

CORRESPONDENCE

Airway, apoptosis, and asthma

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What is 'airway'?

I read with interest the article by Simpson et al. [1] who examined sputum macrophage ingestion of epithelial cells that had been made apoptotic *ex vivo*. This interesting work tallies with cutting edge contributions made by these authors in the field of granulocyte apoptosis and macrophage efferocytosis in asthma and COPD. This time their study apparently paves the way for clinical trial of macrolide antibiotics in the subgroup they define as 'non-eosinophilic asthma', two further areas where the authors are making significant contributions. The authors conclude that their data may explain 'persistent airway neutrophilia' in non-eosinophilic asthma. I think the reader may interpret 'airway' as referring to events equally valid for airway lumen, examined by the authors, and for diseased airway tissue. Intentional or not, this interpretation is underscored by the authors' focus on lumen (and *in vitro*) data in their discussion of clinical relevance. I wish to introduce some caution by highlighting potential dichotomies between airway lumen and tissue. I also have questions regarding *in vivo* relevance of apoptotic epithelial cells studied by Simpson et al. [1].

Death of granulocytes in airway lumen and airway tissues

One obvious aspect is that dead or dying cells, whether by apoptosis or necrosis, would have lost the ability to migrate. Hence, the occurrence of dead cells in diseased bronchial tissues can hardly be revealed by studies of bronchial lumen samples whatever the level of sophistication of applied analyses. So the question is if neutrophil apoptosis, or necrosis, or ingestion by macrophages, in the bronchial lumen, mirrors what may go on in the bronchial tissue? I agree that neutrophil apoptosis may occur in bronchial tissues, even to the extent that phagocytosis of apoptotic neutrophils fails resulting in secondary necrosis of neutrophils and exaggerated inflammation [2], but its importance in disease may not be known. The eosinophil may be an extreme example. None has fully explained why my group and others, including the authors, readily

observe apoptotic eosinophils in the airway lumen (and *in vitro*) whereas apoptotic eosinophils appear to be absent in airway tissues *in vivo* (inside or outside macrophages) (reviewed in [3]). We can speculate on a variety of factors because the richly blood-perfused bronchial mucosa presents an active milieu that is dramatically different from the lumen. However, part of the explanation lies in the observation that eosinophils in asthmatic bronchial mucosa are subject to a necrotic death by which their toxic protein-laden granules become dispersed in the tissue.

Primary necrosis of bronchial tissue eosinophils, clinical control, and subgroups of asthma

Necrosis-induced release of free granules is a true disease event that cannot be explained by tissue handling artifacts [4]. Uller originally demonstrated further that eosinophil necrosis is a primary event that is not preceded by apoptosis [5], which has been confirmed by other groups. Primary necrosis/lysis of eosinophils stands out as a major mode of activation of these cells. Interestingly, it emerges that lysis of bronchial tissue eosinophils is a major factor in clinically uncontrolled asthma [6]. Thus, Volbeda et al. [7] demonstrated that eosinophil granules in the tissue (we all agree that they emanate from lysed eosinophils [6, 8]) more clearly than eosinophil numbers in the bronchial lumen were associated with poor clinical control of asthma. The important necrotic eosinophils occur in the tissue. Admittedly, the free granules may complicate the determination of eosinophils in biopsies. How do you count single granules? Judging from histology graphs this would have been an issue also in the early demonstration of inflammatory subtypes of asthma by Wenzel et al. [9]. The caveat is that non-eosinophilic asthma may not always be fully defined by cell numbers in the bronchial lumen. It may be necessary to determine the degree of eosinophil lysis in the tissue before completely discarding the possibility that the disease, at least in part, is eosinophil-driven.

Lumen or tissue inflammation?

For bronchial tissue-dwelling inflammatory cells there is an alternative mode of elimination to apoptosis followed by phagocytosis. Helene Rosenberg recently highlighted the role of transepithelial route of elimination vs.

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apoptosis in an Editorial in this Journal [10]. Destined for final elimination by mucociliary clearance, eosinophils, and neutrophils leave the bronchial tissue through transepithelial migration into the airway lumen [3]. In accord, resolving inflammation in asthma has been associated with increasing numbers of granulocytes in bronchoalveolar lavage fluid along with depletion of these cells in the bronchial tissue (without evidence of contribution by apoptosis; reviewed in [3]). Conversely, there may be little transepithelial traffic of cells during the development of inflammation. However, the tendency of cells to remain in airway secretions for prolonged times means that sputum samples may not sensitively pick up the waxing and waning of inflammation in chronic asthma. Hence, it is not surprising that cells in sputum samples have successfully defined subgroups of asthma and guided treatments. Yet, the inference from the actual experiments carried out by Simpson et al. [1] would be that the lumen cells have pathogenic effects in asthma. So what is actually known about ability of granulocytes, which dwell in viscous lumen secretions, to adversely engage the bronchial mucosa in asthma? By their occupation of precious space, lumen cells contribute to obstruction, especially in diseased small airways with already compromised by reduced ciliary transport and the presence of mucus-plasma exudates in asthma [11]. But beyond that? Perhaps we can agree that major disease-driving cellular events in asthma likely take place in the bronchial tissues.

