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Loss of size-selectivity at histamine-induced exudation of plasma proteins in atopic nasal airways

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Summary

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Plasma proteins occur in the airway lumen in inflammatory airway diseases. This study tests the hypothesis that airway microvascular-epithelial exudation of plasma proteins, as induced by a non-injurious inflammatory mediator, is characterized by loss of size-selectivity. Using a nasal pool-device, the nasal mucosa of 10 allergic individuals, without current disease, was sequentially exposed to saline and histamine (40 and 400 μg ml^{-1}). Nasal lavage fluid and blood-levels of albumin (69 kD) and α_2-macroglobulin (720 kD) were determined. Histamine produced concentration-dependent exudation of albumin and α_2-macroglobulin. The albumin/α_2-macroglobulin concentration ratio of the saline lavage fluid (baseline) was 40 ± 19. However, at the histamine challenges the ratios were 25 ± 3 and 22 ± 2, respectively, which did not differ from that of circulating plasma (22 ± 2). We conclude that there is minor and size-selective luminal entry of plasma proteins at baseline. However, at concentration-dependent exudative responses to histamine, plasma proteins enter the airway lumen without being sieved. These data indicate that inflammatory stimulus-induced extravasation, lamina propria distribution and paracellular epithelial passage of plasma occur with minimal size-selectivity. Inferentially, the full immunological capacity of plasma proteins may readily be made available at the surface of human intact airway mucosa.

Introduction

The occurrence of albumin and other plasma proteins in the airway lumen is a well-known characteristic of inflammatory airway diseases including asthma and allergic rhinitis (Persson, 1986; Raphael *et al.*, 1991; Van Der Graaf *et al.*, 1991; Persson *et al.*, 1998). The entry of plasma proteins into the airway lumen, in particular their passage across the epithelial lining, is generally considered size-dependent. It is only at ultimately severe conditions like adult respiratory distress syndrome that loss of size-selectivity has been clearly acknowledged (Holter *et al.*, 1986). Yet, data on epithelial passage of macromolecules in the intact guinea-pig trachea suggest the possibility that inflammatory stimulus-induced luminal entry of plasma proteins occurs without sieving and without impeding the functional or structural integrity of the epithelial lining (Persson *et al.*, 1998). Animal data further suggest the existence of a hydrostatic pressure-operated, valve-like epithelial mechanism for luminal entry of plasma macromolecules (Persson *et al.*, 1998). This latter mechanism agrees with observations in animal and human airways showing that the mucosal absorption barrier maintains its tightness at exudation events (Greiff *et al.*, 1991, 1997; Persson *et al.*, 1998).

Acute plasma exudation responses have successfully been determined by the occurrence of fibrinogen and α_2-macroglobulin in airway surface liquids of allergen-challenged human bronchi (Svensson *et al.*, 1995; Persson *et al.*, 1998). The large plasma proteins have appeared even in the absence of statistically significant changes in the levels of albumin, that normally is present on the mucosal surface in variable amounts (Svensson *et al.*, 1995; Persson *et al.*, 1998). Although there is thus some indirect evidence for loss of size-selectivity at airways plasma exudation events, this feature has not been established in controlled experiments involving human airways *in vivo*. This aspect is of significance as the appearance of bulk plasma on the airway surface would be a major first line

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respiratory defence mechanism (Persson *et al.*, 1998). It would also mean that all the pluripotent proteins of the circulation have flooded the mucosal interstices and, potentially, been laid down along important subepithelial and paraepithelial pathways (Persson *et al.*, 1998).

In this study, we have taken advantage of the controlled *in vivo* conditions that are attainable within the human nose in studies of the inflammatory responses of the respiratory tract (Persson *et al.*, 1992). Thus by use of a nasal pool-device (Greiff *et al.*, 1990), we have gently but efficiently removed upper airway surface solutes to create minimal baseline protein levels suitable for determination of induced plasma exudation responses. This device is subsequently used to expose the airway surface with defined concentrations of an exudative mediator for defined periods of time. Furthermore, the non-traumatic nasal pool method involves simultaneous and selective lavage of the airway surface area of interest to efficiently retrieve plasma proteins that have crossed the epithelial lining. The present observations indicate that there is a loss of size-selectivity already at plasma exudation responses evoked by mild inflammatory stimuli. Hence, the concept that all pluripotent proteins of circulating plasma contribute to the innate immunity of intact airway mucosal surfaces is strongly supported by human *in vivo* data.

Materials and methods

Study subjects

Ten patients with seasonal allergic rhinitis, 22–29 years of age (mean age 25 years), were examined out of the pollen season (November) without current symptoms of allergic rhinitis. The patients had no history of general disease or recent nasal disease, and no history of recent drug treatment. The study was approved by the local ethics committee, and informed consent was obtained.

