Allergen-Induced Generation of Mediators in the Mucosa

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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, allergen inhalation, allergic asthma, cardiovascular injury, chemokines, cytokines. — *Environ Health Perspect* 109(suppl 4):553–557 (2001).
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High or persistent exposure to potential allergens, particularly the house dust mite antigens, 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 cells (*3*). Dendritic cells are considered the main antigen-presenting cells in the airways (*5*). For sensitization to occur it is necessary that potential allergens encounter the cells that form an intricate network within epithelial cells (*5,6*). However, the activation of dendritic cells by inhaled antigens normally leads to the induction of inhalation tolerance rather than to allergic inflammation (*5–7*). The mechanisms involved in the disruption of this tolerance in allergic individuals are poorly understood. One hypothesis is that antigen-induced activation of resident cells or other effector cells in the airways may affect the outcome of antigen exposure through the release of substances that provide antigen-presenting cells with specific instruction to direct the response toward an immune reaction (*7,8*). It is therefore important to clarify how inhaled antigens can 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.

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 homogeneity according to standard methods (*3,11–13*). When cultured at high density onto collagen-coated polycarbonate filter inserts, airway epithelial cells conserve their morphology and function and form well-differentiated tight junctions after 7–10 days of incubation (*12*). The inserts are placed into the wells of 24-well culture plates to allow independent access to the apical and to basolateral compartments of the cells and to permit monitoring the transepithelial electrical resistance (*11,12*). The latter is an index of tight junction integrity and can be measured by using a microvolt-ohm meter with a fixed pair of electrodes positioned in the apical and basolateral compartment of the cell monolayers (*11,12*).

In addition to allowing evaluation of antigen trafficking, the isolation of airway epithelial cells from their environment enable the observation of their behavior upon allergen exposure without the effects of interaction with other structural cells and inflammatory cells or their products. Although this represents a limitation for studies designed to investigate the relative importance of the mechanisms involved in airway allergic reactions, which are based on extensive and complex intercellular communications, the use of a pure population of airway epithelial cells is essential for the evaluation of potential innate defects. It is particularly important in the search for primary alterations of bronchial epithelium in allergic asthma that can explain sensitization and localization of the disease to the airways, e.g., abnormal allergen processing (*12,13*).

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

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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 movement (*12*). The intraepithelial 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 aberrantly 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 intraepithelial 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 re-incubated for 2 hr. In the epithelial cells from asthmatics, the migration of Der p1 through the epithelium was evident at concentration 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

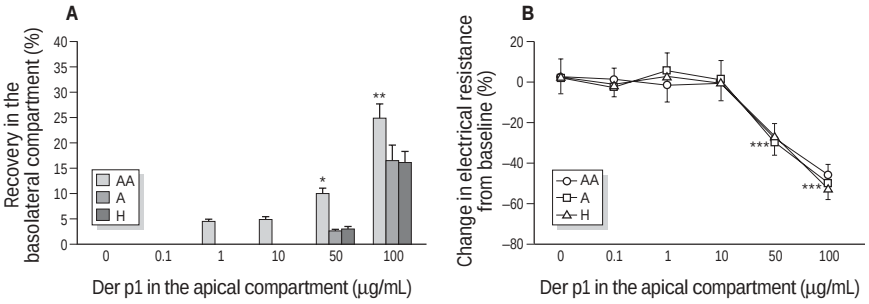

Figure 1. (*A*) Concentration-dependent migration of immunoreactive Der p1 across the monolayers of bronchial epithelial cells from allergic subjects with asthma (AA), allergic subjects without asthma (A) and healthy individuals (H). Cells were cultured onto collagen-coated polycarbonate filter inserts placed into the wells of 24-well culture plates, exposed to the indicated concentrations of Der p1 in the apical compartment for 30 min and reincubated for 2 hr. The amounts of immunoreactive Der p1 recovered in the basolateral compartment of the monolayers are expressed as percentage of the starting concentration in the apical compartment. Der p1 immunoreactivity was evaluated by enzyme-linked immunoabsorbent assay (*12*). (*B*) Change in electrical resistance from baseline under the same experimental conditions. Data are means and standard errors from 5 experiments. *$p < 0.01$ vs A and H; **$p < 0.05$ vs A and H; ***all $p < 0.01$ vs control monolayers exposed to Der p1 diluent.

