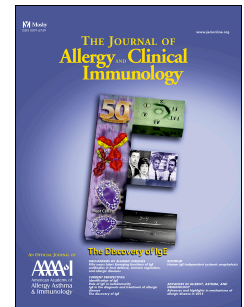


Accepted Manuscript

Airways exudation of plasma macromolecules: in innate defense, epithelial regeneration, and asthma

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PII: S0091-6749(18)31205-3

DOI: [10.1016/j.jaci.2018.07.037](https://doi.org/10.1016/j.jaci.2018.07.037)

Reference: YMAI 13584

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 22 February 2018

Revised Date: 30 June 2018

Accepted Date: 13 July 2018

Please cite this article as: Persson; C, Airways exudation of plasma macromolecules: in innate defense, epithelial regeneration, and asthma, *Journal of Allergy and Clinical Immunology* (2018), doi: 10.1016/j.jaci.2018.07.037.

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Invited review: accepted for publication in JACI, July 2018

Airways exudation of plasma macromolecules: in innate defense, epithelial regeneration,
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I have no conflict of interest.

Abstract.

This review discusses in vivo airway aspects of plasma exudation in relation to current views on epithelial permeability and epithelial regeneration in health and disease.

Microvascular-epithelial exudation of bulk plasma proteins characteristically occurs in asthma being especially pronounced in severe and exacerbating asthma. Healthy human and guinea-pig airways challenged by non-injurious histamine-leukotriene-type autacoids also respond by prompt mucosal exudation of non-sieved plasma macromolecules. Contrary to current beliefs, epithelial permeability in the opposite direction (=absorption of inhaled molecules) has not been increased in asthma and allergic rhinitis or in acutely exuding healthy airways. A slightly increased subepithelial hydrostatic pressure produces such unidirectional outward perviousness to macromolecules. Lack of increased absorption permeability in asthma may further be reconciled with occurrence of epithelial shedding leaving small patches of denuded basement membrane. Counteracting escalating barrier breaks, plasma exudation promptly covers the denuded patches. Here it creates, and sustains, a biologically active barrier involving a neutrophil-rich, fibrin-fibronectin net. Further, in the plasma-derived milieu all epithelial cell types bordering the denuded patch dedifferentiate and migrate from all sides to cover the denuded basement membrane. However, the speedy epithelial regeneration may come at a cost. Guinea-pig *in vivo*-studies demonstrate that patches of epithelial denudation-regeneration are exudation hot spots evoking asthma-like features including recruitment/activation of granulocytes, proliferation of fibrocyte/smooth muscle cells, and basement membrane thickening. In conclusion, non-sieved plasma macromolecules can operate on the intact airway mucosa as potent components of first line innate immunity responses. Exuded plasma also takes center stage in epithelial regeneration. When exaggerated, epithelial regeneration may contribute to inception and development of asthma.

Key words: plasma proteins, innate immunity, airway epithelium, airway microcirculation, epithelial permeability, epithelial regeneration, asthma pathogenesis.

Epithelial barrier dysfunction allowing exaggerated absorption of inhaled molecules is considered a major pathogenic feature of asthmatic and allergic airways. This paradigm is currently supported by observations on epithelial cells in culture¹. By contrast, careful non-invasive in vivo studies have demonstrated lack of increased bronchial permeability to inhaled molecules in individuals with asthma²⁻⁶. Similarly, nasal absorption permeability has not been increased in patients with seasonal or perennial rhinitis⁷⁻¹⁰. This vitro-vivo dichotomy ought to have some bearing on several areas of research. However, the iconoclastic in vivo data demonstrating maintained epithelial barrier function in patients with asthma or allergic rhinitis are not extensively noted in the current literature.

Known features of asthma may contribute to the belief that there must be increased tissue penetration of inhaled molecules in this disease. A prime example is increased shedding of epithelial cells. Clearly, an abnormal loss of the barrier cells in asthma^{11, 12} and rhinitis^{13, 14} would lead to increased penetration of molecules deposited on the airway surface? To make matters worse, molecular biology data produced in vitro are now interpreted to indicate impaired epithelial repair in asthma^{15, 16}. How then may we interpret the patient data on maintained barrier function? Is there a possibility that epithelial barrier restitution somehow is highly efficient in vivo in this desquamative disease? And if so, is exaggerated regeneration, rather than the reputed permeability hypothesis, a pathogenic epithelial aspect of asthma?

Most conspicuous with regard to epithelial permeability may be the observation of increased levels of plasma macromolecules on the airway surface. This feature is now widely considered to reflect a generally increased epithelial perviousness. The inference is that airway mucosal absorption permeability must also be increased in airways where plasma exudation occurs ^{1,17}. Based on evidence cumulating during a century or more, microvascular-epithelial exudation of plasma macromolecules is an established feature of asthma and allergic rhinitis ¹⁸⁻²⁰. However, understanding of epithelial transmission of plasma is limited and so is the potential role of exuded plasma in epithelial regeneration.

Discussing innate immune defense in obstructive lung disease, Curtis et al ²¹ maintain that plasma protein exudation reflects 'microscopic bleedings'. By contrast, several authors have mentioned in passing that plasma exudation can belong to the first line defense of the airway mucosa ^{22,23}. Is it then possible that the epithelial transmission of extravasated plasma is so swift that subepithelial edema does not develop?

Furthermore, has the airway epithelial lining cells such an asymmetry that it can let through bulk plasma macromolecules without allowing increased penetration of inhaled molecules? What mechanism then may allow increased plasma exudation in airways where permeability in the opposite direction is not increased?

This article discusses epithelial barrier functions in vivo with focus on opportunities for plasma macromolecules to be involved in first line defense of the intact airway mucosa, in epithelial regeneration, and in asthma in association with inflammation and exaggerated epithelial regeneration.

Plasma exudation contributing to first line defense of the intact airway mucosa.

Prompt, reversible, and reproducible plasma exudation.

Using challenges with non-injurious histamine-leukotriene-type autacoids, animal and human in vivo experiments have demonstrated how swift plasma exudation responses are evoked in tracheobronchial and nasal airways. Importantly, many autacoid-‘inflammatory’ stimuli increase permeability in airway subepithelial microvessels but are without this effect in the pulmonary circulation ²⁰. Within few minutes after exposure to histamine-type mediators (including leukotrienes, prostaglandins, PAF-acether, bradykinin), plasma protein indices are significantly increased in sampled airway surface liquids in guinea-pig and human airways ²⁴⁻²⁹. This response is dose-dependent, reversible and reproducible. In accord, the exudative response to histamine has been used to assess onset and duration of action of antihistamine drugs in human nasal airways ³⁰.

Non-sieved, non-injurious passage of plasma macromolecules from the sub-epithelial microcirculation to the epithelial surface.

The passage of plasma proteins from the subepithelial microcirculation, through mucosal tissue including basement membranes, and then paracellularly across the intact, pseudostratified epithelial lining cells (Figure 1) involves little sieving. Already an early demonstration of prompt challenge-induced microvascular-epithelial exudation in guinea-pig tracheobronchial airways suggested that plasma macromolecules appeared on the mucosal surface independent of their charge and size ²⁹. The indiscriminate microvascular-epithelial passage of macromolecules explains why the process is called ‘exudation’ rather than ‘transudation’, the latter name rather emphasizing fluid movement ³¹⁻³³. Thus, different-sized plasma proteins have appeared on the challenged but intact guinea-pig and human airway mucosal surface in nearly the same concentration ratios as occur in the circulating blood ^{24, 29, 34, 35}. Similar data have been

reported in asthma by Out et al ³⁶. Accordingly, a large plasma protein such as alpha2-macroglobulin has emerged a well-validated index of plasma exudation in nasal and bronchial airways. Alpha2-macroglobulin has now been determined as such in numerous human in vivo studies ^{34, 36-42}. Further reflecting the non-sieved nature of plasma exudation responses, a tangled and sticky macromolecule such as fibrinogen is also a used index of plasma exudation ^{35, 43}. Yet, the ultrastructure of the epithelial lining has not been affected by the transmission of bulk plasma ⁴⁴⁻⁴⁷.

Figure 1.

Plasma exudation without mucosal edema.

Studies in guinea-pigs and rats indicate that the extravasated plasma is promptly transmitted to the mucosal surface, between and all around epithelial cells ⁴⁶, in the challenged tracheo-bronchial area ^{24, 27, 45, 46, 48}. - Studies in mice are of limited value in this field since this species lacks a bronchial circulation ⁴⁹. - There is no known type of mucosal challenge that results in selective extravasation without the further transmission of plasma across the epithelial lining. Further, the plasma exudation response to autacoids has not been associated with an increase in lymphatic transport of plasma tracers ²⁷. Hence, in agreement with a lack of high permeability edema increased draining of proteins from the mucosal tissues has not occurred. The wet to dry weight ratio of the airway wall has not increased ²⁹, nor has the subepithelial tissue been appreciably expanded to indicate presence of actual edema ^{45, 50}. A morphometric study in allergic rats also demonstrated that the airway narrowing effect of allergen challenge reflected smooth muscle constriction without edema⁵¹. Prolonged stimulation of rat airways with allergens such as house dust mite has increased the number of

subepithelial microvessels, yet with little signs of mucosal edema ⁴⁹. Also in human asthma there is some increased vascularity (reviewed in ⁴⁹) but little evidence for bronchial mucosal edema. Even at sites where the bronchial lumen is filled with mucus and exudate, the adjacent subepithelial mucosal tissue has exhibited no signs of edema ⁵². The bronchoscopic in vivo view is known to give the impression of mucosal edema. However, it is unclear whether the appearance of a thickened mucosa represents edema or is a result of circular smooth muscle impacting on the mucosal tissue at different degrees of bronchoconstriction.

Clearly, plasma exudation involves some deposition of plasma macromolecules in mucosal tissues, most commonly recorded as distribution of Evan's blue dye bound to albumin ⁴⁸. Such observations importantly reflect the capacity of extravasated plasma proteins to contribute to an active mucosal tissue milieu (edema is surplus liquid in the tissue and, therefore, may not be equated with merely the occurrence of Evan's blue-colored tissue).

The basic nature of plasma exudation may be compared to mucus secretion.

The above observations indicate that the basic nature of the plasma exudation process is more physiological than pathophysiological. A non-injurious, reversible, and reproducible response of the airway mucosa, plasma exudation is in several respects comparable to the mucosal secretory response ⁵³. Yet, they are distinctly regulated. Admittedly, histamine-leukotriene-type mediators causing plasma exudation may also be active secretagogues⁵³ suggesting ample opportunities for concomitant occurrence of mucus and plasma proteins on the airway surface. However, the archetypal cholinergic agents causing mucosal secretion are without plasma exudation effects ^{28, 54, 55}.

Furthermore, the reputed neurogenic plasma exudation response, typically evoked by exposure to capsaicin, seems confined to rodents⁵⁶. In human airways capsaicin consistently induces coughing⁵⁷ but no plasma exudation response^{28, 41}. Hence, it is the non-neurogenic plasma exudation responses in rats and guinea-pigs that translate well to human.

Plasma exudation occurs without increased absorption of mucosal surface molecules.

Supporting the physiological aspect, the epithelial barrier against the environment has not been compromised by the exudation of non-sieved plasma. Both in animal and human airways, challenge with histamine-type mediators causes prompt plasma exudation without being associated with any increase in absorption of tracers applied on the mucosal surface^{24, 50, 58, 59}. This feature is important in several respects. One consequence is that the mere increase of plasma proteins on the airway epithelial surface cannot be interpreted as a sign of general perviousness of that epithelial lining. There is also little support for an old belief that plasma exudation may induce shedding when forcing itself into the lumen¹⁸. Hence, any inward permeability change has to be validated by specific data on absorption rates.

Also in the intestine, challenge-induced plasma exudation responses have been demonstrated to occur without being associated with increased absorption permeability^{60, 61 62}. It emerges that plasma exudation and increased penetration of mucosal surface solutes should be considered distinct features.

Both plasma and leukocytes may traverse the airway epithelial lining without compromising its integrity as barrier.

Notes of plasma and leukocyte extravasation as components of tissue responses date from the latter half of the 19-th century. Among central issues discussed already then, Julius Cohnheim (cited in ³²) emphasized that in mucosally lined hollow organs extravasated plasma and leukocytes had access to convenient elimination through epithelial transmission. However, in recent decades the role of trans-epithelial exit in elimination of mucosal leukocytes has largely been ignored. It has been severely eclipsed by focus on the biology of cell apoptosis.

