

# Clinical & Experimental Allergy

## Roles of plasma exudation in asthma and COPD

This editorial discusses the findings of the paper by Khor et al. [2] pp. 1659–67.

C. Persson<sup>1</sup> and L. Uller<sup>2</sup>

<sup>1</sup>Department of Clinical Pharmacology and <sup>2</sup>Department of Experimental Medical Science, Lund University, Lund, Sweden

Forty years ago, Ryley and Brogan demonstrated that the sputum phase of asthmatic sputa contained large amounts of albumin and that glucocorticosteroid treatment reduced the plasma protein content along with improvement in lung function (reviewed in [1]). Khor et al. [2] in this issue of the journal now report that reducing the dose of inhaled steroids is associated with increased bronchial extravasation and exudation of plasma in parallel with clinical deterioration of asthma control. This observation confirms and extends the many previous findings on occurrence of plasma exudation in asthma. Furthermore, it is a timely reminder of a major component of mucosal innate immunity responses that lately has been eclipsed by progress in research on other aspects of immunopathology of airway inflammatory diseases. Plasma exudation may thus be left out of current updates of the immunology of asthma and chronic obstructive pulmonary disease (COPD) [3]; yet, it is a significant feature of both diseases [4]. A further consequence of this neglect may be the persistence of little-founded notions in the field. As briefly discussed here, attention to the bronchial plasma exudation process is needed to distinguish it from so-called 'microscopic bleeding' [5], from general airway permeability changes, and from airway oedema. Further, the findings by Khor et al. [2] should stimulate attention to all the biological activities of exuded plasma in airway tissue and air space. However, it is beyond the scope of this editorial to detail the possible involvement of multipotent plasma-derived protein systems in the immunopathogenesis of asthma and COPD.

### Hallmark of asthma

In the study by Khor et al. [2], plasma exudation is an outstanding feature of reduced asthma control. Khor et al.

[2] thus showed increased plasma exudation without significant increases in inflammatory cells. Nor were any effects on bronchial vascularity, angiogenic sprouts, or expression of vascular endothelial growth factor associated with the worsening of asthma [2]. This is of interest because, together with effects on blood flow, these are the very features that prevail in current discussions of roles of bronchial vasculature in asthma [3, 6, 7]. It is important to acknowledge that the study design [2] involved an initial period of high-dose steroid treatment that may have some degree of carry-over effects on subsequent measurements. Also, it was a dose-reduction study. Hence, the patients who deteriorated still received inhaled steroid albeit only a small dose. Dose-response relationship for airway steroids is notoriously difficult to assess and it cannot be excluded that the remaining low-dose steroid treatment in part explains why so many indices did not change in the studies by Khor et al. [2]. However, this caveat does not detract from the fact that Khor et al. [2] could demonstrate an exclusive association between occurrence of extravasated plasma proteins in bronchial tissues and worsening of clinical asthma. These data support the view of plasma exudation as a hallmark of asthma and suggest important roles of potent pro-inflammatory plasma protein systems that become activated after extravasation into the diseased tissue and air space.

### Plasma exudation without oedema

The inflammatory extravasation of plasma occurs between actively separated endothelial cells (gaps) in post-capillary venules belonging to the bronchial circulation, which brings systemic, oxygenized blood to the airway tissues. The resulting high permeability gaps in the venular wall let out small as well as large plasma proteins. However, the gaps are not usually involved in the extravasation of leucocytes explaining why the exuded plasma may not be associated with increased numbers of inflammatory cells [8]. The extravasated bulk plasma distributes throughout available interstitial spaces where plasma proteins are laid down, as also illustrated by Khor et al.

### Correspondence:

Prof. Carl Persson, Department of Clinical Pharmacology, Lund University Hospital, 221 85 Lund, Sweden.

E-mail: Carl.Persson@med.lu.se

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[2]. Yet, this does not mean that a mucosal oedema has been produced nor has any oedema reached measurable proportions in experimental studies involving challenge-induced airways plasma exudation [8]. Actual bronchial mucosal oedema also remains an unproven component of asthma and COPD.

