

# MECHANISMS OF STEROID ACTION AND RESISTANCE IN INFLAMMATION

## Corticosteroids, eosinophils and bronchial epithelial cells: new insights into the resolution of inflammation in asthma

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### Abstract

Anti-inflammatory therapy in asthma is reliant on corticosteroids, particularly in their inhaled form. However, steroids are rather non-specific in their actions and they also raise concerns regarding compliance and side-effect issues. Furthermore, a small proportion of patients with asthma fail to respond to oral glucocorticoids even at high doses. This article will review the role that steroids and membrane receptor ligation play in the induction of eosinophil apoptosis together with the mechanisms by which corticosteroids enhance the disposal of apoptotic eosinophils by both professional and non-professional phagocytes. Eosinophils are thought to be the major pro-inflammatory effector cell in asthma and their persistence in the airways is probably enhanced by the presence of several asthma-relevant cytokines that prolong eosinophil survival by inhibition of apoptosis (interleukin (IL)-3, IL-5, granulocyte-macrophage colony-stimulating factor, IL-9, IL-13, IL-15). In contrast, a number of signals have been described that accelerate apoptosis in human eosinophils including corticosteroids or ligation of membrane

receptors (CD95, CD45, CD69). Thus, the load of lung eosinophils in asthmatic disease is likely to be related to a balance in the tissue microenvironment between pro- and anti-apoptotic signals. Furthermore, removal of apoptotic eosinophils by phagocytosis by alveolar macrophages or bronchial epithelial cells in a specific receptor-mediated way is as important as the process of apoptosis induction. Corticosteroids enhance the recognition and engulfment of apoptotic eosinophils by macrophages or bronchial epithelial cells. Caspases are key intracellular molecules in the control of apoptosis and defects in caspase-induced apoptosis in eosinophils from steroid-resistant individuals may contribute to the molecular mechanisms underlying glucocorticoid insensitivity in these cells. These findings point the way to new and more targeted anti-inflammatory therapy for asthma and may provide important clues for the development of alternative therapies for glucocorticoid resistance.

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### Introduction

Asthma is a complex disorder characterised by reversible airway obstruction, bronchial hyperresponsiveness and airway inflammation. Key pathological features include infiltration of the airways by activated lymphocytes and eosinophils, damage to and loss of the bronchial epithelium, mast cell degranulation and collagen deposition in the epithelial sub-basement membrane area. These features are often, although not always, seen in patients with mild to moderate disease. In cases of fatal or more severe persistent asthma other pathologies are often seen including occlusion of the airways by mucus, smooth muscle hyperplasia and hypertrophy and goblet cell

hyperplasia. Increased understanding of the nature of the cellular profile of inflammation in asthma and its impact on the airway injury, remodelling and repair will probably lead to novel, more targeted, therapies aimed at reducing or preventing these processes.

Glucocorticoids have powerful anti-inflammatory effects by virtue of their ability to inhibit pro-inflammatory cell recruitment and to down-regulate the production of pro-inflammatory cytokines. Presently, anti-inflammatory therapy in asthma is reliant on corticosteroids, particularly in their inhaled form, and their use is associated with a striking reduction in the numbers of activated eosinophils, mast cells and T cells *in vivo*. However, while efficacious steroids are rather non-specific in their actions, their use

also raises concerns over side-effects and compliance issues, particularly in children and adolescents. Furthermore, a small proportion of patients with asthma fail to respond to oral glucocorticoids even at high doses. Although relatively uncommon, steroid resistance in asthmatic patients places a burden on scarce resources and presents considerable management problems, as few alternative therapies are available. The precise molecular mechanisms underlying glucocorticoid sensitivity remain to be elucidated. However, it has been suggested that alternative splicing of the glucocorticoid receptor (GCR) in infiltrating T cells (Leung *et al.* 1998) and bronchial epithelial cells (Sousa *et al.* 2000) at the mRNA level generates GCR $\beta$ . The latter differs from GCR $\alpha$  in the carboxy-terminal portion of the molecule and does not bind glucocorticoids and thus antagonises the transactivating ability of GCR $\alpha$ . Increased GCR $\beta$  expression has been associated with glucocorticoid-insensitive asthma, although this is rather controversial (Leung & Chrousos 2000). However, what is certain is that a minority of asthmatic patients do not respond well or at all to glucocorticoid therapy. Thus, progress may be made in dealing with these difficult-to-treat patients via the delineation of the mechanisms by which their airway inflammation can be reduced or even prevented.