Nasal lavage fluid sampling

Nasal lavages with and without histamine in the lavage fluid were carried out using a nasal pool-device (Greiff *et al.*, 1990). The nasal pool-device is a compressible plastic container equipped with a nasal adapter. The adapter is inserted into one of the nostrils and the container is compressed by the sitting subject leaning forward in a 60° flexed neck position. The nasal pool-fluid is consequently instilled and maintained in one nasal cavity by the subject herself. When the pressure on the device is released, the fluid returns to the container.

Isotonic saline *per se* followed by isotonic saline and histamine (40 and 400 μg ml^{-1}) were introduced in sequence into the nasal cavity. The fluid was maintained in the nasal cavity for 10 min, and 20 min elapsed between the start of each instillation. To prevent histamine from being retained in the airway, the mucosal surface was irrigated with saline for 30 s, using the nasal pool-technique, immediately after each 10 min challenge. The recovered fluids were centrifuged (105 *g*, 10 min, 4°C), and samples were obtained from the supernatant and frozen (20°C) awaiting analysis.

Blood sampling

Blood samples were collected in association with the nasal lavage series. Thus, 10 ml blood was obtained from each subject using heparinized tubes. The blood samples were centrifuged (500 *g*, 6 min) for separation of plasma. The recovered samples were frozen (–20°C) awaiting analysis.

Analysis

The nasal lavage fluid levels and plasma levels of α_2-macroglobulin were measured using a radioimmunoassay sensitive to 7·8 ng ml^{-1}. Rabbit antihuman α_2-macroglobulin (Dakopatts, Copenhagen, Denmark) was used as antiserum and human serum (Behringwerke Diagnostica, Marburg, Germany) as standard. Human α_2-macroglobulin (Cappel–Organon Teknika, Turnhout, Belgium) was iodinated using the lactoperoxidase method. Tracer and standard and sample, respectively, were mixed with antiserum before adding goat antirabbit antiserum (AstraZeneca, Lund, Sweden). The bound fraction was measured using a γ counter (Pharmacia Upjohn, Uppsala, Sweden). The intra- and inter-assay coefficients of variation were between 3·8 and 6·0% and 3·1–7·2%, respectively.

The nasal lavage fluid levels of albumin were measured using a radioimmunoassay sensitive to 6·2 ng ml^{-1}. Rabbit antihuman albumin (Dakopatts, Copenhagen, Denmark) was used as antiserum and human serum (Calbichem, Sandiego, CA, USA) as standard. Human albumin (Cappel-Organon Teknika, Turnhout, Belgium) was iodinated using the lactoperoxidase method. Tracer and standard or sample, respectively, were mixed with antiserum before adding goat antirabbit antiserum (AstraZeneca, Lund, Sweden). The bound fraction was measured using a γ counter (Pharmacia Upjohn, Uppsala, Sweden). The intra- and inter-assay coefficients of variation were 5–10%.

The plasma levels of albumin were measured using a standard clinical method based on the binding of bromcresol green to albumin and the spectral absorbance of this complex as measured at 630 nm (Doumas & Peters, 1997).

Statistics

Differences in levels of albumin and α_2-macroglobulin, respectively, between saline and histamine lavages were examined using Wilcoxon signed rank test. P-values <0·05 were considered statistically significant. Data are presented as mean ± SEM and as ratios between levels of albumin and α_2-macroglobulin in plasma samples and in nasal lavage fluid samples, respectively.

Results

Nasal lavages with saline demonstrated greater amounts of albumin than α_2-macroglobulin in nasal mucosal surface liquids at this baseline condition. Nasal lavages with histamine (40 and 400 μg ml^{-1}) were associated with dose-dependently increased luminal entry of albumin as well as α_2-macroglobulin (Fig. 1).

The ratio between albumin and α_2-macroglobulin was greater at baseline (saline) nasal lavages than in plasma (Fig. 2). In contrast, observations at histamine-induced plasma exudation revealed ratios between albumin and α_2-macroglobulin virtually identical to that in plasma. The latter observation was true for histamine 40 as well as 400 μg ml^{-1} (Fig. 2).

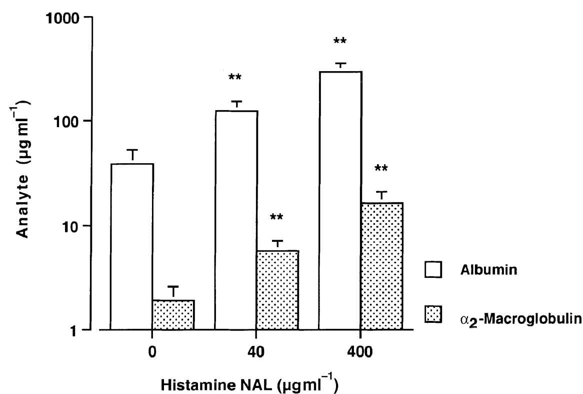

Figure 1 Levels of albumin and α_2-macroglobulin in nasal lavage fluids (NAL) obtained at baseline (saline) and following challenge with histamine. Histamine produced concentration-dependent luminal entry of albumin and α_2-macroglobulin. (**denote $P<0{\cdot}01$).

