

Interaction of Environmental Allergens with Airway Epithelium As a Key Component of Asthma

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Epithelial cells in the airway wall actively interact with environmental antigens/allergens, both in healthy individuals and patients with asthma. In patients with (allergic) asthma, the epithelium is abnormal, showing damaged structures and continuous activation similar to a repair phenotype cell. Epithelial cells bind allergens by a diversity of innate receptors, similar and in part identical to the Toll-like receptor family, which can induce the release of cytokines, chemokines, and growth factors. Protease-containing extracts (house dust mite, fungi) may additionally cause damage of the epithelial cell layer, thereby enhancing the repair phenotype of epithelial cells in patients with asthma. These interactions may result in facilitation of transport of allergens and enhanced presentation to the immune system (Th2-type response). The inflammatory response induces a second phase of Th2-type cytokines and cytotoxic products that will enhance growth factor-mediated airway remodeling, as is found in asthma. An understanding of the largely unknown innate responses of epithelial cells with environmental antigens/allergens may open new treatment modalities for asthma and other airway diseases.

important for removal of the offending agents. From an evolutionary point of view, surface cells (*eg*, epithelial cells) were the first cells that could recognize the non-self environment. In more complex organisms, immune cells (*eg*, antigen presenting cells [dendritic cells representing the major cell type in mucosal surfaces]) also developed the capacity to interact with environmental antigens/allergens to activate the cognate immune system by activating naïve T cells into T helper cells. Mucosal epithelial cells also bear receptors for innate recognition that are actively involved in many defensive functions [1], as discussed later. Inhaled allergens may also contain harmful agents that can damage the epithelial structure. In healthy subjects, these harmful agents are effectively neutralized. However, in patients with asthma, the epithelial cells behave abnormally, showing structural abnormalities [5–8]. Loss of epithelial cells in patients with asthma is followed by an altered type of repair response characterized by enhanced production of cytokines and growth factors [9,10] and enhanced epidermal growth factor receptor (EGFR) and integrin expression [11–13]. The interaction of allergens with epithelial cells in airways of patients with asthma influences the production of chemokines and growth factors and changes the inflammatory response and remodeling of airway wall structures [14,15••]. In this review, the activation and damage of airway epithelial cells by environmental antigens/allergens and its significance in healthy individuals and for the allergenicity in patients with allergic airway diseases (asthma, rhinitis) is discussed.

Introduction

The airways represent the largest epithelial surface of the body that is exposed to many airborne particles, of both pathogenic and nonpathogenic origin. To protect the host against these challenges, an extensive defense system has been developed, ensuring elimination of pathogenic micro-organisms and active removal of particulates of biologic (pollen, fungi, animal dander) and nonbiologic origin (diesel exhaust and dust particles) [1–4]. It is increasingly recognized that epithelial cells can interact with environmental factors (antigens/allergens of biologic origin) through innate recognition systems, possibly

Interaction of Environmental Allergens and Its Effect on General Health Status in a Population

Studies of environmental exposure to air pollution have shown associations with death from cardiovascular disease, chronic obstructive pulmonary disease (COPD), and pneumonia [16]. Daily variations of pollen, as a form of biogenic pollution in The Netherlands, showed associations with daily cardiovascular death and respiratory-disease mortality [17]. Furthermore, it was found that markers such as peripheral eosinophilia, a low forced expiratory volume in 1 second, and positive skin tests

were related to mortality owing to cardiovascular diseases and COPD [18]. Although pollen-induced allergies are common, fatal reactions related to these diseases are extremely rare and do not explain the associations with death found in these studies. It is important to note that smaller-sized pollen particles (less than 10 μm) can be deposited in lower airways and may activate cells in the airway mucosa [19]. These cohort studies suggest that interactions of environmental allergens with the airway surface are common in the population, and may result in serious health effects in susceptible subjects.

