The inhalation of antigens does not normally lead to allergic inflammation, but airway resident cells and their products may affect the outcome of antigen exposure. It is therefore important to elucidate how potential allergens interact with airway epithelial cells and other cells located within and below the epithelium. Some studies have indicated that certain antigens, particularly the major house dust mite antigen Der p1, penetrate the airway epithelium by intracellular transportation or paracellular passage, depending on their concentrations, time of exposure, and ability of the cells to inactivate them. If an antigen possesses proteolytic activity, such as Der p1, and it reaches high concentrations or the exposure is prolonged, the disruption of the tight junction can also favor the transepithelial passage of other antigens. In this way, the antigens can easily encounter the effector cells located between epithelial cells and below the basement membrane. The magnitude of this phenomenon may be more prominent in the airways of asthmatic patients, as their epithelium is more permeable to Der p1 than the epithelium of nonasthmatic patients and releases cytokines after exposure to very low concentrations of this antigen for brief periods. Epithelial cell activation may facilitate the development of allergic mucosal sensitization to Der p1 and contribute to the antigen-induced inflammatory response by affecting the migration and function of dendritic cells, mast cells, and eosinophils. Also, there might be a secondary release of interleukin-6 and endothelin-1, which can have a detrimental effect on the cardiovascular function. Key words: airway epithelium, airway inflammation, allergic inhalation, allergic asthma, cardiovascular injury, chemokines, cytokines. — Environ Health Perspect 109(suppl 4):553–557 (2001).

Allergen-Induced Generation of Mediators in the Mucosa
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The mechanisms by which potential allergens cross the airway epithelium can be explored in vitro by using cell lines (9,10) and monolayers of airway epithelial cells obtained during surgical (9,11) or endoscopic procedures (endobronchial brushing or biopsy) (12,13) from individuals who give informed consent.

The cells from living donors are purified to house dust mite allergens, is a major risk factor for the development of allergic airway diseases (1). Although there is an increased understanding of the mechanisms implicated in the development of allergic airway inflammation once sensitization has occurred, the mechanisms of interaction between antigens and airway resident cells and their role in the sensitization process are still unclear.

The bronchial epithelium is the first barrier encountered by inhaled antigens. Although bronchial epithelial cells express major histocompatibility complex class II molecules (2–4), they cannot process antigen effectively to function as complete accessory molecules (5). Although bronchial epithelial cells of patients with allergic asthma behave differently from normal epithelial cells when exposed to the antigen most often implicated in the pathogenesis of this disease and that this alteration may promote sensitization and the development of allergic responses. Finally, I discuss the mechanisms by which inhaled antigens may potentially affect the cardiovascular function once they have crossed the airway epithelium and caused the activation of airway resident cells.

Models for Studying Antigen Trafficking in Airway Epithelial Cells
The mechanisms by which potential allergens cross the airway epithelium can be explored in vitro by using cell lines (9,10) and monolayers of airway epithelial cells obtained during surgical (9,11) or endoscopic procedures (endobronchial brushing or biopsy) (12,13) from individuals who give informed consent.

The cells from living donors are purified to house dust mite antigen Der p1, penetrate the airway epithelium by intracellular transportation or paracellular passage, depending on their concentrations, time of exposure, and ability of the cells to inactivate them. If an antigen possesses proteolytic activity, such as Der p1, and it reaches high concentrations or the exposure is prolonged, the disruption of the tight junction can also favor the transepithelial passage of other antigens. In this way, the antigens can easily encounter the effector cells located between epithelial cells and below the basement membrane. The magnitude of this phenomenon may be more prominent in the airways of asthmatic patients, as their epithelium is more permeable to Der p1 than the epithelium of nonasthmatic patients and releases cytokines after exposure to very low concentrations of this antigen for brief periods. Epithelial cell activation may facilitate the development of allergic mucosal sensitization to Der p1 and contribute to the antigen-induced inflammatory response by affecting the migration and function of dendritic cells, mast cells, and eosinophils. Also, there might be a secondary release of interleukin-6 and endothelin-1, which can have a detrimental effect on the cardiovascular function. Key words: airway epithelium, airway inflammation, allergic inhalation, allergic asthma, cardiovascular injury, chemokines, cytokines. — Environ Health Perspect 109(suppl 4):553–557 (2001).