### Eosinophils

Eosinophils and their secreted mediators are heavily implicated as effector cells in the pathophysiological changes in the airways in asthma. Eosinophils are thought to play a beneficial role in immunity to parasitic worms (Walsh 1999) and have also been proposed to play a role in host defence against respiratory pathogens (Rosenberg & Domachowske 2001). However, they are also recognised as major effector cells in the inflammatory process underlying much of the pathogenesis of asthma and other allergic diseases (Wardlaw *et al.* 2000). Large numbers of activated tissue eosinophils are typically observed in airway biopsies, sputum and bronchoalveolar lavage fluid obtained from asthmatic patients (Walsh 1999, Wardlaw *et al.* 2000). Eosinophils store and release, on appropriate activation, a wide spectrum of pro-inflammatory mediators including cationic granule proteins, major basic protein (MBP), eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (Walsh 2001a). They have also been shown to synthesise up to 28 cytokines, chemokines and growth factors, many of which are stored in their crystalloid granules (Lacy & Moqbel 2001). It has also recently become clear that eosinophils are not only key effector cells in mediating asthmatic inflammation, but also have a role in presenting allergen to stimulate CD4<sup>+</sup> T-helper (Th) cells (MacKenzie *et al.* 2001). Epithelial damage and loss caused by eosinophil-derived mediators, particularly their highly basic granule proteins – MBP, eosinophil

peroxidase and ECP – is thought to be a major event in asthma pathogenesis (Gleich 2000). Thus it can be appreciated that eosinophils have the ability to make a major contribution to the inflammatory processes underlying asthmatic and allergic disease. Furthermore, it is also becoming increasingly apparent that eosinophil–epithelial cell interactions are an important facet of asthmatic disease in relation to both the initiation and resolution of inflammation (Sexton & Walsh 2002).

### Bronchial epithelial cells

While it is now widely accepted that T cells of the Th2 phenotype orchestrate eosinophilic inflammation through the production of eosinophil-active cytokines and chemokines, airway epithelial cells themselves possess an arsenal of inflammatory mediators. These include cytokines, lipid and peptide products and eosinophil-active chemokines including RANTES, interleukin (IL)-5 and eotaxin 1, 2 and 3. Chemokines are a crucial facet of the interaction between eosinophils and the airway epithelium in asthmatic inflammation (Lukacs 2001). Only eotaxin is eosinophil-specific and its production by the airway epithelium is well established, being regulated by cytokines implicated in asthmatic inflammation including IL-13, IL-4, tumour necrosis factor- $\alpha$  and IL-1 (Lamkhioed *et al.* 1997, Lilly *et al.* 1997, Fujisawa *et al.* 2000, Jedrzkiewicz *et al.* 2000). It is interesting to note that the lung epithelium itself expresses functional CCR3 (Stellato *et al.* 2001), the receptor for RANTES (regulated upon activation, normal T cell expressed and secreted), eotaxin, eotaxin 2 and 3, monocyte chemotactic protein MCP-3 and MCP-4. Although the bronchial epithelium consists of structural non-migratory cells, expression of the CCR3 receptor may represent an auto-regulatory feedback mechanism to monitor chemokine production. Furthermore, eotaxin produced by the epithelium may be sequestered by the CCR3 receptor and presented to infiltrating cells, thereby enhancing their activation, a phenomenon observed with IL-8 and its receptor. Other cytokines make a major contribution to the complexity of epithelial–eosinophil interactions. For example, IL-9 plays a significant role in asthmatic inflammation (Soussi-Gounni *et al.* 2001) and has been shown to stimulate the release of IL-16 and RANTES from bronchial epithelium (Little *et al.* 2001) and will also enhance eosinophil IL-5 receptor expression, differentiation and prolonged survival (Gounni *et al.* 2000). Transgene expression of IL-9 in the lungs of mice resulted in lymphocytic and eosinophilic infiltration of the lung, airway epithelial hypertrophy with mucus production, mast cell hyperplasia and production of IL-4, IL-5 and IL-13 (Temann *et al.* 2002). IL-5 has profound effects on eosinophil accumulation and activation while IL-13 and IL-4 up-regulate eotaxin production by airway epithelial cells via a STAT-6 intracellular signalling

pathway (Matsukura *et al.* 2001). It can be appreciated therefore that the bronchial epithelium can actively promote the attraction and activation of eosinophils. However, under certain conditions, eosinophil interaction with bronchial epithelial cells might contribute to the resolution of inflammation in asthma (Walsh 2001b).

