

Letter to the Editor

Rapid systemic uptake of allergens through the respiratory mucosa

To the Editor:

Little is known about the fate of airborne allergens after deposition on the respiratory mucosa, and the scarce available data are often conflicting.¹ From the 1960s to the 1980s, the predominant idea was that an excessive mucosal permeability is characteristic of the airways of atopic individuals. In contrast, recent data showed a decreased absorption of allergens through the nasal mucosa of patients with allergic rhinitis.^{2,3} Nowadays, the increasing use of local routes for administering immunotherapy has renewed the interest in allergen pharmacokinetics.⁴ Moreover, allergen absorption might play a role in the pathogenesis of “global airway allergy.”⁵ In search of mechanisms of nasobronchial communication, attention is focused on the systemic presence of mediators, such as IL-5⁶ and eotaxin,⁷ which contribute to bone marrow eosinopoiesis and migration of eosinophils from the bone marrow to the sites of inflammation.⁷ Whether these systemic mediators are produced in the airways or rather reflect systemic activation of immune cells after allergen absorption remains unknown. We therefore studied the absorption of macromolecules after nasal and bronchial deposition in a mouse model and assessed the influence of allergic inflammation on the absorption rate.

In a first series of experiments, we applied ovalbumin (OVA; grade V, 1 mg/10 μ L of saline; Sigma, St Louis, Mo) in the nose or trachea of male BALB/c mice, 6 to 8 weeks of age (Harlan, Horst, the Netherlands; $n = 4$ per time point), and measured the serum OVA concentration at 0, 15, and 60 minutes (Ovalbumin ELISA kit; Autogen Bioclear, Wiltshire, United Kingdom). Before the OVA application, a tracheotomy was performed to separate the upper and lower airways, ensuring the selectivity of the deposition in one part of the airway. The esophagus was ligated to prevent gastrointestinal absorption of the protein after nasal application. General anesthesia was maintained during the entire experiment. As early as 15 minutes after nasal and bronchial deposition, OVA was detected in the serum, and the concentration was further increased at 60 minutes after deposition (Fig 1, A). Serum OVA concentrations were higher after bronchial application. Mean serum OVA levels at 1 hour after mucosal deposition were compared with mean serum levels 5 minutes after intravenous injection of the same amount of OVA (considered as the serum concentration after total absorption of the protein), showing that up to 14% of the allergen was absorbed through the respiratory mucosa (data not shown).

OVA was biotinylated (EZ-link Sulfo-NHS-Biotin kit; Pierce, Rockford, Ill) before application in the nose or trachea to demonstrate the migration of allergens from the airway lumen to the airway vessels. Ten minutes after the application, mice were killed, and nasal and lung tissue was fixed in 6% formaldehyde, paraffin embedded, and cut into 6- μ m sections. After deparaffinization and

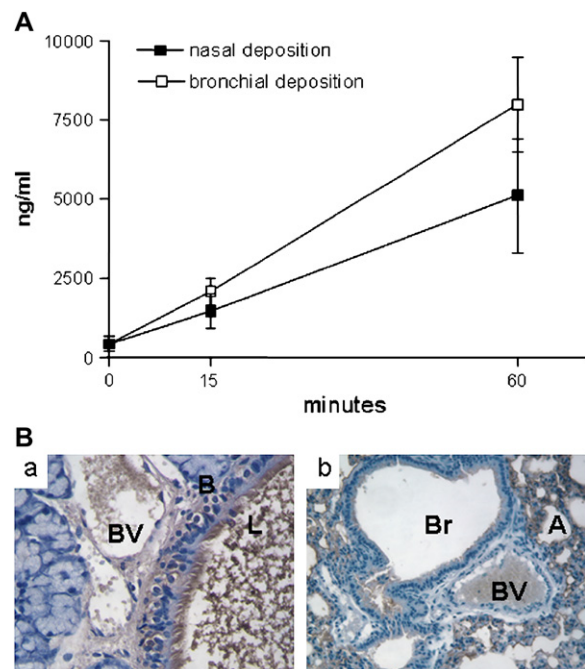


FIG 1. A, Serum OVA concentrations before and at 15 and 60 minutes after nasal or bronchial deposition of 1 mg of OVA to BALB/c mice ($n = 4$ per group). **B,** Histochemical detection of biotinylated OVA in nose (a) and lung (b) tissue of BALB/c mice killed 10 minutes after nasal or bronchial application. B, Basal side of the epithelial layer; L, luminal side of the epithelial layer; BV, blood vessel; A, alveolus; Br, bronchus.