Death of cells through apoptosis followed by engulfment has rapidly gained a hegemonic position as a mechanism responsible for elimination of cells. Apoptosis has been particularly hailed as a mechanism causing resolution of cell-driven airway inflammation. This largely in vitro-data-based view seems unfortunate especially concerning a major granulocyte in asthma, the eosinophil. With apologies to apoptosis proponents it needs underscoring that apoptotic eosinophils have not been demonstrated in human living blood-perfused airway tissues, not even at resolution phases ^{63, 64}. Also, the belief that successful treatment with anti-IL-5 biologicals reflects eosinophil apoptosis is not supported by compelling data^{65, 66}. – Yet, eosinophils do die in asthmatic mucosal tissues. However, their death in vivo is almost exclusively through primary cytolysis, which may cause rather than resolve inflammatory disease conditions ^{64, 67}.

Concomitant with the increasing focus on molecular mechanisms of apoptosis, clinical and experimental *in vivo* data have continued to be publicized indicating that elimination of these cells in asthma occurs through swift migration across the airway epithelium. Epithelial transmission has thus emerged a non-injurious cell exit pathway

at resolving mucosal tissue eosinophilia in asthma⁶³. Also other inflammatory cells appear to use this pathway at inflammation resolution⁶⁸.

The plasticity of mucosal epithelial cell junctions is obviously operating well both at paracellular migration of leukocytes and at plasma exudation. The ability of epithelial cells to transiently yield and still maintain tightness is involved in both. However, the modes of epithelial transmission differ and so do the modes of extravasation. Migration of leukocytes is driven by chemotactic signals separately regulating extravasation, tissue dwelling, and transepithelial passage⁶⁹⁻⁷¹. By contrast, the fate of extravasated plasma seems less to dwell in the tissue but to move promptly to the mucosal surface. It is also of note that the endothelial gaps through which plasma macromolecules escape the circulation are not commonly used by leukocytes^{72, 73}.

Specificity of plasma exudation as a first line innate immune response.

A kind of specificity lies in the strict location and the brief duration of the plasma exudation response. In human nasal as well as the entire human tracheobronchial airways there is a profuse subepithelial microcirculation carrying systemic blood²⁰. At any challenge site postcapillary venules of this circulation can react by prompt formation of high permeability endothelial gaps. This non-injurious response reflects an active process in endothelial cells that is under physiological and pharmacological control^{32, 74, 75}. The gaps may preferentially open in the venular wall facing the epithelium^{72, 76}. At a given challenge burden, the gaps will be open only for a limited time. For example, at a given concentration of histamine, the duration of plasma extravasation may not last more than about 10-20 minutes even in the continued presence of this type of agent^{32, 74}. It is only if the challenge burden increases that the

extravasation will be longer lasting. Challenge with allergen or PAF-acether may cause both an early and a late phase plasma extravasation response^{27, 48 77} Reflecting the time course of extravasation, the plasma proteins then emerge early and late on the mucosal surface at the site of challenge (Figure 1).

Despite its basic physiological nature, plasma exudation is a powerful innate immunity response. It also allows major combat against threatening noxious factors to take place on the tissue surface rather than inside the tissue where the risk of harmful results is so much greater. Plasma exudation contributes to a complex scene of biological activity in vivo. Its indiscriminate nature means that all the potent protein systems of circulating plasma will appear on the mucosal surface with ample opportunities for activation and actions. Whether there is selective activation of individual plasma protein systems depending on the type of exudative challenge agent/insult is not known.

Mediators of plasma exudation in healthy airways.

What factors are causally involved in plasma exudation responses in healthy airways? Since neurogenic plasma exudation may not occur in human airways, a major physiological mechanism has been ruled out. In airways challenged by simple 'irritants' that evoke neurogenic activity the responses may thus be confined to increased mucus secretion and increased blood flow. Plasma exudation steps up the airway response to inhalant insults.

There is a wide range of cell-derived mediators with a known capacity to produce plasma exudation responses in the airways. Histamine-type autacoids including leukotrienes, kinins, and PAF-acether produce prompt plasma exudation effects without

causing epithelial injury, leukotriene C4 and D4 being by far the most potent²⁹. Plasma exudation may depend on release of such mediators from tissue-dwelling mast cells, granulocytes and other cells variably occurring in the normal airway mucosa. Plasma exudation induced by microbial exposure may involve both neutrophil activation¹⁵ and kinins⁷⁸. Environmental challenges that evoke plasma exudation responses may involve epithelial factors operating in local recruitment and activation of inflammatory cells with capacity to release vascular permeability agents⁷⁹. Epithelial factors may also contribute negatively by tonically suppressing airways plasma exudation^{41,80}. It is not known if epithelial cells release such vasoactive mediators *in vivo* that directly cause plasma exudation as a first line airway defense response.

Antimicrobial defense at the surface of an intact airway mucosa.

Based on the present mechanism (Figure 1) it can be inferred that exuded plasma, with its content of immunoglobulins, complement factors, coagulation proteins, and more, may combat microbes deposited on the surface of uninjured mucosal linings. Such a versatile content of multiple profoundly active protein systems may be needed to counteract evasion strategies of pathogens^{81,82}. However, the possibility of a role of airways plasma exudation does not seem to be mentioned in the huge literature that has developed in the field of mucosal surface antimicrobial defense. Instead, the focus is on antimicrobial peptides (AMPs) (some 10 000 pubmed articles on AMPs have appeared in the last two decades). The biology of epithelial-derived AMPs has been explored in detail in cell cultures. Still, their actual role *in vivo* has not been fully assessed, in part because the AMPs are rapidly catabolized^{83,84}. It also seems unlikely that they would survive in a protease-active plasma exudate milieu. Hence, plasma exudation and AMPs may not act together but would be separate antimicrobial mechanisms operating on the

intact mucosal surface. This possibility is now speculative. Not enough may be known about in vivo efficacy of AMPs, and the antimicrobial efficacy of airways exudation of plasma has not yet been subject to any in depth investigation.

Plasma exudation is a first line defense mechanism. However, it also occurs as a sign of tissue engagement at established microbial infections. Mucosal exudation of plasma is thus a general inflammatory feature of virally infected human nasal and bronchial airways^{43, 85}. The observation of exaggerated sputum fibrinogen concentrations in asthmatics suffering from common cold compared to a control group with common cold⁴³ further agrees with the notion that viral induced exacerbations of this disease in part reflect a poor tolerance to viral infection⁸⁶. It has been suggested that both viral and bacterial pathogens increase microvascular permeability in part by activation of 'the contact system' causing the release of the vasoactive autacoid bradykinin⁸⁷. Mechanisms involving endothelial Toll-like receptors 2, 4, and 9 have also been reported to be involved in the increased vascular permeability caused by bacterial infection, but these mechanisms concern pathophysiology of sepsis with no known relevance to mucosal defense⁸⁸.

Innovative in vivo approaches seem required to explore the possibility that disease-inducing microbes deposited on the intact mucosal surface may evoke highly localized plasma exudation responses. A critical aspect concerns the early events prior to any established infection. Is there a window of opportunity for plasma exudation to assist in averting a threatening infection of airway mucosal tissues?

Mechanism of epithelial transmission of extravasated macromolecules.

Transmission of bulk plasma outward across the airway epithelial lining should reflect a mechanism that can accommodate a number of in vivo features (Table 1).

A hydrostatic pressure hypothesis and an approach to testing it.

Considering the aspects listed in Table 1, the idea arises that, somehow, the extravasated plasma itself brings about its own further passage across the epithelial lining. Is it possible that the liberated plasma could increase the local hydrostatic pressure to impact on the basolateral aspects of epithelial cells in such a manner so as to create paracellular pathways to the mucosal surface? Little was known about effects on airway transepithelial macromolecular flux in response to small, non-distorting increases in hydrostatic pressure.

To test the hypothesis of hydrostatic pressure-driven epithelial transmission of macromolecules, intact segments of guinea-pig trachea were dissected. The segments were mounted in organ baths and attached at both ends to liquid-filled tubing using extreme care to avoid edge injuries. By this set-up serosal and mucosal hydrostatic pressures could be regulated separately and effect on epithelial transmission of macromolecular tracers in either direction recorded^{89, 90}. The guinea-pig trachea was considered suited for these experiments because of similarities with human airways including a pseudostratified epithelium and a profuse sub-epithelial microcirculation.

The guinea-pig trachea had also exhibited similar responses to human airways with regard to plasma exudation evoked by allergen and non-neural mediator challenges⁹¹. Additionally, the tracheal cartilage should be an asset since it would counteract confounding distortions of the tissue when the pressure is raised.

In vitro support for the hydrostatic pressure hypothesis.

Raising serosal pressure in the isolated tubal segment of guinea-pig trachea by only 5cm H₂O –markedly less than the microvascular pressure^{47, 74, 92} - caused prompt transepithelial passage of tracer macromolecules from serosal to mucosal surface liquids. This transmission continued at the same increased rate as long as the pressure was increased. Importantly, the transepithelial movement of serosal macromolecules was not associated with increased passage of macromolecular tracers in the opposite direction. Thus, during and after 30 minutes of a serosal pressure challenge mucosal absorption permeability was not increased⁹⁰. In agreement, there was no sign of any damage or structural change of the epithelium⁸⁹. Furthermore, the hydrostatic pressure-induced movement of serosal macromolecules to the mucosal surface was well repeatable and reproducible. Finally, autacoids and drugs, known to either induce or inhibit plasma exudation *in vivo*, were without influence on the hydrostatic pressure-induced epithelial transmission of macromolecules *in vitro*⁸⁹. This latter observation supports the notion that the epithelial passage of extravasated plasma reflects the impact of hydrostatic pressure alone without any need for other effects on the epithelium to facilitate the passage.

In summary, there was tantalizing agreement between experimental hydrostatic pressure-induced transmission of macromolecules in the isolated tracheal tubes and the

in vivo plasma exudation response to histamine-type mediators in animal and human airways. Taken together these observations supported the idea that the extravasated plasma itself, by slightly increasing epithelial basolateral hydrostatic pressures, could create paracellular epithelial macromolecular exit paths. It was proposed that such a mechanism operated at plasma exudation responses in vivo.

The hydrostatic pressure hypothesis confirmed in vivo.

The hydrostatic pressure hypothesis received specific in vivo support. In a segment of rat trachea Serikov et al ⁴⁷ thus demonstrated that an increase in hydrostatic pressure of 4.5cm H₂O in the airway lumen was sufficient to prevent a plasma exudation response in vivo. As with the in vitro experiments, the employment of trachea was probably crucial for the success of the *in vivo* experiments: The applied pressure would not affect the airway tissue layers due to the stabilising effect of cartilage rings. Using scanning electron microscopy Serikov et al ⁴⁷ further confirmed that plasma exudation occurred without affecting epithelial structural integrity ⁴⁶.

Hence, both in vitro and in vivo data support the notion that a slightly increased hydrostatic pressure is the gross operational mechanism involved in the basic plasma exudation response to non-injurious mucosal challenges. Tokuda et al. ⁹³ have reported that an increased hydrostatic pressure gradient from basolateral to apical sides of epithelial cells caused reversible changes in tight junction proteins. However, few studies have determined molecular mechanisms associated with hydrostatic pressure-evoked para-epithelial flux of macromolecular solutes in the airways.

Airways plasma exudation and absorption in asthma and allergic rhinitis

Exudative diseases without increased mucosal absorption permeability

Direct non-invasive measurements of airway permeability seem to have clearly disproved the hypothesis of increased bronchial absorption of inhaled molecules as a basic defect in asthma. Work by leading pulmonary research groups thus demonstrated that absorption of inhaled technetium 99m-labelled DTPA is unrelated to bronchial hyper-responsiveness and that it is not increased in asthma^{2-4, 94}. Lack of increased permeability has been confirmed in studies involving more asthma patient groups and where absorption from different lung zones has been examined⁵. Further, in perennial allergic rhinitis where symptoms were induced by stopping treatment, absorption permeability was not increased⁸.

With dual isotope techniques, determining concomitant mucociliary clearance and absorption of inhaled DTPA, Halpin et al. (cited in references 7,10) reported consistently reduced (sic!) bronchial absorption in asthma compared to health. Similarly, already in 1975 indications of reduced nasal airway absorption in allergic rhinitis were demonstrated⁹. In accord, significantly reduced absorption permeability was observed in seasonal rhinitis along with plasma exudation and allergic symptoms – in these studies absorption tracer concentration, influence of mucociliary clearance, exposed airway surface area, and exposure time were controlled^{7, 10}.

A transient phase of increased absorption permeability may occur in acute asthma⁹⁴. It is possible that extensive denudation is involved as a causative factor in such cases (see below). Challenge with house dust mite, an allergen that stands out as particularly pathogenic, has also caused transiently increased airway absorption in vivo in mice⁹⁵. These data may need confirmation in other species because mice lack a bronchial

microcirculation⁴⁹. Taken together, in vivo data suggest that increased permeability of inhaled molecules is not a basic feature of asthma and allergic rhinitis where, nevertheless, plasma exudation occurs.

The paradigm of increased epithelial permeability in asthma may be unduly strengthened by misrepresentation of in vivo data.