### Eosinophil apoptosis

Apoptosis or programmed cell death is a central and essential process in the resolution of inflammation. Removal of eosinophil infiltrates via apoptosis and concomitant phagocytosis offer considerable potential to ameliorate inflammation (Walsh 2000). Examination of induced sputum has provided evidence that the treatment of exacerbations of asthma with steroids results in the resolution of eosinophilic inflammation by inducing apoptosis in lung eosinophils that are subsequently recognised and phagocytosed by alveolar macrophages (Woolley *et al.* 1996). We have also recently demonstrated that reduced apoptosis in eosinophils present in induced sputum significantly correlates with asthma severity as defined by airflow obstruction and symptom scores (Duncan *et al.* 2001). It has been appreciated for some time that IL-3, granulocyte-macrophage colony-stimulating factor and IL-5 enhance eosinophil survival and therefore eosinophil persistence in the tissues may be prolonged in their presence. Additionally, IL-13 (Luttmann *et al.* 1996, Horie *et al.* 1997), IL-9 (Gounni *et al.* 2000) and IL-15 (Hoontrakoon *et al.* 2002) have all been shown to enhance eosinophil survival. The significance of these findings is emphasised by ample evidence that these cytokines are present in asthmatic airway tissue and that eosinophils isolated from patients with atopic dermatitis and, to a lesser extent inhalant allergy, displayed enhanced survival compared with normal controls (Wedi *et al.* 1997). Thus, there is an evolving hypothesis that the load of lung eosinophils in asthmatic disease is related to a balance in the tissue microenvironment between pro- and anti-apoptotic signals (Walsh 2000).

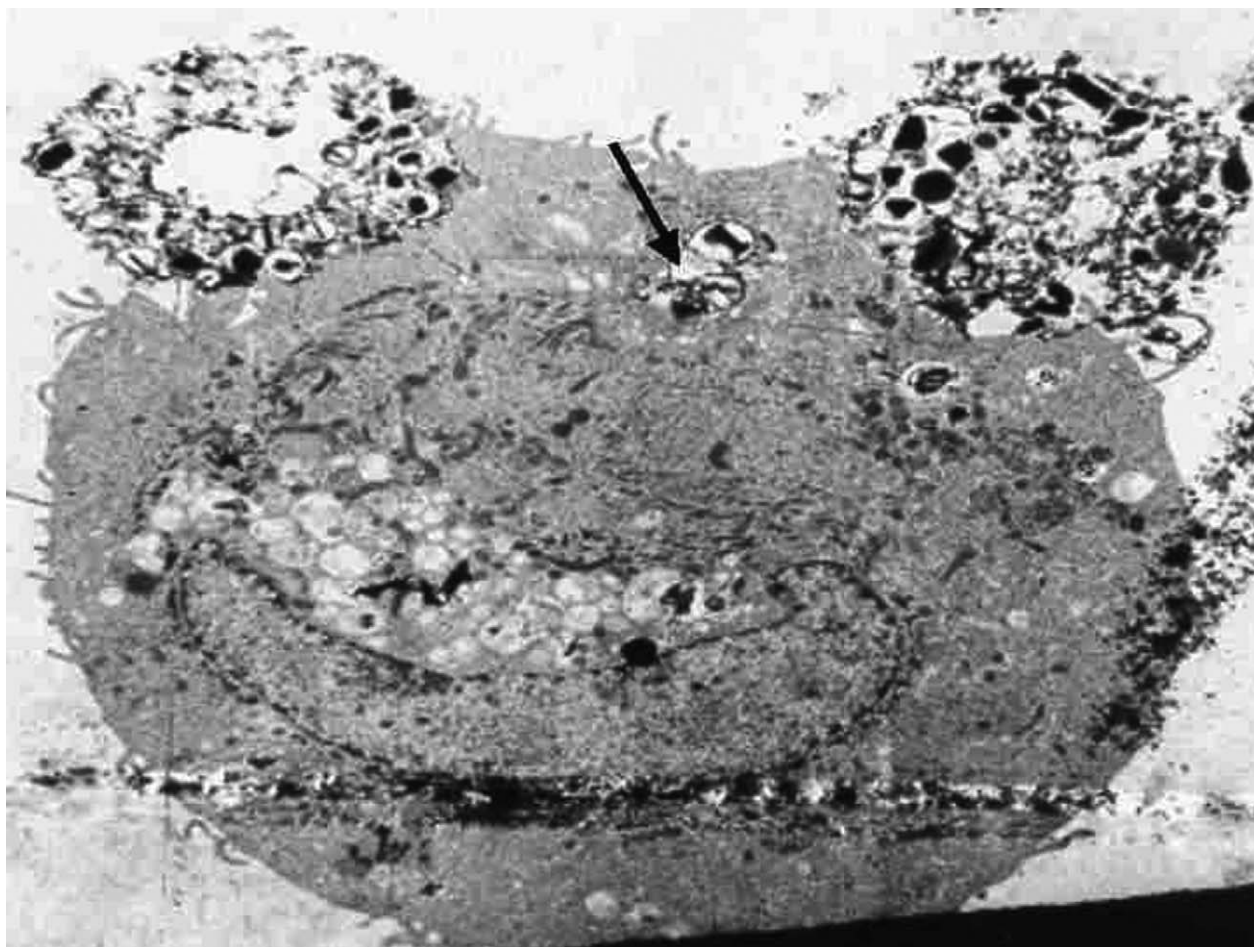
Eosinophils are terminally differentiated end cells which die by apoptosis when cultured *in vitro*, being rapidly recognised and ingested as intact cells by autologous macrophages (Stern *et al.* 1996) or bronchial epithelial cells (Walsh *et al.* 1999, Sexton *et al.* 2001). Eosinophil apoptosis can be induced or accelerated by ligation of membrane receptors by specific mAb for Fas (CD95) (Matsumoto *et al.* 1995, Druilhe *et al.* 1996), CD69 (Walsh *et al.* 1996) and CD45 (Blaylock *et al.* 1999). Glucocorticoids rapidly induce apoptosis in peripheral blood (Meagher *et al.* 1996) and tissue eosinophils (Saunders *et al.* 1999). Nasal polyposis is an inflammatory disorder characterised by an intense infiltration of the respiratory mucosa by eosinophils that is often refractory to treatment with corticosteroids. Interestingly, a recent study demonstrated GCR $\beta$  expression in nasal polyp tissue

