Airway permeability

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Introduction

The upper airway mucosa is a main site for deposition of potentially noxious environmental molecules. As one might expect this mucosa is also well equipped to protect both itself and the rest of the body from harmful influences of foreign material [1]. The regulation of the permeability of 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 absorption barrier uncompromised [5]. Mechanisms involved in the direction-selective paracellular flux of solutes across the intact airway mucosa [6,7] are the basis for the first part of this overview.

If the inhalational insult or disease process are 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 immediately covers the denuded basement membrane [9]. Furthermore, beneath the gel restitution of a new epithelium proceeds speedily [10]. These novel aspects on barrier restitution after shedding are the basis for the second part of the present overview.

Most of the data that will be discussed have been generated in the human nose and the guinea-pig trachea. The focus is primarily on in vivo observations. Explanatory or expanding ex vivo findings have thus been included when the functional in vivo aspects have been first assessed.

An evolving interest in airway barrier function

During the First World War Felix Marchand published his pioneering work on similarities of pathological features in nasal and tracheobronchial mucosa in asthma [11]. He focused on a role of eosinophils, mast cells, and epithelial cells in this disease. Marchand argued against the notion, previously forwarded by Fraenkel [12], that denudation characterizes asthmatic airways. He further regarded the epithelial lining cells not only as a passive barrier but as effector cells potentially driving inflammatory processes. There is now a resurgence of interest in versatile proinflammatory roles of the airway epithelium [13,14] including allergen presentation pathways and subsequent T cell mechanisms which may confer lifelong allergic reactivity [15]. These are important aspects on the airway ‘barrier cells’. However, they are outside the scope of the present discussion.

In the beginning of this century asthma and rhinitis were considered for the first time to be allergic diseases [16]. As a corollary there was then a rapidly growing interest in mucosal penetration of inhaled allergenic substances. It was soon realized that the normal airway-alveolar mucosa would absorb even large allergen molecules. The first specific airway absorption studies involved experiments in the nose [17] avoiding any contribution of alveolar-pulmonary absorption. (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, in the 1960s and onwards, the paradigm was developed that the airways of atopic individuals are characterized by ‘excessive mucosal permeability’ allowing abnormal penetration of inhaled molecules [18-22]. Despite its widely acknowledged attractiveness this notion may now need to be thoroughly re-evaluated. For example, several apparently supporting observations may require reinterpretation. The common presence of plasma proteins on airway mucosal surfaces in asthma and rhinitis has been thought, wrongly as we now see it [5], to be a clear sign of a general hyperpermeability state. Absorption data obtained under less well controlled conditions [23] have been generously interpreted in favour of the hyperpermeability hypothesis or, when failing to support...
the accepted paradigm [24,25] the data may not have received widespread attention. Although not quantitatively confirmed, histological pictures have been publicized showing all the features of the paradigm of hyperpermeability in human allergic airways, including paracellular epithelial ‘gaps’ in the nasal mucosa [26,27] and denuded basement membranes in asthmatic bronchi [28]. As a rule the reductive 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 in vitro cell and cell culture observations [29–31]. Perhaps the notions have developed differently had the early work by Cohen et al. [24] been remembered. Using the Prausnitz-Küstner reaction to assess nasal mucosal absorption of allergen Cohen et al. [24] noted that absorption was much faster and more efficient in healthy subjects than in rhinitis; the poorest absorption rates occurred in those patients who developed an allergic reaction to the topically applied allergen. Recent findings on airway absorptivity in allergic disease confirm and extend the data reported by Cohen et al. 65 years ago. It now seems possible that longstanding exudative, eosinophilic mucositis can be associated with an airway absorption barrier that is even tighter than in health [32].

The current work on the function of the airway absorption barrier is multifaceted. There is interest in the use of nasal application and oral inhalation for systemic absorption of drugs such as peptides, which cannot be ingested because they are destroyed by gastrointestinal juices. This area of research also includes the study of a variety of absorption enhancer molecules-formulations. Endogenous and environmental mechanisms potentially involved in development of frailty and detachment of airway epithelial cells also continue to be examined. The focus is on causative factors such as toxic proteins, oxidants, and proteases as well as physical hydrostatic and osmotic forces. Eosinophils and neutrophils are considered the most important epithelium-damaging effector cells. The vast interest in epithelial damage and shedding reflects the multipotential importance of the airway epithelium in health and in disease processes. However, we think it is equally important to focus the discussion on the epithelial repair processes that ought to be ongoing in asthma and rhinitis. Recent observations in guinea-pig trachea in situ have unravelled some intriguing aspects of the mucosal repair functions. Erjefält et al. [9,10] have thus demonstrated that a denuded airway basement membrane immediately receives a provisional cover that emanates from the microcirculation and that restitution of an epithelial lining may occur exceedingly fast beneath this cover. Epithelial disruption and shedding, even denudation, may, therefore, not be that detrimental to the airway barrier function as we have been inclined to believe.

