

On the physiology of airway absorption of allergens

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Introduction

The human nasal mucosa is a main site for airway deposition of potentially noxious environmental substances. As might be expected, this mucosa is well equipped to protect both itself and the rest of the body from harmful influences of foreign material (1). Regulation of the permeability of the nasal and tracheobronchial mucosa is such that blood plasma may enter the airway lumen without making it any easier for foreign surface molecules to penetrate into the airway tissue (2–4). Thus, circulating humoral defence systems would be allowed to neutralize offending stimuli on the surface of a mucosa that maintains its function as a noncompromised absorption barrier (5). Mechanisms involved in the direction-selective paracellular flux of solutes across the intact airway mucosa (6, 7) are discussed elsewhere in this Supplement.

If the inhalational insult or disease process is severe enough for epithelial lining cells to be shed, even this may occur without a deleterious loss of barrier function. If basal cells remain, they may promptly flatten out and establish cell-to-cell contacts (8). If both columnar and basal cells are removed, a provisional plasma-derived gel may immediately cover the denuded basement membrane (9). Furthermore, beneath the plasma-gel, restitution of a new epithelium proceeds speedily. These novel aspects on barrier functions (10) are also discussed elsewhere in this Supplement.

The focus of the present overview is on absorption across the human airway mucosa *in vivo*. The interest in mucosal absorption abnormalities in airways disease appears to have started with Samuel Meltzers' description in 1910 of "asthma as a phenomenon of anaphylaxis", which was followed by studies on the fate of inhaled material. During 1910–30 many workers demonstrated, in animals and humans, that allergens are significantly absorbed across

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the airway mucosa. During the last several decades hypotheses suggesting that an abnormally increased absorption is pathogenic in airway disease have been disseminated widely. However, actual data in rhinitic and asthmatic airways may not entirely support this view. Rather, there are circumstances under which a decreased absorption of allergenic material may characterize the atopic airway mucosa (see below).

An evolving interest in airway barrier functions

The first specific airway absorption-studies involved experiments on the nasal airway avoiding any contribution of alveolar-pulmonary absorption (11). (The latter route is difficult to distinguish from tracheobronchial absorption in studies involving the lower airways.) Authors interested in allergic and occupational airway diseases have continued to study nasal absorption in human subjects.

Based on select human nasal and animal airway observations, during the 1960s and onwards a paradigm was developed stating that the airways of atopic individuals are characterized by "excessive mucosal permeability" allowing abnormal penetration of inhaled molecules (12–16). Despite its widely acknowledged attractiveness this notion may now need to be re-evaluated, and purportedly supporting observations reinterpreted. For example, the common presence of plasma proteins on airway mucosal surfaces in rhinitis and asthma has been thought, erroneously, (5), to be a sign of general mucosal hyperpermeability. Absorption data obtained under less well-controlled conditions (17) may also have been interpreted generously in favour of the hyperpermeability hypothesis or, when failing to support the accepted paradigms (18, 19), the data may not have received widespread attention. Although not confirmed quantitatively, histological pictures have been published showing all the features of the paradigm of hyperpermeability in human allergic airways, including paracellular epithelial "gaps" in the nasal mucosa (20, 21) and completely denuded basement membranes in asthmatic bronchi (22).

As a rule the reductive and molecular and cellular approaches in medical research are strictly dependent on established paradigms concerning the gross physiological functions in complex *in vivo* biosystems. Hence, it is not surprising that intriguing molecular mechanisms, explaining how the epithelial absorption barrier would become deranged in allergic airway disease, have appeared and

received support from absorption across cultured epithelial layers *in vitro* (23–25).

Perhaps knowledge would have developed differently had the early work by Cohen et al. (18) been recognized (see below).