was almost exclusively found in T cells, macrophages and eosinophils (Hamilos *et al.* 2001). Moreover, these workers observed that treatment with the corticosteroid fluticasone did not suppress eosinophil numbers in some nasal polyp specimens and that this resistance to the effect of fluticasone was associated with increased numbers of GCR $\beta$ -positive eosinophils. The authors concluded that GCR $\beta$  expression, particularly by eosinophils and T cells, is a marker of steroid insensitivity in nasal polyposis. As stated above, eosinophils are highly sensitive to apoptosis induction by corticosteroids that can only be overcome by high concentrations of IL-5 (Walsh & Wardlaw 1997). Thus, induction of GCR $\beta$  might reduce eosinophil sensitivity to steroids, leading to an alteration in the balance between pro- and anti-apoptotic signals and thereby promote eosinophil survival in the tissues of corticosteroid-insensitive patients with asthma.

Furthermore, caspases are key regulators of apoptosis in diverse human cells. Previous work with eosinophils derived from both healthy and asymptomatic allergic individuals has demonstrated an involvement of caspase-3 and -8 in glucocorticoid-induced apoptosis (Zangirilli *et al.* 2000). In contrast, others have reported that dexamethasone-induced apoptosis failed to induce specific caspase-3 and -8 activity in eosinophils compared with spontaneous apoptosis (Zhang *et al.* 2000). We have shown that eosinophils isolated from asthmatic patients with steroid resistance are refractory to steroid-induced apoptosis and that this appears to be due to a defect in the caspase signalling pathways in these cells. Importantly, eosinophils from glucocorticoid-resistant patients are still susceptible to apoptosis induction by ligation of membrane receptors, e.g. CD45 or Fas (G M Walsh & M G Blaylock, unpublished observations). Taken together, these observations indicate potential avenues for the development of novel therapeutic approaches to target eosinophil-induced inflammation in asthma, particularly in those patients who exhibit steroid resistance.

