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Review article

Airway microvascular extravasation and luminal entry of plasma

Lennart Greiff[1], Morgan Andersson[1], Jonas S. Erjefält[2], Carl G. A. Persson[3] and Per Wollmer[4]

Departments of [1]Otorhinolaryngology, Head and Neck Surgery, [2]Neurophysiology, [3]Clinical Pharmacology and [4]Clinical Physiology, University Hospital, Lund/Malmö, Sweden

Correspondence
Lennart Greiff, Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital, SE-221 85 Lund, Sweden
E-mail: lennart.greiff@skane.se

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Summary

Extravasation of plasma from postcapillary venules is a specific *in vivo* response to inflammatory insults. In the nasal and bronchial airways, extravasated plasma has a widespread distribution in the lamina propria, between the epithelial cells and in the airway lumen. This feature, in combination with the fact that the process involves extravasation of bulk plasma, with all peptides and proteins of plasma, indicates that plasma exudation contributes to the dramatic change of the mucosal milieu that characterizes airway inflammation. Accordingly, this process is of key importance to conditions such as allergic rhinitis and asthma. The means by which extravasated plasma participates in mucosal defence is physiological in the sense that it may operate on the surface of the epithelium without impairing its function as an absorption barrier. The flow of plasma into the airway lumen may thus wash away unwanted material from inter-epithelial cell spaces, exuded binding proteins may bind unwanted solutes non-specifically and extravasated immunoglobulins may neutralize allergens. In addition to the role as defence mechanism, extravasated plasma components may act as important pro-inflammatory factors. Furthermore, experimental data as well as observations in natural disease suggest that luminal levels of plasma proteins can be employed as an accessible index reflecting to what degree the airway mucosa is affected by inflammatory processes.

Introduction

The human airway mucosa is a main site for deposition of potentially noxious environmental substances. As would be expected, this mucosa is well equipped to protect both itself and the rest of the body from harmful influences of foreign material. For example, the regulation of its permeability allows plasma to enter the airway lumen without making it easier to foreign surface molecules to penetrate the tissue. Accordingly, circulating humoral defence systems are allowed to neutralize offending stimuli on the surface of a mucosa that maintains its function as absorption barrier uncompromised. This process, i.e. plasma exudation: (i) is a key determinant of the molecular milieu of the inflamed airway mucosa *in vivo*, (ii) can be employed as a specific index reflecting to what degree the airway mucosa is affected by an inflammatory process, and (iii) is a first line airway defence mechanism as well as a potentially pro-inflammatory factor. This brief overview focuses on mechanisms of plasma exudation relevant to upper and lower airway conditions such as allergic rhinitis and asthma.

Extravasation, tissue distribution and luminal entry of plasma

The airway mucosa harbours a profuse superficial microvasculature (Jezierski, 1906; Persson *et al.*, 1998). Accordingly, beneath the airway epithelium, in the nose as well as the bronchi, the microcirculation is very well developed (Fig. 1). The subepithelial microcirculation readily reacts to inflammatory mucosal disturbances. Plasma exudation may thus be produced by actions of various inflammatory mediators on endothelial cells of post-capillary venules (Majno & Palade, 1961; Majno *et al.*, 1961; Pietra *et al.*, 1971). These specific cells are actively separated resulting in formation of grid-like structures or gaps through which plasma leaks into the lamina propria (McDonald *et al.*, 1999). The result is a prompt and very widespread distribution of plasma in the lamina propria, in the