The studies disproving the permeability paradigm are rarely cited in current literature. Instead, little specific data are now serving to support the established notion that asthma is a disease with abnormal penetration of inhaled molecules across the bronchial mucosa. In both human and animal studies observations of increased airway surface levels of plasma proteins are thus commonly reported as a compelling sign of compromised epithelial integrity¹⁷. In view of known features of plasma exudation this interpretation seems unwarranted.

Proteins such as club cell protein (CC16) and surfactant protein D are secreted constituents of airway surface liquids. Reduced levels of these proteins in airway samples, or increased levels in blood and urine, have been reported to support the hypothesis of increased absorption permeability in asthma. However, it has been pointed out that data are inconsistent^{1, 96}. Blood and urine data on CC16 also vary greatly. A recent study additionally demonstrated a puzzling dichotomy: CC16 levels were increased in plasma but reduced in urine after allergen-challenge in asthmatic individuals⁹⁷. Bronchial surface levels of surfactant protein D in severe asthma may be increased as well as decreased^{98, 99} questioning its value as permeability probe. Unsurprisingly, replacement of these indirect indices with direct non-invasive markers

of airway absorption permeability has been strongly recommended ¹. As discussed above absorption of inhaled permeability tracers has not been increased in asthma.

Airway surface proteins in asthma: produced locally or emanating from exuded plasma?

Given the versatility of culture conditions, airway structural cells and leukocytes can exhibit intriguing ability in vitro to produce proteins that also occur in plasma. An uncertainty may, therefore, concern the source of proteins in the airways: whether produced locally or being a component of exuded plasma? There is now a predominance of cell-biology studies over explorative and independent in vivo-research. Hence, the notion of local airway cellular production of plasma proteins is favored, perhaps more than justified with regard to medical importance.

From its non-sieved nature it is predictable ^{100,101} that coagulation and complement proteins will appear on the human airway surface at plasma exudation events. Yet, even in established plasma exudative conditions, such as following airway allergen challenge in atopic individuals ^{100,102}, local production of complement proteins has been forwarded as the preferred source ^{103,104}. As demonstrated in sensitized guinea-pigs and in allergic rhinitis and allergic asthma, allergen challenge has caused both early and late phase plasma exudation responses ^{27,105,106}. Yet, in studies where appearance of complement and coagulation products would reflect this biphasic feature, the role of plasma exudation has not been considered¹⁰⁷.

It is of interest that plasma proteins, such as complement and coagulation proteins ^{108,109}, are considered central in asthma pathogenesis. However, it is surprising that local occurrence of leukocytes with merely a presumed capacity to produce the protein in

question has sufficed to indicate local production - lack of systemic inflammation has similarly been used as an argument to favor a local source^{103, 104}. This kind of evidence/reasoning may not compellingly support a local cellular source. Rather, such widely disseminated arguments likely reflect lack of topical discussions of plasma exudation mechanisms.

Meerschaert et al¹¹⁰ made commendable attempts at identifying the source of increased BAL fluid fibronectin following segmental allergen challenge in asthma. In a small subset of their patients they analysed BAL fluids using an antibody directed at a splice variant of fibronectin considered to be of cellular rather than plasma origin. Possibly reflecting the small number of patients, or methodological issues, the spread in values was large and statistical significance of their intriguing data was not reached. Yet, the authors' message was that the BAL fluid fibronectin was largely derived from cells. To strengthen their argument the authors also focused on significant correlations between fibronectin concentrations and leukocyte numbers in all BAL fluids finding r-values between 0.6 and 0.8. However, they may have missed an opportunity to demonstrate superior correlation between fibronectin and albumin both of which had increased by a factor of 6 day 2 after antigen (mean BAL fluid values compared with antigen day 0). Local production of fibronectin at allergen exposure obviously needed to be confirmed but when the same laboratory later reported BAL fluid fibronectin data following allergen challenge in asthmatics the source of this protein was not considered^{111, 112}. The latter reports included a study where both thrombin activity and fibronectin were determined in BAL fluids obtained 2 days after segmental allergen challenge in asthma¹¹². Thrombin activity correlated nicely with both total protein and fibronectin ($r > 0.8$) supporting the possibility that both thrombin and fibronectin were components of exuded plasma that

would have been produced, accumulated, and remained to a significant extent in airway surface liquids until BAL was carried out 48h after allergen challenge.

Albumin or other plasma protein as biomarker of airways plasma exudation?

Endobronchial allergen challenge in patients with allergic asthma produced significant BAL fluid levels of fibrinogen even when the increased levels of albumin did not reach significance. However, there was correlation between albumin and fibrinogen ($r=0.9$) at the bronchial allergic reaction¹⁰⁰. Varying baseline levels make the relatively small and diffusible albumin molecule a less specific plasma exudation tracer compared to higher weight proteins. In animal tracheobronchial experiments as well as in human nasal studies, cumulated mucosal surface albumin can be washed away creating a suitable baseline condition^{50, 113}. Then albumin is a consistent plasma exudation tracer along with larger molecules including fibrinogen and alpha2-macroglobulin.

In chronic, severe eosinophilic asthma increased sputum levels of alpha2-macroglobulin indicated significant plasma exudation - however, fibrinogen was not increased. Instead, fibrin degradation products were increased⁴⁰. In the chronic disease condition fibrinogen may be trapped as fibrin. When fibrin is deposited and tethered to the airway surface it may not as readily as its degradation products become a component of sputum. These data support the use of alpha2-macroglobulin as biomarker of plasma exudation in chronic disease condition. Local production can be considered concerning proteins that are increased more-fold than the plasma tracer⁴⁰.

Esnault et al¹¹⁴ compared the ratio of their designated BAL fluid protein to albumin and showed that it deviated from the ratio in plasma. Their conclusion was that contribution

from plasma 'leak' was unlikely. However, relatively larger concentrations of the protein were observed in plasma. In such cases work may be required to elucidate potentially confounding factors such as binding, breakdown, and other modes by which exuded proteins may escape detection.

A further confounding factor concerns the possibility that the binding capacity of plasma proteins may bring mucosal tissue-dwelling, cell-derived molecules to the surface at plasma exudation. Thus, in patients with seasonal rhinitis eosinophil-derived proteins were increased in sputum only after histamine inhalations had produced acute bronchial plasma exudation¹¹⁵. Effects of plasma exudation on the distribution of mucosal, cell-derived molecules may need consideration in studies of airway samples.

Plasma exudation and its potentially pathogenic actions in severe asthma.

Although plasma exudation may be a common feature of asthma in general^{40, 116} the best evidence for involvement=occurrence of plasma exudation in asthma may emanate from studies of severe eosinophilic^{40, 117} or neutrophilic¹¹⁸ asthma and from studies where asthma patients are challenged by insults/interventions known to worsen asthma: common cold⁴³, allergen exposure¹⁰², or step-wise reduction of anti-inflammatory treatment^{117, 119}. Plasma exudation indices may even be a dominating inflammatory index of worsening asthma¹²⁰.

'Exudative hyper-responsiveness', determined by exaggerated plasma exudation in response to histamine-type challenges, has been demonstrated in airways subjected to allergic disease and viral infection^{26, 121-123}. Thus, it has occurred in human nasal airways in seasonal allergic rhinitis in adults and children^{122, 123} and in corona virus-

induced common cold²⁶. A common cause of asthma exacerbations viral infections cause plasma exudation in human nasal and bronchial airways^{43,85}. Possibly as a reflection of exudative hyper-responsiveness, particularly large sputum concentrations of fibrinogen were observed in asthmatics in association with natural common cold⁴³.

Leukotrienes (C4 and D4) are several orders of magnitude more potent than other common autacoids as inducers of plasma exudation in guinea-pig tracheobronchial airways²⁹. Eosinophil granule proteins have caused plasma extravasation in the hamster cheek pouch¹²⁴. TNF-alpha has induced a late plasma exudation response in human nasal airways¹²⁵. However, little is known regarding which factors are actually involved in increased plasma exudation in asthma.

Although plasma exudation may not cause edema^{27,45} physical encroachment of airway lumen may still result and aggravate effects of bronchoconstriction¹²⁶. Exposure to plasma proteins may increase mucus production. The exuded plasma may also interact with mucus to increase viscosity and immobilize the airway surface material^{127,128}. This effect would be particularly unwanted in small airways with risk of bronchiolar closure¹²⁹. It has been suggested that mucus plugs in asthmatic airways may be mucus-plasma plugs, but the extent to which plasma contributes to airway plugs remains to be determined in detail.

The exuded plasma proteins offer a Pandora's box with many intact proteins and many of their breakdown molecules offering biological actions of interest. There is established interest in pathogenic roles of complement proteins, coagulation proteins, and kinins in asthma^{108,109,130}. Several plasma proteins may have crucial roles in epithelial

regeneration (discussed below). However, specific roles of individual plasma proteins in disease await the critical tests that trials with selectively targeted interventions will provide.

A maintained airway barrier against environmental challenges at shedding of columnar epithelial cells. A role in innate airway defense?

Although a versatile epithelial barrier is a topical theme, barrier restitution events following different degrees of shedding may not be widely discussed. The most common mode of epithelial shedding is loss of columnar cells. There is interest in apoptosis of epithelial cells but it is not known whether exiting columnar epithelial cells are at any stage of apoptosis. Single columnar cells may leave without a trace since neighboring cells move in squeezing the leaving cell. Such shed single cells may be found in airspace samples with a tapered-off tail. Also sheets of columnar cells are commonly shed in asthma (connected ciliated cells with still beating cilia have been found in asthmatic sputa^{19, 131, 132}). Columnar epithelial cells have some contact with the basement membrane, but it is not known if their removal will cause any plasma exudation. Indeed, a plasma exudation response may not be needed because the remaining basal cells have remarkable transformation capacities.

Following gentle removal selectively of columnar cells from human bronchi or guinea-pig trachea the exposed, normally loose and cobblestone-like basal cells produce a barrier structure within few minutes. They flatten to cover the basement membrane and create interdigitating cell contacts all around¹³³. Hence, limited shedding exclusively of columnar cells may be a normal event serving to rid the airway of cells that are somehow defect. Alternatively, the cells may need to be sacrificed as part of airway

defense operations. Shedding of columnar epithelial cells may not necessarily result in increased absorption permeability nor may it evoke plasma exudation.

Exaggerated plasma exudation reflects in part exaggerated epithelial regeneration in asthma.

Non-sanguineous epithelial denudation in asthma guides in vivo-experiments.

Both plasma exudation and epithelial shedding are increased in asthma, especially in severe disease and at exacerbations ^{11, 18, 19}. There are major aspects to consider in experimental approaches aimed at producing disease-like epithelial denudation in vivo. Extensive shedding of epithelial cells in asthma may not involve any bleeding. Thus, when both columnar and basal cells are shed no bleeding should occur. The shedding in asthma also leaves an apparently intact basement membrane. The discussion below concerns in vivo studies involving guinea-pig airways where the above-mentioned features have been accommodated. Ingrid Erjefält's skilful *in vivo*-interventions, without any surgery and involving physiological baselines ¹³⁴, have been essential both for studies of challenge-induced plasma exudation and for studies of shedding-like denudation in guinea-pig tracheobronchial airways. I apologize for not citing important in vivo research that mostly for given reasons, such as more mucosal damage than involved in asthma-like epithelial shedding, involvement of toxic gases, and employment of a species that lacks a bronchial microcirculation, were not cited.

Epithelial denudation causes plasma exudation that creates a provisional barrier.

In experiments involving gentle, non-sanguineous mechanical denudation in vivo of a patch of dorsal trachea it has been demonstrated that the remaining, intact epithelial basement membrane is not left naked. Locally exuded plasma is promptly spreading

over the surface of the entire denuded basement membrane, but not much further afield.

A plasma-derived gel is thus created to cover the area of interest^{135, 136}. Such gels are not seen in common graphs because they are washed away during preparations for histology examinations.

Mechanisms specifically involved in the plasma exudation response to denudation have not been determined but may in part reflect removal of tonic suppression of microvascular permeability by epithelial factors⁸⁰. The exudation is of much longer duration than the response to histamine-type autacoids¹³⁵. It sustains the cover-gel with fresh plasma until the gel is not needed any more^{135, 136}. The gel is also dislodged as soon as the previously denuded area is fully covered by rapidly migrating regeneration epithelium (discussed below).

The plasma exudation-response to disease-like denudation has several connotations. By its physical appearance, its capacity to trap molecules and particles, and its biological defense capacity the gel is already a coarse barrier. Merely the fibrin/ogen component and associated mechanisms have major immune defense capacities¹³⁷. Consistent with the abundant occurrence of neutrophils in the epithelial regeneration area⁷² fibrin is known to bind neutrophils and other leukocytes as well as a range of cell- and plasma-derived molecules¹³⁸. Through its integrins also eosinophils may exhibit intriguing interactions with fibrin and fibronectin¹³⁹. The continuous supply by exuded plasma provides a highly dynamic *in vivo* milieu suited both for barrier activities and for regeneration of an epithelial cell barrier (Figure 2).

