

CORRESPONDENCE

Regarding comments by Persson et al. (Airway, apoptosis, and asthma) to J. L. Simpson, P. G. Gibson, I. A. Yang, J. Upham, A. James, P. N. Reynolds, S. Hodge and AMAZES Study Research. Impaired macrophage phagocytosis in noneosinophilic asthma. Clin Exp Allergy 2013; 43: 29–35.**S. Hodge and P. N. Reynolds***Lung Research, Department of Thoracic Medicine, Royal Adelaide Hospital, Adelaide, Australia*

We read with interest the comments by Persson et al. and appreciate the opportunity to address the issues raised. Persson has drawn a distinction between inflammatory events in the lumen and the bronchial tissues, and we accept that differences exist. However, we disagree with the comment that ‘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’. Desquamation of inflamed epithelial cells into the lumen is a recognized feature of asthma. Thus, we believe examination of this material is in fact highly relevant. Furthermore, secondary necrosis of desquamated apoptotic cells will result in the release of toxic metabolites that can be expected to have an impact on the local milieu.

We do not dispute that primary necrosis of inflammatory cells, including eosinophils, is of pathogenic relevance in asthma. This of course does not exclude the possible co-existent importance of apoptosis. In support of this proposal, we have previously described excess apoptosis in airways disease (in particular in COPD) and the close association of this finding with defective macrophage function (efferocytosis).

Persson comments that ‘non-eosinophilic asthma’ may not always be defined by cell numbers in the bronchial lumen. This may be true, but somewhat misses the point of our approach. We have used widely accepted definitions of eosinophilic and non-eosinophilic asthma, based on sputum analysis, which provides a pragmatic and clinically useful means of defining a phenotype which has proven a useful strategy already as a basis for treatment decisions. Our work provides evidence that sputum analysis may have even greater utility in guiding treatment.

We were the first to report defective efferocytosis (phagocytosis of apoptotic bronchial epithelial cells) by alveolar macrophages in chronic obstructive pulmonary disease (COPD), associated with evidence of secondary necrosis of the uncleared material [1]. We have since reported similar defects in several other chronic inflammatory lung diseases including bronchiolitis obliterans following lung transplantation. Importantly, the significance of these findings was confirmed by significant correlations between both efferocytosis and the number of apoptotic bronchial epithelial cells and markers of inflammation in COPD [2]. In addition, we found that there was an equally deficient ability of alveolar macrophages from COPD subjects to ingest apoptotic cells, whether we used apoptotic neutrophils or bronchial epithelial cells as targets in our assays [3]. Taken together, these findings support the validity of using bronchial epithelial cells as phagocytic targets in investigations of chronic airways diseases.

The clinical relevance of these findings was further highlighted in our subsequent studies that showed that several macrophage-targeted therapies could improve macrophage efferocytic and phagocytic function and reduce the numbers of apoptotic bronchial epithelial cells *in vivo* using both human COPD subjects and in murine models [2, 4–6]. Importantly, these treatments resulted in a concurrent and significant reduction in markers of inflammation.

Therefore, although we acknowledge the importance of the presence and clearance of inflammatory cells from lung tissue in asthma, the goal of the study by Simpson et al. was to specifically investigate efferocytosis in the airway in non-eosinophilic asthma, a phenotype of asthma that we have described as being quite distinct from the eosinophilic form, with many of the hallmarks of COPD including airway neutrophilia.

Rapid apoptosis and elimination of both apoptotic bronchial epithelial cells and neutrophils from the airway is required for resolution of inflammation. There have

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been several convincing reports of increased rates of bronchial epithelial cell apoptosis in asthma [7, 8]. In fact, as highlighted by Juncadella et al. in a recent edition of *Nature*, clusters of uncleared epithelial cells referred to as 'Creola bodies' in the sputum have been seen in patients with asthma for decades [9]. Contributing to the increased epithelial cell apoptosis are a myriad of apoptosis-stimulating mediators that may be present in the airway in non-eosinophilic asthma including death receptors, cytokines and oxidative stress. We have also recently found an increase in the cytotoxic mediator, granzyme B, in the airways of these patients (Simpson JL, Gibson PG, Yang IA, Upham J, James A, Reynolds PN, Hodge S, AMAZES Study Research Group, unpublished data). It is thus likely that future findings in non-eosinophilic asthma will mirror those from the more extensive investigations that have taken place in other chronic inflammatory lung diseases including COPD. The direct clinical importance of increased rates of lung epithelial cell apoptosis per se has been demonstrated. Inhibition of apoptosis using a caspase inhibitor protected mice from the lethal consequences of endotoxin-associated acute lung injury [10]. A causative link between macrophage dysfunction and consequent accumulation of secondary necrotic material has also been demonstrated. Mice selectively depleted of alveolar macrophages had impaired resolution of inflammation and increased mortality, directly related to increased accumulation of apoptotic material and secondary necrosis [11].

Persson et al. raises the pertinent question of whether neutrophil apoptosis, or secondary necrosis, or ingestion by macrophages, in the bronchial lumen, actually mirrors what may go on in the bronchial tissue. We have found a similar degree of macrophage dysfunction in both the airway and lung tissue of smoke-exposed mice and human COPD subjects [4, 5, 12]. Using macrophage-targeted therapies, we could improve macrophage function in both compartments [4, 5]. The authors themselves previously showed that secondary necrosis of neutrophils (cells with condensed and electron-dense nuclei and ruptured cell membrane) were extensively seen in inflammatory foci both in lung tissue and BAL during intense lung inflammation induced by LPS [13]. This may not only be worsened by the

presence of several mediators that induce apoptosis of, for example, airway epithelial cells (including LPS and TNF) but also prolong the survival of neutrophils by delaying apoptosis and allowing their accumulation at local tissue sites of inflammation. Although these cells can cause damage by release of enzymes such as PAF and PLA2, and finally by their secondary necrosis, controversy persists as to whether this accumulation and sustained activation of neutrophils actually contributes to lung injury [14]. In a model of acute lung injury, prolonging the lifespan of activated neutrophils did not exacerbate acute lung injury, in fact an initial survival benefit was noted when the life span of neutrophils was further prolonged, likely due to an enhanced capacity to clear bacteria as evidenced by the lower bacteria counts in the lung [14]. We argue that what may outweigh all of these relative changes in apoptosis of different cell types is a pan-deficiency in the phagocytic ability of neighbouring macrophages, whether in the airway or lung tissue. Whether the neutrophils are being cleared from the lung tissue by post-egression mucociliary clearance, apoptosis/efferocytosis, or more likely by a combination of both, it is becoming clear that these processes may be together ineffective and contribute to the chronic inflammation in several chronic lung diseases including non-eosinophilic asthma.

There remains a great need for further studies to more clearly identify the reasons for the exact causes of the deficient efferocytosis that is found in a variety of chronic lung diseases; however, current evidence strongly suggests that uncleared apoptotic cells can lead to secondary necrosis and potential tissue damage in several lung diseases, and that targeting the efferocytic function of alveolar macrophages with candidate therapies can lead to a significant improvement in airways inflammation. Although egression is likely to significantly and positively impact resolving acute, self-limiting airway inflammation, the question still remains whether the resultant increased numbers of inflammatory cells in the airway lumen, if not effectively cleared by neighbouring macrophages, will undergo secondary necrosis and perpetuate chronic inflammation in chronic inflammatory diseases such as COPD and potentially in the non-eosinophilic form of asthma.

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