Differences in Allergenicity

Epidemiologic studies have shown considerable differences between exposure to allergens and the capacity to sensitize atopic individuals. However, comparison of allergenicity of allergens by sensitization is complicated by age dependent differences in sensitization patterns. Study of sensitization to fungi are of interest because information on exposure to fungal spores have been studied extensively in the Netherlands, whereas corresponding information on sensitization by skin tests or specific IgE are also available. In our own studies, we found age-dependent sensitization to different fungi with maximum production of IgE at the age of 8 years followed by a strong decline at later ages [20,21]. It was also found that *Alternaria* showed sensitization similar to that of *Cladosporium*, whereas the numbers of spores of *Alternaria* are at least 100 times fewer than those of *Cladosporium*. These differences in allergenicity illustrate the fundamental question concerning the factors and mechanisms that determine the allergenicity of airborne biologic substances. Enzymatic activities have been proposed as factors that may facilitate sensitization to environmental allergens [22, 23]. Furthermore, nonbiologic components (eg, diesel exhaust particles [DEP]) [24–27] and other toxic volatiles of industrial origin, additionally may facilitate entrance of allergens into the airway lumen [28].

Factors in the Allergen

Source that Might Facilitate Allergenicity

Enzymatic activity present in the source of allergens has been proposed as a factor that can augment the permeability of the bronchial epithelium, by either direct damage and/or disruption of epithelial cell contacts, or can activate epithelial cells, resulting in the opening of junctional contacts between epithelial cells. Facilitation of allergen transport across the epithelium will enhance presentation of the allergens to the immunologic system with increased Th2-type responses and IgE antibody formation, in atopic patients. Furthermore, intrinsic activities may be present in allergens that can additionally shift the immunologic response into a Th2-type response (reviewed by Thompson [22]).

Damage to the epithelial cell surfaces were studied in vitro showing morphologic changes of cells in culture and production of cytokines, reflecting both loss of cellular contacts and activation of epithelial cells by a variety of surface receptors. Studies with fungal extracts showed both morphologic changes (eg, shrinking and/or shedding of epithelial cells) demonstrating damage to epithelial cells, which were dependent on the serine proteinase activity [29,30]. Furthermore, it was demonstrated that the production of cytokines was related to the activity and quantity of the proteinases present in the fungal extracts. Stronger cytokine production corresponded with the more sensitizing fungus *Alternaria* whereas *Cladosporium*, with a lower allergenicity, was less active [31].

House dust mite extracts and the different proteinases in these extracts have been studied with respect to release of pro-inflammatory cytokines, changes in permeability, and damaging capacities of epithelial cultures [22,32•]. Both serine proteinases (Der 3, Der p 6, Der p 9) and a cysteine proteinase (Der p 1) caused detachment and activation of epithelial cell cultures [22,33,34]. Der p 1 was shown to be most reactive in disrupting cellular contacts, by degradation of cellular adhesion molecules such as occludin, E-cadherin, and ZO-1, and augmenting mucosal permeability [35••,36,37]. Furthermore, Der p1 was also shown to influence the immunologic response in favor of a Th2-type response [22,38], which appears to be characteristic for this cysteine proteinase and has not been demonstrated for serine proteinases.

It may be proposed that facilitation of allergen transport over the epithelium by proteolytic activity will result in enhanced IgE antibody formation to both the proteinase molecules and the nonprotease components in the allergenic source. This facilitating mechanism may explain why nonenzymatic components, such as Der p 2, can also behave as major allergens in house dust mite extracts. Furthermore, the enzymatic activities may also facilitate IgE antibody responses to different allergens that are inhaled simultaneously [39•]. (A flow-diagram of these interactions are described for fungal allergens and house-dust mites in Fig. 3).