### Swift epithelial passage of bulk plasma

Ingrid and Jonas Erjefält developed sensitive techniques to challenge and lavage guinea-pig trachea and to illustrate the distribution of extravasated plasma including its movement across the epithelial basement membrane and up between and all around epithelial cells in the area of interest [9]. Rather than producing oedema, or being removed by lymphatic transport, much of the plasma thus enters the air space within minutes after its extravasation [8]. Already at minimal increases in the basolateral epithelial hydrostatic pressure, as could be induced by the travelling plasma itself, the tight epithelial lining lets through the extravasated interstitial plasma proteins, again without sieving [4, 8]. Thus, after an acute mediator-induced challenge of the airway mucosa, small plasma proteins such as albumin and 10 times larger plasma proteins can be retrieved from the intact mucosal surface in almost the same concentration ratios as found in circulating plasma. This property explains why large plasma macromolecules, such as  $\alpha$ 2-macroglobulin, are more specific indices of plasma exudation than albumin that in minimal amounts may seep through the vascular-endothelial and mucosal-epithelial barriers also at baseline conditions [8]. Egression of plasma into the air space reflects the plasticity of epithelial cell junctions. Considerable exudation of plasma across the epithelial lining thus occurs without causing epithelial disruption. There is also no increased movement of molecules in the opposite direction explaining why exudative airway disease conditions such as asthma and rhinitis may not exhibit increased absorption abnormalities [8]. Epithelial plasticity is equally impressive when numerous leucocytes egress paracellularly into the air space without leaving any trace that this mode of elimination of inflammatory cells from airway tissue has occurred [10].

Much of the evidence supporting the above description of the microvascular-epithelial exudation of plasma has been generated in guinea-pig trachea and in the accessible human nasal passages in health and disease [4, 8]. Studies addressing translatability of such data to the lower and small airways in asthma and COPD are warranted. The unravelling of mechanisms and roles of respiratory tract plasma exudation would have to go beyond *in vitro* approaches. Moreover, the common mouse models 'of asthma' are not optimal as exuded plasma in the air spaces seems to be lacking [11]. Alternatively, it appears that extravasated plasma and deposition of fibrin can

occur along with many extravasated red blood cells in the alveolar ducts of allergen-challenged and -sensitized mice; such a bleeding response may make you wonder 'What's going on down there anyway?' [12] and how may mouse model findings translate to human asthma?

### Sustained exudation of plasma in large and small airways

In humans, there is a rich subepithelial microvascular network that stretches all the way from trachea to bronchioles; in experimental animals, only the trachea may be thus equipped. Hence, plasma exudation involving the systemic bronchial microcirculation of small airways is a potentially important but rarely studied factor in COPD and severe asthma. Little is also known in detail of factors responsible for sustained microvascular permeability responses in the airways. Histamine/leukotriene-type mediators cause merely a short-lasting spurt of plasma exudation and there is rapid tachyphylaxis to this response. The reputed neurogenic plasma exudation response that happens to be readily evoked in airways of rats and guinea-pigs, has apparently burnt out and has not been convincingly demonstrated in most species including man [8]. Other mediators, similar to allergen challenge, may bring about an acute response followed by a more protracted late-phase plasma exudation response [1, 8]. In *in vivo* studies involving guinea-pig trachea, a sustained plasma exudation response is evoked by such epithelial cell loss that leaves an intact basement membrane 'naked'. Thus, after non-sanguineous epithelial denudation, plasma exudation promptly creates a proteinaceous gel covering the epithelial basement membrane. The exudation-generated repair gel is continuously being supplied by fresh plasma proteins (including fibronectin, fibrinogen, etc.) [13]. The provisional gel matrix remains until substituted by a cover of migrating and repairing mesenchymal-like epithelial cells (reviewed in [14]). The plasma exudation response during the epithelial repair phase is advanced as one of several causative components of epithelial injury-evoked asthma-like conditions [8], and it has lately received attention in explanations of exercise-induced asthma [15]. However, it is for the future to determine in detail what danger, stranger and other signals produce sustained bronchial plasma exudation in disease.

### Mucus-plasma plugs and fibrin formation

One aspect on the occurrence of plasma in the air space concerns its ability to increase viscosity of secretions and, hence, impede mucociliary clearance. This effect likely contributes to producing life-threatening mucus-plasma plugs in asthma [4, 16]. Exuded plasma, including potential activation of the coagulation pathway and the laying

down of fibrin on epithelial surfaces, can also be a major factor in small airways disease in COPD and severe asthma. As demonstrated by endobronchial challenge, fibrinogen, along with albumin and  $\alpha$ 2-macroglobulin, acutely enters the air space of allergic asthmatics at exposure to the relevant allergen [17]. Brims et al. [18] now indicate that there is increased fibrin formation from exuded fibrinogen in stable asthma. These authors further begin to clarify the relative importance of local production and supply through plasma exudation, respectively, of coagulation factors occurring in the asthmatic air space. They also demonstrate that plasma exudation is much increased in severe asthma and that the air space of these patients may exhibit an anti-fibrinolytic milieu, further emphasizing the possibility that fibrin contributes significantly to the severity of the disease [18].