**Acute effects of mediators and allergen**

Topical application of histamine-type mediators (histamine, leukotriene D4, bradykinin, platelet activating factor [PAF]) on the airway mucosa results in immediate extravasation and luminal entry of plasma. An identical acute response is produced by topical allergen in sensitized subjects or in animals [33]. The plasma that is moved into the airway lumen may not have been much sieved. Small and large proteins, including α2-macroglobulin, thus enter the lumen at concentration ratios approaching those existing in the circulation. However, the plasma exudate is distinct from circulating plasma by its rapidly increasing content of protein breakdown products. The plasma-derived peptides and oligo-proteins are not inert. Bradykinins, complement fragments, and fibrinolysis peptides belong to those relatively few plasma-derived molecules whose actions have been partly elucidated.

Plasma proteins may avidly bind interstitial molecules of the airway mucosa. Subepithelial cytokines and other cell-derived mediators may thus be picked up and transported to the airway surface by the plasma exudation process. This possibility has been termed 'lamina propria lavage' [34]. Luminal mediators may emenate directly from activated superficial cells in the airways or they may emenate from subepithelial sources and merely be moved to the surface by an induced plasma exudation response (for example by an allergen challenge procedure). Speculatively, at ongoing airway exudation processes the surface may accumulate and the tissue may be depleted of such cellular release products that bind to plasma proteins.

The luminal entry of bulk plasma is a graded response. This aspect has been examined particularly in the immediate phase (usually within 10–20 min after challenge). The stronger the topical stimulus the more plasma is exuded per unit time. The threshold dose levels of exudative agents that brings about only marginal increases in tissue plasma still produces clear increases in airway luminal plasma [33]. Hence, luminal entry is a rapid and efficient clearance route for extravasated tissue plasma. Lymphatic removal may only have a marginal role at least at exudation responses evoked by acute airway challenges [35]. This also means that the plasma indices of surface samples may accurately assess an
increased permeability response in subepithelial microvessels. This latter aspect is important because it makes it relatively easy to properly examine the microvascular responsiveness to different challenges. Also, the lack of luminal plasma proteins after challenge with neurogenic agents such as capsaicin and nicotine can now be taken as conclusive support for the notion that neurogenic inflammation (plasma extravasation) may not occur in human airways [33].

The human nasal mucosa lends itself to airway specific, well controlled challenge and lavage studies. To take advantage of these possibilities a nasal pool technique has been developed [36]. Using a compressible nasal pool devise it is possible to fill the entire ipsilateral nasal cavity with fluid and solutes that will not be removed by any ciliary activity. The pool technique exposes a large airway mucosal surface area to defined concentrations of agents and tracers. The technique also allows exposure of the same airway mucosal surface area at repeated provocations. After a selected mucosal exposure time the pool fluid, almost quantitatively, is recovered into the device. Thus the exposed mucosal surface is also gently lavaged by the nasal pool fluid providing the opportunity to sample mucosal indices selectively from the area of interest. This gentle lavage procedure can be carried out numerous times in sequence without causing undue changes in mucosal function. It has not been possible to attain similarly controlled experimental conditions in human trancheobronchial airways. However, it is possible that findings in the nose on mucosal functions may be applicable also to the lower airways [33].

Using the nasal pool device Greiff et al. [36] have demonstrated graded exudative effects of different mucosal surface concentrations of histamine. Between 20 μg/mL and 2000 μg/mL this amine produces fivefold to more than 100-fold increases in lavage fluid levels of plasma proteins (albumin to α2-macroglobulin). It appears that bulk plasma exude with all its large proteins and active products is transmitted to the mucosal surface without any appreciable disturbance of the epithelial lining. Several experiments involving human and animal airways have thus demonstrated that the acute plasma exudation response produced by histamine-type mediators or allergen is not associated with any change in the ability of the airway mucosa to absorb small and large hydrophilic solutes. The airway mucosa displays a remarkable asymmetry in that a dramatic increase in the 'outward permeability' occurs without any concomitant or subsequent change in the 'inward permeability' [2–5]. These observations have led to the identification of a role of plasma exudates in airway surface defence also when the mucosa is normally intact [5].