Figure 2.

Epithelial regeneration occurs in an active soup of plasma proteins.

An exclusive in vivo phenomenon the microvascular-epithelial exudation of plasma may not be a well-recognized mechanism in today's topical discussions, which are largely based on data where the dynamic influence of plasma extravasation is absent. For example, it is not widely appreciated that following disease-like epithelial denudation, in guinea-pig trachea, regeneration proteins such as fibronectin and fibrinogen have emanated entirely from extravasated plasma¹³⁵. At epithelial injury sites a novel cell sheet consisting of flattened repair epithelium is thus created in a dynamically active, plasma-derived gel milieu. This is also an active defense milieu. For example, basal cell-like epithelial repair cells may need this protection to compensate for their sensitivity to microbial exposure^{140, 141}. The in vivo exudation milieu seems more important than reflected in the current literature¹⁴².

Epithelial denudation in vivo appears to be exceedingly patchy.

A helicopter view of wholemount airway preparations has revealed numerous tiny denudation spots after in vivo allergen challenge in guinea-pigs. Each denudation patch is a hotspot with intense exudation and repair events^{143 144}. Notably, the area of these spots has been much less than the denudation area revealed by sectioning of such a challenged airway wall¹⁴⁴. Hence, large areas of denudation in histology preparations, although they may reflect epithelial fragility, may in part be sectioning artifacts^{144, 145}.

It is not known in detail what decides patchiness of denudation. Strikingly, this phenomenon is conspicuous even in airways uniformly exposed to noxious agents including allergens and dry air^{143, 146}. Davis and Freed¹⁴⁷ thus carried out segmental dry

air challenges in dogs to produce patchy bronchial denudation. Using a wedged bronchoscope these authors further noted local accumulation of “mucus” and suggested that such material (actual components were not determined) could serve as a surrogate barrier ¹⁴⁷. Mosser et al ¹⁴⁸ demonstrated that only a small fraction of epithelial cells became infected when exposed to rhinovirus. This observation is consistent with a patchy distribution of viral induced epithelial injury. Patchy distribution of epithelial derangement including early signs of epithelial regeneration may be common in publicized histology graphs of diseased airways ¹⁴⁹. Some information on patchy epithelial damage and repair may also be found in studies exploring bronchial mucosal distribution of molecules of interest ^{150, 151}. However, a systematic analysis seems to be lacking.

Speedy restitution of epithelial cell barrier at patchy exudation sites of denudation.

Small-sized areas of epithelial denudation are essential for a speedy repair. At the loss of neighboring epithelium all epithelial cell types bordering a denuded patch will promptly dedifferentiate into poorly differentiated flattened cells. Already during dedifferentiation, cells that previously were fully differentiated secretory and ciliated cells start to migrate. From all sides, such local regeneration cells move speedily in a tethered fashion over the denuded basement membrane ¹³⁶ (Figure 2). Thus, a novel cell sheet consisting of flattened regeneration epithelium is created. The new epithelial cell barrier has reduced paracellular stretches available for passage of molecules possibly explaining part of the reduced absorption demonstrated in asthma and allergic rhinitis (see above). The speedy epithelial restitution requires an intact basement membrane. From its inception it occurs in a dynamic, plasma-derived, gel-like milieu ¹³⁵.

A maintained airway mucosal barrier against environmental challenges at epithelial denudation avoids ever-escalating disease conditions.

When shedding is intense and involves denudation the situation is potentially the start of a spiral of extreme pathogenic consequences. If the barrier against hostile airspace factors is lost, and not restored speedily, ever-increasing inflammation and harm to the airways would be expected to occur. The initial denudation challenge would make it so much easier for additional and more severe challenge-induced effects. In turn, more areas would be denuded and more airway tissues be directly exposed to noxious mucosal surface material. Hence, it is possible that plasma exudation and speedy barrier restitution mechanisms, such as discussed above have evolved to avoid a vicious circle.

A maintained airway barrier in conditions of denudation comes at a cost.

The sustained plasma exudation response is associated with a range of intriguing effects.

The first barrier that is created following epithelial denudation in vivo is the provisional gel cover produced and maintained by sustained plasma exudation. Especially in severe asthma with extensive shedding, epithelial injury-repair processes may thus contribute to the increased plasma exudation recorded in this condition. As discussed above, exaggerated plasma exudation alone may be of pathogenic importance in asthma because of its physical and biological effects. However, plasma exudation is not the only in vivo response to denudation (Figure 2). The epithelial regeneration process in vivo has evoked a range of additional effects that disseminate into the neighborhood of hot spot denudation patches. Such effects include accumulation and activation of granulocytes, increased mucus secretion, and increased proliferation of epithelial and fibroblast/smooth muscle cells ^{72, 136, 152, 153}.

Little support for involvement of epithelial-mesenchymal units (EMU).

The dedifferentiated and migrating regeneration cells may be considered mesenchymal-like. However, they have not been seen to migrate across the plasma-covered basement membrane. Hence, the idea of dissemination of EMU into the lamina propria¹⁵⁴ is not supported. Instead, the dedifferentiated cells have proliferated and eventually undergone redifferentiation to a normal epithelium¹³⁶. Pain et al. have also underscored that evidence for occurrence of EMU in asthma is lacking¹⁵⁴. Thus, the increased numbers of fibrocytes in asthmatic airways^{155, 156} may simply reflect proliferation of these cells occurring in response to signals emanating from inflammatory processes¹⁵⁴ including signals from sites of epithelial denudation-regeneration sites¹³⁶.

Exaggerated epithelial regeneration may contribute inflammatory and remodeling aspects of asthma.

Recruitment and activation of neutrophils are prominent in the regeneration-exudation area. Repeated denudation-regeneration processes further result in reticular basement membrane thickening^{152, 153}. Eosinophil-derived proteins have been suggested to cause part of epithelial shedding in asthma¹⁵⁷. Free eosinophil granules, known to be produced by eosinophil cytolysis and releasing toxic proteins⁶⁷, are also intriguingly associated with epithelial cell loss in asthma¹⁵⁸. In severe asthma eosinophil cytolysis-derived free granules in sputum are a prominent indicator of the disease state¹¹⁷. In guinea-pig airways subjected to denudation, eosinophils already present in the area of interest are activated by primary cytolysis to release clusters of free eosinophil granules⁷². Hence, epithelial regeneration-induced eosinophil cytolysis may be part of a pathogenic circle in asthma¹⁵⁸.

Exaggerated epithelial regeneration both at inception and at severe stages of asthma?

Notably, the asthma-like effects evoked along with epithelial regeneration have been produced in vivo also in airways where no inflammation existed prior to epithelial denudation¹³⁶. It is then of interest that Creola bodies (clusters of desquamated epithelial cells) in aspirated sputum, independent of other factors, was reported to predict development of asthma in wheezing infants¹⁵⁹. Creola bodies in sputum have also been significantly associated with exacerbations of asthma in adults and children^{11, 160}. The occurrence of very large numbers of Creola bodies (up to four hundred clusters in just two smears of sputum¹¹) agrees with a pronounced patchiness of epithelial damage in asthma (cf above).

The multiple effects of epithelial denudation-regeneration alone (Figure 2) support the idea of its operational role both in early initiation of asthma and in severe asthma with exacerbations. The possibility of a pathogenic role of this epithelial process has also been entertained in discussions of exercise-induced asthma¹⁶¹. Further, as demonstrated in guinea-pigs in vivo, plasma exudation and epithelial regeneration responses to airway epithelial denudation have been refractory to steroid treatment¹⁶². This observation is of interest since steroid treatment is not known to prevent development of asthma, and in severe asthma plasma exudation¹¹⁷ along with central disease features may also be refractory in part to steroid treatment effects^{163, 164}.

Therapeutic implications.

Airways plasma exudation reflects not only an active inflammatory process. It is also a significant feature of epithelial regeneration and it has a role in first line innate defense.

Increased bronchial exudation of plasma in asthma may thus reflect a mixture of different types of mucosal events. This diversity has therapeutic implications.

The discovery that beta-agonist bronchodilators have additional microvascular antipermeability actions substantiated the view that venular endothelial permeability is under physiological and pharmacological control¹⁶⁵. Further, beta agonists simultaneously increased the mucosal blood flow¹⁶⁵, which agreed with the view that mucosal blood flow is not a critical component of airways plasma exudation. A limited vascular anti-permeability effect of inhaled beta-agonists has been demonstrated in human healthy bronchi subjected to histamine challenges¹⁶⁶. However, whether these drugs reduce plasma exudation to any significant extent in asthma is not known.

Effective glucocorticoid treatment of asthma reduces plasma exudation¹¹⁷ and it may reduce an exudate hyper-responsiveness¹²³. Reversibly, when the anti-asthma treatment dose of steroids is tapered plasma exudation is increased and asthma is worsened^{119, 120}. The anti-exudative actions of glucocorticoids seem to reflect a general anti-inflammatory efficacy of these drugs rather than direct effects on microvascular permeability. Supporting this view a study in human nasal airways demonstrated that topical glucocorticoid treatment did not reduce histamine challenge-induced plasma exudation¹⁶⁷. These data suggest that glucocorticoid-treated airway mucosa remains vigilant when defense calls for a plasma exudation response. Also, topical glucocorticoid treatment inhibited neither the plasma exudation response nor the speedy epithelial regeneration following epithelial denudation in vivo in guinea-pig trachea¹⁶². Together these in vivo observations tally with glucocorticoids sparing several innate immunity mechanisms, as reported by Schleimer¹⁶⁸. Desired features of an anti-inflammatory,

anti-asthma drug would include efficacy against pathogenic plasma exudation without inhibiting the involvement of this microvascular response when it is expressed as a component of mucosal defense and epithelial regeneration, respectively.

The idea that exaggerated epithelial regeneration qualifies as a causative component of asthma pathogenesis (Table 2) has therapeutic implications. As discussed above there is a risk of ever-escalating disease conditions if epithelial regeneration is not a prompt and speedy process. A desired feature of future treatments would be to find means by which epithelial cell loss can be reduced rather than interfering with mechanisms associated with epithelial regeneration. This goal supports the importance of targeting airway epithelial cells in pharmacological research on epithelial cytoprotection.

Limitations

Exploratory in vivo research often produces unexpected data. If not in accord with accepted notions the observations are not always pursued vigorously. But, they may still be significant. This overview is partly based on in vivo data that disagree with popular research paradigms. Accordingly, the present attempt at understanding airway mucosal features has been little helped by the advanced cell biology research that supports currently leading notions. The ensuing limitation of the present discourse is underscored by its focus on events involving plasma exudation, which is almost exclusively an in vivo phenomenon. Scattered in this article the need for further work is indicated. This need is also implicit by the limited number of research groups involved in generation of some of the data that underpin views presented here.

Conclusion

Independent observations in vivo in patients and guinea-pigs underpin the notions discussed in this review. The notions are novel in the sense that they, more or less explicitly, challenge several current paradigms in airway and asthma research. Inferentially, changing concepts are introduced including a supplementary idea regarding the pathogenesis of asthma (Table 2).

Not long ago increased 'capillary permeability' and extravasation of plasma was considered to reflect vascular injury. This view no longer prevails. Numerous elegant studies have revealed mechanisms of reversible formation of venular endothelial gaps leading to transient extravasation of non-sieved plasma macromolecules. This mode of extravasation occurs in the profuse airway subepithelial microcirculation in response to allergens and other significant inhaled challenges.

Here I have discussed the possibility that the further passage of extravasated, non-sieved plasma across airway epithelial linings is also, in its basic shape, a non-injurious physiological event.

Without causing appreciable edema extravasated plasma macromolecules move between yielding epithelial cells to appear locally on the mucosal surface at the very site of challenge. A slightly increased hydrostatic pressure impacting on basolateral aspects

of epithelial cells seems causally involved in the paracellular epithelial transmission of extravasated plasma. Unless the challenge grows this prompt local exudative response is of brief duration.

Despite the non-sieved nature of the microvascular-epithelial plasma exudation process, absorption of molecules deposited on the mucosal surface is not increased. The epithelial lining also remains structurally intact. These are important features of a first line defense response. They may also explain in part why a disease such as asthma, which exhibits airways plasma exudation, may not display an abnormal inward perviousness.