### ***In vivo* milieu of exuded plasma**

There may be a conceptual problem with lack of specificity of the plasma exudation process; a wide range of inflammatory factors produce exudation and the extravasated plasma may indiscriminately produce a wide range of bioactive molecules. The extravasated plasma thus supplies diseased airways with many of the important regulatory proteins-peptides (fibronectin, fibrinogen-derived proteins and peptides, complement factors, kininogen products, etc.), many of which now being studied as cell products *in vitro*. The exuded plasma is capable of creating a special and highly dynamic, bioactive milieu that may be crucial to our understanding of cellular activities *in vivo* and that may not be fully mimicked by having serum in cell culture media. Studies are warranted to explore if specific activation of individual potent plasma proteins occurs in airways once they have left the circulation compartment, in disease compared with health, in chronic compared with acute exudation, and in asthma compared with COPD?

### **Permeability rather than blood flow determines plasma exudation**

The data generated by Khor et al. [2] support the possibility that plasma exudation is a major cause of asthma morbidity and that its pharmacological inhibition is a significant therapeutic effect in asthma. Further studies have to define to what extent these suggestions are true. Decades of work indicate that steroids are the important anti-exudative drug class in inflammatory airways diseases. Still, the inhibitory mechanism of steroids is not known in detail. Of significance for innate immunity maintenance, steroids may have little effect on acute exudative airway challenges evoked by infection and injury and by mediators that exert direct effects on venular permeability [4, 8]. Such observations further

suggest that an important component of steroid action is indirectly reflecting the general anti-inflammatory efficacy of these drugs to reduce the availability of vasopermeability agents in the diseased airway mucosa. Several drugs may have some effects on microvascular permeability. Through direct effects on venular endothelial cells  $\beta_2$ -agonists can reduce plasma exudation in animal and human airways [4, 8]. Simultaneously, these drugs increase the blood flow. In the little vascularized skin, vasodilatation acts in synergy with increased permeability to increase extravasation of plasma. In the skin, steroid-induced vasoconstriction thus reduces exudation. However, limited blood flow changes in a well-perfused organ such as the airway mucosa may have no effect on the plasma exudation process [4, 8]. Effects on permeability are crucial. However, proof of clinically important anti-exudative actions of  $\beta_2$ -agonists, alone or together with steroids, has yet to be produced.

### **Plasma exudation indices reflect intensity of inflammation**

The readiness by which extravasated plasma enters the air space is actually helpful for the exploration of pharmacological control of airways microvascular permeability. Human bronchial microvascular permeability can thus be well monitored in health and disease through the determination of exudation indices in airway luminal samples [4, 8, 19, 20] such as induced sputum. Greiff and colleagues launched a dual induction protocol, involving an initial challenge to induce plasma exudation followed by sputum induction, to explore the pharmacological regulation of plasma exudation responses in human bronchi [4, 8, 19]. The additional possibility exists that extravasated plasma, with its content of binding proteins, will pick up other released proteins such as cytokines on its way through subepithelial and epithelial interstices and bring them to the surface for easy sampling [4, 8].

Exaggerated plasma exudation responses may develop both as a result of angio-neogenesis and as an increased responsiveness of the venular endothelium. It is of interest that a major cause of exacerbations of asthma and COPD, the common cold viral infections, induces human (nasal) airways microvascular hyperresponsiveness to inflammatory mediators [21]. Speculatively, such a feature could also be involved in the increased plasma exudation occurring in asthmatic individuals at steroid dose reduction [2].

### **Conclusion**

Plasma exudation may not be the first inciting mechanism in the development of airways disease. However, it is a central feature demonstrating that whatever the inflammatory mechanisms, these have reached a level sufficient to produce significant effects on the airway tissue proper.

Thus, the plasma exudation response is specific to inflammation and differs from irritant-induced neural reflex actions evoking effects such as mucus secretion and increased blood flow [4, 8]; both these latter effects are, of course, also increased by inflammatory stimuli. In COPD and asthma, plasma exudation has been associated with disease severity at baseline and at exacerbations [1, 2, 4, 8, 18, 22, 23]. In their studies involving determination of several common inflammatory features, Khor et al. [2] provocatively single out plasma exudation as a particularly important facet of the asthmatic diathesis.

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