Mechanisms of plasma exudation across an intact epithelial lining

From a defence point of view it seemed logical that the epithelial lining should allow the plasma solutes to reach the surface without causing epithelial damage and without loss of the normal mucosal barrier functions. However, what properties of the epithelium and what external influence could produce such a result?

The cumulating in vivo observations indicate that actual secretion may not be involved in the luminal entry of plasma [37]. Although in vitro studies have suggested the albumin may be actively secreted by airway mucosal tissue, this possibility has not been born out in vivo. The in vitro secretion comprises only albumin. Other macromolecular tracers that move along with albumin in the exudation process are not secreted. The in vitro secretion mechanism and the in vivo exudation of plasma are also entirely differentiated by agents which stimulate one and not the other, and by agents that stimulate one and inhibit the other [37]. As a matter of fact, it never occurred to us that secretion could be involved because we had observed that bulk plasma comparable to the collective volume of the cytoplasm of the epithelial lining cells could enter the airway lumen in a few minutes. The only conceivable pathways for this transmission would be extensive paracellular routes. The pattern of response produced by different inducers and inhibitors suggested that the luminal entry of plasma was an obligatory consequence of extravasation. For example, no data suggested that the passage specifically across the epithelial linking was subjected to selective pharmacological regulation. From the in vivo findings it was thus deduced that the extravasated plasma itself somehow might create pathways for its own luminal entry [17]. Physiologists, who have examined water and solute flux across gallbladder and other epithelia [38–40], apparently have not paid attention to the possibility that macromolecular plasma exudates may move into the lumen of cavitary organs through simple and sensitive hydrostatic pressure regulated paracellular mechanisms. The focus has rather been (and still is, [29]) on the possibility that subepithelial hydrostatic pressure and oedema could be a cause of epithelial disruption and sloughing. We formulated a novel hypothesis that could be tested by examining the influence of small increases in subepithelial hydrostatic pressure on airway luminal entry of macromolecular tracers. The test was carried out in vitro employing as undisturbed airway tissue as possible. The guinea-pig trachea was isolated as an intact tube and kept in an organ bath by means that allowed separate regulation of the levels of serosal and mucosal bathing fluids, respectively. Using this model we followed
the movement of fluorescein isothiocyanate labelled dextran (mW 70 000) across the mucosa under conditions when different hydrostatic pressure gradients had been created across the airway mucosa. It was exciting to see the first data showing that a hydrostatic pressure increase of merely 5 cm H2O on the serosal side significantly increased the luminal entry of macromolecules [6]. This increase was induced promptly and was rapidly reversible when the pressure load was taken away. The whole process was well repeatable at 30 min intervals and no change was produced in the epithelial structure or in solute indices such as lactate dehydrogenase that would have been increased if the epithelium had been damaged. The basolateral sides of the cylindrical epithelium of the airway mucosa is particularly sensitive, since application of a pressure load of similar magnitude on the mucosal surface produced no change in the flux of macromolecules in either direction. Also, the function of the mucosa as an absorption barrier was unimpeded during and after the hydrostatic pressure-induced movement of macromolecules to the airway surface [7]. It was further demonstrated that several agents, that either induced or inhibited exudation in vivo, did not affect the hydrostatic pressure-induced luminal entry of macromolecules in the isolated tracheal tube preparation. All these observations [6,7] were in excellent agreement with the previously established characteristics of the airway plasma exudation process in vivo in both guinea-pigs and in human subjects. The hypothesis was advanced that extravasated bulk plasma moves non-injuriously into the airway lumen by slightly increasing the hydrostatic pressure load on the basolateral aspect of the epithelial lining cells.

More detailed histological examination of tissues from in vivo studies indicate that the epithelial passage of bulk plasma is across abundant paracellular routes. The pictorial evidence emerging from studies with tracers of high resolving power thus suggest that plasma macromolecules move between and all around each epithelial cell with no preferred routes observed [41]. This kind of passage means that the burden on each stretch of epithelial apical connections (1 cm² of the mucosal surface may have 40 m of interepithelial contact lines) will be exceedingly small even at pronounced plasma exudation responses. This again is well compatible with the previously established non-injurious nature of mucosal exudation of bulk plasma. The exudation data discussed above suggest a high degree of plasticity of the tight junctions at the apical pole of airway cylindrical epithelium. These junctions appear to have a valve-like function. They readily yield to small hydraulic pressures moving up between the cells and at completion of the mucosal exudation process the junctions apparently resume tightness in a fully reversible way.

