Eur Respir J 1998; 11: 958–970
DOI: 10.1183/09031936.98.11040958
Printed in UK - all rights reserved

European Respiratory Journal
ISSN 0903 - 1936

SERIES 'AIRWAY MUCUS'
Edited by P.K. Jeffery
Number 9 in this Series

Plasma-derived proteins in airway defence, disease and repair of epithelial injury

C.G.A. Persson*+, J.S. Erjefält‡§, L. Greiff#, M. Andersson#, I. Erjefält+, R.W.A. Godfrey‡, M. Korsgren§, M. Linden+, F. Sundler§, C. Svensson#

Plasma-derived proteins in airway defence, disease and repair of epithelial injury. C.G.A. Persson, J.S. Erjefält, L. Greiff, M. Andersson, I. Erjefält, R.W.A. Godfrey, M. Korsgren, M. Linden, F. Sundler, C. Svensson. ©ERS Journals Ltd 1998.

ABSTRACT: One significant characteristic of the airway mucosa *in vivo*, that cannot easily be mimicked *in vitro*, is its microcirculation, which generates a highly dynamic, biologically active milieu of plasma-derived molecules that may pass to the airway lumen *in vivo*.

New data on the mechanisms of airway mucosal exudation indicate that the protein systems of circulating plasma may contribute significantly to the biology and immunology of the lamina propria, its surface epithelium and the luminal surface, not only in injured airways, but also in airways that are activated but display no sign of oedema, epithelial disruption, or increased absorption capacity.

We suggest that present knowledge of the mechanisms of plasma exudation, together with rapidly emerging information (not detailed herein) on receptors, target cells and cellular responses to the plasma-derived molecules, must be considered in any realistic model that investigates "immuno-inflammatory" mechanisms of the airway mucosa.

Eur Respir J 1998; 11: 958–970.

*Dept of Clinical Pharmacology, University Hospital of Lund, Lund, Sweden. +Astra Draco AB, Lund, Sweden. ‡Lung Pathology Unit, Histopathology, National Heart and Lung Institute, Royal Brompton Hospital, London, UK. §Dept of Physiology and Neuroscience, University of Lund, Sweden. #Dept of Otorhinolaryngology, University Hospital of Lund, Sweden.

Correspondence: C. Persson, Dept Clinical Pharmacol, University Hospital, S-221 85 Lund, Sweden
Fax: 46 46 62024

Keywords: Mucosal biology *in vivo,* plasma exudation, plasma proteins

Received: February 11 1998
Accepted for publication February 12 1998

This work was supported by The Swedish Medical Research Council Projects 8308 and 4499, The Vardahl Foundation and Astra Draco, Sweden.

Several basic aspects of airway exudation have been elucidated during the last decade. For example, it has been established that the distribution of extravasated plasma not only includes submucosal tissues, but also the mucosa and its intact airway epithelial lining [1, 2]. Luminal entry of bulk plasma often occurs, as one might expect, in association with epithelial damage and shedding [3, 4]. However, virtually nonsieved plasma exudates can also move between epithelial cells and into the airway lumen even when a structurally and functionally intact epithelial barrier is maintained [2, 5]. Indeed, the luminal entry of plasma is so swift that it is often the case that the process of extravasation does not result in a significant degree of tissue oedema [6]. More important than tissue oedema, therefore, may be the laying down of adhesive and leucocyte-activating proteins, immunoglobulins, growth factors, and other biologically active plasma-derived molecules in the extracellular matrix and on the surface of the airway mucosa as the process of exudation takes place (fig. 1).

Entry of plasma into the airway lumen may seem like a secretory process since it occurs across an intact epithelial lining. Clearly, mucosal exudation, like mucosal secretory activities, qualifies as a first-line respiratory defence mechanism [11]. However, content, mechanisms and routes of luminal entry, inducing factors, inhibitory factors and pharmacological control, make airway mucosal exudation a mechanism entirely distinct from the airway secretory processes described in the previous articles of this review series.

Current studies of mucosal immunology and "inflammation" largely concern the biochemistry of cultured cells dwelling in artificial, yet well-defined, media *ex vivo*. As a consequence, representations of mucosal mechanisms in health and disease have a pronounced, sometimes exclusive, focus on the molecules produced by cells. The present review takes a different but complementary approach and highlights physiological and pathophysiological prerequisites for the participation of plasma proteins in airway mucosal biology. It is envisaged that there is a dynamic

Previous articles in this series: No. 1: P.K. Jeffery, D. Li. Airway mucosa: secretory cells, mucus and mucin genes. *Eur Respir J* 1997; 10: 1655–1662. **No. 2:** W.D. Kim. Lung mucus: a clinician's view. *Eur Respir J* 1997; 10: 1914–1917. **No. 3**: L.D. Martin, L.G. Rochelle, B.M. Fischer, T.M. Krunkosky, K.B. Adler. Airway epithelium as an effector of inflammation: molecular regulation of secondary mediators. *Eur Respir J* 1997; 10: 2139–2146. **No. 4:** R. Wu, Y.H. Zao, M.M.J. Chang. Growth and differentiation of conducting airway epithelial cells in culture. *Eur Respir J* 1997; 10: 2398–2403. **No. 5:** K.C. Kim, K. McCracken, C.Y. Shin, M. Jo, C.J. Lee, K.H. Ho. Airway goblet cell mucin: its structure and regulation of secretion. *Eur Respir J* 1997; 10: 2644–2649. **No. 6:** J.H. Widdicombe. S.J. Bastacky, D.X-Y. Wu, C.Y. Lee. Regulation of depth and composition of airway surface liquid. *Eur Respir J* 1997; 10: 2892–2897. **No. 7:** M. King. Experimental models for studying mucociliary clearance. *Eur Respir J* 1998; 11: 222–228. **No. 8:** T. Miyata, H. Kai, Y. Isohama, K. Takahama. Current opinion of muco-active drug research: strategies and problems. *Eur Respir J* 1998; 11: 480–491.

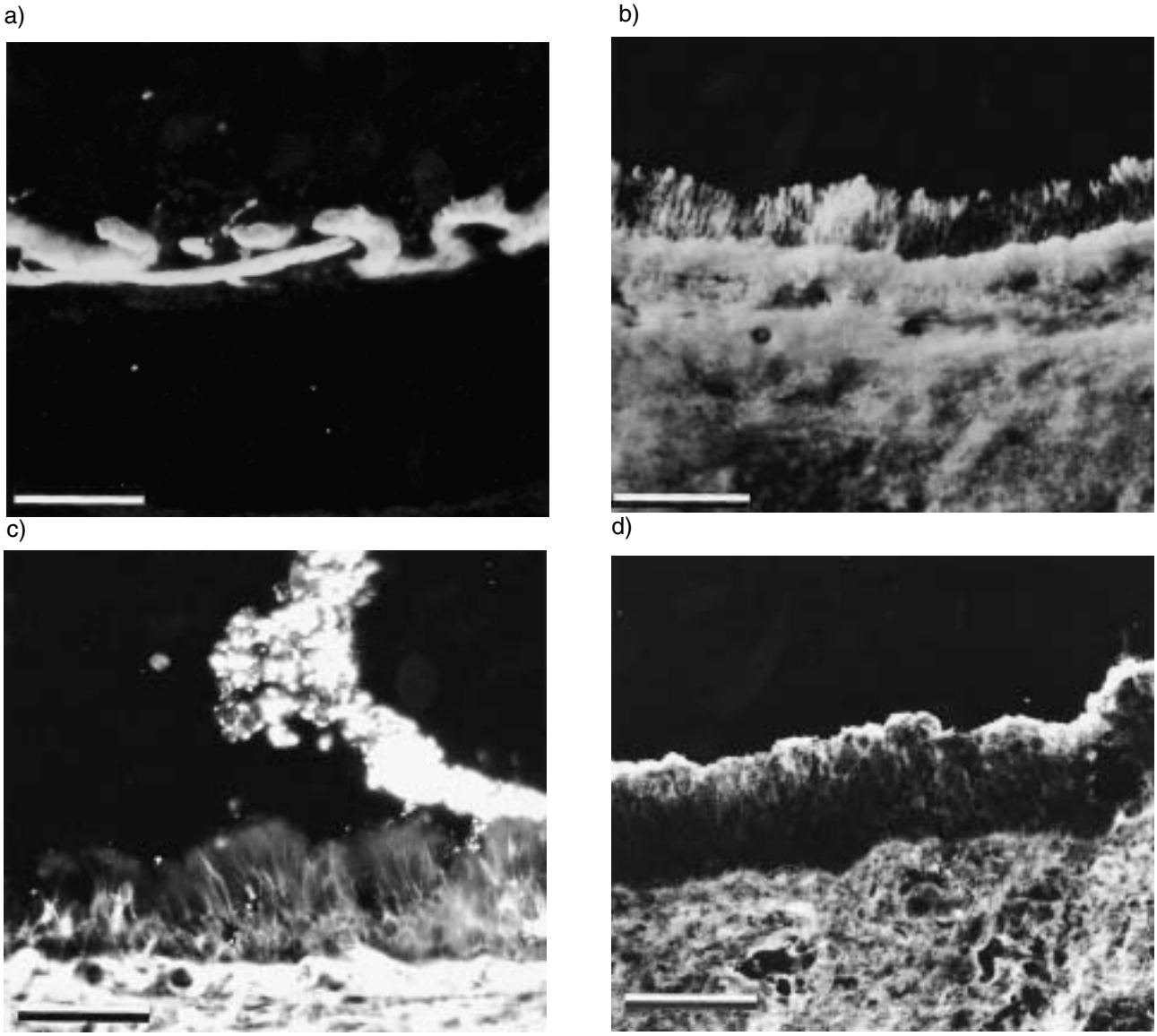

Fig. 1. – a) microvessels containing colloidal gold (diameter 5 nm) are seen just beneath the airway epithelium. Such a profuse and superficial microcirculation is present in guinea-pig trachea (illustrated) and in human nasal and tracheobronchial airways. b) 5 min after mucosal exposure to allergen or leukotriene-type mediators, bulk plasma (colloidal gold) is distributed in the entire lamina propria and epithelium and has entered the airway lumen (not shown). Fluorescent micrographs demonstrate fibrin(ogen) immunoreactivity in tracheal mucosa: c) 1 h after allergen challenge; and d) 5 h after toluene diisocyanate (TDI) challenge (see also figure 2). This adhesive and inflammatory protein is laid down in the extracellular matrix of the mucosa, including, the space around epithelial cells, and on the mucosal surface. Data are from references [7–9] and unpublished observations. Internal scale bars: a) and b) 100 μm; c) and d) 50 μm.

interplay between plasma-derived molecules, their receptors and airway epithelial cells and their secretions *in vivo*, which affect airway defence or induce disease.