quenching of the endogenous peroxidase, sections were incubated with streptavidin conjugated with peroxidase, followed by DAB substrate (Dako, Carpinteria, Calif). Unlabelled OVA was applied in control mice. We could demonstrate the presence of OVA not only at the luminal side of the nasal mucosa but also subepithelially and in the submucosal blood vessels (Fig 1, B). After endobronchial application, biotinylated OVA was present in the bronchi, in the alveoli, and in pulmonary blood vessels (Fig 1, B). Control sections were negative.

In a second series of experiments, we studied the effect of OVA-induced allergic airway inflammation on the systemic penetration of allergens. In OVA-sensitized mice OVA could not be used for the absorption study because of interference of OVA-specific antibodies of the allergic mice with the ELISA. Therefore horseradish peroxidase (HRP; Sigma), which has a molecular weight (40 kd) that is comparable with that of OVA (45 kd), was administered at the same dose as OVA in prior experiments. The absorption of HRP was compared between naive mice and mice with OVA-induced allergic airway inflammation, according to our previously described protocol.⁸ HRP (1 mg/10 μ L) was applied in the nasal cavity or bronchi, as described above, and detected in serum by means of ELISA. Again, lower airway absorption was higher than nasal absorption. No significant differences were found

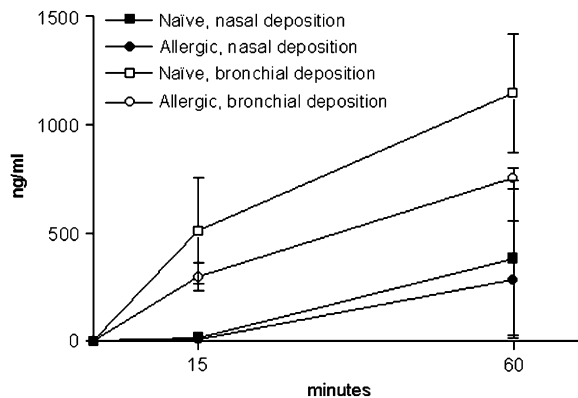


FIG 2. Serum HRP concentrations before and at 15 and 60 minutes after nasal or bronchial deposition in BALB/c mice with or without allergic airway inflammation (n = 4 per group).

in the magnitude of the absorption between allergic and naive mice, with a trend toward decreased absorption in the allergic mice (Fig 2).

The absorption of macromolecules shown in our experiments can probably be generalized for most human airborne allergens because they are generally smaller in size than OVA, ranging from 10 to 40 kD. Although the mechanism of allergen absorption remains to be studied, epithelial transcytosis is most likely, as previously described for HRP in studies with electron microscopy.⁹ Lower airway absorption is consistently higher than nasal absorption in our experiments, which is probably due to the high bronchoalveolar surface area and the short distance between alveolar lumina and pulmonary capillaries. Our observations on nasal allergen absorption are in line with recent data of Passalacqua et al⁴ showing absorption of intranasally administered radiolabeled Par j 1 with a plasma peak between 1 and 2 hours after administration. However, as mentioned by the authors, gastrointestinal absorption was not excluded in their study, and the majority of radioactivity in plasma was caused by free iodide and small degradation products. In our murine study gastrointestinal absorption was prevented by means of ligation of the esophagus, and a tracheotomy allowed us to study absorption after nasal or bronchial allergen application separately. Passalacqua et al⁴ showed a more rapid clearance of allergens from the nose to the upper pharynx in allergic subjects compared with that seen in control subjects, which also could contribute to the lower absorption rate that we observed in allergic mice.

In conclusion, we show remarkably rapid and high absorption of OVA through the respiratory epithelium in both the nose and lower airways. Through the systemic presence of allergens, an immunologic cascade can be initiated in the circulation, leading to the generation of inflammation both at the site of allergen deposition and at a distance.

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