Early observations suggesting reduced or unchanged absorption in allergy

In 1930 Cohen et al. (18) studied absorption of ragweed pollen insufflated into the noses of normal and allergic subjects, all of whom had had their arms passively sensitized with serum obtained from one patient allergic to ragweed. Their intriguing observations suggested the possibility that the more the mucosa react to an inflammatory/allergic stimulus the less of the allergenic material is absorbed. In each of their 50 normal subjects they saw a local skin reaction within 30 min of the nasal application of allergen. However, in atopic patients with rhinitis and asthma (none was sensitized to ragweed) only 4–7 of 12 showed positive skin reactions, and it occurred later than in normal subjects. Furthermore, when pollen was administered to two ragweed-sensitive patients, a strong local nasal reaction occurred, but there was no indication at all that the ragweed allergenic material had been absorbed in these patients. Cohen et al. (18) also stated: "In each instance when no absorption was demonstrated, ragweed extract was injected into the passively sensitised area at the end of the test period to assure us of the presence of antibodies in the area and of the patient's ability to react."

By contrast, during the 1960s, Leskowitz et al. advanced the notion that "excessive mucosal permeability exists in atopic individuals" (12, 13, 26). Thus it was thought that a defect barrier to inhaled antigen should constitute a difference between health and allergic disease. When testing this hypothesis more directly, Kontou-Karakitsos et al. (19) actually confirmed the finding by Cohen et al. (18) and showed that non-atopic individuals absorbed airway allergens more readily, or at least as readily, as those subjects who were atopic but not sensitive to the employed nasal test allergen.

Working with sensitized guinea-pigs, Richardson et al. (27) found that the inhaled antigen (horseradish peroxidase; HRP) had only a superficial distribution and did not penetrate the epithelial layer. The allergen (HRP) produced bronchoconstriction and it is likely that prompt plasma exudation also occurred in these animals. Again, one may hypothesize that mucosal responses occurring at the very

site of allergen impaction might have contributed to the lack of penetration. This explanation is supported by the fact that in later work HRP was shown consistently to penetrate the epithelium, but these studies were carried out in animals which had not been sensitized to this particular tracer protein.

Reports of increased airway absorption

During the 1970s and into the 1980s Boucher, Hogg, Pare, Ranga, and associates (15, 16, 28, 29) carried out a series of animal studies forwarding the hypothesis, previously championed by Salvaggio and Leskowitz (12), of an inflammatory stimulus-induced "hyperpermeability" of the airway mucosa. These workers studied the mucosal absorption permeability exclusively. However, they reasoned that the increase in mucosal permeability was probably bidirectional and, therefore, they considered the well documented presence of plasma proteins in asthmatic sputa (30, 31) as convincing support for their hypothesis (15, 16, 28, 29).

Using HRP as an inhaled tracer protein and various types of inflammatory and mediator provocations Boucher, Hogg, Pare et al. (15, 16, 28, 29) reported increased intraepithelial penetration of HRP and suggested that epithelial tight junctions had been disrupted. In 1984 the earlier, more qualitative, morphological work was extended with a significant quantitative electron microscopy study (32). However, that study could not confirm the previously proposed structural disturbance of epithelial junctional complexes in guinea-pig airways. The apparent inconsistencies may be illustrated by an excerpt from a discussion on the pathology of the airway epithelium in asthma by Hogg and Walker: "This change in permeability is maximal when the epithelium looks the least disrupted and returns to normal when the epithelium appears to be very disrupted" (33).

Reactive oxygen products, surfactant active agents, occupational and other toxic chemicals may cause acute epithelial damage and thereby increase the absorption of solutes across the airway mucosa (3, 25, 34, 35). Eosinophilic proteins (29) and certain viral infections (36) may also produce epithelial damage and thus acutely increase the inward perviousness of the mucosa. If airway absorption of inhaled material is increased this may cause significant adverse effects because subepithelial cells and different airway end-organs will be abnormally exposed to potentially harmful environmental agents. However, in rhinitis and asthma increased absorption may not be a major problem.