Extravasation of plasma from the mucosal microcirculation

In human nasal and tracheobronchial airways and in guinea-pig trachea there is a profuse network of microvessels immediately beneath the pseudostratified epithelium (figs. 1, 3). When the airways are subjected to an inflammatory insult, the endothelium of the post-capillary venules of the bronchial microvasculature is promptly affected by agents that alter its permeability [3, 4, 6, 11]. Mediators such as bradykinin, histamine, leukotriene D_4, platelet activating factor (PAF)-acether and others, applied topically to the airway mucosa, produce a marked increase in bronchial venular permeability [6, 11]. The formation of small interendothelial pores is associated with increased permeability [3, 12, 13] (fig. 3). Through these pores bulk plasma is driven into the extravascular space by hydrostatic pressure forces. Local colloid osmotic pressure gradients are thus abolished [14] and the lamina propria becomes endowed with macromolecules. Complete distribution of plasma into the extracellular matrix of the subepithelial zone may be accomplished during the first minute after mucosal challenge [7]. Virtually "nonsieved" plasma also traverses the epithelial basement membrane and moves up between surface epithelial cells, gaining entry into the airway lumen [1, 6, 7] (fig. 1).

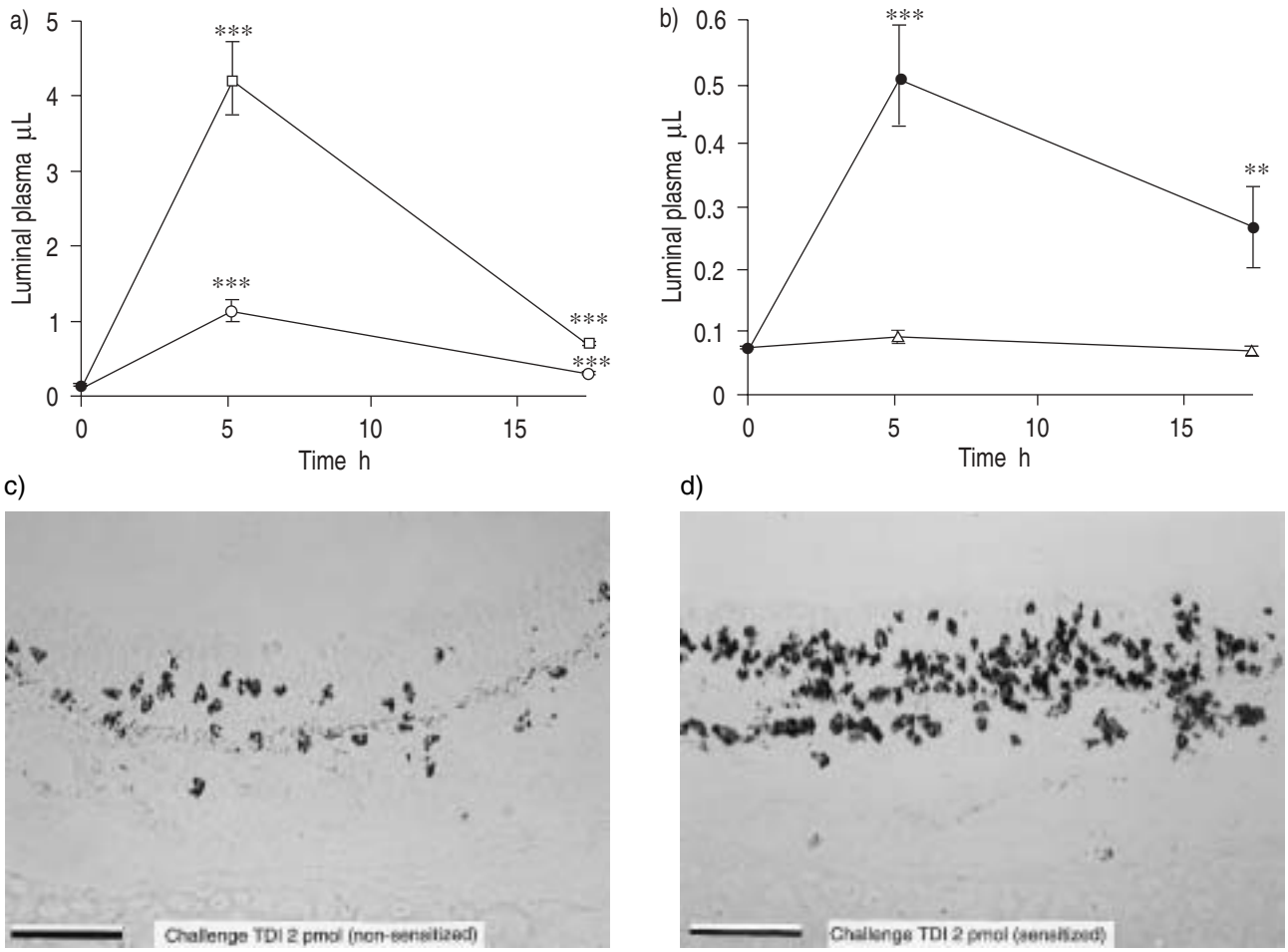

Fig. 2. – a) Toluene diisocyanate (TDI) applied at concentrations of 200 pmol (□) or 20 pmol (○) on the airway mucosa of previously unexposed guinea-pigs causes dose-dependent and sustained plasma exudation responses. b) after repeated exposure to 20 pmol of TDI (●) the airway mucosa develops an increased sensitivity and responds significantly to the exceedingly low dose of 2 pmol, whereas unsensitized mucosa (Δ) shows no response. This response is associated with the marked mucosal eosinophilia shown in the micrographs from c) nonsensitized and d) sensitized mucosa. Data are from references [6, 9–10] and unpublished observations. Internal scale bars=60 µm.

The nonsieved nature of exuded plasma

The maintenance of plasma-like concentrations by different-sized proteins in the airway lumen indicates that molecules ranging from albumin (60 kDa) to α_2-macroglobulin (700 kDa) may all move equally and swiftly across all the barriers that exist between the venular compartment and the luminal surface of the airway mucosa (fig. 4) [1, 6. 15]. As a corollary, the acute bronchial exudative response to allergen challenge may be best measured by the increase in bronchoalveolar lavage (BAL) fluid levels of large proteins such as α_2-macroglobulin [16]. BAL albumin levels are already high and variable at baseline; albumin is, therefore, not always a reliable index of bronchial exudation of plasma [15].

Due to unknown degrees of concentration (or dilution) the actual volumes of the plasma exudate entering the airway lumen have not been assessed. A cautious estimate is that less than 1 mL of bulk plasma enters the nasal passages during an acute allergic reaction with symptoms. The limited volume that is exuded per unit time further underscores the notion that its content of bioactive proteins and peptides is probably of greater importance than whether or not oedema develops.

Bioactive molecules of the plasma exudate

In airway conditions where there is exudation, the lamina propria, the epithelial basement membrane, the epithelial lining and the mucosal lumenal surface are endowed with pluripotent protein systems that are normally, in less active forms, retained within the vascular compartment [15] (fig. 5). The extravasated plasma contains adhesive and leucocyte-activating proteins such as fibrin(ogen) and fibronectin [4, 19–21]. These plasma-derived proteins are laid down along pathways [7], which include the paracellular epithelial routes through which leucocytes pass to the lumen (fig. 1). The complement family and the coagulation cascade of bioactive molecules have been extensively examined [22, 23] and their potential roles in the airway mucosa deserve attention. For instance, fibrin degradation products act as potent growth factors on a variety of cells [24, 25].

Other examples of plasma-derived mediators include kinins, immunoglobulins, proteases and antiproteases. Plasma further contains cytokines that, despite their low concentrations, may well exert biological actions [26]. Through its capacity to target other molecules [27], α_2-macroglobulin may have profound influences on cytokines and other