### Removal of apoptotic cells

While much attention has rightly been paid to the study of the mechanisms by which pro-inflammatory cells such as the eosinophil can be induced to become apoptotic, it must be remembered that removal of cellular corpses by phagocytosis is as vital a process as apoptosis itself (Savill & Fadok 2000). Failure to do so will result in disintegration of the apoptotic cell via a process termed secondary necrosis and the subsequent uncontrolled leakage of the dying cell's contents, resulting in a propagated inflammatory response. Indeed many pathological conditions are thought to be due to either a failure in the apoptotic process, phagocytic clearance of apoptotic cells, or both. The macrophage is considered to be one of the most important cells involved in apoptotic cell removal and

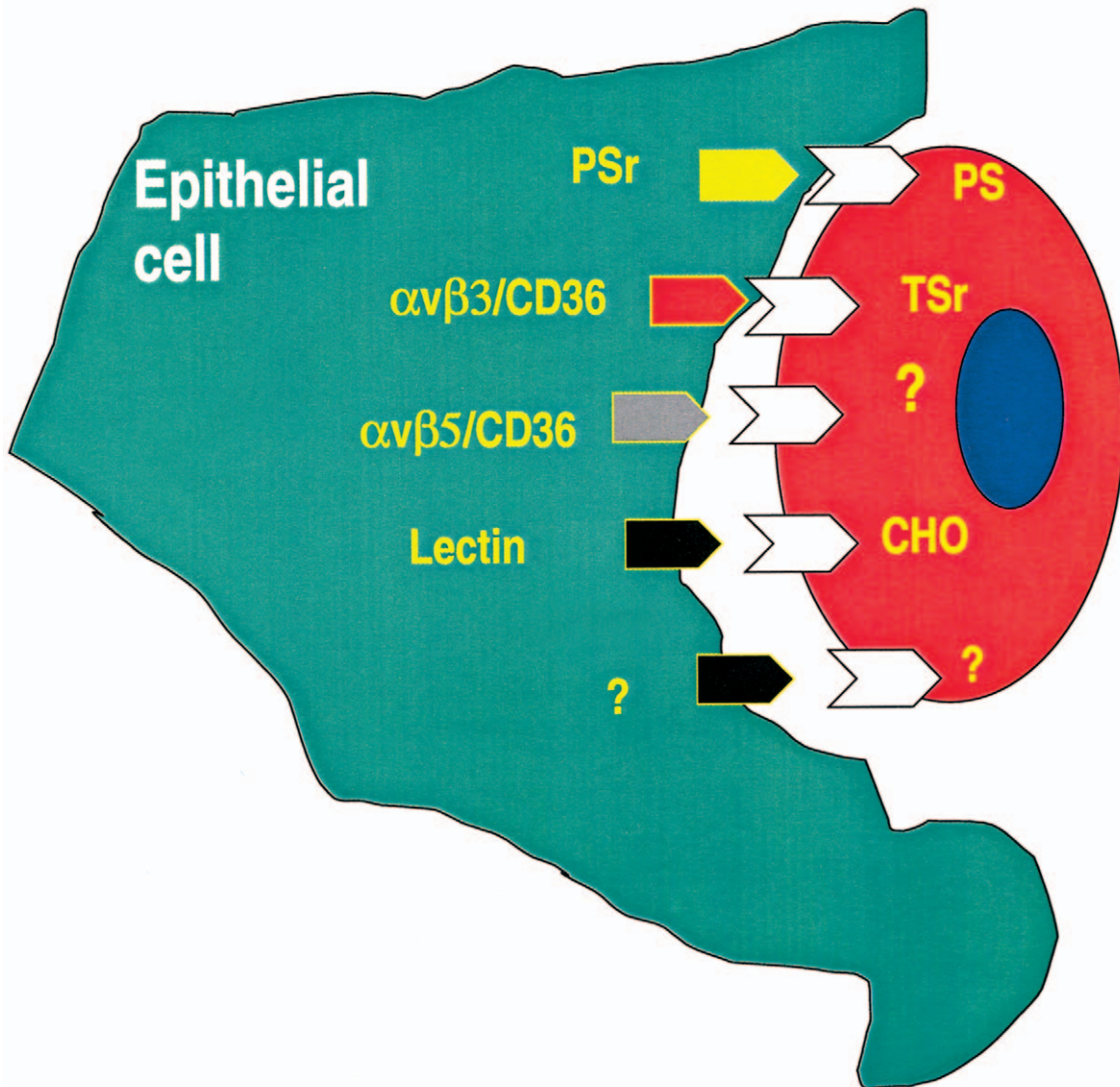


**Figure 1** Electron micrograph demonstrating apoptotic eosinophil ingestion by A549 bronchial epithelial cell. The arrow demonstrates evidence of partial digestion of an apoptotic eosinophil, i.e. a phagosome containing a degenerated eosinophil including an eosinophil granule (from Sexton *et al.* 2001 with permission). Original magnification  $\times 5000$ .