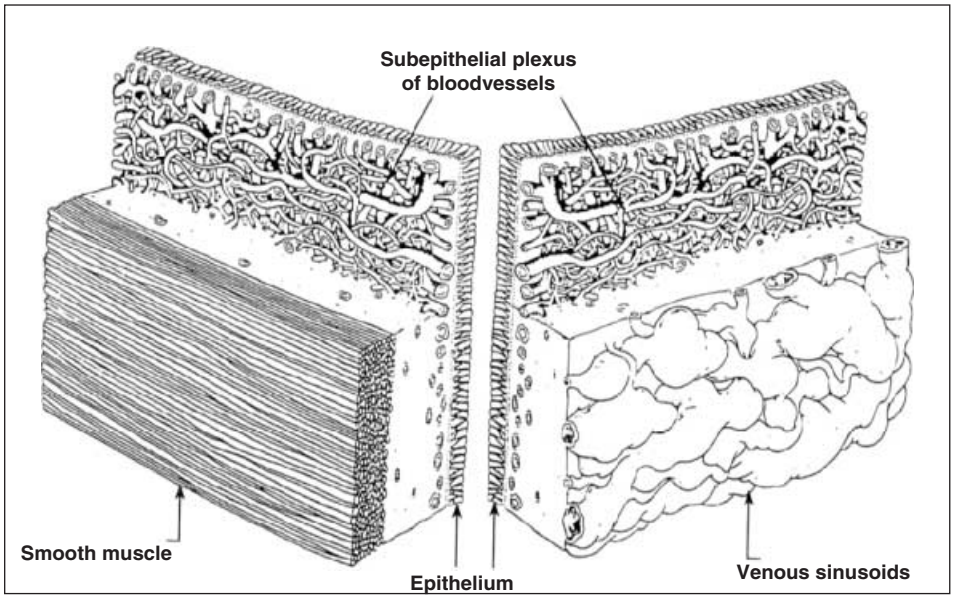

Figure 1 Illustration of the subepithelial network of microvessels in the nasal (right) and bronchial (left) airways (Persson *et al.*, 1998). Apparent differences comprise the presence of sinusoids in the nasal airway whereas such structures are absent in the bronchial airways. Note, however, the similar presence and distribution of subepithelial microvessels.

epithelium (between epithelial cells), and also in the airway lumen (Erjefält *et al.*, 1995; Persson *et al.*, 1998) (Fig. 2).

The extravasation of plasma occurs along hydrostatic pressure gradients formed between the microvasculature and the lamina propria. The extravasated plasma may move also across the epithelium aided by pressure-operated mechanisms, allowing epithelial cells to separate transiently and plasma to move into the airway lumen via para-cellular routes. This hypothesis is supported by observations *in vivo* showing a para-cellular appearance of plasma tracers at exudative conditions (Erjefält *et al.*, 1995, Fig. 3) and by observations *in vitro* showing that application of a slight hydrostatic pressure on the serosal aspect of guinea-pig tracheal preparations readily produces lumenal entry of macromolecules (Persson *et al.*, 1990; Gustafsson & Persson, 1991). Furthermore, it has recently been shown that luminal entry of plasma *in vivo* is sensitive to experimental changes in airway luminal pressure (Berg *et al.*, 2003).

Topical administration of N-nitro-L-arginine methyl ester (L-NAME), a false substrate for nitric oxide (NO) synthase, produces dose-dependent mucosal exudation (luminal entry) of plasma in guinea-pig tracheal airways (Erjefält *et al.*, 1994). Furthermore, in the human nasal airway topical nitroprusside, a NO-donor drug, reduces histamine-induced plasma exudation (Greiff *et al.*, 1995). These observations with NO-active drugs, together with demonstrations of a presence of NO-synthase in the airway mucosa (Kobzik *et al.*, 1993) and of removal of the epithelium *in vivo* as a potent plasma exudation stimulus (Erjefält *et al.*, 1996), suggest the possibility that epithelial NO tonically suppresses the microvascular permeability as part of an endogenous control mechanism. In this context, it is conceivable that mediators and other pro-inflammatory factors may produce plasma exudation by inhibiting such a mechanisms in addition to having effects directly on postcapillary venules.

Exudation of bulk plasma with its bioactive peptide and proteins

A range of disease factors and inflammatory mediators have been demonstrated to produce dose-dependent luminal entry of plasma proteins. Such factors include allergen and infectious agents as well as histamine, bradykinins, leukotrienes, platelet activating factor (PAF), eosinophil cationic protein (ECP), tryptase, tumour necrosis factor-α (TNFα) etc. (reviewed in Persson *et al.*, 1998). Common to these stimuli is that they indirectly or directly produce dose-dependent extravasation and luminal entry of different-sized plasma proteins (Fig. 4).