Increased absorption across the airway mucosa

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 [3,42,44,45]. Proteases, eosinophilic proteins [30], and certain viral infections [43] may also produce epithelial damage and thus increase the inward perversiveness 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 environment agents.

An increase in airway permeability is actually desired when systemic drugs, such as insulin and other peptides, are applied on the airway mucosa for systemic treatment purposes. To this end absorption enhancers are used [44]. However, to be acceptable for repeated topical airway application, absorption enhancers should not cause longlasting epithelial changes nor should they cause other significant airway mucosal effects. An absorption enhancer mechanism should thus involve significantly increased airway absorption for a well defined period of time without inducing significant adverse mucosal reactions. Oxygen radicals as generated by H2O2, surfactant-active agents such as dioctylsodiumsulphosuccinate, water-absorbing particles such as starch beads, and sodium caprate increase absorption of hydrophilic molecules across airway mucosa [3,42,44,45]. All these agents also produce plasma exudation responses [3,42, unpublished observations]. Indeed, as demonstrated with a range of histamine-type mediators it appears much easier to increase exudation than absorption [5].

The intensity and duration of airways plasma exudation may quantitate such adverse mucosal processes which are of significant inflammatory nature [46]. Hence, we suggest that the plasma exudation response may reflect to what extent the absorption enhancement is achieved at the expense of other less desired airway actions. If absorption enhancers are not devoid of an exudative effect this latter action should be shortlasting or it may reflect a serious adverse effect of such pharmaceutical agents.

Airway mucosal absorptivity in disease

Non-specific hyperresponsiveness in the human nose may be assessed as abnormally increased challenge-induced symptoms (blockade, 'secretion', sneezes, and irritation-pain) at challenge with methacholine or histamine [47]. By employment of proper challenge stimuli we may get additional information as to the selective
responsiveness of sensory nerves, the secretory apparatus, the microcirculation, etc. [33,47]. An increased penetration of challenge agents might equally explain increased plasma exudation responses already in animals that have not been sensitized to this agent [48]. The acute exposure to TDI also involves structural epithelial changes but only a marginal increase in the absorption of luminal molecules. When the animals have become sensitized to TDI they respond with exudative eosinophilic inflammation to exceedingly low doses (<1 nL/animal) of this agent [49]. TDI-asthma-rhinitis is associated with plasma exudation and hyperresponsiveness [50] but whether this occupational disease is associated with any change in airway absorption now remains unknown.

It has recently been demonstrated that the common cold viruses may produce significant disease symptoms, hyperresponsiveness, and exudation of plasma macromolecules without causing appreciable increases in the human nasal airway absorption permeability. Coronavirus-induced nasal infection is thus associated with increased plasma exudation responses to histamine [51]. This disease characteristic probably reflects true changes in the responsive end-organ (microcirculation) since increased penetration of the challenge agents may not apply [51]. Severe airway infections caused by human influenza virus may be associated with extensive airway epithelial damage and shedding [43] but the extent of increased mucosal absorptivity under these conditions now remains little studied.

Using the controlled conditions that are offered by the nasal pool technique [36] Greiff et al. [32] have observed that the nasal absorption of a small hydrophilic tracer (CrEDTA) is abnormally slow in subjects with allergic rhinitis. 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. In a separate study similar findings have now been obtained concerning peptide absorption across the allergic nasal mucosa [52]. During the Swedish pollen season rhinitic individuals also develop a significantly increased responsiveness to histamine challenge expressed as abnormally increased plasma exudation effects [53]. Since absorption of histamine may be decreased in these patients the recorded hyperresponsiveness may be an underestimation of the change that had occurred in the airway microcirculation. It may not be feasible to have perfectly controlled conditions for studies of absorption across a defined bronchial mucosal surface in vivo in man. However, in a recent study Halpin et al. [54] made serious attempts to correct for mucociliary transport of the inhaled absorption tracer and could demonstrate that the absorption permeability in asthma may be reduced. It appears that a new paradigm on airway tightness in allergic inflammation is under development.

**Speedy restitution of epithelium after shedding in vivo**

During different time periods, separated by about a centennium, asthma has been characterized as a desquamative disease with denuded bronchial basement membranes. Denudation has even been considered a hallmark of asthma and many research groups have lately employed denuded airways in in vitro contractility experiments to somehow mimic the asthmatic condition. It seems clear that in chronic inflammatory airway disease epithelial damage and shedding are increased. However, this may not necessarily mean that the airways will exhibit denuded basement membranes. A key question that may not have received sufficient attention concerns the epithelial repair- or restitution-process that would be set in motion as soon as shedding occurs.