At epithelial shedding, diverse mechanisms are operating to maintain tightness and prevent undue penetration of mucosal surface material. Even when both columnar and basal epithelial cells are shed (denudation) the naked basement membrane will promptly receive a provisional barrier of exuded plasma. The activated plasma forms a gel-like structure involving a leukocyte-rich fibrin-fibronectin net covering the basement membrane. The gel remains and is continuously supplied by fresh plasma until a new cover of epithelial regeneration cells is established. In a dynamic plasma-derived molecular milieu, ciliated and secretory epithelial cells surrounding denuded spots dedifferentiate into flattened, rapidly migrating regeneration cells. Thus, airways with patchy denudation sites are not without barriers or may only transiently exhibit a reduced absorption barrier. Barrier restitution is potentially prompt even in severe conditions with extensive, but patchy, denudation of epithelium.

Prompt and speedy epithelial regeneration in severe asthma counteracts the risk of severely escalating barrier breaks. However, epithelial regeneration processes also evoke disease-like effects. In experimental in vivo studies the mere process of denudation/regeneration on top of an uninjured basement membrane has caused plasma exudation, granulocyte recruitment/activation, mucus secretion, proliferation of fibroblast/smooth muscle cells, and thickening of reticular basement membrane. Hence, exaggerated epithelial regeneration, occurring in plasma exudation hot spots, seems worthy of consideration as a factor contributing not only to a speedy epithelial regeneration and a maintained absorption barrier but also to inception and worsening of asthma.

What is currently known:

The bronchial mucosa in vivo in asthma exhibits exudation of plasma and epithelial shedding. Yet there is no increased absorption of inhaled molecules.

The above three features can be reconciled thanks to additional in vivo-observations:

- The asymmetry and the plasticity of the epithelial lining allows outward passage of macromolecules without altering its tightness as a barrier towards molecules deposited on the mucosal surface (the epithelial transmission of non-sieved plasma macromolecules appears to be driven by a small, sub-edematous increase in hydrostatic pressure transiently and non-injuriously impacting on the basolateral aspect of the pseudostratified epithelial lining cells).
- Epithelial regeneration mechanisms following shedding operate promptly to reconstitute barrier functions in vivo. Even at denudation the naked but intact basement membrane will be promptly covered, first by a plasma-derived fibrin-fibronectin net with continuous supply by fresh plasma and leukocytes. This

cover is a coarse physical and highly potent biological barrier. Furthermore, it provides a milieu in which all types of epithelial cells bordering the denuded area dedifferentiate into speedily migrating regeneration cells, which are tethered together. In the likely event of quite patchy denudation in vivo, a cellular barrier will thus speedily replace the provisional plasma-derived barrier leaving little opportunity for increased absorption of inhaled molecules. However, the regeneration sites are also inflammatory and remodeling hotspots suggesting the possibility that exaggerated epithelial regeneration at multiple denudation sites contributes to development and worsening of asthma.

What more we need to know:

- The basic features of airways plasma exudation indicate that it has roles in first line defense on the surface of an intact mucosa in health and disease. Hence, we need to know further details of involved mechanisms and how its in vivo-functions fare by comparison with other defense mechanisms operating at the mucosal surface including mucus secretion and production of antimicrobial peptides.
- We need to define in detail several additional features of plasma exudation in asthma including: To what extent does plasma exudation actually occur? Is there an exudative hyper-responsiveness to challenge with microvascular permeability agents? To what extent does exuded plasma contribute to airway plugs? What is the relative contribution of cell-derived versus plasma-derived same proteins in asthma including coagulation and complement molecules? On that note, is plasma exudation an 'all-in' defense/disease mechanism or are there conditions when individual plasma protein systems are selectively activated?

- Plasma exudation in response to asthma-like epithelial denudation is distinguished by sustained occurrence providing both physical and biological protection. We need to know what plasma-derived molecules are essential for epithelial regeneration and whether any cell-derived molecule is also essential in this process in vivo? We need to know the extent of patchiness of epithelial denudation and how we can define such hot spots in asthma? How do inflammatory and remodeling responses evoked by epithelial regeneration events fare compared to other such mechanisms in vivo at inception of asthma? At development of asthma? And at exacerbations of asthma?

References.

1. Georas SN, Rezaee F. Epithelial barrier function: at the front line of asthma immunology and allergic airway inflammation. *J Allergy Clin Immunol* 2014; 134:509-20.
2. Elwood RK, Kennedy S, Belzberg A, Hogg JC, Pare PD. Respiratory mucosal permeability in asthma. *Am Rev Respir Dis* 1983; 128:523-7.

3. O'Byrne PM, Dolovich M, Dirks R, Roberts RS, Newhouse MT. Lung epithelial permeability: relation to nonspecific airway responsiveness. *J Appl Physiol Respir Environ Exerc Physiol* 1984; 57:77-84.
4. Chan TB. Pulmonary epithelial permeability in normal individuals and asthmatic patients. *Ann Acad Med Singapore* 1985; 14:462-4.
5. Del Donno M, Chetta A, Foresi A, Gavaruzzi G, Ugolotti G, Olivieri D. Lung epithelial permeability and bronchial responsiveness in subjects with stable asthma. *Chest* 1997; 111:1255-60.
6. Persson C, Uller L. Epithelial perviousness in allergic airways disease. *J Allergy Clin Immunol* 2017; 140:1211.
7. Greiff L, Andersson M, Svensson C, Lundin S, Wollmer P, Persson CG. Reduced airway absorption in seasonal allergic rhinitis. *Am J Respir Crit Care Med* 1997; 156:783-6.
8. Greiff L, Andersson M, Svensson J, Wollmer P, Lundin S, Persson CG. Absorption across the nasal airway mucosa in house dust mite perennial allergic rhinitis. *Clin Physiol Funct Imaging* 2002; 22:55-7.
9. Kontou-Karakitsos K, Salvaggio JE, Mathews KP. Comparative nasal absorption of allergens in atopic and nonatopic subjects. *J Allergy Clin Immunol* 1975; 55:241-8.
10. Greiff L, Wollmer P, Svensson C, Andersson M, Persson CG. Effect of seasonal allergic rhinitis on airway mucosal absorption of chromium-51 labelled EDTA. *Thorax* 1993; 48:648-50.
11. Naylor B. The shedding of the mucosa of the bronchial tree in asthma. *Thorax* 1962; 17:69-72.

- 994 12. Montefort S, Djukanovic R, Holgate ST, Roche WR. Ciliated cell damage in the
 995 bronchial epithelium of asthmatics and non-asthmatics. *Clin Exp Allergy* 1993;
 996 23:185-9.
- 997 13. Cruz AA, Naclerio RM, Proud D, Togias A. Epithelial shedding is associated with
 998 nasal reactions to cold, dry air. *J Allergy Clin Immunol* 2006; 117:1351-8.
- 999 14. Berger G, Bernheim J, Ophir D. Epithelial shedding of the inferior turbinate in
 1000 perennial allergic and nonallergic rhinitis: a riddle to solve. *Arch Otolaryngol*
 1001 *Head Neck Surg* 2007; 133:78-82.
- 1002 15. Kicic A, Stevens PT, Sutanto EN, Kicic-Starcevic E, Ling KM, Looi K, et al.
 1003 Impaired airway epithelial cell responses from children with asthma to rhinoviral
 1004 infection. *Clin Exp Allergy* 2016; 46:1441-55.
- 1005 16. Sweerus K, Lachowicz-Scroggins M, Gordon E, LaFemina M, Huang X, Parikh M, et
 1006 al. Claudin-18 deficiency is associated with airway epithelial barrier dysfunction
 1007 and asthma. *J Allergy Clin Immunol* 2017; 139:72-81 e1.
- 1008 17. Loser S, Lloyd CM, Gregory LG. Reply. *J Allergy Clin Immunol* 2017; 140:1211-2.
- 1009 18. Dunnill MS. The pathology of asthma, with special reference to changes in the
 1010 bronchial mucosa. *J Clin Pathol* 1960; 13:27-33.
- 1011 19. Persson CG. Centennial notions of asthma as an eosinophilic, desquamative,
 1012 exudative, and steroid-sensitive disease. *Lancet* 1997; 350:1021-4.
- 1013 20. Persson CG, Svensson C, Greiff L, Anderson M, Wollmer P, Alkner U, et al. The use
 1014 of the nose to study the inflammatory response of the respiratory tract. *Thorax*
 1015 1992; 47:993-1000.
- 1016 21. Curtis JL, Freeman CM, Hogg JC. The immunopathogenesis of chronic obstructive
 1017 pulmonary disease: insights from recent research. *Proc Am Thorac Soc* 2007;
 1018 4:512-21.

- 1019 22. Schleimer RP, Berdnikovs S. Etiology of epithelial barrier dysfunction in patients
1020 with type 2 inflammatory diseases. *J Allergy Clin Immunol* 2017; 139:1752-61.
- 1021 23. Tomazic PV, Birner-Gruenberger R, Leitner A, Obrist B, Spoerk S, Lang-Loidolt D.
1022 Nasal mucus proteomic changes reflect altered immune responses and epithelial
1023 permeability in patients with allergic rhinitis. *J Allergy Clin Immunol* 2014;
1024 133:741-50.
- 1025 24. Erjefalt I, Persson CG. Inflammatory passage of plasma macromolecules into
1026 airway wall and lumen. *Pulm Pharmacol* 1989; 2:93-102.
- 1027 25. Svensson C, Baumgarten CR, Pipkorn U, Alkner U, Persson CG. Reversibility and
1028 reproducibility of histamine induced plasma leakage in nasal airways. *Thorax*
1029 1989; 44:13-8.
- 1030 26. Greiff L, Andersson M, Akerlund A, Wollmer P, Svensson C, Alkner U, et al.
1031 Microvascular exudative hyperresponsiveness in human coronavirus-induced
1032 common cold. *Thorax* 1994; 49:121-7.
- 1033 27. Erjefalt I, Greiff L, Alkner U, Persson CG. Allergen-induced biphasic plasma
1034 exudation responses in guinea pig large airways. *Am Rev Respir Dis* 1993;
1035 148:695-701.
- 1036 28. Halldorsdottir H, Greiff L, Wollmer P, Andersson M, Svensson C, Alkner U, et al.
1037 Effects of inhaled histamine, methacholine and capsaicin on sputum levels of
1038 alpha 2-macroglobulin. *Thorax* 1997; 52:964-8.
- 1039 29. Persson CG, Erjefalt I, Andersson P. Leakage of macromolecules from guinea-pig
1040 tracheobronchial microcirculation. Effects of allergen, leukotrienes, tachykinins,
1041 and anti-asthma drugs. *Acta Physiol Scand* 1986; 127:95-105.

- 1042 30. Greiff L, Andersson M, Svensson C, Persson CG. Topical azelastine has a 12-hour
1043 duration of action as assessed by histamine challenge-induced exudation of alpha
1044 2-macroglobulin into human nasal airways. *Clin Exp Allergy* 1997; 27:438-44.
- 1045 31. Persson CG. Role of plasma exudation in asthmatic airways. *Lancet* 1986; 2:1126-
1046 9.
- 1047 32. Persson CG. Plasma exudation and asthma. *Lung* 1988; 166:1-23.
- 1048 33. Chung KF, Rogers DF, Barnes PJ, Evans TW. The role of increased airway
1049 microvascular permeability and plasma exudation in asthma. *Eur Respir J* 1990;
1050 3:329-37.
- 1051 34. Greiff L, Andersson M, Erjefalt JS, Svensson C, Persson CG. Loss of size-selectivity
1052 at histamine-induced exudation of plasma proteins in atopic nasal airways. *Clin*
1053 *Physiol Funct Imaging* 2002; 22:28-31.
- 1054 35. Svensson C, Alkner U, Pipkorn U, Persson CG. Histamine-induced airway mucosal
1055 exudation of bulk plasma and plasma-derived mediators is not inhibited by
1056 intravenous bronchodilators. *Eur J Clin Pharmacol* 1994; 46:59-65.
- 1057 36. Out TA, van de Graaf EA, Jansen HM. Permeability or local production of
1058 immunoglobulins and other inflammatory proteins in asthma. *Eur Respir J Suppl*
1059 1991; 13:148s-55s.
- 1060 37. Nocker RE, Out TA, Weller FR, de Riemer MJ, Jansen HM, van der Zee JS. Induced
1061 sputum and bronchoalveolar lavage as tools for evaluating the effects of inhaled
1062 corticosteroids in patients with asthma. *J Lab Clin Med* 2000; 136:39-49.
- 1063 38. Van Rensen EL, Hiemstra PS, Rabe KF, Sterk PJ. Assessment of microvascular
1064 leakage via sputum induction: the role of substance P and neurokinin A in
1065 patients with asthma. *Am J Respir Crit Care Med* 2002; 165:1275-9.

