

EDITORIAL

Eating their way out of trouble: selective uptake of apoptotic eosinophils by bronchial epithelial cells

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Role of eosinophils in allergic inflammation and asthma

Central to the inflammatory hypothesis in asthma is the role of the eosinophil and its capacity to elaborate and release a series of highly cytotoxic and pro-inflammatory mediators [1]. The regulation of eosinophil production and function has been extensively studied and much is now known about its selective recruitment, priming, activation and prolonged survival by cytokines predominantly encoded on chromosome 5q_{31–34} (the IL-4 gene cluster) [2, 3]. Three major eosinophilopoietic cytokines (IL-3, IL-5, GM-CSF (granulocyte macrophage-colony stimulating factor)) lead to the maturation and terminal differentiation of eosinophils within the bone marrow [4] and influence their recruitment to sites of inflammation. This latter process involves adhesion to endothelial cells, which is mediated via an interaction between vascular cell adhesion molecule-1 (VCAM-1) and the eosinophil-restricted very late activation antigen-4 [5] and intracellular adhesion molecule-1 (ICAM-1) with lymphocyte function associated antigen [6]. Eosinophils migrate in response to a range of chemoattractants, including chemokines, complement components and lipid mediators [2, 5, 7], and express the cognate receptors for a variety of factors including IL-8, regulated on activation, normal T cell expressed and secreted (RANTES), eotaxin, IL-1, IL-2, IL-3, IL-4, IL-5, IL-9, IL-16, GM-CSF, IFN- γ , Ig and TNF- α [8]. These cells are thus equipped to respond to a wide range of inflammatory stimuli. Eosinophil activation and/or cytolysis leads to the release of a unique repertoire of highly histotoxic substances including cationic proteins (eosinophil-derived neurotoxin, eosinophil peroxidase, eosinophilic cationic protein, major basic protein) [9] and metalloproteases and eosinophils are a major source of cysteinyl-leukotrienes that contribute to bronchoconstriction, microvascular leak and bronchial hyper-responsiveness [10].

The resolution of eosinophil inflammation

The elimination of airway eosinophils is thought to rely on two major processes. Firstly, it is clear that antigen challenge can induce a rapid and highly co-ordinated exit of large numbers of eosinophils into the airway lumen (estimated at up to 35 000 cells/min/cm²) [11]. This event is likely to be driven by the polarized release of chemoattractants from the apical surface of epithelial cells [12] and appears to cause little if any immediate damage to the epithelium [11, 13, 14].

In addition, attention has focussed on the potential role of cytolysis and in particular apoptosis in the resolution of

eosinophilic inflammation [15–17]. In this respect it is of interest that many cytokines that are associated with tissue eosinophilia *in vivo* can inhibit the rate of constitutive cell death *in vitro* [18]. The hypothesis that the inhibition of apoptotic cell death may underlie certain forms of chronic eosinophilic inflammation is supported by the observation that corticosteroids are potent inducers of eosinophil apoptosis both *in vitro* and *in vivo* [19, 20]. In contrast, neutrophils are resistant to the pro-apoptotic effects of corticosteroids, an effect that may relate to the differential expression of glucocorticoid- α and - β (GR- α and GR- β) mRNA between these two cell types [21] and the capacity of GR- β to act as a dominant negative inhibitor of GR- α activity [22].

Under *in vitro* conditions apoptotic eosinophils are rapidly engulfed by inflammatory macrophages, a process that would be predicted to prevent secondary necrosis and result in the resolution of airway inflammation [15, 23]. This view has been challenged, however, by *in vivo* studies where eosinophil apoptosis could be detected only in cells present in the airway lumen [14]. Moreover, corticosteroids were shown to have no effect on the extent of eosinophil apoptosis within the airway wall and failed to affect the rate of eosinophil entry into the lumen. While these data may not take full account of the very rapid and efficient coupling of granulocyte apoptosis to engulfment *in vivo* [24], they suggest the possibility that trans-endothelial migration of eosinophils or residency within the airway wall may confer an 'apoptosis-resistant' phenotype on these cells. This process would be predicted to be relatively selective for the eosinophil since apoptotic neutrophils are readily apparent in a number of inflammatory conditions [25].

Modulation of eosinophil survival by endothelial cells

Endothelial cells were initially thought to play a passive 'barrier' role in vascular physiology, with their active functions limited to aiding coagulation. However, numerous studies have now demonstrated a pivotal role for these cells in regulating metabolism (e.g. angiotensin converting enzyme expression, serotonin degradation), inflammation (e.g. TNF- α , VCAM and ICAM expression) and vascular tone (e.g. NO (nitric oxide), prostacyclin and endothelin production) [26]. Their strategic positioning also enables endothelial cells to influence the priming and activation status of peripheral blood leucocytes as they transmigrate into an inflammatory focus [6, 27]. This effect is mediated in part through the ability of endothelial cells to express a wide range of cytokines including IL-1, IL-5, IL-6, IL-8, monocyte chemotactic

protein and the colony-stimulating factors GM-CSF, G-CSF and M-CSF [28]. In conjunction with other endothelial cell-derived stimuli (e.g. platelet activating factor, eicosanoids and nitric oxide), these factors are thought to play a central role in modulating the inflammatory process.

With reference to the transmigrating eosinophil, recent preliminary data indicate that human pulmonary artery endothelial cells produce a factor(s) that delays eosinophil apoptosis *in vitro* [29]. This factor, which is discrete from GM-CSF, IL-5 and IL-3, has a similar efficacy in survival terms to GM-CSF but is entirely eosinophil-selective in that it has no effect on neutrophil longevity. The precise identity of this survival factor and whether it is elaborated *in vivo* remains to be determined. These studies do, however, emphasize the intimate cross-talk that exists between structural 'barrier' cells in the lung and the influxing inflammatory cells. Indeed, such interactions may be crucial to the regulation of chronic inflammatory [30]. They also illustrate the fundamental differences that exist between the apoptotic programming of neutrophils and eosinophils.