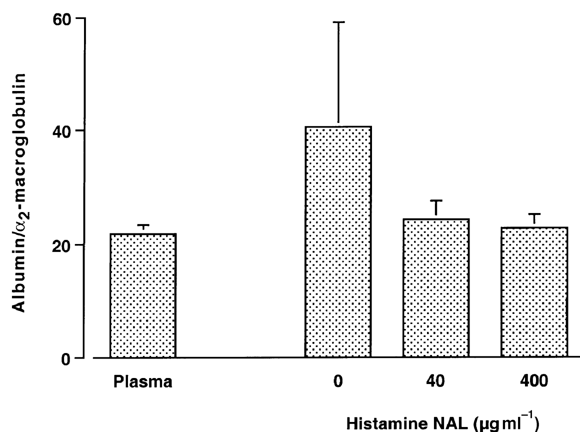

Figure 2 Concentration ratios between levels of albumin and α_2-macroglobulin in plasma samples and in nasal lavages fluids (NAL) collected at baseline (saline) and after challenge with histamine. Histamine-induced plasma exudation is characterized by loss of size-selectivity as reflected by similar albumin/α_2-macroglobulin ratios in NAL and circulating plasma.

Discussion

This study confirms the graded plasma exudation effect of topical histamine in human nasal mucosa and demonstrates that mild to moderate plasma exudation responses involve extravasation, mucosal tissue passage, and luminal entry of albumin and a more than 10 times larger protein, α_2-macroglobulin, without alteration of the concentration ratio that these two proteins hold in circulating blood. Thus, it is anticipated that practically all circulating plasma proteins, irrespective of size, can readily be made available to combat inflammatory insults on the surface of an intact airway mucosa.

The nasal pool method used in this study has been validated previously with regard to gentleness to the airway mucosa, challenge effects, reproducibility and almost quantitative retrieval of instilled fluid into the nasal cavity (Greiff *et al.*, 1990). It has also been used to demonstrate onset, peak and duration of the exudative action of histamine in the human nose (Greiff *et al.*, 1990). Furthermore, the nasal pool technique was instrumental to the demonstration that pronounced plasma exudation responses to histamine did not change the absorption rate across the human airway mucosa (Greiff *et al.*, 1991; Persson *et al.*, 1998). These previous findings contributed to the present study design. Also, the two proteins selected for analysis in this study have previously been employed in assessment of plasma exudation responses in human nasal as well as bronchial airways (Persson, 1986; Raphael *et al.*, 1991; Van Der Graaf *et al.*, 1991; Svensson *et al.*, 1995; Persson *et al.*, 1998). Their sizes comprise most of the immunologically interesting molecules of circulating plasma. The local airway production/secretion of the present proteins is probably negligible especially by comparison with the amounts exuded during the 10 min of histamine exposures that were applied in this study. The similar concentration ratios found in the present histamine-exposed airway lumen and in blood samples may actually corroborate the plasma origin of albumin and α_2-macroglobulin.

Observations in animals indicate that bulk plasma, after its extravasation, is first distributed in the lamina propria. Within seconds the plasma proteins then move across the epithelial basement membrane and up all around epithelial cells towards the lumen (Persson *et al.*, 1998). Thus, plasma proteins can be retrieved on the airway surface within a minute after a topical inflammatory but non-injurious challenge. At challenge with epithelium-damaging agents such as H_2O_2 luminal entry of plasma is even more immediate (Persson *et al.*, 1998). Indeed, at loss of epithelial cells the denuded area will promptly be covered by a plasma-derive fibrin-fibronectin gel that is continuously supplied with bulk plasma until restitution of an epithelial cell cover has taken place (Persson *et al.*, 1998). Thus, plasma exudation evoked by injurious challenges to the airway mucosa has a much prolonged time course as compared with the brief spurt of plasma exudation induced by histamine (Persson *et al.*, 1998). The histamine-type mediators also produce their plasma exudation responses without affecting the passage of hydrophilic

solutes from the lumen into the mucosa (Greiff *et al.*, 1991; Persson *et al.*, 1998). To explain such a valve-like function of the airway mucosa, experimental data suggest that a small increase in the hydrostatic pressure load (<5 cm H_2O) on the basolateral aspect of the intact epithelial lining, such as could be caused by the extravasated plasma itself, is sufficient to reproducibly move macromolecules into the airway lumen without altering the absorption capacity of that same airway mucosa (Persson *et al.*, 1998). This hydraulic epithelial mechanism is not directly influenced by histamine-type inflammatory mediators that thus cannot selectively influence the luminal entry of already extravasated plasma (Persson *et al.*, 1998). The present observation of loss of size-selectivity is compatible with the previously proposed mechanism of epithelial passage of plasma macromolecules.