The ratio in plasma between albumin, a small protein, and α_2-macroglobulin, one of the largest plasma proteins, is about 20:1. In baseline nasal lavages it is usually 40:1 indicating that the minor baseline luminal entry of plasma proteins that occurs is size-selective favouring smaller proteins (Fig. 4). However, at exudative conditions the ratio is the same as in plasma (Fig. 4). These observations indicate that there is a loss of endothelial–epithelial size-selectivity at inflammatory stimulus-induced plasma exudation, and that the process involves luminal entry of non-sieved, bulk plasma (Greiff *et al.*, 2002a).

The fact that extravasation and luminal entry of plasma reflects bulk exudation implicates that all growth factors, adhesive and cytokine binding factors, immunoregulatory factors, and pro-inflammatory factors of plasma are distributed in the airway mucosa (reviewed in Persson *et al.*, 1998) (Fig. 5). The result is a dramatic change of the mucosal molecular milieu

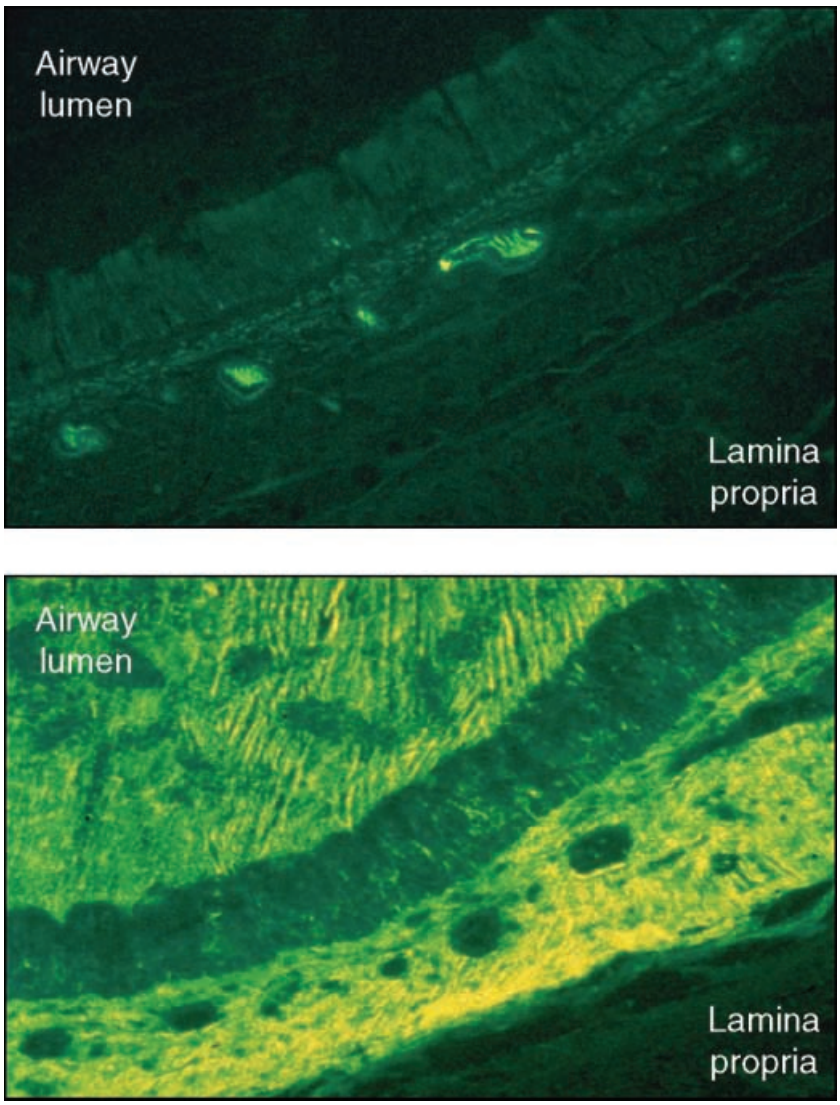

Figure 2 Plasma labelled with fluorescent macromolecules (fluorescein-isothiocyanate-dextran, molecular weight 60 000 da) and detected in the guinea-pig bronchial airway by fluorescence microscopy. At baseline (upper panel) plasma is present in the subepithelial microcirculation. At exudation (lower panel) a widespread distribution of extravascular plasma is seen in the lamina propria and in the airway lumen.