Modulation of eosinophil function and fate by bronchial epithelial cells

The capacity of eosinophils to damage bronchial epithelial cells is well recognized and is believed to be one of the major factors contributing to airway damage in asthma [1]. A number of studies suggest that this interaction is not all one sided and that bronchial epithelial cells can also influence eosinophil function. Hence bronchial epithelial cells elaborate many pro-inflammatory molecules including GM-CSF, eotaxin, RANTES and macrophage inflammatory protein-1 α , all of which are powerful chemoattractants for eosinophils [31]. Likewise, eosinophils bind directly to bronchial epithelial cells and in certain circumstances this interaction leads to marked degranulation [32]. Bronchial epithelial cells have also been shown to prolong eosinophil longevity again through the production of GM-CSF [33].

So, how does the bronchial epithelial cell defend itself against such attack and can it fight back? The capacity of these cells to propel inflammatory cells towards the larynx by mucociliary transport may well help [34], as will the production of several anti-inflammatory molecules, including prostaglandin E₂ and α 1-antitrypsin [35]. Bronchial epithelial cells also express Fas-L and other agents such as TNF- α that are capable of inducing eosinophil apoptosis [36]. This suggests that bronchial epithelial cells have evolved a fairly extensive raft of barrier, humeral and cell:cell interaction mechanisms to defend themselves against granulocyte attack.

In the current issue of *Clinical and Experimental Allergy*, Sexton et al. [37] provide a further insight into the relationship between airway epithelial cells and eosinophils. Firstly, using confocal microscopy, scanning and transmission electron microscopy (EM) they confirm their earlier observation that bronchial epithelial cells cultured from either large or small airways display a capacity to recognize and phagocytose apoptotic eosinophils [38]. While the phagocytic capacity of epithelial cells has been described in a number of other settings (e.g. in the renal tubule and involuting mouse

mammary gland [39, 40]) the apparent specificity of this event is intriguing. Hence, the current paper reveals that when tested under identical conditions, quiescent- and cytokine-stimulated bronchial epithelial cells are unable to recognize or ingest apoptotic neutrophils. This effect is ascribed to the lack of expression of the mannose receptor on epithelial cells. As might be predicted, the phagocytic uptake of apoptotic eosinophils involves integrin (α v β 3, α 5 β 5, CD36), phosphatidylserine receptor- and lectin-dependent interactions [37, 38, 41]. Of note, the avidity of epithelial cells for apoptotic eosinophils can be further enhanced by prior treatment with the corticosteroid dexamethasone [41], IL-1, TNF- α or ligation of CD44 [37], effects all similar to those reported by Dransfield and colleagues examining macrophage uptake of apoptotic neutrophils [42, 43]. The study by Sexton and colleagues also confirms that monocyte-derived macrophages ingest apoptotic eosinophils more avidly than apoptotic neutrophils [42].

Taken together these studies have a number of important implications for our understanding of eosinophilic inflammation. First, they suggest that the transmigration of eosinophils across the vascular endothelium and then across the epithelial surface into the airway lumen may have a major influence on the apoptotic programming of eosinophils. Indeed, the 'pro-survival' environment within the micro-compartment of the airway wall may be so intense as to prevent apoptotic cell death entirely. A different balance appears to exist, however, in the airway lumen where apoptotic eosinophils are seen in far greater numbers especially during the resolution phase of an asthma exacerbation [44]. A number of factors may contribute to this, not least a relative defect in the capacity of macrophages within the lumen to ingest apoptotic cells. It is possible therefore that bronchial epithelial cells have developed the capacity to phagocytose apoptotic eosinophils to provide an additional tier of defence against these highly toxic cells; if left, secondary necrosis may ensue creating further havoc.

The limitations of Sexton's study relate firstly to its *in vitro* setting and the use of a monolayer cell culture system. The stringency of the wash protocol employed following the initial eosinophil and bronchial epithelial cell co-culture and the inability of trypsin to affect the phagocytic index suggest that eosinophil adhesion, as opposed to phagocytosis, has been fully excluded. Studies examining the capacity of other non-professional phagocytes (e.g. fibroblasts, smooth muscle cells, dendritic cells and hepatocytes) to engulf apoptotic neutrophils [45] have proven difficult to replicate and hence it is important that independent validation of this work is provided. Moreover, this study does not address the relative contribution of bronchial epithelial cells and other non-professional phagocytes to the removal of apoptotic cells *in vivo* [15]. If phagocytic uptake of apoptotic eosinophils really does represent a major clearance pathway for these cells, this should be apparent in bronchial biopsy specimens.

As with all good studies, the work by Sexton and co-workers opens up more questions than it answers; not least the relative contribution of bronchial epithelial cell phagocytosis of apoptotic eosinophils to the clearance of airway lumen eosinophils *in vivo*, whether bronchial epithelial cells can ingest apoptotic eosinophils if presented at their basal or baso-lateral (non-luminal) surface and the (presumably profound) effects of epithelial damage on this process.

Whether the increased phagocytic uptake induced by corticosteroid treatment underlies any of the therapeutic efficacy of these agents is also unclear. However, the fact that the uptake of apoptotic eosinophils by bronchial epithelial cells is seemingly so selective and can be regulated gives encouragement that this pathway may offer a useful therapeutic target.

Acknowledgements

The work in the authors' laboratory is supported by the Wellcome Trust, MRC, Asthma UK, British Lung Foundation, Papworth Hospital R&D Committee and the Isaac Newton Trust.

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