- 1066 39. Greiff L, Andersson M, Svensson C, Akerlund A, Alkner U, Persson CG. Effects of
1067 two weeks of topical budesonide treatment on microvascular exudative
1068 responsiveness in healthy human nasal airways. *Eur Respir J* 1997; 10:841-5.
- 1069 40. Brims FJ, Chauhan AJ, Higgins B, Shute JK. Coagulation factors in the airways in
1070 moderate and severe asthma and the effect of inhaled steroids. *Thorax* 2009;
1071 64:1037-43.
- 1072 41. Greiff L, Andersson M, Svensson C, Nilsson M, Erjefalt I, Erjefalt JS, et al. Topical
1073 nitroprusside may reduce histamine-induced plasma exudation in human nasal
1074 airways. *Allergy* 1995; 50:593-7.
- 1075 42. Van de Graaf EA, Out TA, Roos CM, Jansen HM. Respiratory membrane
1076 permeability and bronchial hyperreactivity in patients with stable asthma. Effects
1077 of therapy with inhaled steroids. *Am Rev Respir Dis* 1991; 143:362-8.
- 1078 43. Pizzichini MM, Pizzichini E, Efthimiadis A, Chauhan AJ, Johnston SL, Hussack P, et
1079 al. Asthma and natural colds. Inflammatory indices in induced sputum: a
1080 feasibility study. *Am J Respir Crit Care Med* 1998; 158:1178-84.
- 1081 44. Luts A, Sundler F, Erjefalt I, Persson CG. The airway epithelial lining in guinea
1082 pigs is intact promptly after the mucosal crossing of a large amount of plasma
1083 exudate. *Int Arch Allergy Appl Immunol* 1990; 91:385-8.
- 1084 45. Rogers DF, Alton EW, Aursudkij B, Boschetto P, Dewar A, Barnes PJ. Effect of
1085 platelet activating factor on formation and composition of airway fluid in the
1086 guinea-pig trachea. *J Physiol* 1990; 431:643-58.
- 1087 46. Erjefalt JS, Erjefalt I, Sundler F, Persson CG. Epithelial pathways for luminal entry
1088 of bulk plasma. *Clin Exp Allergy* 1995; 25:187-95.

- 1089 47. Serikov VB, Jang YJ, Widdicombe JH. Estimate of the subepithelial hydrostatic
 1090 pressure that drives inflammatory transudate into the airway lumen. *J Appl*
 1091 *Physiol* (1985) 2002; 92:1702-8.
- 1092 48. Olivenstein R, Du T, Xu LJ, Martin JG. Microvascular leakage in the airway wall
 1093 and lumen during allergen induced early and late responses in rats. *Pulm*
 1094 *Pharmacol Ther* 1997; 10:223-30.
- 1095 49. Wagner EM, Jenkins J, Schmieder A, Eldridge L, Zhang Q, Moldobaeva A, et al.
 1096 Angiogenesis and airway reactivity in asthmatic Brown Norway rats.
 1097 *Angiogenesis* 2015; 18:1-11.
- 1098 50. Erjefalt I, Luts A, Persson CG. Appearance of airway absorption and exudation
 1099 tracers in guinea pig tracheobronchial lymph nodes. *J Appl Physiol* (1985) 1993;
 1100 74:817-24.
- 1101 51. Du T, Xu LJ, Lei M, Wang NS, Eidelman DH, Ghezzi H, et al. Morphometric changes
 1102 during the early airway response to allergen challenge in the rat. *Am Rev Respir*
 1103 *Dis* 1992; 146:1037-41.
- 1104 52. Malmstrom K, Lohi J, Sajantila A, Jahnsen FL, Kajosaari M, Sarna S, et al.
 1105 Immunohistology and remodeling in fatal pediatric and adolescent asthma.
 1106 *Respir Res* 2017; 18:94.
- 1107 53. Rogers DF. Airway mucus hypersecretion in asthma: an undervalued pathology?
 1108 *Curr Opin Pharmacol* 2004; 4:241-50.
- 1109 54. Greiff L, Svensson C, Andersson M, Persson CG. Effects of topical capsaicin in
 1110 seasonal allergic rhinitis. *Thorax* 1995; 50:225-9.
- 1111 55. Kaulbach HC, White MV, Igarashi Y, Hahn BK, Kaliner MA. Estimation of nasal
 1112 epithelial lining fluid using urea as a marker. *J Allergy Clin Immunol* 1993;
 1113 92:457-65.

- 1114 56. Germonpre PR, Joos GF, Pauwels RA. Characterization of the neurogenic plasma
1115 extravasation in the airways. *Arch Int Pharmacodyn Ther* 1995; 329:185-203.
- 1116 57. Midgren B, Hansson L, Karlsson JA, Simonsson BG, Persson CG. Capsaicin-induced
1117 cough in humans. *Am Rev Respir Dis* 1992; 146:347-51.
- 1118 58. Erjefalt I, Persson CG. Allergen, bradykinin, and capsaicin increase outward but
1119 not inward macromolecular permeability of guinea-pig tracheobronchial mucosa.
1120 *Clin Exp Allergy* 1991; 21:217-24.
- 1121 59. Greiff L, Wollmer P, Pipkorn U, Persson CG. Absorption of ⁵¹Cr EDTA across the
1122 human nasal airway barriers in the presence of topical histamine. *Thorax* 1991;
1123 46:630-2.
- 1124 60. Gustafsson B, Persson CG. Allergen-induced mucosal exudation of plasma into rat
1125 ileum and its inhibition by budesonide. *Scand J Gastroenterol* 1992; 27:587-93.
- 1126 61. Persson CG, Gustafsson B, Erjefalt JS, Sundler F. Mucosal exudation of plasma is a
1127 noninjurious intestinal defense mechanism. *Allergy* 1993; 48:581-6.
- 1128 62. McHugh KJ, Svensjo E, Persson CG. Exudative and absorptive permeability in
1129 different phases of an experimental colitis condition. *Scand J Gastroenterol* 1996;
1130 31:900-5.
- 1131 63. Persson C, Uller L. Transepithelial exit of leucocytes: inflicting, reflecting or
1132 resolving airway inflammation? *Thorax* 2010; 65:1111-5.
- 1133 64. Persson C, Ueki S. Lytic eosinophils produce extracellular DNA traps as well as
1134 free eosinophil granules. *J Allergy Clin Immunol* 2017;141:1164.
- 1135 65. Persson C. Eosinophil apoptosis-inducing drugs risk worsening rather than
1136 resolving asthma. *J Allergy Clin Immunol* 2015; 135:1662.
- 1137 66. Bochner BS, Kiwamoto T, Katoh T, Zhu Z, Tiemeyer M. Reply: To PMID 25497369.
1138 *J Allergy Clin Immunol* 2015; 135:1662-3.

67. Persson C, Uller L. Theirs but to die and do: primary lysis of eosinophils and free eosinophil granules in asthma. *Am J Respir Crit Care Med* 2014; 189:628-33.
68. Persson C, Uller L. Resolution of leucocyte-mediated mucosal diseases. A novel in vivo paradigm for drug development. *Br J Pharmacol* 2012; 165:2100-9.
69. Liu L, Mul FP, Kuijpers TW, Lutter R, Roos D, Knol EF. Neutrophil transmigration across monolayers of endothelial cells and airway epithelial cells is regulated by different mechanisms. *Ann N Y Acad Sci* 1996; 796:21-9.
70. Adcock IM, Caramori G. Chemokine receptor inhibitors as a novel option in treatment of asthma. *Curr Drug Targets Inflamm Allergy* 2004; 3:257-61.
71. Nourshargh S, Hordijk PL, Sixt M. Breaching multiple barriers: leukocyte motility through venular walls and the interstitium. *Nat Rev Mol Cell Biol* 2010; 11:366-78.
72. Erjefalt JS, Sundler F, Persson CG. Eosinophils, neutrophils, and venular gaps in the airway mucosa at epithelial removal-restitution. *Am J Respir Crit Care Med* 1996; 153:1666-74.
73. Baluk P, Bolton P, Hirata A, Thurston G, McDonald DM. Endothelial gaps and adherent leukocytes in allergen-induced early- and late-phase plasma leakage in rat airways. *Am J Pathol* 1998; 152:1463-76.
74. Grega GJ, Adamski SW. The role of venular endothelial cells in the regulation of macromolecular permeability. *Microcirc Endothelium Lymphatics* 1988; 4:143-67.
75. Svensjo E, Arfors KE, Raymond RM, Grega GJ. Morphological and physiological correlation of bradykinin-induced macromolecular efflux. *Am J Physiol* 1979; 236:H600-6.

- 1163 76. McDonald DM. Endothelial gaps and permeability of venules in rat tracheas
1164 exposed to inflammatory stimuli. *Am J Physiol* 1994; 266:L61-83.
- 1165 77. O'Donnell SR, Erjefalt I, Persson CG. Early and late tracheobronchial plasma
1166 exudation by platelet-activating factor administered to the airway mucosal
1167 surface in guinea pigs: effects of WEB 2086 and enprofylline. *J Pharmacol Exp*
1168 *Ther* 1990; 254:65-70.
- 1169 78. Imamura T, Tanase S, Szmyd G, Kozik A, Travis J, Potempa J. Induction of vascular
1170 leakage through release of bradykinin and a novel kinin by cysteine proteinases
1171 from *Staphylococcus aureus*. *J Exp Med* 2005; 201:1669-76.
- 1172 79. Cromwell O, Hamid Q, Corrigan CJ, Barkans J, Meng Q, Collins PD, et al. Expression
1173 and generation of interleukin-8, IL-6 and granulocyte-macrophage colony-
1174 stimulating factor by bronchial epithelial cells and enhancement by IL-1 beta and
1175 tumour necrosis factor-alpha. *Immunology* 1992; 77:330-7.
- 1176 80. Erjefalt JS, Erjefalt I, Sundler F, Persson CG. Mucosal nitric oxide may tonically
1177 suppress airways plasma exudation. *Am J Respir Crit Care Med* 1994; 150:227-
1178 32.
- 1179 81. Blom AM, Hallstrom T, Riesbeck K. Complement evasion strategies of pathogens-
1180 acquisition of inhibitors and beyond. *Mol Immunol* 2009; 46:2808-17.
- 1181 82. Egesten A, Frick IM, Morgelin M, Olin AI, Bjorck L. Binding of albumin promotes
1182 bacterial survival at the epithelial surface. *J Biol Chem* 2011; 286:2469-76.
- 1183 83. van Wetering S, Sterk PJ, Rabe KF, Hiemstra PS. Defensins: key players or
1184 bystanders in infection, injury, and repair in the lung? *J Allergy Clin Immunol*
1185 1999; 104:1131-8.

- 1186 84. Lecaille F, Lalmanach G, Andrault PM. Antimicrobial proteins and peptides in
 1187 human lung diseases: A friend and foe partnership with host proteases.
 1188 *Biochimie* 2016; 122:151-68.
- 1189 85. Akerlund A, Greiff L, Andersson M, Bende M, Alkner U, Persson CG. Mucosal
 1190 exudation of fibrinogen in coronavirus-induced common colds. *Acta Otolaryngol*
 1191 1993; 113:642-8.
- 1192 86. Uller L, Persson C. Viral induced overproduction of epithelial TSLP: Role in
 1193 exacerbations of asthma and COPD? *J Allergy Clin Immunol* 2018.
- 1194 87. Oehmcke-Hecht S, Kohler J. Interaction of the Human Contact System with
 1195 Pathogens-An Update. *Front Immunol* 2018; 9:312.
- 1196 88. Khakpour S, Wilhelmsen K, Hellman J. Vascular endothelial cell Toll-like receptor
 1197 pathways in sepsis. *Innate Immun* 2015; 21:827-46.
- 1198 89. Persson CG, Erjefalt I, Gustafsson B, Luts A. Subepithelial hydrostatic pressure
 1199 may regulate plasma exudation across the mucosa. *Int Arch Allergy Appl*
 1200 *Immunol* 1990; 92:148-53.
- 1201 90. Gustafsson BG, Persson CG. Asymmetrical effects of increases in hydrostatic
 1202 pressure on macromolecular movement across the airway mucosa. A study in
 1203 guinea-pig tracheal tube preparations. *Clin Exp Allergy* 1991; 21:121-6.
- 1204 91. Persson CG, Erjefalt JS, Greiff L, Andersson M, Erjefalt I, Godfrey RW, et al.
 1205 Plasma-derived proteins in airway defence, disease and repair of epithelial
 1206 injury. *Eur Respir J* 1998; 11:958-70.
- 1207 92. Intaglietta M. Microvascular pressure measurements by cannulation:
 1208 independency of concentration gradients and deviations in micro-servonulling.
 1209 *Microvasc Res* 1971; 3:396-9.