Recent data on normal human airway barrier functions

Studies employing small polar absorption tracers such as ^{99m}Tc -DTPA (mol wt 492 dalton) and ^{51}Cr -EDTA (mol wt 372 dalton), likely to be absorbed via paracellular epithelial pathways, suggest similar absorption properties of the nasal and bronchial mucosa (37). However, there may be important interindividual variability in absorption rate across the airway mucosa (38, 39). A variability has been demonstrated particularly with larger molecules such as polysucrose (mol wt 15 000 dalton) (39), which may be more similar to the size of allergens. Thus, normal human nasal airways may exhibit a 25-fold difference in polysucrose penetration (39). In these experiments tracer concentration, mucosal surface area of exposure and time of tracer exposure were well and accurately controlled. Hence, the healthy nasal mucosa may absorb allergen-sized molecules at highly varying rates. However, almost nothing is known about the impact of this normal variability on either the general defence capacity of the airway mucosa or the incidence of airway disease.

Airway mucosal absorptivity in disease

Nonspecific hyperresponsiveness in the human nose may be assessed as abnormally increased challenge-induced symptoms (sneezes, secretion, blockage and irritation) at challenge with methacholine and histamine (40). By employment of proper challenge stimuli we may obtain additional information as to the selective responsiveness of sensory nerves, the secretory apparatus, the microcirculation, etc. (41–43). An increased penetration of challenge agents might equally explain nonspecific and the latter specific end-organ types of airway hyperresponsiveness. However, it is doubtful whether increased penetration and absorption may apply in hyperresponsive airways.

It has been demonstrated recently that the common cold viruses may produce hyperresponsiveness and exudation of plasma, along with significant disease symptoms, without causing appreciable increases in the human nasal airway absorption-permeability. Corona virus-induced nasal infection is thus associated with increased plasma exudation responses to histamine (43). This disease characteristic probably reflects true changes in the responsive end-organ (microcirculation) since increased penetration of the challenge agent may not apply. Severe airway infections, caused by the human influenza virus, may be associated with

extensive airway epithelial damage and shedding (36) but the extent of increased mucosal absorptivity under these conditions remains little studied.

Using the controlled conditions that are offered by the “nasal pool-technique” (44), we have observed that the nasal absorption of a small hydrophilic tracer (^{51}Cr -EDTA, mol wt 372 dalton) is abnormally slow in subjects with allergic rhinitis (45). Thus, late into the Swedish birch pollen season, when eosinophilic, exudative inflammation would have been present for several weeks, the allergic airway mucosa exhibited an increased functional tightness. Furthermore, we have recently employed desmopressin (mol wt 1067 dalton) as nasal absorption tracer and demonstrated that patients with seasonal allergic rhinitis, developing a sustained symptomatic disease at natural allergen exposure, absorb this tracer to a lesser degree than outside the pollen season (46). Desmopressin may reflect how well peptides and small allergens are absorbed across the human nasal mucosa.

It may not be feasible to have perfectly controlled conditions for studies of absorption across a defined bronchial mucosal surface *in vivo* in man. Thus, neither the exposed mucosal surface area nor the surface concentrations of the absorption-tracers can be monitored in these studies. Ilowite et al. (47) have reported that asthmatic subjects absorb inhaled tracer molecules more readily than control subjects, but this result was evident only when the data had been corrected for variations in mucociliary clearance rates as determined on a separate day. However, in a more recent study Halpin et al. (48) made simultaneous corrections for mucociliary transport of the inhaled tracer and could demonstrate that the absorption permeability in asthma is reduced. Indeed, most studies on mucosal absorptivity in asthma demonstrate that this disease may have at least a normally tight airway lining, and that absorption rates of inhaled tracers do not correlate to hyperresponsiveness (49).

It appears that a new paradigm on airway tightness in allergic inflammation is warranted.