Nonproteolytic enzymatic components have been shown to be important allergens (eg, alcalase from *Bacillus subtilis* [subtilisin], carbohydrases, and nonhydrolytic enzymes including enzyme inhibitors). From the family of the carbohydrases, α -amylase of fungal origin was shown to be an important occupational allergen in the baking industry [40,41] that may induce both early and late reactions after inhalation in sensitized patients [42]. In vitro cultures of epithelial cells (A549) with a highly purified α -amylase shows the induction of interleukin (IL)-6 and IL-8 cytokines with maximum production at 25 $\mu\text{g}/\text{ml}$ and morphologic changes (shrinking and shedding) of epithelial cells at levels above 200 $\mu\text{g}/\text{ml}$, indicating loss of cellular contacts (Fig. 1). The observation that cytokine production starts at concentrations lower than morpho-

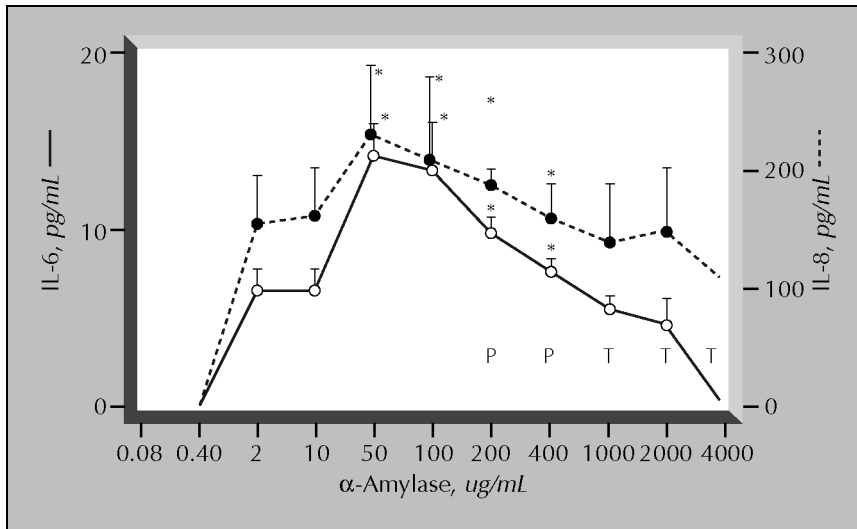


Figure 1. Morphologic changes and production of interleukin (IL)-6 and IL-8 by A549 cells in the presence of increasing concentrations of high-pressure liquid chromatography-purified α -amylase (Courtesy of Prof Dr G.S.P. Groot, DSM Gist-Brocades, Research and Development, Delft, The Netherlands). Cells were cultured until confluency. Culture conditions, morphologic observation, and cytokine quantification were performed as described by Kauffman *et al.* [31]. *—significantly enhanced production of cytokines; *hatched line*—production of IL-8; *p*—shrinking or partial detachment; *solid line*—production of IL-6; *T*—total/complete detachment of epithelial cells.

logic changes do suggests that cell surface receptors are involved that interact with the carbohydrase α -amylase in a similar manner as has been found for protease-activated receptors (PAR). It may be hypothesized that carbohydrase-activated receptors (CARs) are present on epithelial cells, with a mechanism similar to that described for PARs.

Nonenzymatic Interactions with Airway Epithelial Cells

Epithelial activation by fungal extracts using either heat-treatment or protease inhibitors showed that these interactions were dependent on the presence of serine proteinases [30,31]. With house dust mite extracts, using column chromatography separated fractions, cytokine production by the low-molecular weight fractions was (in contrast to the protease-containing fractions) heat- and proteinase-inhibitor resistant, suggesting innate recognition by epithelial cells [32•]. Additionally, we have shown that the major nonprotease allergen Der p 5 (recombinant Der p5, provided by M. Chapman), strongly induced cytokine production by epithelial cells [43]. Fractionated grass- and tree-pollen extract also strongly activated epithelial cells in a protease-independent way, whereas heating induced even stronger cytokine production [32]. Recently, it was demonstrated that activation of epithelial cells by pollen extracts is in part induced by a platelet-activating factor (PAF)-like substance found in pollen extracts [44–46]. In addition to nonenzymatic interactions, fresh pollen extracts also induced a protease-dependent damage to *in vitro* cultures of epithelial cells [47]. Preliminary results with potato extracts in our laboratory (not published) showed similar heat-resistant activation (production of IL-6 and IL-8), with epithelial cells (A549 cells). Together, these observations suggest that epithelial cells can interact with environmental components based both on enzymatic activities of the allergen and interaction with innate recognition receptor systems of unknown nature.