- 1210 93. Tokuda S, Miyazaki H, Nakajima K, Yamada T, Marunaka Y. Hydrostatic pressure
 1211 regulates tight junctions, actin cytoskeleton and transcellular ion transport.
 1212 *Biochem Biophys Res Commun* 2009; 390:1315-21.
- 1213 94. Lemarchand P, Chinnet T, Collignon MA, Urzua G, Barritault L, Huchon GJ.
 1214 Bronchial clearance of DTPA is increased in acute asthma but not in chronic
 1215 asthma. *Am Rev Respir Dis* 1992; 145:147-52.
- 1216 95. Steelant B, Farre R, Wawrzyniak P, Belmans J, Dekimpe E, Vanheel H, et al.
 1217 Impaired barrier function in patients with house dust mite-induced allergic
 1218 rhinitis is accompanied by decreased occludin and zonula occludens-1
 1219 expression. *J Allergy Clin Immunol* 2016; 137:1043-53 e5.
- 1220 96. Lakind JS, Holgate ST, Ownby DR, Mansur AH, Helms PJ, Pyatt D, et al. A critical
 1221 review of the use of Clara cell secretory protein (CC16) as a biomarker of acute or
 1222 chronic pulmonary effects. *Biomarkers* 2007; 12:445-67.
- 1223 97. Stenberg H, Wadelius E, Moitra S, Aberg I, Ankerst J, Diamant Z, et al. Club cell
 1224 protein (CC16) in plasma, bronchial brushes, BAL and urine following an inhaled
 1225 allergen challenge in allergic asthmatics. *Biomarkers* 2017:1-10.
- 1226 98. Emmanouil P, Loukides S, Kostikas K, Papatheodorou G, Papaporfyriou A, Hillas
 1227 G, et al. Sputum and BAL Clara cell secretory protein and surfactant protein D
 1228 levels in asthma. *Allergy* 2015; 70:711-4.
- 1229 99. Mackay RM, Grainge CL, Lau LC, Barber C, Clark HW, Howarth PH. Airway
 1230 Surfactant Protein D Deficiency in Adults With Severe Asthma. *Chest* 2016;
 1231 149:1165-72.
- 1232 100. Salomonsson P, Gronneberg R, Gilljam H, Andersson O, Billing B, Enander I, et al.
 1233 Bronchial exudation of bulk plasma at allergen challenge in allergic asthma. *Am*
 1234 *Rev Respir Dis* 1992; 146:1535-42.

- 1235 101. Andersson M, Michel L, Llull JB, Pipkorn U. Complement activation on the nasal
 1236 mucosal surface--a feature of the immediate allergic reaction in the nose. *Allergy*
 1237 1994; 49:242-5.
- 1238 102. Svensson C, Gronneberg R, Andersson M, Alkner U, Andersson O, Billing B, et al.
 1239 Allergen challenge-induced entry of alpha 2-macroglobulin and tryptase into
 1240 human nasal and bronchial airways. *J Allergy Clin Immunol* 1995; 96:239-46.
- 1241 103. Wang Y, Miwa T, Ducka-Kokalar B, Redai IG, Sato S, Gullipalli D, et al. Properdin
 1242 Contributes to Allergic Airway Inflammation through Local C3a Generation. *J*
 1243 *Immunol* 2015; 195:1171-81.
- 1244 104. Krug N, Tschernig T, Erpenbeck VJ, Hohlfeld JM, Kohl J. Complement factors C3a
 1245 and C5a are increased in bronchoalveolar lavage fluid after segmental allergen
 1246 provocation in subjects with asthma. *Am J Respir Crit Care Med* 2001; 164:1841-
 1247 3.
- 1248 105. Lopuhaa CE, de Riemer MJ, Out TA, Sjamsoedin DH, Aalberse RC, Jansen HM, et al.
 1249 Comparison of allergen-induced late inflammatory reactions in the nose and in
 1250 the skin in house dust mite-allergic patients with or without asthma. *Int Arch*
 1251 *Allergy Immunol* 2003; 130:266-74.
- 1252 106. Elovsson S, Smailagic A, Erjefalt I, Zackrisson C, Eriksson C, Wang X. Evaluation of
 1253 nasal barrier dysfunction at acute- and late-phase reactions in a guinea pig model
 1254 of allergic rhinitis. *Vascul Pharmacol* 2005; 43:267-76.
- 1255 107. Thwaites RS, Gunawardana NC, Broich V, Mann EH, Ahnstrom J, Campbell GA, et
 1256 al. Biphasic activation of complement and fibrinolysis during the human nasal
 1257 allergic response. *J Allergy Clin Immunol* 2018.
- 1258 108. Wills-Karp M. Complement activation pathways: a bridge between innate and
 1259 adaptive immune responses in asthma. *Proc Am Thorac Soc* 2007; 4:247-51.

- 1260 109. de Boer JD, Majoor CJ, van 't Veer C, Bel EH, van der Poll T. Asthma and
1261 coagulation. *Blood* 2012; 119:3236-44.
- 1262 110. Meerschaert J, Kelly EA, Mosher DF, Busse WW, Jarjour NN. Segmental antigen
1263 challenge increases fibronectin in bronchoalveolar lavage fluid. *Am J Respir Crit*
1264 *Care Med* 1999; 159:619-25.
- 1265 111. Becky Kelly EA, Busse WW, Jarjour NN. A comparison of the airway response to
1266 segmental antigen bronchoprovocation in atopic asthma and allergic rhinitis. *J*
1267 *Allergy Clin Immunol* 2003; 111:79-86.
- 1268 112. Terada M, Kelly EA, Jarjour NN. Increased thrombin activity after allergen
1269 challenge: a potential link to airway remodeling? *Am J Respir Crit Care Med* 2004;
1270 169:373-7.
- 1271 113. Greiff L, Pipkorn U, Alkner U, Persson CG. The 'nasal pool' device applies
1272 controlled concentrations of solutes on human nasal airway mucosa and samples
1273 its surface exudations/secretions. *Clin Exp Allergy* 1990; 20:253-9.
- 1274 114. Esnault S, Kelly EA, Sorkness RL, Evans MD, Busse WW, Jarjour NN. Airway factor
1275 XIII associates with type 2 inflammation and airway obstruction in asthmatic
1276 patients. *J Allergy Clin Immunol* 2016; 137:767-73 e6.
- 1277 115. Greiff L, Andersson M, Svensson C, Linden M, Wollmer P, Persson CG.
1278 Demonstration of bronchial eosinophil activity in seasonal allergic rhinitis by
1279 induced plasma exudation combined with induced sputum. *Thorax* 1999; 54:33-
1280 6.
- 1281 116. Schoonbrood DF, Lutter R, Habets FJ, Roos CM, Jansen HM, Out TA. Analysis of
1282 plasma-protein leakage and local secretion in sputum from patients with asthma
1283 and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1994;
1284 150:1519-27.

- 1285 117. Pizzichini MM, Pizzichini E, Clelland L, Efthimiadis A, Mahony J, Dolovich J, et al.
 1286 Sputum in severe exacerbations of asthma: kinetics of inflammatory indices after
 1287 prednisone treatment. *Am J Respir Crit Care Med* 1997; 155:1501-8.
- 1288 118. Desai D, Gupta S, Siddiqui S, Singapuri A, Monteiro W, Entwistle J, et al. Sputum
 1289 mediator profiling and relationship to airway wall geometry imaging in severe
 1290 asthma. *Respir Res* 2013; 14:17.
- 1291 119. Persson C, Uller L. Roles of plasma exudation in asthma and COPD. *Clin Exp*
 1292 *Allergy* 2009; 39:1626-9.
- 1293 120. Khor YH, Teoh AK, Lam SM, Mo DC, Weston S, Reid DW, et al. Increased vascular
 1294 permeability precedes cellular inflammation as asthma control deteriorates. *Clin*
 1295 *Exp Allergy* 2009; 39:1659-67.
- 1296 121. Erjefalt I, Persson CG. Increased sensitivity to toluene diisocyanate (TDI) in
 1297 airways previously exposed to low doses of TDI. *Clin Exp Allergy* 1992; 22:854-
 1298 62.
- 1299 122. Svensson C, Andersson M, Greiff L, Alkner U, Persson CG. Exudative
 1300 hyperresponsiveness of the airway microcirculation in seasonal allergic rhinitis.
 1301 *Clin Exp Allergy* 1995; 25:942-50.
- 1302 123. Meyer P, Andersson M, Persson CG, Greiff L. Steroid-sensitive indices of airway
 1303 inflammation in children with seasonal allergic rhinitis. *Pediatr Allergy Immunol*
 1304 2003; 14:60-5.
- 1305 124. Minnicozzi M, Duran WN, Gleich GJ, Egan RW. Eosinophil granule proteins
 1306 increase microvascular macromolecular transport in the hamster cheek pouch. *J*
 1307 *Immunol* 1994; 153:2664-70.
- 1308 125. Widegren H, Korsgren M, Andersson M, Greiff L. Effects of TNFalpha on the
 1309 human nasal mucosa in vivo. *Respir Med* 2007; 101:1982-7.

- 1310 126. Hutt G, Wick H. [Contraction of circular muscle, thickness of inner lining and their
1311 mutual interference in the constriction of tubular transverse sections]. *Pflugers*
1312 *Arch Gesamte Physiol Menschen Tiere* 1954; 258:311-4.
- 1313 127. Aitken ML, Verdugo P. Donnan mechanism of mucin release and conditioning in
1314 goblet cells: the role of polyions. *Symp Soc Exp Biol* 1989; 43:73-80.
- 1315 128. Innes AL, Carrington SD, Thornton DJ, Kirkham S, Rousseau K, Dougherty RH, et
1316 al. Ex vivo sputum analysis reveals impairment of protease-dependent mucus
1317 degradation by plasma proteins in acute asthma. *Am J Respir Crit Care Med* 2009;
1318 180:203-10.
- 1319 129. Sturton G, Persson C, Barnes PJ. Small airways: an important but neglected target
1320 in the treatment of obstructive airway diseases. *Trends Pharmacol Sci* 2008;
1321 29:340-5.
- 1322 130. Proud D. The kinin system in rhinitis and asthma. *Clin Rev Allergy Immunol*
1323 1998; 16:351-64.
- 1324 131. Naylor B, Railey C. A Pitfall in the Cytodiagnosis of Sputum of Asthmatics. *J Clin*
1325 *Pathol* 1964; 17:84-9.
- 1326 132. Montefort S, Roberts JA, Beasley R, Holgate ST, Roche WR. The site of disruption
1327 of the bronchial epithelium in asthmatic and non-asthmatic subjects. *Thorax*
1328 1992; 47:499-503.
- 1329 133. Erjefalt JS, Sundler F, Persson CG. Epithelial barrier formation by airway basal
1330 cells. *Thorax* 1997; 52:213-7.
- 1331 134. Erjefalt IA, Wagner ZG, Strand SE, Persson CG. A method for studies of
1332 tracheobronchial microvascular permeability to macromolecules. *J Pharmacol*
1333 *Methods* 1985; 14:275-83.

- 1334 135. Erjefalt JS, Erjefalt I, Sundler F, Persson CG. Microcirculation-derived factors in
1335 airway epithelial repair in vivo. *Microvasc Res* 1994; 48:161-78.
- 1336 136. Erjefalt JS, Erjefalt I, Sundler F, Persson CG. In vivo restitution of airway
1337 epithelium. *Cell Tissue Res* 1995; 281:305-16.
- 1338 137. Delvaeye M, Conway EM. Coagulation and innate immune responses: can we view
1339 them separately? *Blood* 2009; 114:2367-74.
- 1340 138. Mosesson MW. Fibrinogen and fibrin structure and functions. *J Thromb Haemost*
1341 2005; 3:1894-904.
- 1342 139. Johansson MW, Annis DS, Mosher DF. $\alpha(M)\beta(2)$ integrin-mediated
1343 adhesion and motility of IL-5-stimulated eosinophils on periostin. *Am J Respir*
1344 *Cell Mol Biol* 2013; 48:503-10.
- 1345 140. Plotkowski MC, Zahm JM, Tournier JM, Puchelle E. *Pseudomonas aeruginosa*
1346 adhesion to normal and injured respiratory mucosa. *Mem Inst Oswaldo Cruz*
1347 1992; 87 Suppl 5:61-8.
- 1348 141. Jakiela B, Brockman-Schneider R, Amineva S, Lee WM, Gern JE. Basal cells of
1349 differentiated bronchial epithelium are more susceptible to rhinovirus infection.
1350 *Am J Respir Cell Mol Biol* 2008; 38:517-23.
- 1351 142. Cyranoski D. Surgeon commits misconduct. *Nature* 2015; 521:406-7.
- 1352 143. Erjefalt JS, Korsgren M, Nilsson MC, Sundler F, Persson CG. Association between
1353 inflammation and epithelial damage-restitution processes in allergic airways in
1354 vivo. *Clin Exp Allergy* 1997; 27:1344-55.
- 1355 144. Erjefalt JS, Korsgren M, Nilsson MC, Sundler F, Persson CG. Prompt epithelial
1356 damage and restitution processes in allergen challenged guinea-pig trachea in
1357 vivo. *Clin Exp Allergy* 1997; 27:1458-70.