Albumin and α_2-macroglobulin represent the general and specific binding capacity of plasma proteins (Peterson & Venge, 1987; James, 1990). The present data thus suggest the possibility that extravasated plasma may bind various pro-inflammatory factors occurring in the tissue and move them to the airway surface for clearance through mucociliary transport mechanisms. Rinsing mucosal interstices, including the para-epithelial spaces, would be a significant component of the role of the present plasma exudation mechanism in innate immunity. However, the most important implication may be that adhesive, leucocyte-activating, growth factor active, complement active, or otherwise biologically active plasma proteins will promptly operate not only in the mucosal tissue but also on the surface of an insulted but still intact airway mucosa. The possibility that plasma-derived proteins from time to time may dominate the active biophase *in vivo*, not only in the damaged and repairing airway mucosa but also in non-injurious inflammatory conditions, is of interest. Yet, the complex contribution of plasma-derived molecules to the inflammatory biology of the human airway mucosa may not have received wide attention. This may in part reflect the absence of plasma exudate in currently employed test systems. Thus, only poor plasma exudation responses seem to apply for mouse models of allergic airway diseases (Persson *et al.*, 1997). Also, the major focus on 'inflammation' *in vitro* naturally highlights proteins that are generated by cells in culture rather than highlighting the similarly active proteins that emanate from the microcirculation *in vivo*. It is of note that the guinea-pig trachea *in vivo* may well reflect the human airway mucosa as regards plasma exudation responses (Persson *et al.*, 1998).

In conclusion, this study has demonstrated that the acute plasma exudation response to local human airway provocation with a non-injurious exudative mediator involves loss of size-selectivity. Hence, bulk plasma may promptly enter the airway lumen. These *in vivo* data underscore the importance of considering the complex and dynamic contribution of plasma-derived molecules in any realistic model that investigates immuno-inflammatory mechanisms of the airway mucosa.

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References

Doumas BT, Peters T. Serum and urine albumin: a progress report on their measurement and clinical significance. *Clin Chim Acta* (1997); **258**: 3–20.

Greiff L, Pipkorn U, Alkner 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.

Greiff L, Wollmer P, Pipkorn U, Persson CGA. Absorption of ^{51}Cr-EDTA across the human nasal mucosa in the presence of topical histamine. *Thorax* (1991); **146**: 630–632.

Greiff L, Lundin S, Svensson C, Andersson M, Wollmer P, Persson CGA. Reduced airway absorption in seasonal allergic rhinitis. *Am J Respir Crit Care Med* (1997); **156**: 783–786.

Holter JF, Weiland JE, Pacht ER, Gadek JE, Davis WB. Protein permeability in the adult respiratory distress syndrome. Loss of size-selectivity of the alveolar epithelium. *J Clin Invest* (1986); **78**: 1513–1522.

James K. Interactions between cytokines and α_2-macroglobulin. *Immunol Today* (1990); **11**: 163–166.

Peterson CG, Venge P. Interaction and complex-formation between the eosinophil cationic protein and α_2-macroglobulin. *Biochem J* (1987); **245**: 781–787.

Persson CGA. Role of plasma exudation in asthmatic airway. *Lancet* (1986); **2**: 1126–1129.

Persson CGA, Svensson C, Greiff L. *et al.* The use of the nose to study the inflammatory response in the respiratory tract. *Thorax* (1992); **47**: 993–1000.

Persson CGA, Erjefält JS, Korsgren M, Sundler F. The mouse trap. *TIPS* (1997); **18**: 465–467.

Persson CGA, Erjefält JS, Greiff L. *et al.* Contribution of plasma-derived molecules to mucosal immune defence, disease and repair in the airways. *Scand J Immunol* (1998); **47**: 302–313.

Raphael GD, Igarashi Y, White M V, Kaliner M A. The pathophysiology of rhinitis V. Cources of protein in allergen-induced nasal secretions. *J Allergy Clin Immunol* (1991); **88**: 33–42.

Svensson C, Grönneberg R, Andersson M. *et al.* Allergen challenge-induced exudation of α_2-macroglobulin across human nasal and bronchial microvascular–epithelial barriers. *J Allergy Clin Immunol* (1995); **96**: 239–246.

Van Der Graaf EA, Out TA, Roos CM, Jansen HM. Respiratory membrane permeability and bronchial hyperreactivity in patients with stable asthma. *Am Rev Respir Dis* (1991); **143**: 362–368.