Innate Interactions of Epithelial Cells by Inhaled Allergens

Enzymatic interactions

Activation of epithelial cells may be dependent on enzymatic disruption of cellular contacts or recognition by innate receptor systems. As described earlier, proteases have been shown in various sources of allergens (*eg*, fungi, house dust mites, cockroaches). Der p1 was shown to be active in disrupting cellular contacts by destruction of cellular adhesion molecules such as occludin, E-cadherin, and ZO-1, and serine peptidases from house dust mites can degrade occludin [36,48]. Disruption of cellular contacts directly activates cellular signaling, owing to changes in the cellular cytoskeleton, and induce cytokine production [49]. Concentrations of proteases that disrupt cellular contacts may also induce cellular death by apoptosis [50] or direct cell death [31,50,51]. However, although most of the enzymes (proteases and carbohydrases) can detach epithelial cells from the culture surface, dose-response curves generally show cytokine production at concentrations before any morphologic changes can be observed [31,32•]. This is demonstrated for the cytokine production by increasing dosages of trypsin (a well-defined protease), showing IL-6 and IL-8 cytokine production at low concentrations (Fig. 2). Loss of cellular contacts, reflected by morphologic changes (shrinking and epithelial shedding), is found at higher dosages. In a similar manner, changes in epithelial paracellular conductance were shown with Der p 1 concentrations at low dosages that did not yet induce cell detachment, suggesting that changes in permeability may occur without physical damage of the epithelial mucosa [52]. The low-dose protease-induced activation of epithelial cells, as described earlier, suggests that interaction with PARs may be involved. PAR-1, PAR-2, and possibly PAR-4 have been shown on airway epithelial cells and were shown to activate epithelial cells in producing anti-inflammatory PGE₂ and pro-inflammatory cytokines [53–55]. Preliminary data with serine proteases of fungal origin also showed activation of PAR receptors [31].

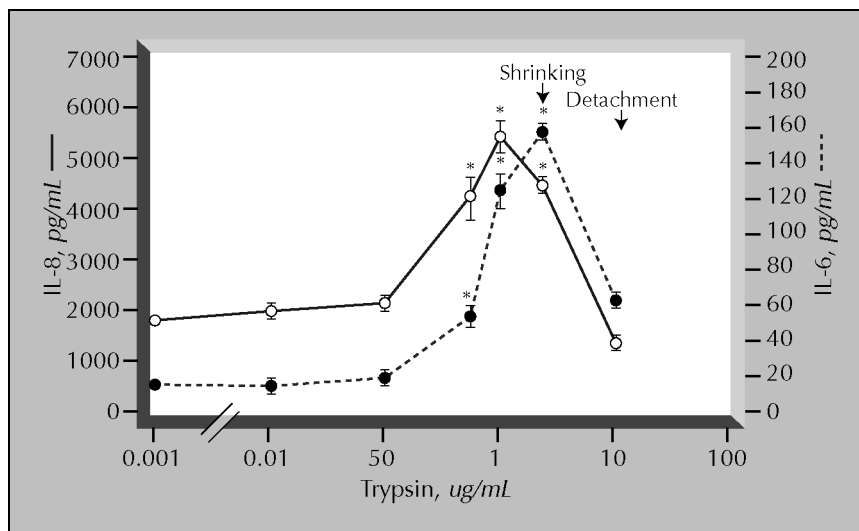


Figure 2. Production of interleukin (IL)-8 and IL-6 and corresponding changes in morphology of epithelial cells (A549) in the presence of increasing concentrations of trypsin. Culture conditions, morphologic observations, and cytokine quantification as described by Kauffman *et al.* [31]. Phases of shrinking and detachment are indicated. *—significantly enhanced production of cytokines; hatched line—production of IL-8; solid line—production of IL-6.