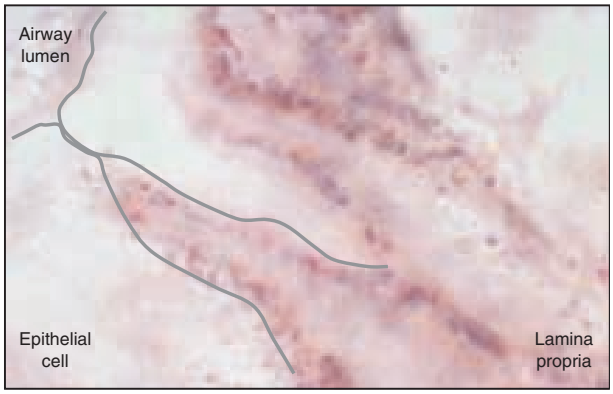

Figure 3 Plasma labelled with gold particles (diameter 5 nm) and detected in the guinea-pig bronchial airway by autometallographic silver intensification (Erjefält *et al.*, 1995). Lines indicate lateral borders of epithelial cells. Note the exclusively para-cellular appearance of plasma (dark tracer particles) at inflammatory stimulus-induced exudation.

in vivo. Accordingly, at airway inflammation, the extravasated plasma must be viewed as a major determinant of that milieu. Conceivably, such a contribution may in part explain why experimental findings *in vitro* sometimes do not translate well into complex *in vivo* situations.

Non-injurious unidirectional luminal entry of plasma

There is a brief phase after the extravasation from the microcirculation when the lamina propria is flooded with plasma. Promptly, within a minute, the exudate then moves across the epithelium into the airway lumen. Although this process involves movement of bulk plasma, its luminal entry does not *per se* damage the structure of the epithelium even as assessed by electron microscopy (Wihl & Mygind, 1977; Erjefält *et al.*, 1995; Greiff *et al.*, 1997a). As a further substantiation of

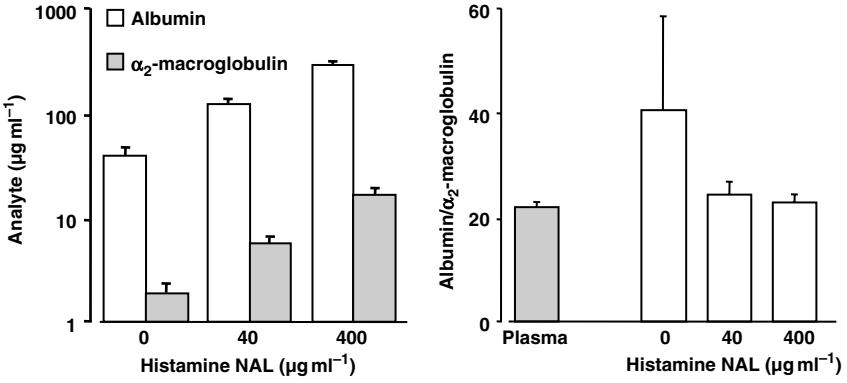

Figure 4 Luminal entry of different-sized plasma proteins at human nasal histamine challenges (left panel). Ratio between nasal lavage fluid levels albumin and α_2-macroglobulin at experimental histamine challenges (right panel). Note that the ratio at exudation is identical to that in plasma, indicating that the process of plasma exudation involves bulk plasma.

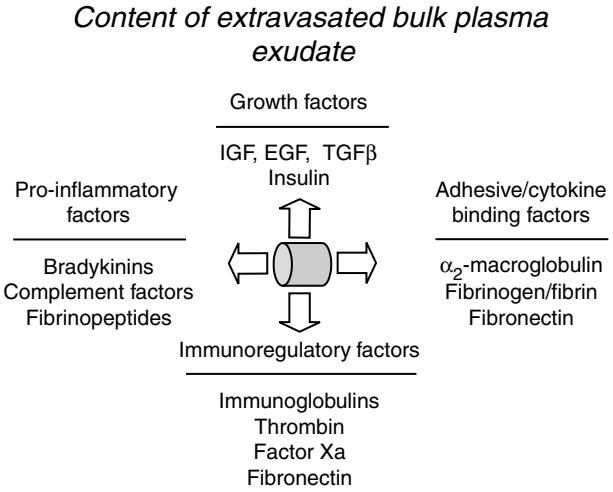

Figure 5 Examples of plasma-derived peptides and proteins in the plasma exudate. Such growth factors, adhesive and cytokine binding factors, immunoregulatory factors, and pro-inflammatory factors are distributed in the airway mucosa in airway inflammation and this process results in a dramatic change of the mucosal milieu.