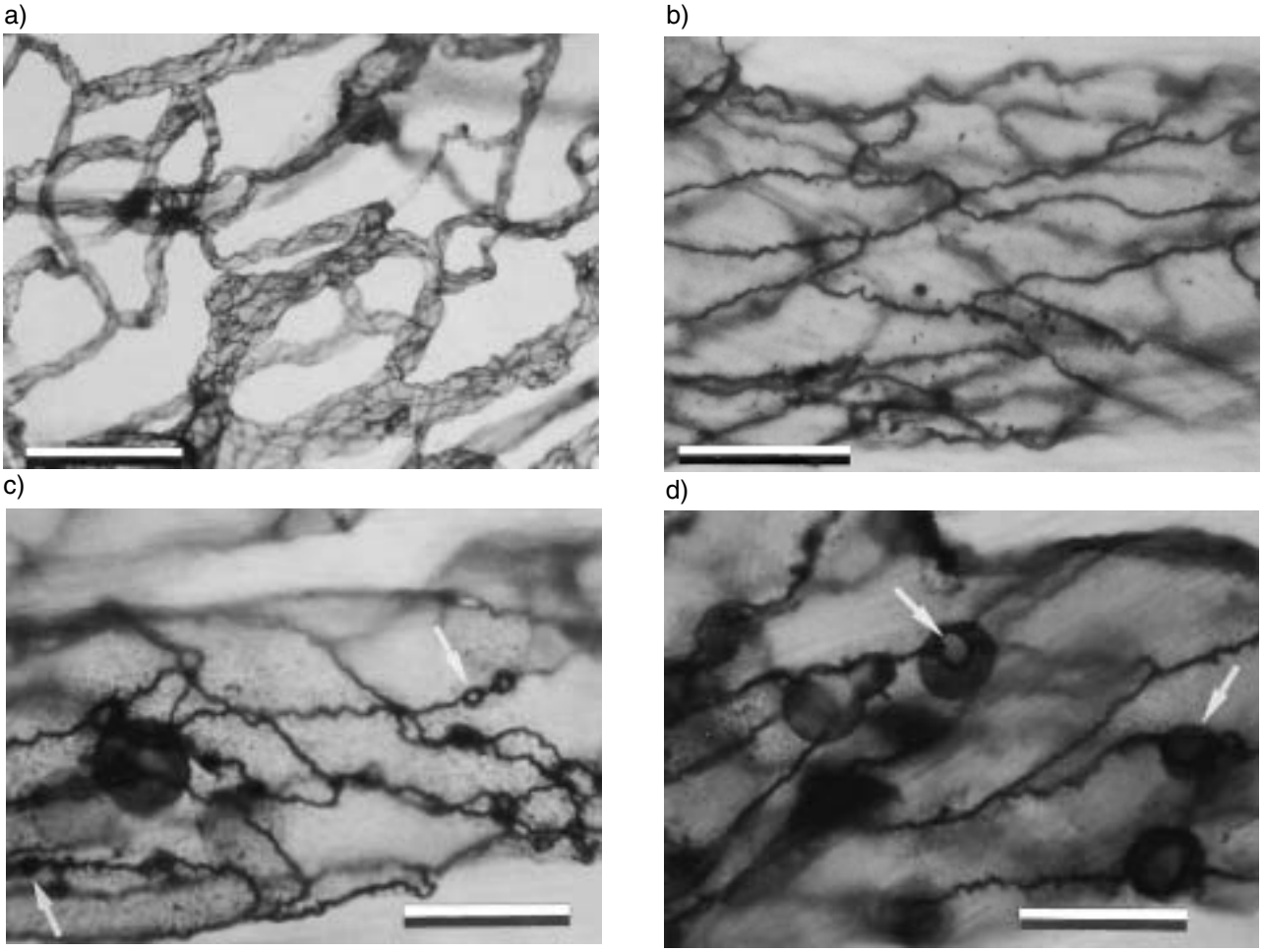

Fig. 3. – Light micrographs demonstrating subepithelial venules in airway mucosal wholemount preparations. The endothelial cell borders are outlined as silver-stained black lines. They are intact in baseline conditions (a, b). In airways challenged with inflammatory mediators the endothelial cells become distinctly separated producing small round holes or pores (arrows; c) through which bulk plasma exits. If the inflammatory response has the capacity to recruit leucocytes, these will also exit across the venular wall (arrows; d) but not through the pores. Data are from reference [3] and unpublished observations (see also references [12, 13]. Internal scale bars: a) 150 μm; b), c) and d) 20 μm.

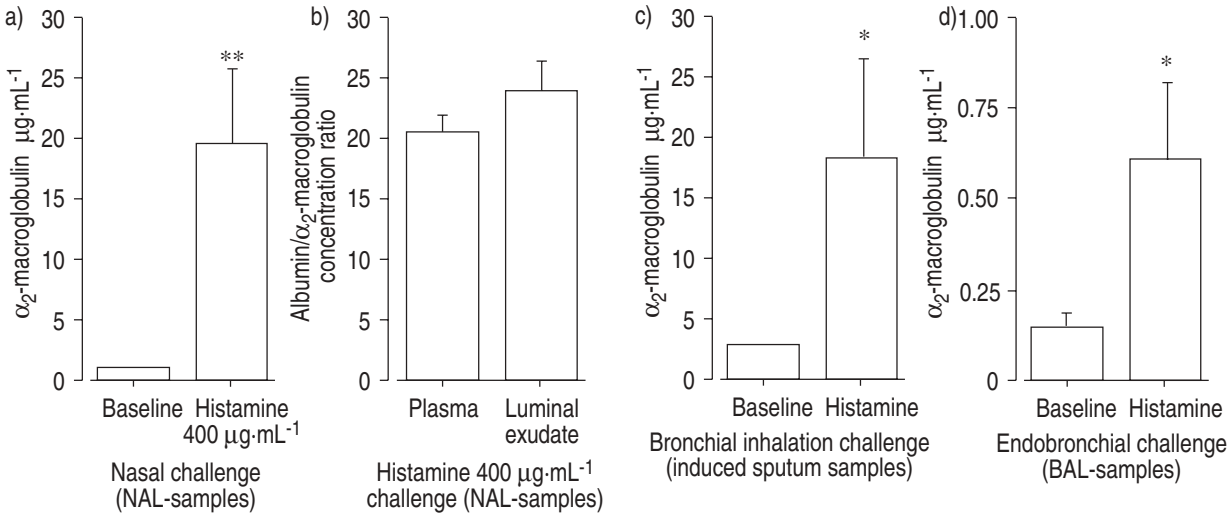

Fig. 4. – Acute human airway exudation of bulk plasma. a) histamine (400 μg·mL^{-1}) causes mucosal exudation of albumin (not shown) and α_2-macroglobulin in human nasal airways. b) the mucosal exudation response involves little sieving of the plasma macromolecules, as indicated by similar concentration ratios between albumin (MW 69 kDa) and α_2-macroglobulin (MW 700 kDa) in plasma and on the surface of the histamine-challenged airway. Note that histamine, even at 2,000 μg·mL^{-1}, may not affect the nasal mucosa as an absorption barrier [5]. Histamine inhalation and endobronchial allergen application also cause prompt bronchial mucosal exudation of α_2-macroglobulin as determined in samples of c) induced sputum and d) bronchial lavage, respectively. Data are from references [16–18] and published observations. *, **: $p<0.05$, $p<0.01$ *versus* baseline. NAL: nasal airway lavage; BAL: bronchoalveolar lavage.

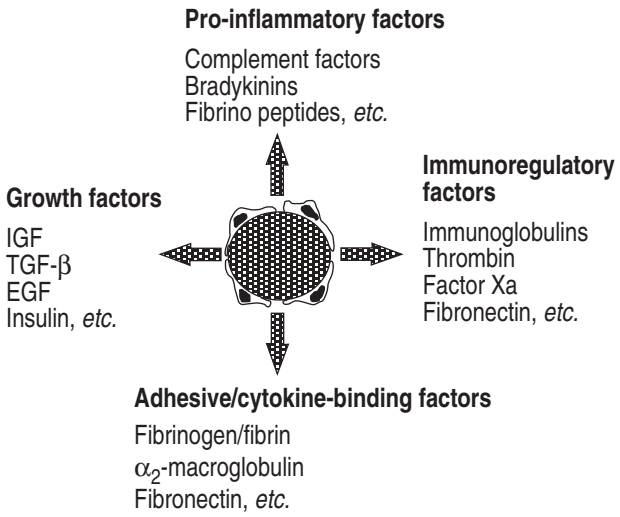

Fig. 5. – The exudation process will rapidly and efficiently distribute a variety of plasma-derived, bioactive molecules throughout the extracellular matrix in the subepithelial tissue, the epithelium, and airway lumen.

cell-derived proteins in the airways (see below). Taken together, an overwhelming variety of plasma-derived molecules, including as yet unidentified proteins and peptides, will participate in the process of airway inflammation which normally serves to protect the host by a transient defence reaction [2] or, if inappropriate and chronic, may be injurious [3, 15].

Epithelial passage of bulk plasma

Extravasated bulk plasma readily traverses a normal airway epithelial lining [1]. Accordingly, the acute extravasation response may not lead to mucosal oedema [6] or to increases in airway lymph node levels of plasma proteins; the exudation process may, thus, occur and yet not be detectable by the commonly used analytical methods [6, 28, 29].

Contrary to current beliefs, luminal entry of bulk plasma causes no disturbances of the pseudostratified epithelial lining, which remains uncompromised, both structurally (as assessed by light microscopy and transmission electron microscopy), and functionally (determined by absorption of small and large molecules) [2, 5]. In an as yet unexplained manner, the extravasated macromolecules pass through the epithelial tight junctions situated at the apicolateral borders of the epithelial cells. It is these specialized membrane structures that are the final selectively permeable barrier to the passage of the plasma into the airway lumen [30] (fig. 6). As with the alveolar lining epithelium, it is the "tightness" of the epithelial tight junctions that largely determines whether tissue oedema will follow bulk extravasation of plasma. Whilst the occurrence of luminal entry of bulk plasma, without impeding the capacity of the mucosa to act as an absorption barrier, is a potent first line respiratory defence response [2], questions remain with regard to the mechanisms involved. How could the airway epithelium, famous for its tightness, suddenly let through molecules the size of α_2-macroglobulin?

One of the few possibilities compatible with the many observations on luminal entry of bulk plasma in both animals and humans was that the extravasated plasma itself had caused its own epithelial passage, by mechanisms involving an increased basolateral pressure load on the epithelial surface cells [32]. This hypothesis (fig. 5) was testable. It required a system in which the epithelial barrier could be examined separately from that of the microcirculation; otherwise the mucosa should remain as it is *in vivo*. Such a test system was achieved in the carefully isolated tracheal tube preparations, maintained in an organ bath and mounted such that serosal and mucosal surface solutions were separated, allowing studies of the influence of a hydrostatic pressure load on either side of the epithelium. Extreme care was required to avoid edge trauma yet still provide control over hydrostatic pressure changes and sampling procedures [32].

Observations made in the intact tube preparation [5, 32] support the above hypothesis by demonstrating that: 1) the extravasating bulk plasma moving between the columnar epithelial cells needs to increase the local hydrostatic pressure by only a few cmH_2O to create ubiquitous paracellular passage routes for macromolecules into the lumen (paracellular stretches of 30–40 m would be available for this passage per square centimetre of mucosal surface); 2) the luminal entry operates without the need for the pharmacological effects of mediators that act directly on the epithelial barrier [32]; and 3) the hydraulic ("plasma exudate-epithelial") mechanism of luminal entry of macromolecules exhibits reversibility, repeatability and direction-selectivity in agreement with the *in vivo* observations [5].