Generally, PAR receptors are exposed on the basolateral site of the epithelial cells, suggesting important interactions with tissue (cell)-derived enzymes such as mast cell tryptase (PAR-2) and thrombin (PAR-1) (Walls *et al.*, personal communication) and are more strongly expressed in patients with asthma [56]. Protease-induced cytokine production by epithelial cells was shown to be dependent on the activation of NF κ B and NF-AT [57,58]. With higher concentrations of proteases, cytokine production may continue (especially IL-8) during the phase of loss of cellular contacts (shrinking), whereas during the phase of detachment, cytokine production may even decrease below baseline levels [30,31].

Nonenzymatic recognition of allergens by airway cells

The activation of epithelial cells by low-molecular weight antigens of house dust mites and by grass, tree pollen, and potato extract indicate that epithelial cells can recognize many foreign substances by innate receptor systems that have been poorly described. Innate recognition of the respiratory epithelial cells has been described for substances derived from micro-organisms in their defensive role to prevent infection (recently reviewed [1,3,51]). It may be proposed that evolution has equipped airway epithelial cell receptors that can recognize substances of biologic, nonpathogenic origin (*eg*, grass and tree pollen, animal dander).

Innate recognition of antigens/allergens involves families of pattern-recognition receptors (PRRs), such as Toll-like receptors (TLRs), CD14, and scavenger receptors (SR), which can recognize different molecular structures present on bacterial and fungal surfaces, the so-called pathogen-associated molecular patterns (PAMPs) [1,3,59]. Most studies concerning this interaction have been performed with dendritic cells in relation to bacterial antigens such as lipopolysaccharide (LPS), lipoteichoic acid (LTA), and C-lectin carbohydrate receptors. Most of these agents were also shown to activate epithelial cells, indicating the presence of innate receptor sys-

tems on epithelial cells. Furthermore, the experimental results with grass and tree pollen and house dust mite extracts suggest that there are more innate receptor systems that can activate epithelial cells [32,43]. Recently, a new type of receptor was demonstrated for LTA from *Staphylococcus aureus* interacting with the platelet-activating factor receptor (PAFR) on epithelial cells. This interaction resulted in the activation of surface-bound matrix-metalloproteinases (a disintegrin and metalloproteinase), ADAM-10, followed by release of epithelial growth factor (EGF) and mucin production [60••]. It is important to mention that one of the new members of this ADAM family, ADAM-33, was recently shown to be associated with asthma and bronchial hyperresponsiveness, indicating the importance of these cell-surface-bound metalloproteinases in the development of asthma [61••]. The observation that pollen extracts also contain pro-inflammatory eicosanoid-like substances (*eg*, PAF, prostaglandin E₂ [PGE₂], and leukotrienes [44–46]) opens a new way for nonenzymatic interaction of epithelial cells with pollen extracts. The observation that protease-independent activation was found also in higher molecular weight fractions (compared with PAF-like substances) of pollen [32•] suggests that different types of interaction may be proposed. It can be expected that similar interaction mechanisms may be operative for other nonenzymatic substances in different allergenic sources. The general activation of epithelial cell lines and primary cells with these allergens suggests that this innate recognition is common in the population and may actively participate in the cleaning and removal of bio-organic substrates from the airways.

Innate Recognition, Enzyme-mediated Activation, and Damaging Agents

In healthy airways, the offending agents are effectively neutralized. In cases of damage and loss of epithelial cells by proteolytic enzymes, the repair mechanism rapidly restores the denuded areas [62], resulting in transient periods of