In vitro observations

As in other areas of medicobiological research, *in vitro* studies involving epithelial cell cultures completely dominate the current work on mucosal permeability. Beautiful regulatory mechanisms are being unravelled and multifacetedly documented in these experimental test systems. The caveat is that translation *in vivo* may not be possible. Thus,

the *in vitro* pharmacology of tight junction dissociation may for the most part be without appropriate support from critical *in vivo* data (50).

Similarly, there has been a longstanding explosion of *in vitro* studies dealing with epithelium-derived relaxing factors but all attempts at corroborating such an epithelial function *in vivo* have obviously failed (51).

The possibility that allergens with enzymatic activity might damage the epithelium and increase airway absorption has received considerable attention (52). Apparently supporting this notion, house dust mite allergens produce epithelial damage and increased mucosal perviousness *in vitro* through enzymatic activity, in a model where bovine bronchial epithelium was prepared and mounted in a two-chamber system, and the presence of house dust mite allergen increased flux of radio-iodine labelled albumin from the apical to the baso-lateral aspect (53, 54). However, in a recent study involving symptomatic patients with perennial house dust mite allergic rhinitis, the absorption capacity of the airway mucosa did not differ from that recorded in healthy subjects (55).

Several factors may explain why the findings differ, although epithelial derangement may well occur both *in vitro* and *in vivo*. Exclusive to the *in vivo* condition epithelial injury is associated with instantaneous and high-speed repair processes. Hence, epithelial restitution alone might prevent the development of a deranged absorption barrier in blood-perfused tissue (56). Perennial allergic rhinitis is also associated with microvascular–epithelial exudation that may neutralize the protease activity of house dust mite allergens already on the airway surface.

Effects of glucocorticosteroid treatment

The complex mucosal milieu of inflamed airways, where activities of cells may serve both proinflammatory and defence/repair promoting processes, makes it of importance to consider the balance of actions of pluripotent glucocorticosteroids under *in vivo* conditions in particular. In a guinea-pig model of epithelial damage-repair (57) highly efficient anti-inflammatory topical doses of budesonide had no effect on the high-speed restitution process. Furthermore, budesonide did not inhibit the associated plasma exudation or the recruitment of leucocytes to the repair area (57). In human nasal airways, glucocorticosteroid treatment may not prevent the plasma exudation response that is mounted in defence (58) as opposed to its anti-exudative potency in rhinitis and asthma (31, 59). Hence, it appears that

glucocorticosteroids may not interfere with fundamental mucosal tissue responses if these are serving a defence and repair process. This selectivity may also explain the beneficial clinical effect of glucocorticosteroids on epithelial shedding and tissue remodelling as an indirect effect caused by the inhibition of the preceding allergic inflammation. Indeed, it has been reported that the epithelial structure may improve by topical glucocorticosteroid treatment (60), but there are as yet no reports on functional correlates in terms of changes in airway absorption barrier functions that would suggest whether or not allergen absorption is affected by topical glucocorticosteroid treatment.

Conclusions

Offending stimuli, such as allergens, occupational agents, microorganisms, mechanical irritants, etc. landing on the

airway mucosa activate the mucosal defence systems, including mucosal exudation of plasma. The means by which exuded plasma is active in mucosal defence are physiological in the sense that the plasma exudate may operate on the surface of the epithelium without impairing its function as an absorption barrier. For example, the flow of plasma into the airway lumen may wash away unwanted material from interepithelial cell spaces; exuded albumin will bind unwanted solutes nonspecifically; exuded immunoglobulins will neutralize allergens. If epithelial damage is inflicted, *in vivo* restitution mechanisms may see to it that absorption barriers of the airway mucosa are well maintained. This *in vivo* function is supported by actual observations in disease. It is likely that defence mechanisms, exclusive to the airway mucosa *in vivo*, including plasma exudation and epithelial repair (as well as secretion) explain why the airway mucosa remains functionally tight in inflammatory diseases.

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