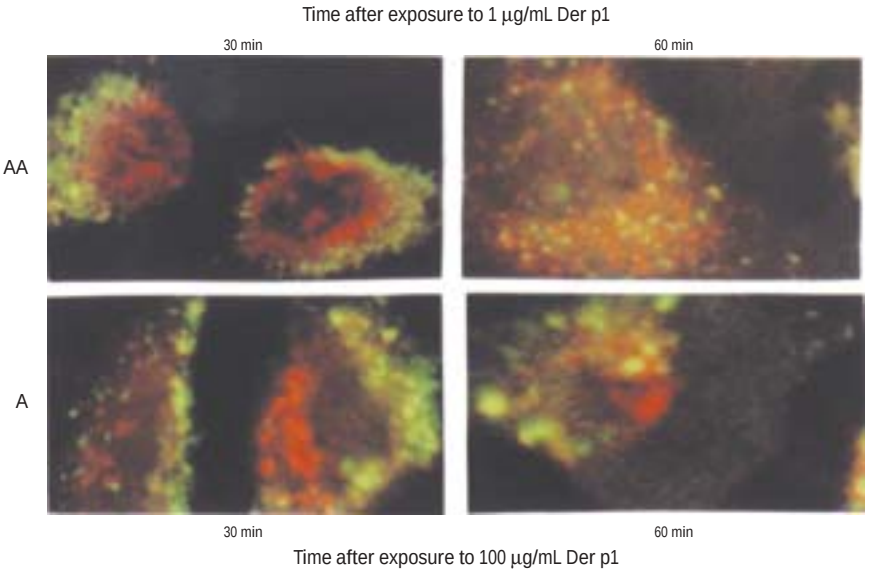

Figure 2. Localization of Der p1 in the endosomal and lysosomal compartments of bronchial epithelial cells from one allergic subject with asthma (AA) and one allergic subject without asthma (A) at the indicated time points following 30-min exposure to 1 and 100 µg/mL Der p1. For these experiments, cells were grown on glass coverslips and Der p1 was labeled with fluorescein isothiocyanate (FITC) before being added to the cells. After exposure, cells were rinsed in phosphate-buffered saline (PBS) and fixed in methanol/acetic acid. Fixed cells were immersed for 15 min in blocking solution containing 5% goat serum and 0.2% Triton-X in PBS, and then incubated for 1 hr with either a mouse anti-cathepsin D antibody (endosomal marker) or the mouse anti-CD63 antibody (lysosomal marker). The cells were rinsed repeatedly in PBS, incubated with Texas-red-labeled secondary antibodies, rinsed, and mounted before analysis by fluorescence microscopy. The yellow staining at 30 and 60 min indicates colocalization of Der p1 in the endosomal and lysosomal compartment, respectively (original magnification: ×600). The results are representative of six separate experiments.

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*).