High or persistent exposure to potential allergens, particularly the house dust mite antigen Der p1, penetrate the airway epithelium by intracellular transportation or paracellular passage, depending on their concentrations, time of exposure, and ability of the cells to inactivate them. If an antigen possesses proteolytic activity, such as Der p1, and it reaches high concentrations or the exposure is prolonged, the disruption of the tight junction can also favor the transepithelial passage of other antigens. In this way, the antigens can easily encounter the effector cells located between epithelial cells and below the basement membrane. The magnitude of this phenomenon may be more prominent in the airways of asthmatic patients, as their epithelium is more permeable to Der p1 than the epithelium of nonasthmatic patients and releases cytokines after exposure to very low concentrations of this antigen for brief periods. Epithelial cell activation may facilitate the development of allergic mucosal sensitization to Der p1 and contribute to the antigen-induced inflammatory response by affecting the migration and function of dendritic cells, mast cells, and eosinophils. Also, there might be a secondary release of interleukin-6 and endothelin-1, which can have a detrimental effect on the cardiovascular function. Key words: airway epithelium, airway inflammation, allergic inhalation, allergic asthma, cardiovascular injury, chemokines, cytokines. — Environ Health Perspect 109(suppl 4):553–557 (2001).

Antigen Trafficking and Aberrant Permeability of Asthmatic Epithelium
Studies employing cultured bronchial epithelial cells or epithelial cell lines have indicated that certain antigens expressing proteolytic reaction (7,8). It is therefore important to clarify how inhaled antigens interact with airway epithelial cells and other resident cells and if the modalities of this interaction are abnormal in individuals with allergic diseases.

In this article, I discuss the potential mechanisms by which antigens may cross the epithelium and reach their targets in the bronchial mucosa in order to initiate and reiterate allergic responses. I review the results of studies indicating that bronchial epithelial cells of patients with allergic asthma behave differently from normal epithelial cells when exposed to the antigen most often implicated in the pathogenesis of this disease and that this alteration may promote sensitization and the development of allergic responses. Finally, I discuss the mechanisms by which inhaled antigens may potentially affect the cardiovascular function once they have crossed the airway epithelium and caused the activation of airway resident cells.

This article is based on a presentation at the Workshop on Inhaled Environmental/Occupational Irritants and Allergens: Mechanisms of Cardiovascular and Systemic Responses held 31 March to 2 April 2000 in Scottsdale, Arizona, USA.

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activity, such as the major house dust mite allergen Der p1 (14), can cross the epithelium by disruption of tight junctions and cell detachment (9–11).

An investigation conducted on bronchial epithelial cells from allergic individuals with and without asthma has shown that in asthmatic patients Der p1 can also cross the epithelium by intracellular migration (12). The intracellular localization of the antigen was observed within 30 min after the exposure to 1 µg/mL Der p1 in the bronchial epithelial cells of both asthmatic and nonasthmatic subjects. After further 30 min of incubation, Der p1 was still detectable in the internal vesicular bodies or membrane sheets of cells from asthmatics but disappeared in the cells of allergic subjects who did not have asthma. These results suggested that bronchial epithelial cells of asthmatic patients may be abnormally permeable to certain allergens and that this may be due to an innate or acquired inability to destroy or inactivate these molecules.

Further studies with epithelial cells from allergic and healthy donors have revealed that an intracellular transportation of Der p1 occurs also in epithelial cells from individuals without asthma if the concentration of the allergen is sufficiently high and the time of exposure sufficiently long to overcome the degradation or inactivation process.

In the experiments illustrated in Figure 1A, bronchial epithelial cells were exposed for 30 min to increasing concentrations of Der p1 in the apical compartment and then reincubated for 2 hr. In the epithelial cells from asthmatics, the migration of Der p1 through the epithelium was evident at concentrations as low as 1 µg/mL. With concentrations of Der p1 equal to or greater than 50 µg/mL, a transepithelial migration of the antigen was also detectable in the cell cultures from allergic nonasthmatic donors and from healthy controls. The electrical resistance of the monolayers progressively decreased after exposure to concentrations of Der p1 greater than 10 µg/mL (Figure 1B), indicating that disruption of the integrity of tight junctions by Der p1 could at least partly account for the movement of Der p1 from the apical to the basolateral compartment at those concentrations.