Epithelial shedding driven airway inflammation without epithelial apoptosis

Simpson et al. [1] used epithelial cells as the apoptotic target cell for engulfment by the sputum macrophages. By their strategic position and immuno-inflammatory response to environmental challenges, bronchial epithelial cells receive attention as major effector cells in asthma. Asthmatic epithelium may exhibit abnormally low production of antiviral interferons [12, 13], and overproduce upstream inflammatory cytokines [13] involving a range of effects including interactions with dendritic cells [14]. Preceding the current focus on epithelial cells as effectors at the interface between innate and adaptive pathogenic immunology, we developed an interest in repair processes subsequent to epithelial shedding that occurs without damage to the basement membrane (as in asthma). Original *in vivo* studies (together with Jonas and Ingrid Erjefält) on non-sanguineous loss of pseudostratified epithelial cells, without epithelial cell apoptosis or necrosis, actually provided basic aspects of epithelial restitution following shedding (reviewed in [15]). Our discovery of promptness and speed of restitution, participating epithelial cells (also ciliated and secre-

tory cells that previously were considered end-differentiated cells became repair cells), the role of plasma-derived fibrin-fibronectin cover of basement membrane etc [15] have apparently become accepted (also by the authors, see introduction in reference [16]) as established knowledge. We have seen fewer acceptances of our equally well-documented findings on potential pathogenic importance of simple shedding without apoptosis: Our hypothesis (first published in this Journal; [17]) stated that epithelial shedding-restitution processes alone could produce many of the defining inflammatory and remodelling features of asthmatic bronchi: plasma exudation, hypersecretion, recruitment, and activation of neutrophils and eosinophils, as well as proliferation of fibroblast and smooth muscle cells [17]. In accord, Creola bodies (clusters of desquamated epithelial cells) in aspirated sputum, independent of other factors, predicted development of asthma in children [18]. The speed by which denuded areas were covered in our experimental *in vivo* studies rather provided an explanation as to why increased bronchial absorption permeability has been so difficult to demonstrate in asthma (another field where *in vitro* paradigms remain little supported by clinical *in vivo* data, reviewed in [15]).

Apoptosis of epithelial cells?

Simpson et al. [1] may have used epithelial cells partly as surrogate for neutrophils. They also champion the idea that epithelial apoptosis is an important disease feature and that macrolide antibiotics desirably increase macrophage phagocytosis of these cells ([1], but more detailed in reference [16]). However, what evidence, beyond bronchial brushing data, demonstrates (preferably by gold standard electron microscopy) that epithelial apoptosis occurs to an important extent in asthma? Further, is apoptosis then a significant event prior to, or at shedding? Shedding of infected epithelial cells occurs, but to what extent are these cells alive, apoptotic or necrotic? Is this shedding a pathogenic event, or is suicidal sacrifice of single infected epithelial cells rather an antiviral defence mechanism? This latter possibility is suggested by instantaneous closure of gaps as single cells leave the epithelial lining [15]; it is further compatible with the finding that the number of shed rhinovirus-infected ciliated cells was unrelated to the common cold symptoms [19]. Also, loss of single epithelial cells appears to be the most common type of shedding in asthma (reviewed in [15]). If apoptosis is largely an event occurring in the airway lumen, what is then the evidence that macrophage engulfment of apoptotic epithelial cells occurs and even overwhelms the phagocytic activity of macrophages (cf. [16])? Or are shed epithelial cells merely an innocent component of the lumen debris?

Conclusion

I think these are relevant questions in a topical field where paradigms may be under development or where currently accepted views may not be fully supported by *in vivo* data. Importantly, this discussion does not question any aspect of the impressive clinical efficacy of macrolide antibiotics in chronic lung diseases. On this note, clinical efficacy of steroids in these diseases has not been reduced by inability of investigators to con-

firm *in vivo* the reputed steroid-induced effects on apoptosis. Based largely on *in vitro* data, without compelling translational support, it has somehow become 'general knowledge' that steroids increase apoptosis of eosinophils and lymphocytes and decrease apoptosis of neutrophils (reviewed in [3]). Simpson et al. [1] also make unreferenced statements on such effects of steroids without any reservation regarding clinical relevance.

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