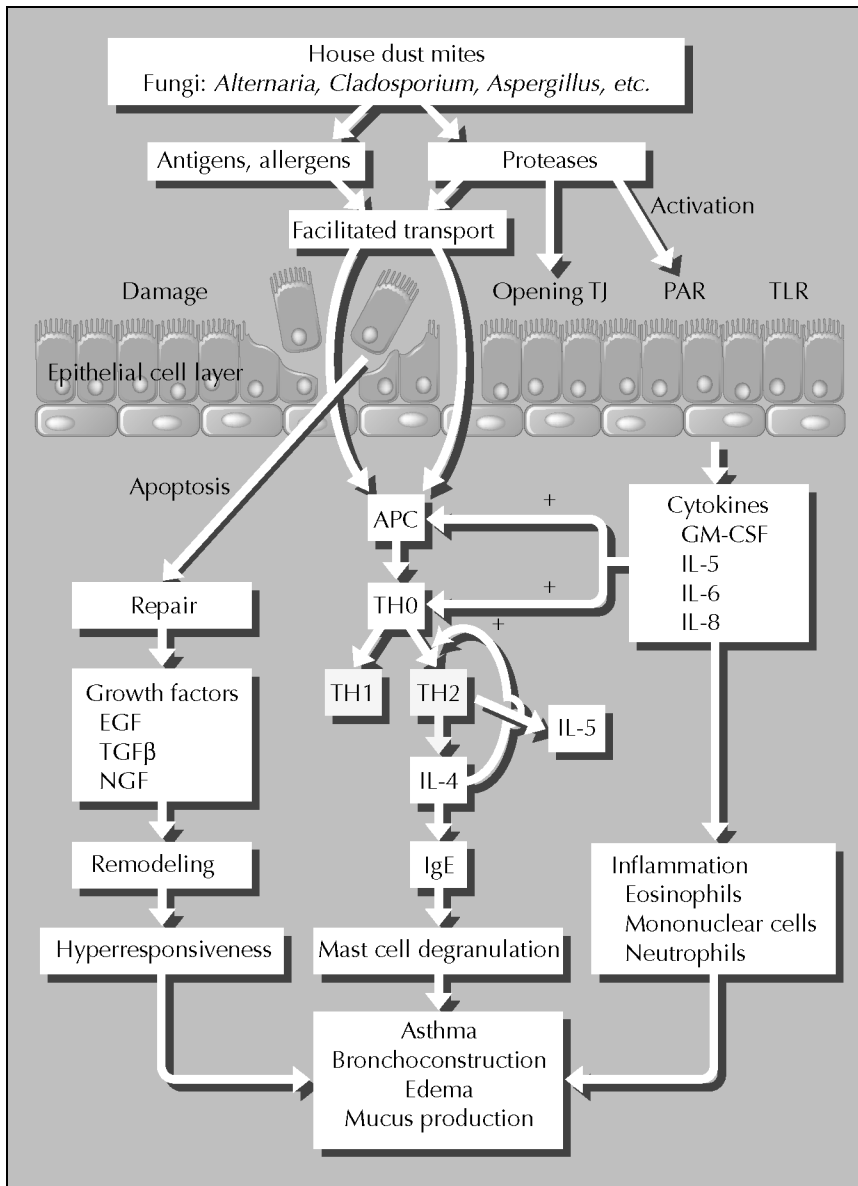


Figure 3. Proteases of fungal and mite origin are shown to interact with epithelial cells, either by activation of PAR receptors or damage of the epithelial surface by disruption of cellular contacts resulting in opening of the tight-junctions or detachment of epithelial cells from the airway surface (*top*). Both processes result in a facilitated transport of allergens and allergens over the epithelial surface, thereby increasing the immunologic recognition by antigen-presenting cells (APC) (eg, dendritic cells). Release of GM-CSF (specifically released by epithelial cells from asthmatic patients; see text) and IL-6 may promote the differentiation of immature dendritic cells and naïve lymphocytes to a Th2-type response, resulting in IgE-mediated mast cell activation (*center*). Damage of the epithelial surface and interaction with specific receptors (eg, the ADAM family members) initiates the release of growth factors as part of a repair response. Abnormal production of growth factors, owing to chronic repair state or altered reactivity of fibroblasts or smooth muscle cells, may result in remodeling of the airway structures (activation of the epithelial-mesenchymal trophic unit). Remodeling of airways has been linked to parameters of activation of asthma (eg, airway obstruction and hyperresponsiveness) (*left*). Pro-inflammatory cytokines and chemokines released by activated epithelial cells promote the attraction of Th2-type lymphocytes, monocytes, and eosinophils. Eosinophils may enhance the asthmatic state by releasing tumor growth factors (TGF- β), different cytokines (eg, GM-CSF, IL-4, IL-5), and release of cytotoxic products (eosinophil-derived neurotoxin, major-basic protein, eosinophil peroxidase, erythropoietin) that may cause additional damage to the epithelial surface, enhancing and promoting the repair stage conditions of the epithelial surface. EGF—epithelial growth factor; GM-CSF—granulocyte-macrophage colony-stimulating factor; IL—interleukin; NGF—nerve growth factor; PAR—protease-activated receptors; TJ—tight junction; TLR—Toll-like receptors.