- 1358 145. Ordonez C, Ferrando R, Hyde DM, Wong HH, Fahy JV. Epithelial desquamation in
1359 asthma: artifact or pathology? *Am J Respir Crit Care Med* 2000; 162:2324-9.
- 1360 146. Davis MS, Schofield B, Freed AN. Repeated peripheral airway hyperpnea causes
1361 inflammation and remodeling in dogs. *Med Sci Sports Exerc* 2003; 35:608-16.
- 1362 147. Davis MS, Freed AN. Repeated hyperventilation causes peripheral airways
1363 inflammation, hyperreactivity, and impaired bronchodilation in dogs. *Am J Respir*
1364 *Crit Care Med* 2001; 164:785-9.
- 1365 148. Mosser AG, Brockman-Schneider R, Amineva S, Burchell L, Sedgwick JB, Busse
1366 WW, et al. Similar frequency of rhinovirus-infectible cells in upper and lower
1367 airway epithelium. *J Infect Dis* 2002; 185:734-43.
- 1368 149. Laitinen LA, Heino M, Laitinen A, Kava T, Haahtela T. Damage of the airway
1369 epithelium and bronchial reactivity in patients with asthma. *Am Rev Respir Dis*
1370 1985; 131:599-606.
- 1371 150. Lackie PM, Baker JE, Gunthert U, Holgate ST. Expression of CD44 isoforms is
1372 increased in the airway epithelium of asthmatic subjects. *Am J Respir Cell Mol*
1373 *Biol* 1997; 16:14-22.
- 1374 151. Spina D, Rigby PJ, Paterson JW, Goldie RG. Autoradiographic localization of beta-
1375 adrenoceptors in asthmatic human lung. *Am Rev Respir Dis* 1989; 140:1410-5.
- 1376 152. Persson CG, Erjefalt JS, Erjefalt I, Korsgren MC, Nilsson MC, Sundler F. Epithelial
1377 shedding--restitution as a causative process in airway inflammation. *Clin Exp*
1378 *Allergy* 1996; 26:746-55.
- 1379 153. Erjefalt JS, Persson CG. Airway epithelial repair: breathtakingly quick and
1380 multipotentially pathogenic. *Thorax* 1997; 52:1010-2.

- 1381 154. Pain M, Bermudez O, Lacoste P, Royer PJ, Botturi K, Tissot A, et al. Tissue
1382 remodelling in chronic bronchial diseases: from the epithelial to mesenchymal
1383 phenotype. *Eur Respir Rev* 2014; 23:118-30.
- 1384 155. Nihlberg K, Larsen K, Hultgardh-Nilsson A, Malmstrom A, Bjermer L, Westergren-
1385 Thorsson G. Tissue fibrocytes in patients with mild asthma: a possible link to
1386 thickness of reticular basement membrane? *Respir Res* 2006; 7:50.
- 1387 156. Gizycki MJ, Adelroth E, Rogers AV, O'Byrne PM, Jeffery PK. Myofibroblast
1388 involvement in the allergen-induced late response in mild atopic asthma. *Am J*
1389 *Respir Cell Mol Biol* 1997; 16:664-73.
- 1390 157. Motojima S, Frigas E, Loegering DA, Gleich GJ. Toxicity of eosinophil cationic
1391 proteins for guinea pig tracheal epithelium in vitro. *Am Rev Respir Dis* 1989;
1392 139:801-5.
- 1393 158. Persson C. Primary lysis of eosinophils in severe desquamative asthma. *Clin Exp*
1394 *Allergy* 2014; 44:173-83.
- 1395 159. Yamada Y, Yoshihara S, Arisaka O. Creola bodies in wheezing infants predict the
1396 development of asthma. *Pediatr Allergy Immunol* 2004; 15:159-62.
- 1397 160. Ogata H, Motojima S, Fukuda T, Makino S. [Creola bodies and the eosinophil
1398 cationic protein in sputum in acute asthmatic attacks with respect to their clinical
1399 significance]. *Arerugi* 1990; 39:1567-75.
- 1400 161. Anderson SD, Kippelen P. Airway injury as a mechanism for exercise-induced
1401 bronchoconstriction in elite athletes. *J Allergy Clin Immunol* 2008; 122:225-35;
1402 quiz 36-7.
- 1403 162. Erjefalt JS, Erjefalt I, Sundler F, Persson CG. Effects of topical budesonide on
1404 epithelial restitution in vivo in guinea pig trachea. *Thorax* 1995; 50:785-92.

- 1405 163. Wenzel SE, Szefer SJ, Leung DY, Sloan SI, Rex MD, Martin RJ. Bronchoscopic
1406 evaluation of severe asthma. Persistent inflammation associated with high dose
1407 glucocorticoids. *Am J Respir Crit Care Med* 1997; 156:737-43.
- 1408 164. Louis R, Schleich F, Barnes PJ. Corticosteroids: still at the frontline in asthma
1409 treatment? *Clin Chest Med* 2012; 33:531-41.
- 1410 165. Erjefalt I, Persson CG. Pharmacologic control of plasma exudation into
1411 tracheobronchial airways. *Am Rev Respir Dis* 1991; 143:1008-14.
- 1412 166. Greiff L, Wollmer P, Andersson M, Svensson C, Persson CG. Effects of formoterol
1413 on histamine induced plasma exudation in induced sputum from normal subjects.
1414 *Thorax* 1998; 53:1010-3.
- 1415 167. Greiff L, Andersson M, Svensson C, Alkner U, Persson CG. Glucocorticoids may not
1416 inhibit plasma exudation by direct vascular antipermeability effects in human
1417 airways. *Eur Respir J* 1994; 7:1120-4.
- 1418 168. Schleimer RP. Glucocorticoids suppress inflammation but spare innate immune
1419 responses in airway epithelium. *Proc Am Thorac Soc* 2004; 1:222-30.

Legend. Figure 1.

This drawing represents three major steps involved in airway microvascular-epithelial exudation of non-sieved plasma proteins. Following local mucosal challenges autacoids induce high permeability gaps between endothelial cells in postcapillary venules (I). The venules belong to a profuse subepithelial microcirculation distinct from the pulmonary circulation²⁰. Through the gaps plasma macromolecules enter interstitial spaces where an increased hydrostatic pressure (II) will impact on basolateral aspects of epithelial cells. A pressure increase of only 5cm H₂O is sufficient for inducing para-epithelial transmission of plasma macromolecules (III). The exudation occurs without prior edema formation, without sieving, and without compromising the epithelial barrier. In accord, histamine-challenged human airway mucosa *in vivo* exudes albumin (Alb) and alpha2-macroglobulin (a2M) at a concentration ratio (a2M/Alb) that agrees with that in circulating plasma^{34, 36}. Further, absorption of molecules deposited on the mucosal surface is not increased. The extravasation of plasma (I) is an active endothelial cell event under physiological and pharmacological control^{74, 75}. The ensuing epithelial passage (III) appears to be a hydrostatic pressure-driven hydraulic mechanism driven by the extravasated plasma itself (II). The microvascular-epithelial exudation of non-sieved plasma macromolecules is a prompt, reversible, repeatable, and usually short-lasting innate immune response.

Legend. Figure 2.

This drawing illustrates events occurring in vivo following epithelial denudation leaving an uninjured basement membrane. No bleeding occurs but the basement membrane is not left naked. Plasma exudation produces, and continuously supplies, a gel-like cover involving a fibrin-fibronectin net. Also leukocytes, especially neutrophils, are recruited and activated. Eosinophils already present in the airway are activated by primary cytotoxicity. The gel serves as a highly active, provisional barrier and milieu for speedy epithelial regeneration. All epithelial cell types bordering the denuded patch, notably including both secretory and ciliated cells, promptly dedifferentiate into flattened regeneration cells. As in micrographs of asthmatic bronchi, basal cells may not readily be distinguished from regeneration cells. Loosely linked the latter migrate from all sides over the denuded patch at a speed of several $\mu\text{m}/\text{min}$. When a new cell cover is complete, plasma exudation stops, the plasma-derived gel dislodges, and proliferation plus redifferentiation towards a normal epithelium begin in the previously denuded area. The epithelial regeneration in vivo alone induces recruitment and activation of granulocytes, hyperexudation, hypersecretion, proliferation not only of epithelial cells but also of fibrocyte/smooth muscle cells, and thickening of epithelial reticular basement membrane. In desquamating asthma multiple patches of epithelial regeneration would thus contribute to known pathogenic inflammatory and remodeling effects.

DCC = dedifferentiating ciliated cell internalising or shedding its cilia, flattening, and migrating.

DSC = dedifferentiating secretory cell releasing its secretory granules, flattening, and migrating.

1480 DRC = dedifferentiated, mesenchymal-like regeneration cells migrating speedily.

1481 IEG = venular inter-endothelial gaps through which non-sieved plasma proteins

1482 extravasate.

1483 N = neutrophils

1484 E = eosinophils undergoing cytolysis and liberating its protein-rich granules.

1485 PFSM = proliferating fibrocyte/ smooth muscle cells.

1486 TRBM = thickened reticular basement membrane.

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Box 1.

Features that define the basic physiology of plasma exudation.

Direction and injury aspects of plasma exudation:

- The outward paracellular, epithelial transmission of macromolecules should be a unidirectional perviousness allowing extravasated plasma proteins to appear locally on the challenged airway mucosal surface without being associated with any increase in inward permeability.
- Although plasma exudation is an important response to epithelial denudation its basic nature involves transmission of macromolecules across pseudostratified airway epithelial linings without compromising the ultrastructure nor the functional tightness of the epithelial lining cells as an absorption barrier.

Epithelial transmission of bulk plasma preventing formation of sub-epithelial edema:

- The epithelial exudation mechanism transmits mucosal challenge-induced, extravasated plasma macromolecules so swiftly that the increased vascular high permeability will not result in edema in the sub-epithelial area of interest.
- Autacoids and drugs known to cause and inhibit, respectively, vascular permeability should not affect the epithelial transmission of extravasated plasma. Indeed, reducing the ability of the epithelial transmission of plasma would promote development of sub-epithelial edema.

Time aspects and reproducibility:

- The epithelial transmission of plasma macromolecules should occur promptly within few minutes after their extravasation. With a short delay it represents the time course of extravasation from the profuse network of sub-epithelial post-capillary venules.
- Thus, the epithelial component of the exudation mechanism sees to it that the mucosal challenges, which increase sub-epithelial venular leakage of plasma macromolecules, will automatically be followed by appearance of extravasated plasma proteins on the mucosal surface at the very site of challenge.
- The plasma exudation response is fully reversible and repeatable.

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Box 2.**Changing concepts of epithelial permeability and regeneration.**

Currently acknowledged permeability concept (largely based on observations in cell cultures and on misrepresentation of *in vivo* data):

- Asthma is characterized by increased permeability to inhaled molecules.

The present permeability concept (based on actual observations *in vivo* in patients):

- Asthma is characterized by unchanged or even decreased permeability to inhaled molecules.

Collateral evidence regarding plasma exudation and epithelial regeneration supporting and expanding 'The present permeability concept':

Epithelial features *in vivo*, summarized below (I-IV), agree with a well-maintained barrier function in airways that, nevertheless, exhibit epithelial shedding and/or plasma exudation.

- I. Reflecting a pronounced asymmetry of the intact pseudostratified epithelium increased airways exudation of non-sieved plasma macromolecules is not associated with increased permeability in the opposite, inward direction. (Specific data obtained in guinea-pig and human airways).
- II. At asthma-like, non-sanguineous denudation a well sustained, plasma exudation-derived active barrier, involving a leukocyte-rich fibrin-fibronectin net, promptly covers the naked basement membrane. (Specific data obtained in guinea-pig airways).
- III. In the dynamic exudate gel cover all types of epithelial cells, bordering the denuded area, promptly dedifferentiate into flattened epithelial regeneration cells that migrate speedily in a tethered fashion over the basement membrane. (Specific data obtained in guinea-pig airways).
- IV. At exposure to inhaled insults, which cause epithelial shedding in asthma, the ensuing denudation areas may be exceedingly patchy. This limitation is important since epithelial regeneration cells are produced all around individual denudation patches. Dedifferentiated epithelial cells will thus rapidly replace provisional covers of plasma exudate-derived gels in small-sized denuded spots. (Specific data obtained in guinea-pigs and with support from observations on patchy denudation in canine airways and asthma).

A novel concept of asthma pathogenesis (based on observations *in vivo* in asthma indicating that epithelial shedding is not associated with increased absorption permeability and on observations *in vivo* in guinea-pigs indicating that epithelial regeneration processes alone produce neutrophil recruitment and activation, lytic activation of eosinophils, plasma exudation, increased mucus secretion, proliferation of fibrocyte/smooth muscle cells, and reticular basement membrane thickening):

- Exaggerated epithelial regeneration at multiple denudation patches, rather than increased epithelial permeability, is pathogenic in asthma.

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