Neurogenic exudative inflammation - burnt out?

Using a compressible nasal "pool device", a well-defined area of airway mucosal surface can be kept in contact with known concentrations of agents and tracers. After the decided length of exposure time the instilled fluid is readily recovered into the "pool device" providing the opportunity to quantify exclusively from the area of interest [33]. As a lavage procedure the nasal pool method can be repeated *ad libitum* apparently without causing disturbances to the nasal mucosa. Exudative responses can, thus, be examined to advantage in the nose. Furthermore, the findings in the nose may also reflect those that occur in the human tracheobronchial tree [17].

The contractile and secretologue effects of the neurotransmitter acetylcholine, and its analogues (*e.g.*, methacholine, carbachol) are virtually without exudative effects in nasal and bronchial airways of animals and humans [6, 34]. In human nose, irritants such as nicotine and capsaicin, evoke pain and mucin secretion (assessed as luminal fucose), but no plasma exudation [35, 36] (fig. 7). Thus, at maximal secretory doses [35] in healthy subjects as well as in patients suffering from allergic rhinitis we could not detect a trace of neurogenic exudation [35, 36] (unpublished observations). Admittedly, these observations cannot disprove the possibility that large doses of capsaicin may still be associated with leakage of plasma, as has been reported recently [32]. The negative nasal findings have recently been extended to human bronchi, in which inhalation of histamine was found to increase the luminal entry of α_2-macroglobulin by several times, whereas significant

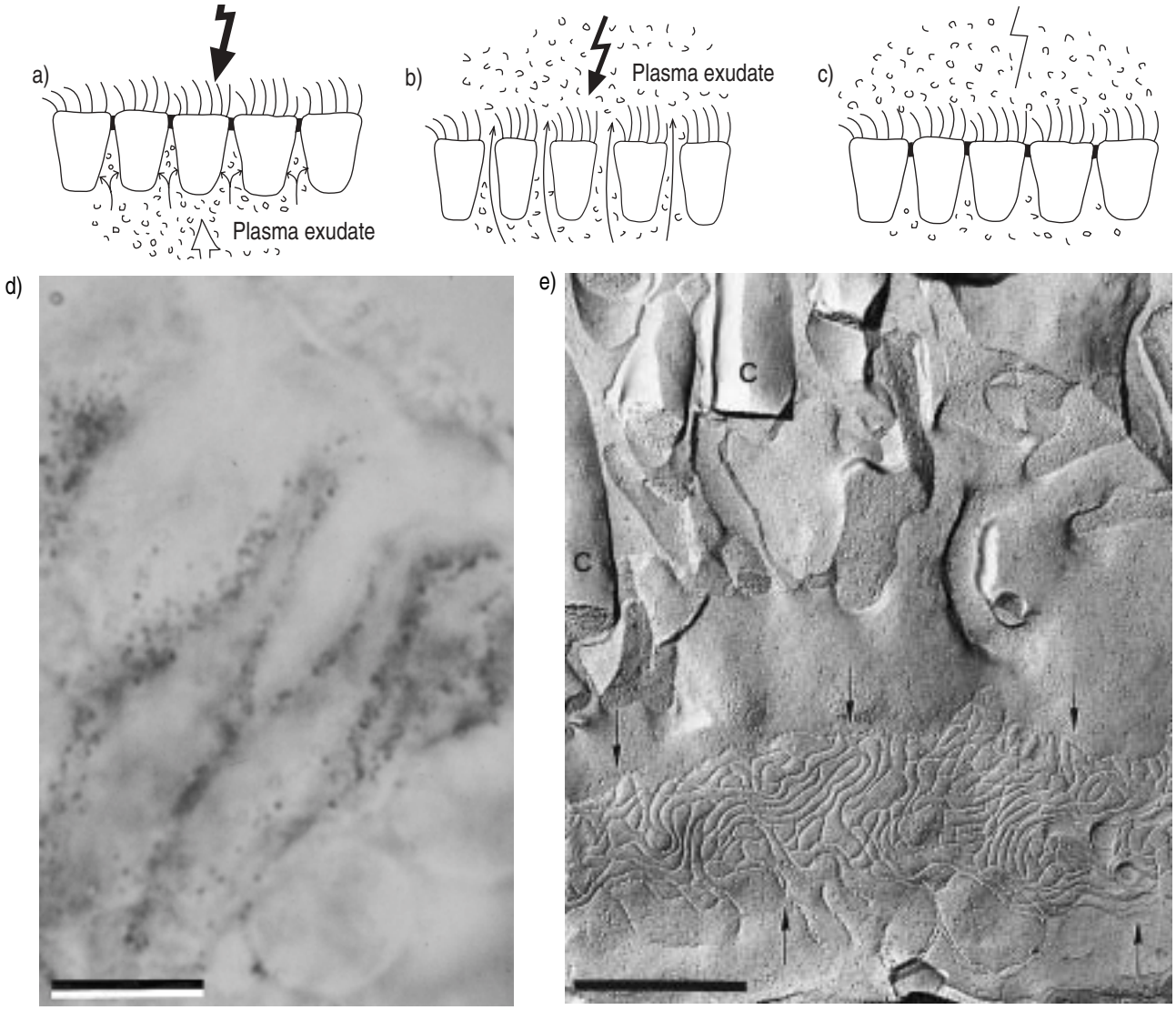

Fig. 6. – a) after extravasation, bulk plasma moves up between epithelial cells in the challenge area. Through a slight hydrostatic pressure load (<5 cmH_2O) on the basolateral aspects of the epithelial cells, potentially caused by the extravasated plasma itself. b) unidirectional flux of macromolecules into the airway lumen is effected. c) thus, bulk plasma appears on the mucosal surface without loss of epithelial integrity. The hypothesis is that the intact airway epithelial lining has a hydraulically operated valve function, in agreement with a wide range of *in vivo* data and directly supported by findings in intact airway tube preparations (see text). d) a light micrograph illustrates extravasated gold particles between and around epithelial cells a few minutes after local allergen challenge of the airway mucosa of sensitized guinea-pigs. The exceedingly small extracellular space in the tight junctional area makes it almost impossible to observe the passage of plasma tracers in this apical paracellular region. e) example of a tight junctional belt as seen by freeze fracture-transmission electron microscopy found in the ciliated epithelium of a human main bronchus. The belt is comprised of many interconnecting strands/grooves (arrows) indicating by the "count strand hypothesis" of CLAUDE [31] that the barrier formed by the junction is relatively "tight". (Data from references [7, 30] and unpublished observations). C: cilia. Internal scale bars: d) 6 μm; e) 0.5 μm.

cough-inducing capsaicin inhalations were completely lacking in this effect [37] (measured as concentrations of α_2-macroglobulin in induced sputum). Such human data (fig. 7) are in contrast to findings in the airways of healthy guinea-pigs: here agents such as capsaicin produce pronounced exudation of bulk plasma into the airway lumen [39, 40]. Whilst rodent data are now the basis for a widely acknowledged notion of "neurogenic airways inflammation", we suggest that the phenomenon of neurogenic airway inflammation is more relevant to rodents than to humans.

Exudative agents

In contrast to neuropeptides, leukotriene-type mediators (histamine, bradykinin, leukotriene D_4, PAF-acether *etc*) produce graded exudative responses over a wide range of concentrations in both guinea-pig and human airways [6, 11, 33, 39–42] (cf. figs. 4 and 7). There is also the possibility that exudative agents act through inhibition of mucosal nitric oxide that may tonically suppress airways plasma exudation [43]. Eosinophil granule proteins increase vascular permeability in the hamster cheek pouch [44], and in guinea-pig trachea (unpublished observations by Erjefält *et al*.). Certain cytokines, proteases and fibrinolytic peptides may also induce a prompt exudative response as will any agent that has a capacity to release vasoactive agents *in vivo*. A variety of autacoids cause extravasation of plasma in the systemic microvascular beds of many organs, including the airways [45, 46]. It should be noted that the pulmonary microvasculature, in