Erjefält et al. [8–10] recently have examined effects induced by and following from gentle epithelial cell removal in vivo in guinea-pig trachea. The employed in vitro model mimics epithelial shedding by not causing bleeding, or damage to the basement membrane. Two important findings are the promptness and the high speed by which epithelial restitution starts and proceeds, respectively. The immediate in vivo responses to denudation are several-fold. The microcirculation responds by exuding bulk plasma and, with little delay, large numbers of neutrophils are extravasated (no bleeding occurs). Thus, a plasma-derived fibrin-fibronectin gel rich in neutrophils soon covers the denuded basement membrane. This provisional cover is maintained and continuously supplied with plasma until a new tight epithelium has been established. The intact epithelial cells bordering the denuded area also respond immediately after loosing their neighbour cells. Secretory and ciliated cells (and probably also basal cells) dedifferentiate, flatten and migrate over the membrane. The migration rate is particularly fast during the first minutes after denudation. The speed of migration, most likely aided by in vivo-specific factors, is so high (~3 μm/min) that shedding, even of clusters of epithelial cells, would result in de-epithelialized basement membranes only for quite brief periods of time. Hence, epithelial shedding even to the extent of denudation of limited areas...
may occur in vivo with little consequence to the mucosal barrier functions. Defence and protection during the restitution process would be well catered for by the neutrophil-rich plasma-derived gel. This gel, with its content of plasma-derived migration-promoters such as fibronectin, fibrin and growth factors is obviously a suitable supramembranal milieu for high speed epithelial restitution. (In vitro studies dealing with epithelial repair demonstrate only relatively slow events.)

Complete denudation with loss of both columnar and basal cells may not be the most common kind of shedding. Columnar cells may rather more easily be shed [55,56] and thus leave a cobble-stone surface of basal cells behind. What happens to the basal cells when they loose their columnar neighbours? This question is currently being addressed by experimental approaches involving both animal and human airways [8]. It appears that the basal cells promptly undergo extensive flattening and that they establish extensive contact with each other. Airway basal cells may thus be well suited to keep up the barrier function at shedding of ciliated and secretory cells. The new flat epithelium that is established after shedding or denudation consists of cells that have a larger apical surface than normal columnar epithelium. Hence, a reduced length of paracellular stretches per unit mucosal area would be available for solute absorption. This structural change might explain the observations of reduced absorption in desquamative airway diseases. It is a separate matter that the limited sequelae to epithelial cell removal may also change our view on the role of epithelial shedding in respiratory defence.

Conclusion

The intact airway epithelium has tight cell to cell contacts at the apical pole of the cylindrical cells. However, the tight mucosa still absorbs small molecules through paracellular routes. Even proteins are absorbed but at slow rates. In exudative eosinophilic allergic disease the airway absorptivity may not be increased. It can even be decreased. This novel notion of mucosal tightness in inflammation is compatible with recent observations in two other areas of research: 'Mechanisms of mucosal exudation of plasma' and 'Mechanisms of restitution of epithelial lining cells after shedding'. When the intact airway mucosa is exposed to inflammatory agents including allergen, it responds with mucosal exudation of 'bulk' plasma. Aided by a self-sustained hydraulic pressure, the large plasma proteins enter the airway lumen without increasing the airway absorption ability. This paracellular valve-like mechanism of the normal columnar epithelium makes luminal entry of plasma a significant first line defence mechanism [5]. Sustained inflammatory processes cause epithelial damage, and epithelial shedding may be extensive in rhinitis and asthma. If only columnar cells are shed the remaining basal cells may promptly flatten out and strengthen the barrier. If denudation occurs the basement membrane is immediately covered by a plasma-derived gel. The gel also provides a special milieu in which bordering ciliated, secretory, and basal cells dedifferentiate, flatten and migrate speedily. A new epithelial lining is established in such a milieu and at such high speed that epithelial shedding should probably also be considered a well functioning airway defence process. The new findings on basal cell responses at columnar cell losses and on re-epithelialization in a plasma-derived gel in vivo after denudation may in part explain why increased absorption permeability has not been widely demonstrated in allergic and other inflammatory airway diseases. Perhaps the clues to explain increased absorption tightness in asthma and rhinitis can also be found among mucosal exudation and repair mechanisms as they evolve under proper in vivo conditions.

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