaut E et al. Carbon nanotubes have a deleterious effect on the nose: the first in vitro data. *Rhinology* 2011;**49**:445–452.
 84. Hammad H, Lambrecht BN. Dendritic cells and airway epithelial cells at the interface between innate and adaptive immune responses. *Allergy* 2011;**66**:579–587.
 85. Huvenne W, Callebaut I, Reekmans K, Hens G, Bobic S, Jorissen M et al. *Staphylococcus aureus* enterotoxin B augments granulocyte migration and survival via airway epithelial cell activation. *Allergy* 2010;**65**:1013–1020.
 86. Sachse F, Becker K, von Eiff C, Metze D, Rudack C. *Staphylococcus aureus* invades the epithelium in nasal polyposis and induces IL-6 in nasal epithelial cells in vitro. *Allergy* 2010;**65**:1430–1437.
 87. Sachse F, Becker K, Basel TJ, Weiss D, Rudack C. IKK-2 inhibitor TPCA-1 represses nasal epithelial inflammation in vitro. *Rhinology* 2011;**49**:168–173.
 88. Semlali A, Jacques E, Rouabhi M, Milot J, Laviolette M, Chakir J. Regulation of epithelial cell proliferation by bronchial fibroblasts obtained from mild asthmatic subjects. *Allergy* 2010;**65**:1438–1445.
 89. Vinhas R, Cortes L, Cardoso I, Mendes VM, Manadas B, Todo-Bom A et al. Pollen proteases compromise the airway epithelial barrier through degradation of transmembrane adhesion proteins and lung bioactive peptides. *Allergy* 2011;**66**:1088–1098.
 90. Pichavant M, Charbonnier AS, Taront S, Brichet A, Wallaert B, Pestel J et al. Asthmatic bronchial epithelium activated by the proteolytic allergen Der p 1 increases selective dendritic cell recruitment. *J Allergy Clin Immunol* 2005;**115**:771–778.
 91. Roschmann KI, van Kuijen A, Luiten S, Jonker MJ, Breit TM, Fokkens WJ et al. Purified Timothy grass pollen major allergen Phl p 1 may contribute to the modulation of allergic responses through a pleiotropic induction of cytokines and chemokines from airway epithelial cells. *Clin Exp Immunol* 2012;**167**:413–421.
 92. Nathan AT, Peterson EA, Chakir J, Wills-Karp M. Innate immune responses of airway epithelium to house dust mite are mediated

- through beta-glucan-dependent pathways. *J Allergy Clin Immunol* 2009;**123**:612–618.
93. van Drunen CM, Reinartz S, Wigman J, Fokkens WJ. Inflammation in chronic rhinosinusitis and nasal polyposis. *Immunol Allergy Clin North Am* 2009;**29**:621–629.
 94. Mattoli S, Marini M, Fasoli A. Expression of the potent inflammatory cytokines, GM-CSF, IL6, and IL8, in bronchial epithelial cells of asthmatic patients. *Chest* 1992;**101**:27S–29S.
 95. Tomee JF, van Weissenbruch R, de Monchy JG, Kauffman HF. Interactions between inhaled allergen extracts and airway epithelial cells: effect on cytokine production and cell detachment. *J Allergy Clin Immunol* 1998;**102**:75–85.
 96. Ito T, Wang YH, Duramad O, Hori T, Delespesse GJ, Watanabe N et al. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med* 2005;**202**:1213–1223.
 97. Miyata M, Hatsushika K, Ando T, Shimokawa N, Ohnuma Y, Katoh R et al. Mast cell regulation of epithelial TSLP expression plays an important role in the development of allergic rhinitis. *Eur J Immunol* 2008;**38**:1487–1492.
 98. Zhou B, Comeau MR, De Smedt T, Liggett HD, Dahl ME, Lewis DB et al. Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol* 2005;**6**:1047–1053.
 99. Al-Shami A, Spolski R, Kelly J, Fry T, Schwartzberg PL, Pandey A et al. A role for thymic stromal lymphopoietin in CD4(+) T cell development. *J Exp Med* 2004;**200**:159–168.
 100. Angkasekwinai P, Park H, Wang YH, Wang YH, Chang SH, Corry DB et al. Interleukin 25 promotes the initiation of proallergic type 2 responses. *J Exp Med* 2007;**204**:1509–1517.
