

EDITORIAL

Eosinophil–epithelial cell interactions: a special relationship?

The airway epithelium is composed primarily of ciliated, non-ciliated and basal cells which, under normal circumstances, provide a protective barrier against the external environment. This protective role is enhanced by other mechanisms including mucous secretion and mucociliary clearance together with the release of mediators which may have either pro-inflammatory or protective functions. It is now widely accepted that a key facet of the airway inflammation crucial to the pathogenesis of all forms of asthma is eosinophil-mediated damage to the bronchial epithelium [1]. Eosinophil–epithelial interactions are thought to make a major contribution to asthmatic airway inflammation for two primary reasons.

Firstly, lung tissue damage can manifest itself as a consequence of an inappropriate accumulation of eosinophils and the subsequent release of their highly toxic granule proteins. In particular, deposition of eosinophil major basic protein has been heavily implicated in epithelial damage and loss, an event thought to be important in the development of airway hyperresponsiveness [2]. In addition, release of eosinophil granule-associated products such as chemokines and cytokines at local sites of inflammation is likely to be of significant relevance to both paracrine and autocrine functions [3].

Secondly, a large number of studies have demonstrated that airway epithelial cells are potent sources of pro-inflammatory substances including GM-CSF, IL-1 β , IL-6, IL-11, IL-16 and IL-18, together with the chemokines RANTES, eotaxin, macrophage inflammatory protein-1 α (MIP-1 α) and IL-8 [4]. The profound effects exerted by GM-CSF on eosinophil function include inducing their prolonged survival through the inhibition of apoptosis. Additionally, chemokines such as eotaxin, RANTES and MIP-1 α can attract eosinophils to sites of ongoing asthmatic inflammation.

Eotaxin is a potent and specific eosinophil chemoattractant which has been implicated as a major pro-inflammatory mediator in asthma [5]; it has been shown to be produced by lung epithelial cells stimulated with IFN- γ in the presence of IL-1 and TNF α [6]. Furthermore, *in vivo* expression of eotaxin and MCP-4 mRNA in the lungs of asthmatic subjects was particularly evident in the bronchial epithelium and inflammatory cells and eotaxin mRNA levels were significantly correlated to the numbers

of eosinophils present in the airway tissue of these patients [7]. There is considerable evidence that IL-5 also plays a pivotal role in promoting eosinophil functions with selective effects on their production, maturation and activation [8]. IL-5 has been detected in bronchial mucosal biopsies of patients with either atopic or non-atopic asthma and its levels were closely related to eosinophil numbers and severity of symptoms [9–11]. IL-5 is thought to be produced by several cell types, including eosinophils [12].

Recently, both epithelial cell lines and primary cultures of human nasal and bronchial epithelial cells have been shown to constitutively express IL-5 mRNA or protein which was upregulated following stimulation with TNF- α . IL-5 protein was detected in bronchial biopsies from normal healthy airways and in the supernatants of the adenocarcinoma epithelial cell line A549 after stimulation with TNF- α and IFN- γ [13]. Thus, epithelial derived IL-5 may have significant effects on eosinophil activation and persistence at local sites of inflammation.

Other histopathological features of asthma include sub-basal membrane thickening, hypertrophy and hyperplasia of both smooth muscle cells and subepithelial glands, events thought to result directly from the activity of matrix metalloproteinases (MMP) in airway interstitial tissue. Significant quantities of mRNA transcripts for MMP-9 (gelatinase-B) have been detected in bronchial biopsies from asthmatic subjects, the majority of which were expressed by eosinophils [14], and eosinophil stimulation with TNF- α markedly upregulates their ability to release MMP-9 *in vitro* [15]. Furthermore, MMP-9 has been reported to play an important role in eosinophil migration through the basement membrane underlying the endothelium [16]. Thus, the significant quantities of MMP-9 secreted by eosinophils might contribute to asthma pathogenesis by instigating pathological changes; they may also weaken epithelial cell-cell junctions through their capacity to cleave the adhesion molecules responsible for maintaining the integrity of the lung epithelium, thereby contributing to the loss of epithelial integrity that is characteristic of asthma.

In this regard it is interesting to note that in a murine model of asthma, antigen inhalation by sensitized animals was associated with infiltration of the lungs by eosinophils accompanied by an increase in the levels of MMP-2

(gelatinase-A) and MMP-9 in bronchial washings from these animals. Importantly, administration of an inhibitor to MMP-2 decreased airway responsiveness and reduced eosinophil infiltration of the airway wall and lumen [17]. These findings not only emphasize the importance of MMP-2 and MMP-9 in governing the infiltration of eosinophils into the asthmatic lung but also raise the possibility that inhibition of MMP activity might represent a therapeutic strategy for asthma.

Further interaction between eosinophils and ligands expressed by epithelial cells might result in eosinophil adhesion followed by mediator release and/or their degranulation and concomitant damage to the bronchial epithelium. It seems reasonable to assume that eosinophil interaction with bronchial epithelial cells is under the control of adhesion receptors in a manner analogous to that observed for eosinophil adhesion to endothelial cells [18]. However, examination of the literature reveals that *in vitro* studies of the adhesion molecules responsible for eosinophil–epithelial interactions do not appear to be as clear cut as those reports which elucidated the adhesive mechanisms responsible for eosinophil adhesion to endothelial cells. This is most likely a reflection of the diverse provenance of the bronchial epithelial cells used for these studies. Some workers used epithelial cell lines such as BEAS-2B or A549 whilst others have studied primary cultures of bronchial epithelial cells derived from lung or nasal tissue explant cultures or commercially sourced bronchial epithelial cells.