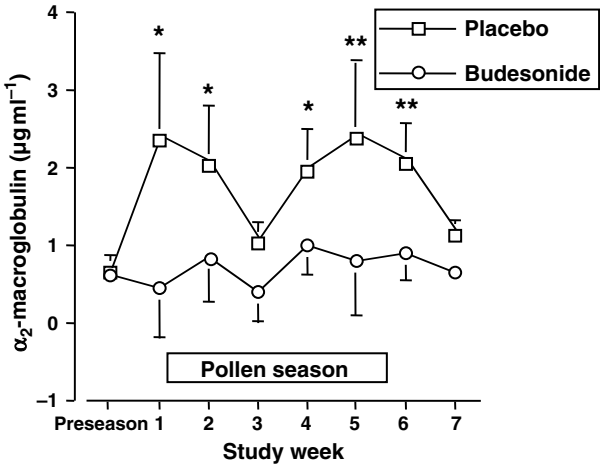

Figure 6 Levels of α_2-macroglobulin in nasal lavages samples obtained at baseline (preseason) and at seasonal allergen exposure with and without treatment with a topical glucocorticosteroid. Allergen exposure is associated with increased levels of α_2-macroglobulin, indicating plasma exudation, and this response is attenuated by the above treatment, reflecting indirect effects on mediator-releasing inflammatory cells.

maintained epithelial integrity, luminal entry of plasma is not associated with any increased airway absorption, i.e. the perviousness of the epithelium to hydrophilic tracers and peptides that enter the mucosa from the luminal compartment is unaffected (Greiff *et al.*, 1991). Also, airway conditions characterized by exudative inflammation may even be associated with unaffected or even reduced absorption permeability (Elwood *et al.*, 1983; Greiff *et al.*, 1997b, 2002b). The physiological, non-injurious mechanisms involved in extravasation and luminal entry of bulk plasma in the airways are the basis for the proposal that this process is a first line defence mechanism of the airway mucosa (Persson *et al.*, 1991).

Plasma exudation in airway disease

In addition to its role as an airway mucosal defence mechanism, the plasma exudate may be a pathogenetic factor in rhinitis and asthma. Physical properties of the plasma exudate may, e.g. interfere with hydration of the airways and impair mucociliary transport. Furthermore, and probably of greater importance, the potent plasma peptide and protein systems (above) may sustain and perpetuate airway inflammatory processes. Also, an increased exudative responsiveness may develop (Greiff *et al.*, 1994a; Svensson *et al.*, 1995a; Meyer *et al.*, 1999). Such an increased proclivity of the airway subepithelial microcirculation to respond with plasma exudation appears to be a characteristic feature of inflammatory airway diseases.

The luminal entry of plasma is specific to inflammatory stimuli and extends to threshold inflammatory conditions. Accordingly, it is appropriate and convenient to monitor this response on the mucosal surface by analysing levels of plasma proteins in lavage fluids and sputum (Fig. 6, Persson *et al.*, 1998). α_2-Macroglobulin may be particularly valid as such an index as it is specific to plasma and present in airway surface liquids at baseline only in minute concentrations. Increased concentrations of plasma proteins, or plasma-derived activities, in airway mucosal surface liquids have been demonstrated in,

e.g. allergic rhinitis (Naclerio *et al.*, 1983; Svensson *et al.*, 1994) and asthma (Van der Graaf *et al.*, 1991) (Fig. 6), reflecting how the airway mucosa is affected by an on-going inflammatory process.