 101. Corrigan CJ, Wang W, Meng Q, Fang C, Eid G, Caballero MR et al. Allergen-induced expression of IL-25 and IL-25 receptor in atopic asthmatic airways and late-phase cutaneous responses. *J Allergy Clin Immunol* 2011;**128**:116–124.
 102. Takahashi K, Hirose K, Kawashima S, Niwa Y, Wakashin H, Iwata A et al. IL-22 attenuates IL-25 production by lung epithelial cells and inhibits antigen-induced eosinophilic airway inflammation. *J Allergy Clin Immunol* 2011;**128**:1067–1076.
 103. Rankin AL, Mumm JB, Murphy E, Turner S, Yu N, McClanahan TK et al. IL-33 induces IL-13-dependent cutaneous fibrosis. *J Immunol* 2010;**184**:1526–1535.
 104. Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L et al. IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. *Proc Natl Acad Sci U S A* 2007;**104**:282–287.
 105. Prefontaine D, Nadigel J, Chouiali F, Audusseau S, Semlali A, Chakir J et al. Increased IL-33 expression by epithelial cells in bronchial asthma. *J Allergy Clin Immunol* 2010;**125**:752–754.
 106. Louten J, Rankin AL, Li Y, Murphy EE, Beaumont M, Moon C et al. Endogenous IL-33 enhances Th2 cytokine production and T-cell responses during allergic airway inflammation. *Int Immunol* 2011;**23**:307–315.
 107. Wills-Karp M, Rani R, Dienger K, Lewkowich I, Fox JG, Perkins C et al. Trefoil factor 2 rapidly induces interleukin 33 to promote type 2 immunity during allergic asthma and hookworm infection. *J Exp Med* 2012;**209**:607–622.
 108. Nikolaidis NM, Wang TC, Hogan SP, Rothenberg ME. Allergen induced TFF2 is expressed by mucus-producing airway epithelial cells but is not a major regulator of inflammatory responses in the murine lung. *Exp Lung Res* 2006;**32**:483–497.
 109. Reinartz SM, van Ree R, Versteeg SA, Zuidmeer L, van Drunen CM, Fokkens WJ. Diminished response to grass pollen allergen challenge in subjects with concurrent house dust mite allergy. *Rhinology* 2009;**47**:192–198.
 110. Vroiling AB, Duinsbergen D, Fokkens WJ, van Drunen CM. Allergen induced gene expression of airway epithelial cells shows a possible role for TNF-alpha. *Allergy* 2007;**62**:1310–1319.
 111. Vroiling AB, Jonker MJ, Breit TM, Fokkens WJ, van Drunen CM. Comparison of expression profiles induced by dust mite in airway epithelia reveals a common pathway. *Allergy* 2008;**63**:461–467.
 112. Oldenbeuving NB, KleinJan A, Mulder PG, Lumley P, de Groot EJ, van Drunen CM et al. Evaluation of an intranasal house dust mite provocation model as a tool in clinical research. *Allergy* 2005;**60**:751–759.
 113. Mattila P, Renkonen J, Toppila-Salmi S, Parviainen V, Joenvaara S, Alff-Tuomala S et al. Time-series nasal epithelial transcriptomics during natural pollen exposure in healthy subjects and allergic patients. *Allergy* 2010;**65**:175–183.
 114. Van Rijt RL, Vos N, Willart M, Muskens F, Tak PP, van der Horst C et al. Persistent activation of dendritic cells after resolution of allergic airway inflammation breaks tolerance to inhaled allergens in mice. *Am J Respir Crit Care Med* 2011;**184**:303–311.
 115. Holgate ST, Holloway J, Wilson S, Bucchieri F, Puddicombe S, Davies DE. Epithelial-mesenchymal communication in the pathogenesis of chronic asthma. *Proc Am Thorac Soc* 2004;**1**:93–98.
 116. Rinia AB, Kostamo K, Ebbens FA, van Drunen CM, Fokkens WJ. Nasal polyposis: a cellular-based approach to answering questions. *Allergy* 2007;**62**:348–358.
 117. Spits H, Di Santo JP. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat Immunol* 2011;**12**:21–27.
 118. Allakhverdi Z, Delespesse G. Hematopoietic progenitor cells are innate Th2 cytokine-producing cells. *Allergy* 2012;**67**:4–9.
 119. Mjosberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B et al. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CCR2 and CD161. *Nat Immunol* 2011;**12**:1055–1062.