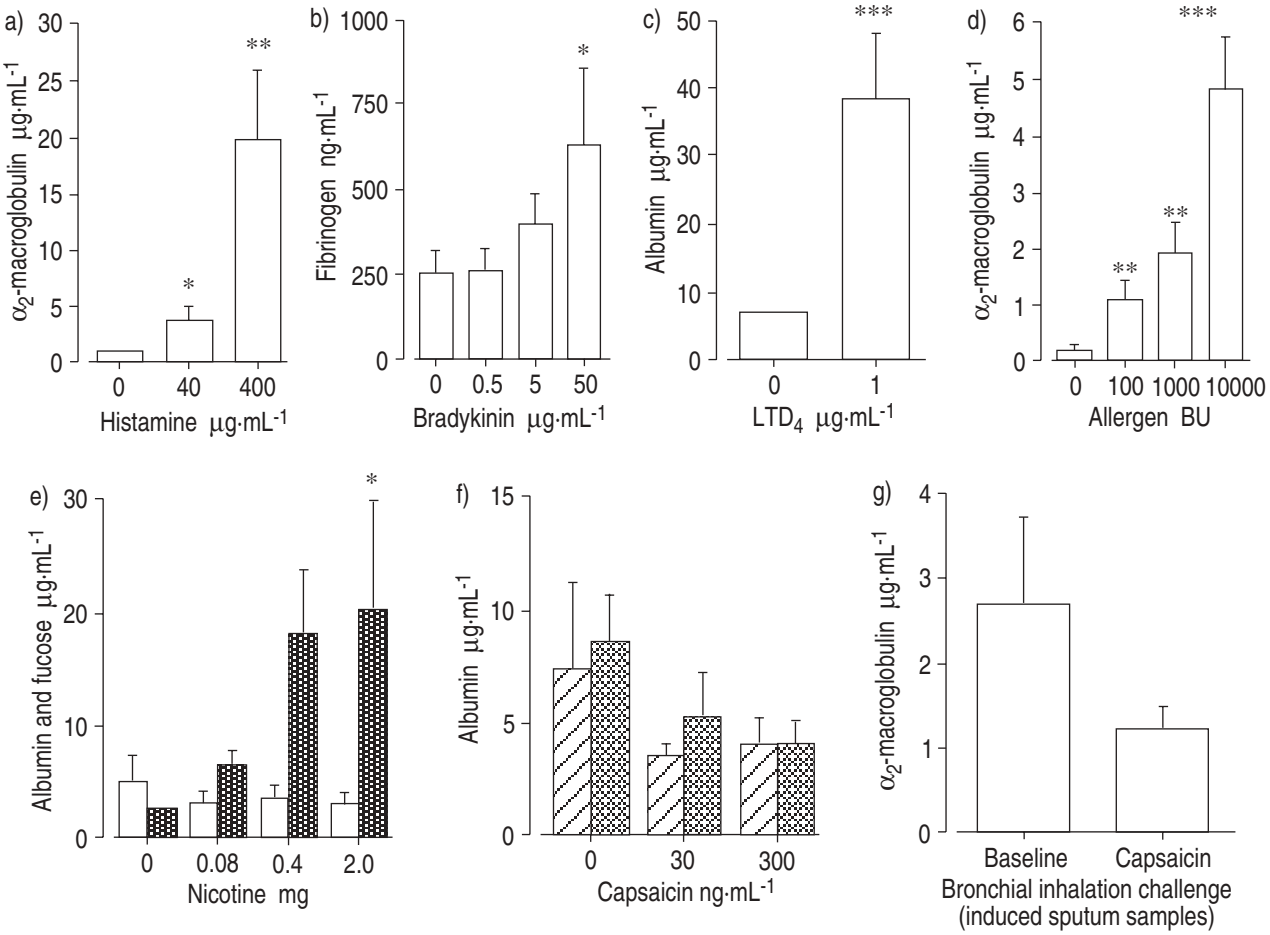

Fig. 7. – Inflammatory mediators such as: a) histamine; b) bradykinin; and c) leukotriene D_4 (LTD_4) are effective exudative agents in human nasal airways, as is d) allergen in allergic individuals. However, at doses which produce pain and mucus secretion, neurogenic agents such as: e) nicotine (: albumin; : fucose) and f) capsaicin (: prior to season; : late in season) have not induced plasma exudation in the human nose. g) inhalation of capsaicin causes strong cough responses but bronchial mucosal exudation of plasma is not detected. (Compare with figure 4, where histamine inhalation-induced exudation is given as a positive control). Data are from references [16, 17, 33, 35–37] and unpublished observations. *, **, ***: $p<0.05$, $p<0.01$, $p<0.001$. NAL: nasal airway lavage; BAL: bronchoalveolar lavage.

contrast to that of the bronchial (systemic) microcirculation, is insensitive to several common inflammatory mediators such as histamine [14, 46].

Nasal and endobronchial challenge experiments have demonstrated that the human airway mucosa responds to allergen with prompt luminal entry of bulk plasma, which includes α_2-macroglobulin and fibrinogen [16, 18, 21]. Allergen challenge in subjects with allergic asthma produces both immediate and late-phase responses during which there is plasma exudation [16, 47]. In guinea-pigs, challenge of the tracheal mucosa by allergen (in sensitized animals) or PAF-acether also produces similar dual plasma exudation responses [40, 48, 49]. The immediate phase is largely over within 1 h. It is followed by a "late phase" of airway exudation that peaks about 5 h after challenge. Single dose topical treatment with clinically effective anti-asthma steroids prevents the late phase exudation [10, 50].

In contrast to allergens, the small molecular weight occupational chemical, toluene-diisocyanate (TDI), evokes plasma exudation responses in airways that have not previously been exposed to TDI. Within the dose range of 20–200 pmol, TDI produces dose-dependent plasma exudation into guinea-pig airways [6, 8]. These doses, applied to a defined surface area of the large airway of guinea-pigs, may be compared to the accepted occupational exposure level which corresponds to a daily human body burden of about 100 pmol TDI. Following a single dose of TDI, exudation starts promptly, peaks 5 h after challenge, and continues for about 15 additional hours (fig. 2). Longstanding epithelial and smooth muscle abnormalities ensue; tracheobronchial tone and the number of secretory cells are significantly increased several weeks after the single TDI challenge [8].

Guinea-pigs that receive repeated challenges with 20 pmol of TDI given to large tracheobronchial airways develop an increased inflammatory responsiveness to TDI [9]. Thus, challenge with exceedingly low doses of TDI (2 pmol), in sensitized animals, is associated with a marked and sustained exudative response and a pronounced eosinophilia (fig. 2). This TDI-induced exudative response, in contrast to that observed in nonsensitized animals, is inhibited by glucocorticoid pretreatment [9]. The mediators involved in plasma exudation evoked by such low and occupationally relevant doses of TDI have not yet been determined.

"Induced exudation" to monitor airway inflammation

Many molecules are bound by albumin and, for example, α_2-macroglobulin has a demonstrated capacity to bind cytokines [27] and eosinophil cationic protein (ECP) [51]. Hence, it is anticipated that the exudative process may bring with it significant numbers of markers of inflammation (*i.e.*, pro-inflammatory mediators present in the extracellular compartment of the inflamed mucosa), to the airway surface [15]. New observations may already support this hypothesis. Acute exudations of α_2-macroglobulin, induced by nasal histamine challenges in subjects with allergic rhinitis, have been associated with significantly increased luminal levels of IL-6 [52]. WOOLLEY *et al*. [53], examining effects of allergen challenge in allergic asthma, have recently observed increased luminal levels of granulocyte-monocyte colony stimulating factor (GM-CSF) in the bronchi along with decreased mucosal tissue levels of this cytokine. We speculate that these data may, in part, be explained by their transport during plasma exudation, which is probably evoked by the allergen challenge [16], bringing tissue cytokines to the airway lumen [15].

Both in the nose and the bronchi, histamine challenge-induced exudation has been instrumental in the demonstration of elevated eosinophil cationic protein (ECP), otherwise not evident in lavage and sputum samples. The "induced exudation" method has, thus, uncovered eosinophilic activity in the nasal mucosa of allergic children [54]. Equally, the "induced exudation" disclosed a significant bronchial eosinophilic activity (*i.e.*, increased sputum ECP levels) in adult subjects suffering from seasonal allergic rhinitis [55].

One basic functional aspect of our hypothesis is that the process of exudation normally serves to rid the airway mucosa of potentially harmful molecules [2, 15]. Another inference is that plasma exudation may be an important determinant of bioactive molecules of the airway mucosa *in vivo*, not only in its own right, but also with regard to local distribution of cell-derived molecules. Finally, one of the practical corollaries is that the dual induction method, "induced exudation" followed by induced sputum, may prove to be a valuable procedure in the future, which could provide an insight into the nature of bronchial inflammation in distinct airway diseases and conditions [55].

Epithelial shedding-induced plasma exudation

Epithelial shedding to the point of denudation, may induce several tissue responses that may primarily serve to heal damaged areas. Potentially the most important repair and defence-promoting response organ during the first phases of repair is the mucosal microcirculation. Within a minute after epithelial shedding *in vivo*, interendothelial permeability-pores are formed along the venular wall (fig. 3) and a pronounced, local exudative responses is initiated [3, 4]. Extravasated bulk plasma immediately enters the airway lumen at the site of denudation where a gel-like network of fibrin and fibronectin fibres, with other included plasma proteins, is formed on the denuded but intact basement membrane [4, 58]. Thus, the local extracellular milieu will be endowed promptly with plasma-derived molecules such as fibronectin (fig. 8) having defence, and repair-promoting activities [20]. Another tissue response, which is initiated by epithelial shedding, is the local recruitment and activation of leucocytes (fig. 3) including the activation of eosinophils in the airway wall [3, 58]. During the first few hours after epithelial shedding *in vivo* there is increased exudation of plasma that continues until a new epithelial cell cover is present. Then, there is increased proliferation both in the epithelium and in subepithelial fibroblasts and smooth muscle cells at the site of the lesion [58].

There is a wealth of data (and concepts) arising from experimental systems *in vitro*: cell culture studies totally dominate the current epithelial research scene. This may be unfortunate, as the *in vivo* events in this field of investigation (epithelial injury-restitution processes and their sequelae) may not readily be mimicked experimentally *in vitro* where epithelial cells dwell in culture media. These are some of the recently unravelled features of epithelial restitution following epithelial shedding as they occur in the relevant *in vivo* milieu [58, 63]. Allergen challenge-induced epithelial patches of injury are dynamic foci where concomitant epithelial shedding and repair processes occur in an exudate-derived molecular milieu [59, 60] (fig. 8). Eosinophil cytolysis and large numbers of clusters of free eosinophil granules (Cfegs) are also associated with these sites [59, 64]. The patchy accumulation of activated leucocytes and potent plasma-derived proteins agrees with findings of conglomerates of shed epithelia, necrotic leukocytes, Cfegs and plasma proteins in airway discharge material in asthma and allergic rhinitis [65–68]. Another clinical inference is that the early appearance of a continuous lining following epithelial restitution after injury may in part explain the findings of an unchanged, or even a decreased, airway absorption permeability in allergic airway disease [3, 69]. The pluripotential roles of events associated with epithelial shedding-restitution in the pathogenesis of airway diseases have been reviewed elsewhere [61, 62].

Role of plasma exudation in disease

Even if extravasation of plasma into the airway tissue frequently does not produce mucosal oedema, there are several other sequelae to be considered [65, 70]. As discussed above, plasma is an important source furnishing the mucosal extracellular matrix with protein components having multiple biological activities, without which airway inflammation may not develop. Because of its physical properties and interactions, a plasma exudate may impede the patency of the lower airway passages in several ways, which have been described [65, 70]. Exuded plasma may accumulate in the airways, particularly during late night and early morning hours [17]. It contributes to so-called "mucous plugs" that are actually "plasma-mucous plugs" [71]. Against a background of mucosal thickening and stagnated exudate mucous in the lumen, an attack of asthma may cause severe and life-threatening obstruction of the bronchi, even with moderate bronchial smooth muscle contraction [70]. SCHOONBROOD *et al.* [56], who examined plasma proteins in sputum samples, concluded that plasma leakage in asthma correlates with airway responsiveness.