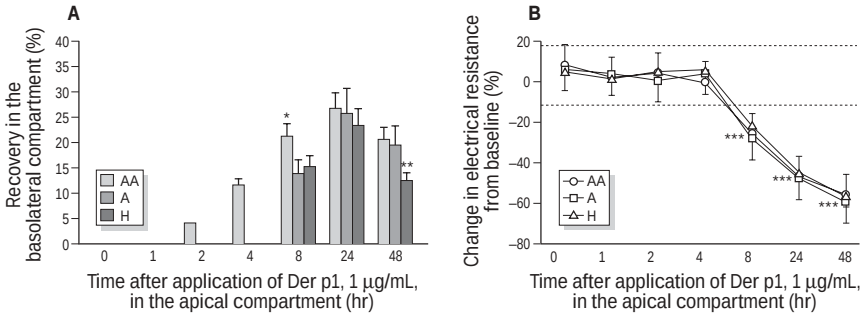

Figure 3. (*A*) Time-dependent migration of immunoreactive Der p1 across the monolayers of cells from allergic subjects with asthma (AA), allergic subjects without asthma (A) and healthy individuals (H). Cells were cultured onto collagen-coated polycarbonate filter inserts placed into the wells of 24-well culture plates and exposed to 1 µg/mL Der p1 in the apical compartment for the indicated times. The amounts of immunoreactive Der p1 recovered in the basolateral compartment of the monolayers are expressed as percentage of the starting concentration in the apical compartment. Der p1 immunoreactivity was evaluated by enzyme-linked immunosorbent assay (*12*). (*B*) Change in electrical resistance from baseline under the same experimental conditions. The dashed lines indicate the range of changes in electrical resistance detected in control monolayers exposed for the same periods of time to the diluent of 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.

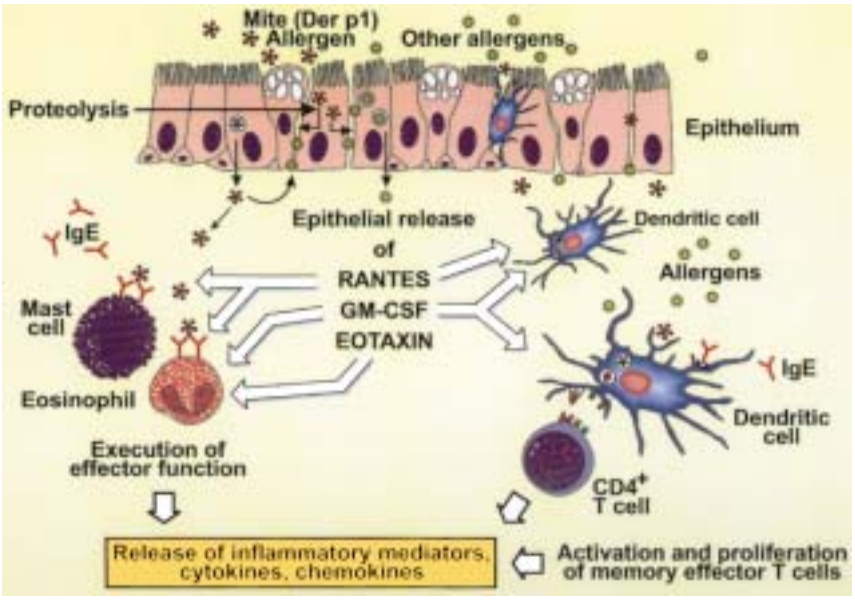

Figure 4. Schematic summary of how Der p1 and other antigens might cross the bronchial epithelium by the intracellular and paracellular pathways, reach target cells within and below the epithelium, and cause sensitization and allergic inflammation both directly and through the release of epithelial cell–derived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