Mucosal exudation of plasma in relation to inflammatory cell activities

One aspect of the plasma exudation process relates to the possibility that plasma proteins, during their passage through interstitial spaces of the airway mucosa, may bind and move cell products to the mucosal surface. Plasma proteins, notably α_2-macroglobulin, have thus been demonstrated to bind mediators, such as ECP, as well as many cytokines (Peterson & Venge, 1987). Accordingly, we have observed that histamine challenge-induced plasma exudation may transport cellularly released, subepithelial molecules including mediators and cytokines into the airway lumen (Meyer *et al.*, 1999). The luminal appearance of inflammatory cell indices may thus occur secondary to the extensive lamina propria distribution and epithelial passage of extravasated bulk plasma, and histamine challenges may be used as an experimental measure to produce this effect. Also, besides the possibility that the plasma exudation process may increase the airway luminal levels of mediators and cytokines, the luminal levels of these cell products may be reduced by various treatments through a mechanism involving anti-permeability effects. Accordingly, it may be useful to put luminal observations on cellular indices into a context describing also the degree of plasma exudation.

Pharmacological control of the plasma exudation response

Microvascular anti-permeability effects of β_2-agonists were demonstrated already during the 1970s. Numerous groups have since then shown that β_2-agonists have a capacity to reduce inflammatory stimulus-induced plasma exudation, including observations involving human nasal and bronchial airways. For example, in a study involving the human nasal airway, high doses of terbutaline inhibited allergen challenge-induced plasma exudation (Svensson *et al.*, 1995b). Furthermore, in a study involving healthy subjects, formoterol attenuated challenge-induced bronchial plasma exudation responses (Greiff *et al.*, 1998). The effect of β_2-agonists is likely mediated through receptors located on the permeability regulating endothelial cells of the postcapillary venules (Baluk & McDonald, 1994), and drugs acting at this level may be classified as 'anti-permeability drugs' (reviewed in Persson *et al.*, 1998). Additional substances within this class of drugs are prostacyklins and (topical) NO-donors.

Anti-histamines have also been shown to attenuate plasma exudation responses. Specifically, this class of drugs reduces acute allergen challenge-induced plasma exudation in allergic rhinitis (Bousquet *et al.*, 1988; Naclerio *et al.*, 1990; Greiff *et al.*, 2002). It is likely that this effect reflects mediator antagonism at the H1-receptor level on the endothelial cells of postcapillary venules. Mediator antagonism is thus an additional mechanism by which a pharmacological treatment can influence inflammatory stimulus-induced plasma exudation.

Glucocorticosteroid treatment is a key regimen in the management of allergic rhinitis and asthma. In both these conditions, glucocorticosteroids have been demonstrated to reduce the airway mucosal output of plasma (Fig. 6) (Van der Graaf *et al.*, 1991; Svensson *et al.*, 1994; Meyer *et al.*, 2003). Animal airway studies have shown prompt anti-exudative effects of glucocorticosteroids even at brief mucosal exposure to the drug, suggesting direct effects on the microcirculation (Miller-Larsson & Brattsand, 1990). In contrast, in human airways, the anti-exudative effect appears to be mediated almost exclusively by effects on the mediator-releasing inflammatory cells rather than by actions on the microcirculation. For example, treatment with a clinical dose of a topical glucocorticoid has little effect on histamine-induced nasal mucosal exudation of plasma (Greiff *et al.*, 1994b). In allergic rhinitis and asthma, it is thus likely that the anti-exudative effect of glucocorticosteroid effect reflects inhibition of the inflammatory processes that fuel and perpetuate airway inflammation.

Conclusions

Offending stimuli deposited on the airway mucosa may produce plasma exudation. The widespread mucosal distribution of such extravasated plasma and the fact that this process involves extravasation of bulk plasma with all its potent peptides and proteins contribute to the dramatic change of the mucosal molecular milieu that characterizes airway inflammation. Experimental data as well as observations in natural diseases suggest that luminal levels of plasma proteins can be employed as an accessible index reflecting to what degree the airway mucosa is affected by inflammatory processes *in vivo*. The means by which the plasma exudates participates in mucosal defence are physiological in the sense that it may operate on the surface of the epithelium without impairing its function as an absorption barrier. The flow of plasma into the airway lumen may thus wash away unwanted material from inter-epithelial cell spaces, exuded binding proteins may bind unwanted solutes non-specifically, and extravasated immunoglobulins may neutralize allergens. In addition, extravasated plasma components may act as important pro-inflammatory factors.

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