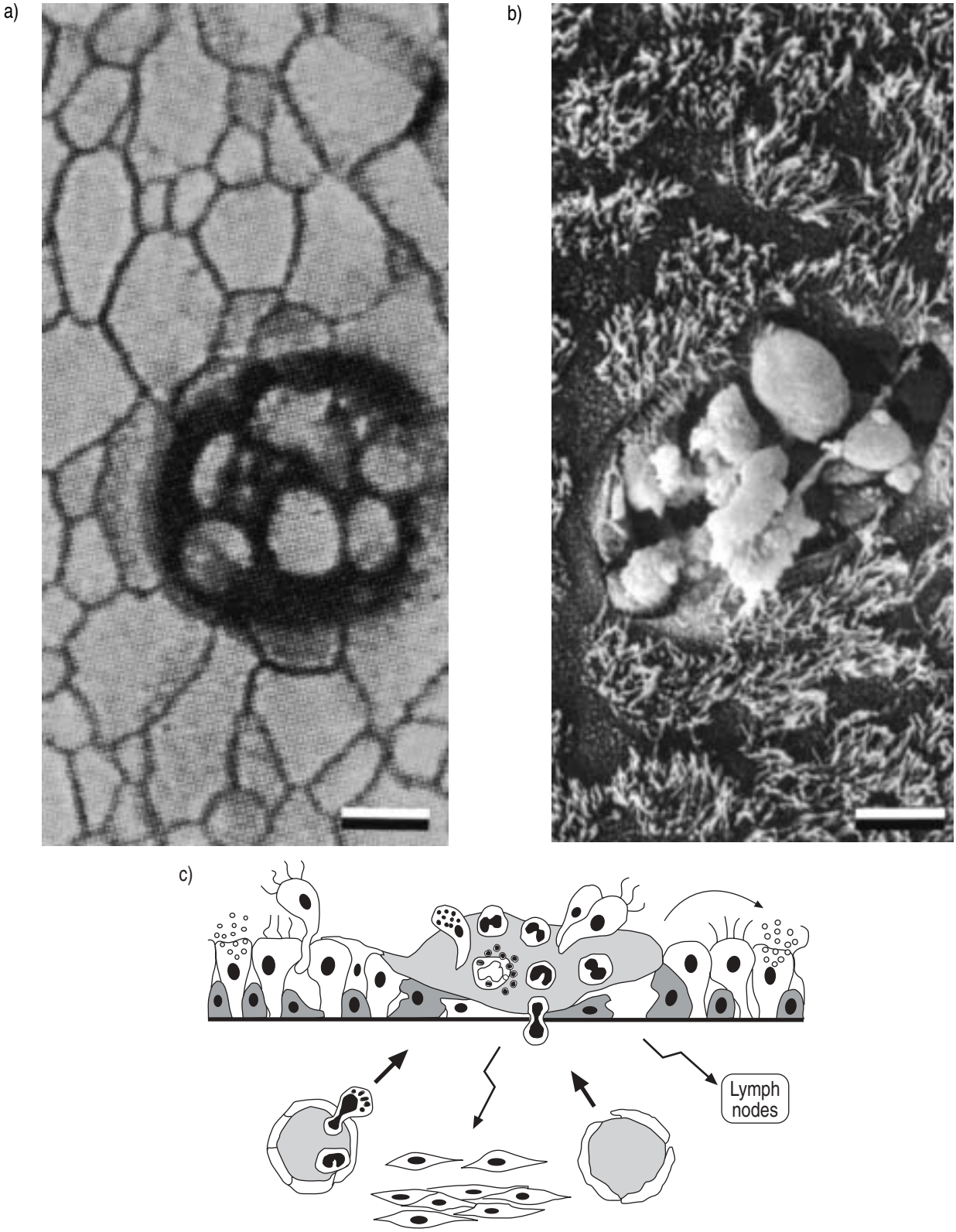

Fig. 8. – Epithelial injury-repair in guinea-pig allergic airways 1 h after allergen exposure. The regular mosaic pattern of epithelial cells is interrupted by patches of epithelial injury-repair processes. This is shown by light microscopy (aerial view) in: a) wholemount preparation where apical epithelial cell borders have been silver-stained; and b) by scanning electron microscopy. c) schematic depiction of the epithelial injury-repair process and its sequalae. Damage may proceed apically while rapid repair covers the basement membrane with restitutional cells. The injury-repair sites are intense and dynamic foci of inflammatory processes. The molecular milieu may be dominated by plasma-derived proteins and peptides. Activated neutrophils and eosinophils occur here together with epithelial cell debris in a fibrin-fibronectin gel. The sequelae to simple epithelial shedding *in vivo* further include remodelling effects involving not only the epithelium, but also fibroblasts, smooth muscle cells and regional lymph nodes. (Data and concepts are from references [3, 4, 57–62].

Beside the pathogenic potential of the exuded molecules, these may also serve in defence, repair and the prevention of infection. For example, bulk plasma exudation occurring at the height of corona virus-induced common cold [72] may contribute to the acute inflammation, but can also favour a rapid resolution of this condition. The role of exuded plasma may be exaggerated in inflammatory airway diseases, because in these conditions the endothelium may be particularly sensitive to mediators that increase the venular permeability. This exudative hyp-erresponsiveness has been demonstrated as an abnormally large exudative response to histamine challenges in allergic and infectious rhinitis [73, 74]. However, exudative hyperresponsiveness is not a consistent feature of inflammatory airway diseases [42].

Antiexudative effects of drugs

The venular endothelial cell is a target both for exudative agents and for molecules that produce antiexudative effects by direct vascular antipermeability mechanisms [14, 42]. Antihistamines, therefore, have a predictive antiexudative efficacy in airways where a significant histamine-induced tone exists. Similarly, leukotriene-inhibitory drugs would be effective antiexudative agents under conditions when leukotrienes contribute significantly to airway exudation. β_2-agonists, chromones and theophyllines reduce inflammatory challenge-induced plasma exudation responses in guinea-pig airways [11, 28]. This action may reflect direct functional antagonism of the venular wall since the effect appears not to depend upon which mediator is used to evoke the exudation. Furthermore, these antiasthma drugs may not reduce airway mucosal blood flow [28].

The long duration of action of topical formoterol given to animal airways was first observed in respect of its potent anti-exudative effect [75] (fig. 9). In subjects with seasonal allergic rhinitis, topical nasal applications of high doses of terbutaline reduced allergen challenge-induced levels of plasma proteins (α_2-macroglobulin) and mast cell mediators (tryptase) in fluid lavaged from the mucosa [18]. However, due to lack of human data it is not known whether any drug other than steroids exerts clinically significant antiexudative effects in the airways. Antiexudative drug effects (fig. 9) that reduce the functional reactivity of the mucosa to inflammatory stimuli, may be desirable in the treatment of asthma and other inflammatory airway conditions. However, the exudative response, required for respiratory defence and repair, should not be impeded during such treatment.

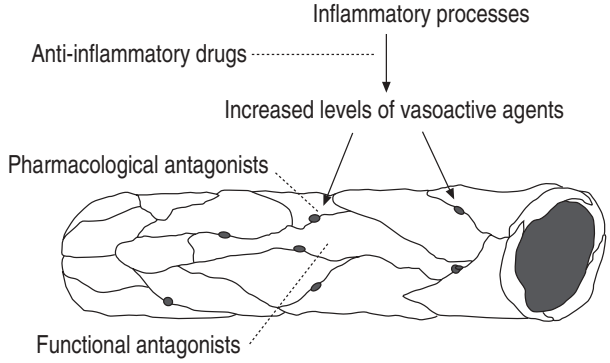

Fig. 9. – Different mechanisms by which drugs may reduce airways plasma exudation. Anti-inflammatory drugs, such as steroids, inhibit inflammatory processes and may, thus, indirectly prevent the increased microvascular permeability. Pharmacological antagonists, such as antihistamines, inhibit the venular permeability effect of a single mediator. Functional antagonists, such as β_2-agonists, act on the venular endothelium making it generally less responsive to the vasoactive agents.

The antiexudative efficacy of systemic steroid treatment was reported when the plasma proteins began to be det-ermined in airway discharges in asthma and rhinitis. Not-ably, the steroid-induced inhibition of exudation occurred without concomitant reduction in indices of secretion pre-sent in sputum [76]. A poor antisecretory effect of steroids supports the notion that plasma exudation closely reflects airway inflammation [17, 77]. As reviewed elsewhere [77] airways exudate not only differs from epithelium-derived secretions through its content, but the processes behind these two mucosal outputs also have a qualitatively distinct pharmacology and physiology regarding both their induction and inhibition.

Van de Graaf *et al.* [78] have demonstrated that maintenance treatment with an inhaled steroid significantly reduces the plasma exudation that occurs in chronic nonallergic asthma. By determination of a large plasma protein (fibrinogen) and a plasma-derived mediator (bradykinin) in nasal lavage fluid. Svensson *et al.* [79] have demon-strated topical steroid-mediated inhibition of plasma ex- udation and, hence, inhibition of its derived peptides in seasonal allergic rhinitis. Interestingly, steroids are not universally effective. Lack of antiexudative effects both in the common cold [80] and on acute exposure to histamine-like mediators [81] and inflammatory chemicals [9] can be interpreted as a poor capacity of steroids to prevent plasma exudation when it is acting as an airway defence mechanism. Steroids may also be ineffective against airway exudation due to epithelial shedding [82] and, therefore, may not adversely impede the prompt and very rapid restitution of epithelial cells over the airway basement membrane that occurs *in vivo* after shedding [83]. Taken together, these findings suggest that topical airway steroids manage to achieve a useful balance between their effects on exudative inflammation and lack of interference with the processes of defence and repair. Such experimental observations may explain, in part, why long-term treatment with these potent anti-inflammatory drugs in asthma and rhinitis may be associated with unchanged or even reduced frequency of airway infections [83].