cytokine and growth factor production. Furthermore, the simultaneous production of PGE₂ limits the inflammatory response to a nonclinical state. In patients with asthma, however, the epithelial cells behave abnormally, showing more extensive damage with shedding of columnar and basal epithelial cells. [5,7]. Damage of the airway surface is followed by a repair response, characterized by rapid flattening of neighboring cells, followed by proliferation, differentiation, and renewed polarization of the epithelial cells [14,62,63]. The higher susceptibility to environmental agents/allergens in asthma may be due to inherited deficiencies in the repair response following damage by environmental or inflammatory stress. Epithelial dysfunctioning during the repair process may be associated with delayed apoptosis or proliferation of epithelial cells [15••,64•]. Impaired regeneration alters the epithelial phenotype characterized by enhanced and continuous production of cytokines and growth factors and expression of

growth factor receptors [65,66]. The continuous release of growth factors (EGF, tumor growth factor- β , tumor necrosis factor- α) will stimulate the formation of myofibroblasts and the production of matrix proteins [15••,67], resulting in airway wall remodeling (Fig. 3). Chemokines released by epithelial cells of patients with asthma may induce a Th2-type inflammatory response, with infiltrations of Th2 lymphocytes, eosinophils, and neutrophils, in patients with atopic asthma. Although the release of GM-CSF by epithelial cells may facilitate the formation of a Th2-type response by influence of the maturation of dendritic cells [68], production of IL-6 can directly modulate the differentiation and survival of Th2 cells [69,70] (Fig. 3). This Th2-type inflammation causes a second phase of production of cytokines and deposition of cytotoxic proteins beneath the epithelial cell layer. Release of IL-4 and IL-13 will induce the release of growth factors by epithelial cells [71], thereby further enhancing the remodeling of the airway tissue [72].

Furthermore, toxic products from eosinophils and release of mast cell tryptase [73] may additionally cause damage and activation of epithelial cells. In this way, the Th2-type response may add in an autocrine way to the chronicity of asthma and corresponding airway remodeling. The contribution of innate recognition of allergens by functionally altered epithelial cells in asthma fits within the current discussion on the role of atopy in asthma and the necessity for other possible etiologic mechanisms [74••,75]. Further elucidation of the mechanisms of innate recognition and its place in the development of asthma will create new changes for therapeutic intervention.

Conclusions

Epithelial cells of the airways are activated by environmentally inhaled allergens of nonpathogenic origin. Activation of epithelial cells can be induced by enzymatic activities present in these allergens (proteases, carbohydrases). Certain, more aggressive enzymes (eg, Der p 1 and serine proteases of fungal origin) may damage the epithelial surface followed by repair reactions and intensifying remodeling of the airway tissue. Furthermore, allergens can activate epithelial cells by innate recognition systems, using a variety of innate receptors (eg, PAR, Toll-like receptors, CD14, PAFR), the role of which is largely unexplored for allergenic components. The epithelial cells in patients with asthma react to this damage and activation by production of pro-inflammatory cytokines that are directly responsible for the inflammatory response, attraction of Th2-type lymphocytes, and repair-induced release of growth factors that are responsible for airway remodeling. The allergen-epithelial cell-induced inflammatory response will induce a second phase of Th2-type response with release of Th2 cytokines (IL-4 and IL-13), whereas toxic products from eosinophils and neutrophils and mast cell products (tryptase) will additionally enhance the inflammatory and remodeling reactions.

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