Bronchial epithelial cells from asthmatic patients showed intracellular localization of Der p1 at 30 and 60 min after exposure to concentrations of Der p1 as low as 1 µg/mL for 30 min. At these time points, the antigen could be respectively detected in the endosomal and lysosomal compartments (Figure 2). The same pattern of intracellular localization of the allergen was observed in epithelial cells of allergic individuals without asthma only after exposure to 100-fold higher concentrations of Der p1 (Figure 2). This indicated that an intracellular migration of the antigen could occur in nonasthmatic bronchial epithelial cells in addition to the paracellular migration due to tight junction disruption.

Another set of experiments suggested that exposure time was an additional important factor affecting the ability of Der p1 to penetrate the bronchial epithelium from different donors. When exposure to low concentrations of Der p1 (1 µg/mL) was prolonged and greater than 4 hr, a transepithelial movement of the antigen could be detected also in the monolayers from allergic nonasthmatic subjects and from healthy donors (Figure 3A). This was at least partly due to a disruption of the tight junctions by Der p1, as it was paralleled by a progressive reduction in the transepithelial resistance (Figure 3B).

The experiments reported above indicate that Der p1 can cross the airway epithelium...
via both transcellular and paracellular movements. Nonasthmatic epithelium can resist the intracellular passage of low concentrations of Der p1 that reach its surface for a short time. The mechanism of this resistance and the reason it is defective in asthmatic epithelium remain unknown, but asthmatic epithelial cells may have an intrinsic defect in intracellular antiproteases defenses as the intracellular transport of Der p1 is abolished by antiproteases (12,13).

In the same experiments, brief exposure to high concentrations of an antigen with proteolytic activity or prolonged exposure to low concentrations caused disruption of the tight junctions in bronchial epithelial cells from asthmatic patients and also in the cells from nonasthmatic individuals. If this event also occurs in vivo, it may favor penetration into the airway mucosa of other antigens devoid of proteolytic activity, thereby increasing the probability that they can encounter antigen-presenting cells and cause sensitization (Figure 4). Such a phenomenon would explain why sensitization to house dust mite antigens is frequently associated with sensitization to multiple antigens (15).

![Image](image_url)

Figure 3. (A) Time-dependent migration of immunoreactive Der p1 across the monolayers of cells from allergic subjects with asthma (AA), allergic subjects without asthma (A) and healthy individuals (H). Cells were cultured onto collagen-coated polycarbonate filter inserts placed into the wells of 24-well culture plates and exposed to 1 µg/mL Der p1. Data are means and standard errors from 6 experiments. *p < 0.05 versus A and H; **p < 0.05 versus AA and A; *** all p < 0.01 versus control monolayers exposed to Der p1 diluent.

![Image](image_url)

Figure 4. Schematic summary of how Der p1 and other antigens might cross the bronchial epithelium by the intracellular and paracellular pathways, reach target cells within and below the epithelium, and cause sensitization and allergic inflammation both directly and through the release of epithelial cell–derived mediators.

Antigen-Induced Epithelial Cell Activation and Mediator Release

Some studies have revealed that exposure of airway epithelial cells to Der p1 results in the release of proinflammatory cytokines and chemokines, particularly granulocyte–macrophage colony-stimulating factor (GM-CSF) (9,12,13), interleukin (IL)-6 (9), and the protein regulated-on-activation-normal-T cell-expressed-and-secreted (RANTES) (13). The release of these mediators from bronchial epithelial cells of asthmatic patients is associated with the intracellular transport of the allergen and occurs after brief exposure to low concentrations of Der p1 (12,13).