In general, findings from several studies suggest that eosinophil adhesion to bronchial epithelial cells is mediated by the $\beta 2$ and $\alpha 4$ integrins binding to unidentified counter structures on the epithelium. For example, eosinophil adhesion to the alveolar carcinoma cell line A549 was variable despite high expression of ICAM-1 following TNF α or IFN γ stimulation [19]. Enhanced adhesion to the A549 cells was only found following stimulation of eosinophils with the non-physiological activator phorbol myristate acetate [20]. In contrast the BEAS-2B epithelial cell line has been shown to express both ICAM-1 and VCAM-1 [19,21] following stimulation with cytokines, although only one of these studies reported inhibition of eosinophil adhesion with a monoclonal antibody against VCAM-1 [21]. However, a role for ICAM-1 in eosinophil–epithelial cell interactions cannot be ruled out since a study which used commercially obtained cytokine-stimulated normal human bronchial epithelial cells demonstrated ICAM-1-dependent adhesion [22]. The functional importance of eosinophil adhesion to bronchial epithelial cells was shown by the observation of eosinophil degranulation in the presence of a bronchial epithelial cell line when both cell types were activated with TNF or IL-5 [23].

Although the foregoing suggests that eosinophil–epithelial

cell interactions are predominantly pro-inflammatory, there is evidence that under certain circumstances these interactions might promote the resolution of asthmatic inflammation. For example, prostaglandin E2 is the predominant prostanoid secreted by the airway epithelium and has been shown to inhibit eosinophil aggregation [24] or degranulation [25]. While a recent report demonstrated that prostaglandin E2 can prolong eosinophil survival *in vitro*, this effect was only marginal when compared with IL-5 [26]. Furthermore, prostaglandin E2 has recently been shown to inhibit the proliferative response of bone marrow derived eosinophil precursors in naïve or ovalbumin sensitized and challenged mice [27].

Apoptosis or programmed cell death is a vital aspect of the resolution of inflammation. Apoptotic cells display membrane-associated changes which are recognized by phagocytes that rapidly engulf them before they can release their pro-inflammatory mediators. There is accumulating evidence that induction of apoptosis in eosinophils and their subsequent engulfment by macrophages might play an important role in the resolution of asthmatic or allergic inflammation and a range of signals which induce eosinophil apoptosis has been described (reviewed in [28]). It is becoming apparent that a number of non-professional phagocytes including dendritic cells, smooth muscle cells and lung fibroblasts also have the capacity to recognize and ingest apoptotic cells [29]. Although the bronchial epithelium is generally considered to be the target for cell damage and loss by eosinophil derived mediators, we have recently made the novel observation that human small airway epithelial cells also have the capacity to ingest apoptotic eosinophils. This is a specific lectin- and integrin-mediated process which was enhanced by the pro-inflammatory cytokines IL-1 α and TNF α [30].

We have extended these observations to show that alveolar epithelial cells also ingest apoptotic eosinophils but these cells predominantly recognize and engulf apoptotic eosinophils via the $\alpha v \beta 5$ integrin, a receptor not used by macrophages for recognition of apoptotic cells [29]. Importantly we have also found that corticosteroids upregulated the capacity of bronchial epithelial cells to engulf apoptotic eosinophils indicating a further important function for this class of drugs (Sexton DW, Blaylock MG & Walsh GM, submitted). These *in vitro* observations offer a provocative additional pathway for the clearance of lung eosinophils in asthma.

Another potential route by which the bronchial epithelium might modulate eosinophilic inflammation is via the Fas/Fas-ligand pathway which is responsible for initiating apoptosis in diverse cell types. Eosinophils express low but consistent levels of Fas antigen whose ligation by either the Fas ligand or anti-Fas monoclonal antibodies induces their apoptosis [31,32], while a recent study demonstrated

expression of Fas ligand by human airway epithelial cells [33]. It is tempting to speculate that in the appropriate circumstances eosinophil interaction with bronchial epithelial cells might result in apoptosis induction and subsequent eosinophil clearance by both alveolar macrophages and also by bronchial epithelial cells. Thus, expression of Fas and Fas ligand in the airway epithelium might play an important modulating role in the inflammatory response commonly found in asthmatic lungs.

In addition to eosinophils, neutrophils are often present at sites of allergic inflammation. Neutrophil granules contain significant quantities of serine proteases of which the most important is elastase. The release of these proteolytic enzymes as part of an inflammatory response can degrade the connective tissue matrix, a process which is thought to be a key pathophysiological mechanism in lung disease, including the development over time of pulmonary emphysema in chronic asthma. The activity of protease inhibitors is therefore vital for maintaining tissue integrity by modulating the activity of these enzymes. Human plasma contains a range of serine proteinase inhibitors which are members of the serpin superfamily. Of these, α -1-antitrypsin is the prototypic member and its primary function is the inhibition of neutrophil elastase activity. During tissue injury or inflammation, when elevated proteinase activity is present, the role of proteinase inhibitors seems to be particularly important and this is reflected by an increase in their plasma concentration as part of the acute phase reaction. Although the liver appears to be the primary source of α -1-antitrypsin it has also been shown to be synthesized by extrahepatic cells, including lung epithelial cells [34]. It is therefore of interest that the study of Johansson *et al.* [35] in the current issue of *Clinical & Experimental Allergy* demonstrated that eosinophils also secrete α 1-antitrypsin inhibitor which was found to be present in their specific granules. Although the levels detected were low – about 50 ng per five million eosinophils – α -1-antitrypsin released by eosinophils recruited to tissue sites of inflammation may play an important role in counteracting the tissue damage induced by neutrophil-derived proteinase activity which is a common feature of chronic lung disease.

Although the relationship between eosinophils and the epithelium in asthmatic disease has traditionally been considered a pro-inflammatory one, the more recent findings outlined here give new insights into the potential and intriguing role of epithelial-eosinophil interactions in the resolution of lung inflammation.

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