Conclusion

Current studies of immuno-inflammatory mechanisms of airway mucosa largely concern the functions and biochemistry of cultured cells dwelling in defined, but artificial, *ex vivo* solutions. Unfortunately, the *in vitro* data do not always translate well in to the dynamic situation *in vivo* [84]. Important aspects of airway inflammation, defence and repair (and airway drugs) may, thus, have to be unravelled directly in the complex, but relevant, biosystems that include an active microcirculation; *i.e.*, in human airways *in vivo* [17] and animals models [85]. Recent examples of discoveries *in vivo*, that have not been predicted by *in vitro* research paradigms, include activation of airway mucosal eosinophils, through a regulated cytolytic

mechanism [64], and the processes of airway epithelial shedding and restitution which may be associated with disease [61, 62]. It is of note that these eosinophilic and epithelial events occur in close association with airways exudation.

An active microcirculation generates a highly dynamic, biologically active milieu of plasma-derived molecules. New data on the mechanisms of airway exudation suggest that the protein systems of circulating plasma may contribute significantly to the biology and immunology of the airway mucosal tissue, yet the mucosal surface, not only in injured airways, but also in activated airways, may display no sign of oedema, no sign of epithelial disruption and no sign of increased absorption capacity. The extent or lack of tissue oedema may depend critically on the plasticity of the epithelial tight junctions, which readily allow the movement of plasma to the airway lumen. The present considerations together with the rapidly growing quantity of information (not detailed herein) on receptors, target cells and cellular actions of the plasma-derived molecules and the interactions of plasma with the epithelium-derived secretions, which have formed the focus of the present series, may have to be considered in realistic models of "immuno-inflammatory" mechanisms of the airway mucosa.

Appendix: definitions

Airway exudation comprises: 1) extravasation of bulk plasma from postcapillary venules belonging to the airway mucosal microcirculation that carries systemic blood; and 2) the further movement of nonsieved plasma exudate into the extracellular matrix of the lamina propria, across the basement membrane, between epithelial cells, and into the airway lumen.

In contrast to the "transudation" of protein-poor fluid the mucosal exudate is typically nonsieved and contains all the large plasma proteins.

Airway exudation does not encompass cell traffic that is regulated by separate mechanisms. Although luminal entry of bulk plasma occurs without comprising integrity and the normal barrier function of the epithelial lining, airway exudation is entirely distinct from airway secretory processes.

Acknowledgement: The authors would like to thank P.K. Jeffery for his helpful discussion and encouragement.

References

1. Persson CGA, Erjefält I. Inflammatory leakage of macromolecules from the vascular compartment into the tracheal lumen. *Acta Physiol Scand* 1986; 126: 615–616.
2. Persson CGA, Erjefält I, Alkner U, *et al.* Plasma exudation as a first line respiratory mucosal defence. *Clin Exp Allergy* 1991; 21: 17–24.
3. Erjefält JS, Sundler F, Persson CGA. Eosinophils, neutrophils and venular gaps in the airway mucosa at epithelial removal-restitution. *Am J Respir Crit Care Med* 1996; 153: 1666–1674.
4. Erjefält JS, Erjefält I, Sundler F, Persson CGA. Microcirculation-derived factors in airway epithelial repair *in vivo*. *Microvasc Res* 1994; 48: 161–178.
5. Persson CGA, Andersson M, Greiff L, *et al*. Airway permeability. *Clin Exp Allergy* 1995; 23: 807–814.
6. Erjefält I, Persson CGA. Inflammatory passage of plasma macromolecules into airway tissue and lumen. *Pulm Pharmacol* 1989; 2: 93–102.
7. Erjefält JS, Erjefält I, Sundler F, Persson CGA. Epithelial pathways for luminal entry of bulk plasma. *Clin Exp Allergy* 1995; 25: 187–195.
8. Persson CGA, Gustafsson B, Luts A, Sundler F, Erjefält I. Toluene diisocyanate produces an increase in airway tone that outlasts the inflammatory exudation phase. *Clin Exp Allergy* 1991; 21: 715–724.
9. Erjefält I, Persson CGA. Increased sensitivity to toluene diisocyanate (TDI) in airways previously exposed to low doses of TDI. *Clin Exp Allergy* 1992; 22: 854–862.
10. Erjefält I, Persson CGA. Effects of budesonide on allergen- and toluene diisocyanate (TDI)-induced late phase airway exudation of bulk plasma. *Am J Respir Crit Care Med* 1995; 151: A223.
11. Persson CGA, Erjefält I, Andersson P. Leakage of macromolecules from guinea-pig tracheobronchial microcirculation. Effects of allergen, leukotrienes, tachykinins and anti-asthma drugs. *Acta Physiol Scan* 1986; 127: 95–105.
12. Hultström D, Svensjö E. Intravital and electron microscopic study of bradykinin-induced vascular permeability changes using FITC-dextran as a tracer. *J Pathol* 1979; 129: 125–133.
13. McDonald M. Endothelial gaps and permeability of venules in rat tracheas exposed to inflammatory stimuli. *Am J Physiol* 1994; 266: L61–83.
14. Grega GJ, Persson CGA, Svensjö E. Endothelial cell reactions to inflammatory mediators assessed *in vivo* by fluid and solute flux analysis. *In*: Ryan US, ed. Endothelial Cells. Boca Raton, FL, USA, CRC, 1988; pp. 103–122.
15. Persson CGA, Erjefält JS, Andersson M, Greiff L, Svensson C. Extravasation, lamina propria flooding and lumenal entry of bulk plasma exudate in mucosal defence, inflammation, and repair. *Pulm Pharmacol* 1996; 9: 129–139.
16. Svensson C, Grönneberg R, Andersson M, *et al*. Allergen challenge-induced entry of alpha-2-macroglobulin and tryptase into human nasal and bronchial airways. *J Allergy Clin Immunol* 1995; 96: 239–246.
17. Persson CGA, Svensson C, Greiff L, *et al.* I. Editorial. The use of the nose to study the inflammatory response of the respiratory tract. *Thorax* 1992; 47: 993–1000.
18. Svensson C, Greiff L, Andersson M, Alkner U, Grönneberg R, Persson CGA. Antiallergic actions of high topical doses of terbutaline in human nasal airways. *Allergy* 1995; 50: 884–890.
19. Tang L, Eaton JW. Fibrin(ogen) mediates acute inflammatory responses to biomaterials. *J Exp Med* 1993; 178: 2147–2136.
20. Roman J, McDonald JA. Fibronectins and fibronectin receptors in lung development, injury, and repair. Crystal RG, West JB *et al*., eds. Lung: Scientific Foundations, 2nd ed. Philadelphia, Lippincott 1997; pp. 737–757.
21. Salomonsson P, Grönneberg R, Gilljam H, *et al*. Bronchial exudation of bulk plasma at allergen challenge in allergic asthma. *Am Rev Respir Dis* 1992; 146: 1535–1542.
22. Schwaeble WJ, Whaley K. Complement and antigen-antibody complexes. *In*: Kay AB, ed. Allergy and Allergic Diseases. London, Blackwells, 1997; pp. 307–323.
23. Kaplan AP, Silverberg M, Reddigari S. Intrinsic coagula-