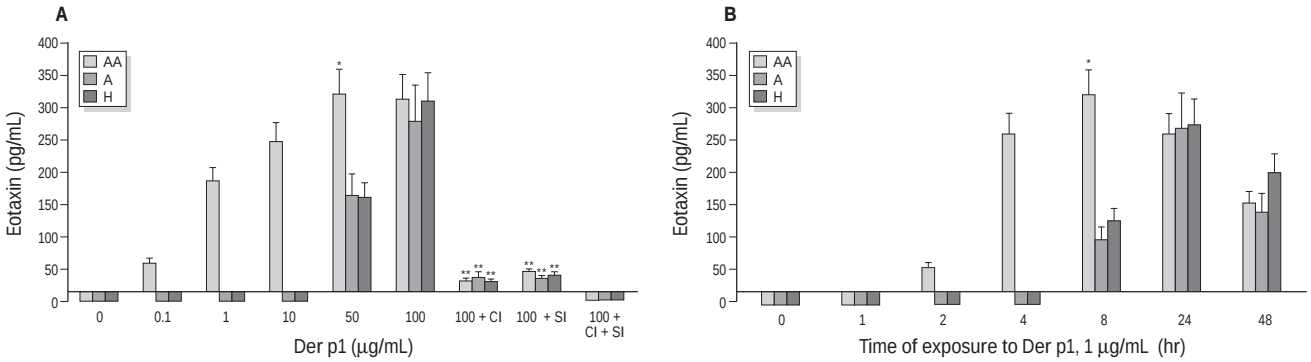

Figure 5. (*A*) Concentration-dependent release of immunoreactive eotaxin in monolayers of cells from allergic subjects with asthma (AA), allergic subjects without asthma (A), and healthy individuals (H) after exposure to the indicated concentrations of Der p1 for 30 min and reincubation for 4 hr in the presence or absence of the cysteine protease inhibitor (CI) E64, 10 μM, and the serine protease inhibitor (SI) α_1-antiprotease, 100 nM. (*B*) Time-dependent release of immunoreactive eotaxin in the same monolayers exposed for the indicated times to 1 μg/mL Der p1. Cells were cultured onto collagen-coated polycarbonate filter inserts placed into the wells of 24-well culture plates and Der p1 was applied to the apical compartment. Eotaxin immunoreactivity was evaluated in the culture medium by a specific radioimmunoassay (*20*). Der p1-induced immunoreactivity was obtained by subtraction of the immunoreactivity detected in control monolayers exposed to the diluent of Der p1. The horizontal line indicates the detection limit of the assay (14.5 pg/mL). Data are means and standard errors from 5 experiments. *$p < 0.01$ versus A and H; **$p < 0.001$ versus cells exposed to 100 μg/mL Der p1 alone.

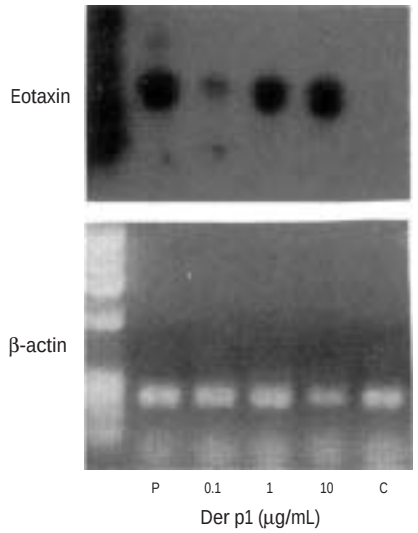

Figure 6. Demonstration of eotaxin messenger RNA (*19,20*) in cultured bronchial epithelial cell monolayers of one patient with allergic asthma exposed to the indicated concentrations of Der p1 or its diluent as negative control (C) for 30 min and reincubated for 2 hr. Positive controls (P) were cells incubated for 4 hr with tumor necrosis factor-α, 10 ng/mL. Total cellular RNA (1-μg aliquots) was extracted and reverse transcribed into complementary DNA. The complementary DNA was amplified by the polymerase chain reaction procedure using upstream and downstream primers complementary to positions 168-187 (GGGCCAGC-TTCTGTCCCAAC) and positions 369-392 (TTATGGCTTTGGAGTTGGAGATTT) of the 5′ and 3′ ends of the human eotaxin gene. Aliquots of 5 μL of each resulting polymerase chain reaction product were analyzed by Southern blot hybridization with a [^{32}P]UTP-labeled human eotaxin antisense riboprobe. The blot was exposed on X-Omat film for 12 hr at –70°C. The polymerase chain reaction products obtained after amplification of the complementary DNA with primers specific for the β-actin gene are reported to show comparable lane loading. The data presented here represent 3 separate experiments.

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 antiprotease 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 antiprotease defense 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 antiproteases reduce the risk of antigen sensitization?
- What intracellular events lead to mediator release after exposure of the airway

epithelium to antigens that do not express proteolytic activity?
- To what extent can the mediators released by airway epithelial cells as a result of antigen exposure affect cardiovascular function?
- Can antigens directly affect the cardiovascular function through their enzymatic activities once they have crossed the epithelium and reached the circulation?

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