much effort has been expended in increasing our knowledge of the receptor-mediated mechanisms responsible for this process. Glucocorticoids such as dexamethasone and hydrocortisone have been shown to markedly increase the avidity of human monocyte-derived macrophages for apoptotic neutrophils, eosinophils and Jurkat T cells (Liu *et al.* 1999). Many lines of evidence suggest an important role for non-professional phagocytes in the recognition and removal of apoptotic cells (Platt *et al.* 1998). These include dendritic cells and structural cells such as fibroblasts or hepatocytes. Our own work has established that both primary cultures of human small airway epithelial cells (Walsh *et al.* 1999) and the alveolar epithelial cell line A549 (Sexton *et al.* 2001) are capable of phagocytosing apoptotic, but not freshly isolated, eosinophils. Both small airway epithelial cells and A549 cells remained viable after eosinophil ingestion and went on to digest their apoptotic cell meal (Fig. 1). Recognition and phagocytosis of apoptotic eosinophils was a specific event under the

control of integrin, lectin and phosphatidylserine membrane receptors (summarised in Fig. 2). Importantly, we also demonstrated that the corticosteroid dexamethasone increased both the percentage of bronchial epithelial cells engulfing apoptotic eosinophils and in particular the number of apoptotic eosinophils ingested by each epithelial cell, adding another potential facet to the anti-inflammatory properties of corticosteroids in asthma therapy (Sexton *et al.* 2001). This effect was seen with both small and large airway cells and also with the A549 epithelial cell line. Increased receptor expression did not appear to be responsible for increased avidity for apoptotic eosinophils by bronchial epithelial cells following dexamethasone treatment. There are a number of potential explanations for this observation. These include the participation of, as yet, unidentified recognition receptors, or a conformational change in the receptors under investigation which increased their affinity for ligand in a manner analogous to that described for activated





**Figure 2** Receptor-mediated mechanisms involved in the recognition of apoptotic eosinophils (in red) by bronchial epithelial cells (PS=phosphatidylserine; PSr=phosphatidylserine receptor; TSr=thrombospondin receptor; CHO=carbohydrate; ? unidentified receptor interactions).

leukocyte, integrin-dependent, interaction with endothelial cells. Furthermore, in macrophages, glucocorticoid-augmented phagocytosis of apoptotic neutrophils was characterised by recruitment of paxillin and pyk2 to focal contacts and a down-regulation of p130 Cas, a key adaptor molecule in integrin adhesion signalling. Increased phagocytic avidity for apoptotic neutrophils was mirrored by higher levels of active Rac and enhanced cytoskeletal activity (Giles *et al.* 2001). Similar processes may be involved in the enhanced phagocytosis of apoptotic eosinophils by bronchial epithelial cells following treatment with corticosteroids.

Phagocytosis of apoptotic cells not only safely disposes of potential sources of myriad pro-inflammatory substances but also has profound effects on the phagocyte. In macrophages, apoptotic cell uptake prevents the release of phlogistic agents such as eicosanoids or pro-inflammatory cytokines and promotes the release of anti-inflammatory cytokines (Voll *et al.* 1997, Fadok *et al.* 1998, Mai-Lan *et al.* 2002). Engulfment of apoptotic eosinophils also induces an anti-inflammatory cytokine and mediator secretory profile in the macrophage (Stern *et al.* 1996). This effect of apoptotic cell ingestion on macrophages is potent; when both apoptotic and necrotic cells are

phagocytosed, it is the anti-inflammatory macrophage cytokine profile that prevails (Cocco & Ucker 2001). Whether epithelial cells, like macrophages, can be programmed to produce an anti-inflammatory cytokine and mediator secretory profile following ingestion of apoptotic eosinophils is presently under investigation.

## Conclusions

Corticosteroids exert their potent anti-inflammatory effects in asthma on resident and infiltrating cells. It is becoming clear that important additional facets to their therapeutic efficacy include induction of apoptosis in eosinophils and the promotion of increased avarice of resident macrophages and bronchial epithelial cells for apoptotic eosinophils, thereby promoting their rapid and safe clearance. The recognition and ingestion of apoptotic eosinophils by alveolar macrophages and bronchial epithelial cells may promote an anti-inflammatory cytokine secretion profile in these cells. These findings point the way to novel therapies for asthma therapy that are more targeted and which may also benefit those patients with glucocorticoid-resistant disease.

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