In the experiments illustrated in Figure 5A–B, we exposed bronchial epithelial cells from different donors to either increasing concentrations of Der p1 for 30 min or to a fixed concentration of Der p1 (1 µg/mL) for various periods of time and evaluated the release of immunoreactive eotaxin. The pattern of eotaxin immunoreactivity was consistent with the results of the experiments on the transepithelial migration of the antigen discussed above. Thus, exposure to low concentrations of Der p1 for brief periods induced an appreciable eotaxin release only in the cultures of epithelial cells from asthmatic patients. Higher concentrations of the antigen or a prolonged exposure was required to induce a similar effect in the cultures of cells from nonasthmatic subjects. Der p1 activity was partially inhibited by a cysteine protease inhibitor (CI) and by a serine protease inhibitor (SI) naturally produced by human lung (16,17). It was abolished by a combination of these protease inhibitors.

As previously described for GM-CSF and RANTES release (12,13), eotaxin release induced by Der p1 was due to increased transcription from the eotaxin gene (Figure 6).

The observation that Der p1 induces mediator release from airway epithelial cells in vitro is in keeping with the results of studies demonstrating the in vivo production of GM-CSF, RANTES, and eotaxin by the bronchial epithelium during natural or experimental exposure to house dust mite allergens and other allergens in asthmatics (18–21). These epithelial cell–derived mediators can promote sensitization and amplify ongoing allergic reactions. RANTES is a chemotactic factor for dendritic cells (22) and mast cells (23). GM-CSF enhances the ability of dendritic cells to differentiate and present antigens effectively and may contribute to direct the response to house dust mite antigens toward an immune reaction (3,5,8). Finally, RANTES, GM-CSF, and eotaxin all upregulate eosinophil chemotaxis or eosinophil activation and mediator release (21,24) (Figure 4).
To investigate the mechanisms by which Der p1 induces transcription from GM-CSF, RANTES, and eotaxin genes in epithelial cells, we reasoned that the promoter/enhancer regions of these genes all contain one or more binding sites for the nuclear factor (NF-κB) (18,21,25,26). We therefore tested the ability of Der p1 to promote NF-κB-induced gene transcription. We found that this antigen favors the nuclear translocation of NF-κB and its binding to DNA through degradation of the NF-κB cytoplasmic inhibitor IκBα and that Der p1-induced NF-κB DNA-binding activity is followed by gene transcription with a time-course suggesting a causal relationship (13).

However, the effect of Der p1 on cytokine release from airway epithelial cells is not specific, as an allergen-induced release of GM-CSF and IL-8 has been demonstrated with timothy grass pollen and birch pollen (9). Induction of cytokine release by these antigens was not associated with any proteolytic activity.

Potential Effect of Antigen Inhalation on Cardiovascular Function

Antigen inhalation can cause an anaphylactic reaction in sensitized individuals (27) if the dose of antigen inhaled is sufficiently high or if the level of sensitization is such that it elicits a generalized reaction upon exposure to relatively low amounts of the antigen.

However, inhaled allergens may have a detrimental effect on cardiovascular function by some subtler and less expected mechanisms. The antigen-induced release of IL-6 from airway epithelial cells (9) may represent one of these mechanisms. In fact, increased concentrations of circulating IL-6 as a result of acute and chronic airway inflammation are known to induce the release of acute-phase reactants from hepatocytes, and these in turn induce an increase in blood viscosity and promote thrombus formation (28,29).

The production of endothelin-1 from airway epithelial cells as a result of the antigen-induced release of GM-CSF (30) may also represent an important event affecting cardiovascular function. If this peptide is released in sufficient amounts from the airway epithelium to reach the circulation, it can cause vasoconstriction and ventricular arrhythmias (31,32).

Issues Requiring Further Investigation

Several aspects of antigen delivery and its effect on the cardiovascular function need further investigation:

- What are the mechanisms involved in the uptake of antigens by airway epithelial cells? Do antigens bind to a specific receptor located on the surface of airway epithelial cells?
- Why are bronchial epithelial cells from asthmatic individuals more permeable to certain antigens than normal epithelial cells? Does this depend on an innate or acquired defect of the anti-protease protection system? If so, do other pathologic conditions exist in which this defect can be demonstrated or is it specific to asthma? Because cigarette smoke reduces anti-protease defenses in the airways (16,17) and potentiates the Der p1-induced increase in permeability of bronchial epithelium (11), are smokers more susceptible to antigen sensitization than other persons? Would treatment with anti-proteases reduce the risk of antigen sensitization?
- What intracellular events lead to mediator release after exposure of the airway...
Allergen-induced airway mediators

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