tion/bradykinin-forming cascade. *In*: Kay AB, ed. Allergy and Allergic Diseases. London, Blackwells, 1997; pp. 324–339.
24. Blombäck B, Blombäck M, Edman P, Hessel B. Human fibrinopeptides: isolation, characterization and structure. *Biochim Biophys Acta* 1966; 115: 371–380.
25. Gray AJ, Reeves JT, Harrisson NK, Winlove P, Laurent GJ. Growth factors for human fibroblasts in the solute remaining after clot formation. *J Cell Sci* 1990; 96: 271–274.
26. Grainger DJ, Kemp PR, Metcalfe JC, *et al*. The serum concentration of active transforming growth factor-B is severely depressed in advanced atherosclerosis. *Nature Med* 1995; 1: 74–79.
27. Borth W, Feinman RD, Gonias SL, Quigley JP, Strickland DK, eds. Biology of α_2-Macroglobulin, Its Receptor, and Related Proteins. New York, New York Academy of Science, 1994.
28. Erjefält I, Persson CGA. Pharmacological control of plasma exudation into tracheobronchial airways. *Am Rev Respir Dis* 1991; 143: 1008–1014.
29. Erjefält I, Luts A, Persson CGA. Appearance of airway absorption and exudation tracers in guinea-pig tracheobronchial lymph nodes. *J Appl Physiol* 1993; 74: 817–824.
30. Godfrey RWA, Severs NJ, Jeffery PK. Freeze-fracture morphology and quantification of human bronchial epithelial tight junctions. *Am J Respir Cell Mol* 1992; 6: 453–458.
31. Claude P. Morphological factors influencing transepithelial permeability: a model for the resistance of the zonula occludens. *J Membr Biol* 1978; 39: 219–232.
32. Persson CGA, Erjefält I, Gustafsson B, Luts A. Subepithelial hydrostatic pressure may regulate plasma exudation across the mucosa. *Int Arch Allergy Appl Immunol* 1990; 92: 148–153.
33. Greiff L, Alkner U, Pipkorn U, Persson CGA. The "nasal pool" device applies controlled concentrations of solutes on human nasal airway mucosa and samples its surface exudations/secretions. *Clin Exp Allergy* 1990; 20: 253–259.
34. Prescott T, Kaliner MA. Vascular mechanisms in rhinitis. *In*: Busse WW, Holgate ST, eds. Asthma and Rhinitis. Oxford, Blackwells 1995; pp. 777–790.
35. Greiff L, Erjefält I, Wollmer P, *et al*. Effects of nicotine on the human nasal airway mucosa. *Thorax* 1993; 48: 651–655.
36. Greiff L, Svensson C, Andersson M, Persson CGA. Effects of topical capsaicin in seasonal allergic rhinitis. *Thorax* 1995; 225–229.
37. Haldursdottir H, Greiff L, Wollmer P, *et al*. Effects of inhaled histamine, methacholine and capsaicin, on sputum levels of α_2-macroglobulin. *Thorax* 1997; 52: 964–968.
38. Sanico AM, Atsuta S, Proud D, Togias A. Dose-dependent effects of capsaicin nasal challenge: *in vivo* evidence of human airway neurogenic inflammation. *J Allergy Clin Immunol* 1997; 100: 632–641.
39. Persson CGA, Erjefält I, Sundler F. Airway microvascular and epithelial leakage of plasma induced by PAF-acether and capsaicin. *Am Rev Respir Dis* 1987; 135: A401.
40. Asakura K, Narita S, Kojima T, Saito H, Kataura A. Changes in nasal airway resistance and secretory response in the guinea-pig after nasal challenge with capsaicin and histamine. *Eur Arch Otorhinolaryngol* 1994; 251: 224–228.
41. Svensson C, Baumgarten CR, Pipkorn U, Alkner U, Persson CGA. Reversibility and reproducibility of histamine-induced plasma leakage in the human nasal airways. *Thorax* 1989; 44: 13–18.
42. Berman AR, Liu MC, Wagner EM, Proud D. Dissociation of bradykinin induced plasma exudation and reactivity in the peripheral airways. *Am J Respir Crit Care Med* 1996; 154: 418–423.
43. Erjefält JS, Erjefält I, Sundler F, Persson CGA. Mucosal nitric oxide may tonically suppress airways plasma exudation. *Am J Respir Crit Care Med* 1994; 150: 227–232.
44. Minnicozzi M, Durán WN, Gleich GJ, Egan RW. Eosinophil granule proteins increase microvascular macromolecular transport in the hamster cheek pouch. *J Immunol* 1994; 153: 2664.
45. Chung KF, Barnes PJ, eds, Pharmacology of the Respiratory Tract. New York, Dekker, 1993.
46. Hurley JV. Types of pulmonary microvascular injury. *Ann NY Acad Sci* 1992; 384: 269–286.
47. Liu MC, Hubbard WC, Proud D, *et al.* Immediate and late inflammatory responses to ragweed antigen challenge in the peripheral airways in asthmatic airways. *Am Rev Respir Dis* 1991; 144: 51–58.
48. Erjefält I, Greiff L, Alkner U, Persson CGA. Allergen-induced biphasic plasma exudation responses in guinea-pig large airways. *Am Rev Respir Dis* 1993; 148: 695–701.
49. O'Donnell SR, Erjefält I, Persson CGA. Early and late tracheobronchial plasma exudation factor administered to the airway mucosal surface in guinea-pigs: effects of WEB 2086 and enprofylline. *J Pharmacol Exp Ther* 1990; 254: 65–70.
50. Persson CGA. Airway microcirculation, epithelium, and glucocorticoids. *In*: Schleimer RP, Busse WW, O'Byrne PM, eds. Inhaled Glucocorticoids in Asthma, Mechanisms and Clinical Actions. New York, Dekker, 1997; pp. 167–201.
51. Peterson CGB, Venge P. Interaction and complex-formation between the eosinophil cationic protein and α_2-macroglobulin. *Biochem J* 1987; 245: 781–787.
52. Persson CGA, Alkner U, Andersson M, Greiff L, Linden M, Svensson C. Histamine challenge-induced "lamina propria lavage" and mucosal output of IL-6 in human airways. *Eur Respir J* 1995; 8: Suppl. 19, 125s.
53. Woolley KL, Ådelroth E, Woolley MJ, Jordana M, O'Byrne PM. Effects of allergen challenge on eosinophils, eosinophil cationic protein and granulocyte-macrophage colony-stimulating factor in mild asthmatics. *Am J Respir Crit Care Med* 1995; 151: 1915–1924.
54. Greiff L, Andersson E, Andersson M, *et al.* Mucosal output of α_2-macroglobulin and ECP in children with seasonal allergic rhinitis (Abstract). *Am J Respir Crit Care Med* 1997; 155: A979.
55. Greiff L, Andersson M, Svensson C, Linden M, Wollmer P, Persson CGA. Dual induction of plasma exudation and sputum unravels bronchial eosinophil activity in seasonal allergic rhinitis. *Thorax* 1998; in press.
56. Schoonbrood DFM, Lutter R, Habets FJM, Roos CM, Jansen HM, Out TA. Analysis of plasma protein leakage and local secretion in sputum from patients with asthma and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1994; 150: 1519–1527.
57. Persson CGA, Erjefält JS. Airway epithelial restitution following shedding and denudation. *In*: Crystal RG, West JB, Weibel ER, Barnes PJ, eds. The Lung: Scientific Foundations, 2nd ed. New York, Raven, 1997; pp. 2611–2627.
58. Erjefält JS, Erjefält I, Sundler F, Persson CGA. *In vivo* restitution of airway epithelium. *Cell Tissue Res* 1995;

281: 305–316.
59. Erjefält JS, Korsgren M, Nilsson MC, Sundler F, Persson CGA. Association between inflammation and epithelial damage-restitution processes in allergic airways *in vivo*. *Clin Exp Allergy* 1997; 27: 1344–1355.
60. Erjefält JS, Korsgren M, Nilsson MC, Sundler F, Persson CGA. Prompt epithelial damage- and restitution-processes in allergen challenged guinea-pig trachea. *Clin Exp Allergy* 1997; 27: 1458–1470.
61. Persson CGA, Erjefält JS, Erjefält I, Korsgren M, Nilsson M, Sundler F. Epithelial shedding-restitution as a causative process in airway inflammation. *J Clin Exp Allergy* 1996; 26: 746–755.
62. Erjefält JS, Persson CGA. Airway epithelial repair: breathtakingly quick and multipotentially pathogenic. *Thorax* 1997; 52: 1010–1012.
63. Wilhelm DL. Regeneration of tracheal epithelium. *J Pathol Bacteriol* 1953; 95: 543–550.
64. Persson CGA, Erjefält JS. Eosinophil lysis and free granules: an *in vivo* paradigm for cell activation and drug development. *Trends Pharmacol Sci* 1997; 18: 117–123.
65. Dunnill MS. The pathology of asthma with special reference to the change in the bronchial mucosa. *J Clin Pathol* 1960; 13: 27–33.
66. Naylor B. The shedding of the mucosa of the bronchial tree in asthma. *Thorax* 1962; 17: 69–72.
67. Persson CGA. Centennial notions of asthma as an eosinophilic, desquamative, exudative, and steroid-sensitive disease. *Lancet* 1977; 350: 1021–1024.
68. Erjefält JS, Greiff L, Andersson M, Persson G, Svensson C, Persson CGA. Eosinophil lysis and clusters of free eosinophil granules (Cfegs) in rhinitis and asthma. (Abstract). *Am J Respir Crit Care Med* 1997; 155: A977.
69. Greiff L, Andersson M, Svensson C, Lundin S, Wollmer P, Persson CGA. Reduced airway absorption in seasonal allergic rhinitis. *Am J Respir Crit Care Med* 1997; 156: 783–786.
70. Persson CGA. Role of plasma exudation in asthmatic airways. *Lancet* 1986; 2: 1126–1129.
71. Jeffery PK. Pathology of asthma. *Br Med Bull* 1992; 48: 23–29.
72. Åkerlund A, Greiff L, Andersson M, Bende M, Alkner U, Persson CGA. Mucosal exudation of fibrinogen in corona virus-induced common cold. *Acta Otolaryngol (Stockh)* 1993; 113: 642–648.
73. Svensson C, Andersson M, Greiff L, Alkner U, Persson CGA. Exudative hyperresponsiveness of the airway microcirculation in seasonal allergic rhinitis. *Clin Exp Allergy* 1995; 25: 942–950.
74. Greiff L, Andersson M, Åkerlund A, *et al*. Microvascular exudative hyperresponsiveness in human corona virus-induced common cold. *Thorax* 1994; 49: 121–127.
75. Erjefält I, Persson CGA. Long duration and high potency of antiexudative effects of formoterol in guinea-pig tracheobronchial airways. *Am Rev Respir Dis* 1991; 144: 788–791.
76. Keal EE. Biochemistry and rheology of sputum in asthma. *Postgrad Med J* 1971; 47: 171–177.
77. Persson CGA. Airway mucosal exudation of plasma. *In*: Takishima T, Shimura S, eds. Airway Secretion. Physiological Bases for the Control of mucous Hypersecretion. New York, Dekker, 1994; pp. 451–468.
78. Van de Graaf EA, Out TA, Roos CM, Jansen HM. Respiratory membrane permeability and bronchial hyperreactivity in patients with stable asthma. Effects of therapy with inhaled steroids. *Am Rev Respir Dis* 1991; 143: 362–368.
79. Svensson C, Klementsson H, Andersson M, Pipkorn U, Alkner U, Persson CGA. Glucocorticoid-induced attenuation of mucosal exudation of bradykinins and fibrinogen in seasonal allergic rhinitis. *Allergy* 1994; 49: 177–183.
80. Farr BM, Gwaltney JM Jr, Hendley JO. A randomized controlled trial of glucocorticoid prophylaxis against experimental rhinovirus infection. *J Infect Dis* 1990; 162: 1173–1177.
81. Greiff L, Andersson M, Svensson C, Åkerlund A, Alkner U, Persson CGA. Effects of two weeks' topical budesonide treatment on microvascular exudative responsiveness in healthy human nasal airways. *Eur Respir J* 1997; 10: 841–845.
82. Erjefält JS, Erjefält I, Sundler F, Persson CGA. Effects of topical budesonide on epithelial restitution *in vivo* in guinea-pig trachea. *Thorax* 1995; 50: 785–792.
83. Schleimer RP, Busse WW, O'Byrne PM, eds. Inhaled Glucocorticoids in Asthma. Mechanisms and Clinical Actions. New York, Dekker, 1997.
84. Persson CGA. Discoveries in complex biosystems. *Nature Biotech* 1997; 15: 927.
85. Persson CGA, Erjefält JS, Korsgren M, Sundler F. The mouse trap. *Trends Pharmacol Sci* 1997; 18: 465–467.