REVIEW

Eosinophil Apoptosis and Clearance in Asthma

Garry M. Walsh

School of Medicine and Dentistry, University of Aberdeen, Scotland, UK.
Corresponding author email: g.m.walsh@abdn.ac.uk

Abstract: Asthma is an increasingly common respiratory condition characterized by reversible airway obstruction, bronchial hyper-responsiveness and airway inflammation with a clear unmet need for more effective therapy. Eosinophilic asthma is a phenotype of the condition that features increased blood or sputum eosinophils whose numbers correlate with disease severity. Several lines of evidence are now emerging, which implicate increased persistence of eosinophils in the lungs of patients with asthma as a consequence of inhibition of and defects in the apoptotic process, together with impaired apoptotic cell removal mechanisms. This article will update our knowledge of the mechanisms controlling eosinophil apoptosis and clearance, together with evidence implicating defects in apoptosis and pro-inflammatory cell removal in asthma. Recent developments in novel therapies for asthma that target eosinophil apoptotic and/or clearance pathways will also be discussed.

Keywords: eosinophil, apoptosis, phagocytic removal

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Introduction

Asthma is now one of the most common chronic diseases in developed countries and is characterized by reversible airway obstruction, airway hyper-responsiveness (AHR) and airway inflammation. Asthma pathology results in fundamental structural changes to the airway including goblet cell hyperplasia, airway smooth muscle hypertrophy and subepithelial fibrosis.[1] Inhaled glucocorticoids (GC) remain the gold standard of therapy for asthma because of their potent anti-inflammatory properties that primarily reduce AHR, disease exacerbations and hospitalizations, while improving lung function and quality of life.[2] However, GC have well-documented side effects and they are symptomatic medications that require lifetime therapy for the majority of patients with asthma, whose symptoms usually return upon GC withdrawal.[3] Moreover, concerns remain over patient compliance to therapy, while variations in the clinical response of asthmatics to inhaled GC therapy are common. A significant subgroup of asthmatic patients respond poorly or not at all to high-dose inhaled or systemic GC treatment.[4] These considerations highlight the need for the development of more effective and safe anti-inflammatory therapy for asthma.

Eosinophil involvement in inflammatory conditions affecting the skin, gastrointestinal tract and upper and lower airways is well documented.[5] Eosinophilic asthma is a phenotype of the condition characterized by increased blood or sputum eosinophils whose numbers correlate with disease severity.[6] Infiltrating tissue eosinophils release their potent pro-inflammatory arsenal, including granule-derived basic proteins, lipid mediators, cytokines and chemokines. These contribute to airway inflammation and lung tissue remodeling that includes epithelial cell damage and loss, airway thickening, fibrosis and angiogenesis.[7] More recent evidence suggests that in addition to their role as degranulating effector cells, eosinophils have the capacity to act as antigen-presenting, cells resulting in T cell proliferation and activation, thereby propagating inflammatory responses.[8,9]

This article will update our knowledge of the mechanisms controlling eosinophil apoptosis together with the evidence implicating defects in apoptosis and pro-inflammatory cell removal in asthma. Recent developments in novel therapies for asthma that target eosinophil apoptotic and/or clearance pathways will also be discussed.

Role of Eosinophil Apoptosis in Asthma

Asthma represents one of the most prevalent diseases affecting humans, for which eosinophils have an established role in disease pathogenesis. In healthy individuals, eosinophils are present in the circulation in low numbers and are rarely found in the lung, being mostly confined to the tissues surrounding the gut. Eosinophil accumulation in the asthmatic lung is complex, involving their maturation in and release from the bone marrow, adhesion to and transmigration through the post-capillary endothelium, and then their chemotaxis to and activation/degranulation at inflammatory foci.[10] The processes controlling eosinophil accumulation are of obvious importance and represent potential therapeutic targets for antagonism of their accumulation in asthma.[11] However, apoptosis and the disposal of apoptotic cells by phagocytic removal (efferocytosis) is a vital aspect of inflammation resolution in all multi-cellular organisms. Thus, a balance in the tissue microenvironment between pro- and anti-apoptotic signals is likely to greatly influence the load of lung eosinophils in the asthmatic lung. Eosinophils have a limited life-span. In the circulation for 8–18 hours and in the tissues for 3–4 days, as like neutrophils they are terminally-differentiated cells programmed to undergo apoptosis in the absence of viability-enhancing stimuli. Eosinophil persistence in the airways is enhanced by the presence of several asthma-relevant cytokines that prolong eosinophil survival by inhibition of apoptosis. The roles of IL-3, IL-5, IL-9, IL-13, IL-15 and GM-CSF in this regard are well established,[12–15] and there is ample evidence that all of these elements are present in the asthmatic lung in significant quantities.[16] Thymic stromal protein (TSLP), IL-25 and IL-33 represent a triad of cytokines released by airway epithelial cells in response to various environmental stimuli or by cellular damage. They act in concert to drive Th2 polarization through overlapping mechanisms causing remodeling and pathological changes in the airway walls, suggesting pivotal roles in the pathophysiology of asthma. All 3 have been shown to have a number of effects on eosinophil function, including enhancement of their viability through the inhibition of apoptosis.[17–19] Eosinophil interactions

with proteins of the extracellular matrix are likely to contribute to their persistence within the tissues. For example, integrin-mediated eosinophil adhesion to fibronectin[20,21] results in the autocrine production of viability-enhancing cytokines GM-SCF, IL-3 and IL-5. These interactions between multiple cytokines and extracellular matrix components antagonize eosinophil programmed cell death, thereby prolonging their longevity for weeks.

Nitric oxide (NO) released from human tissues is associated with pro-inflammatory effects with potentially important implications in asthma, where levels in exhaled air correlate with clinical symptoms of asthma, sputum eosinophilia and markers of eosinophil activation.[22,23] In addition to potential pro-inflammatory effects, there is evidence that NO induces in vitro human eosinophil apoptosis both in the absence and presence of IL-5 or GM-CSF via c-Jun-N-terminal kinase (JNK)[24] or caspase 6 and 3 activation.[25] More recently, NO-induced eosinophil apoptosis has been shown to be mediated via reactive oxygen species, JNK and late mitochondrial permeability transition.[26] NO release in the asthmatic lung may act as a counter regulatory mechanism to limit eosinophilia in inflamed lungs, indeed the degree of apoptosis in sputum eosinophils was found to positively correlate with exhaled NO in children.[27]

The desire to understand how the process of apoptosis can be harnessed in the quest for novel asthma therapy has led to much interest in furthering our understanding of the triggers and intracellular mechanisms controlling apoptosis induction in human eosinophils.[28,29] For example, several studies have demonstrated that ligation of membrane receptors including Fas (CD95)[30,31] CD69,[32] siglec-8,[33,34] CD30[35] and CD45[36] induce eosinophil apoptosis in vitro. Interestingly, eosinophil expression of the latter receptor is elevated in patients with asthma compared with non-asthmatic controls.[37] Mice sensitised with ovalbumin (OVA) develop lung inflamation including airway eosinophilia following an OVA inhalation challenge. This process represents a widely used animal model of asthma. The administration of anti-Siglec-5 accelerates eosinophil apoptosis in an OVA murine asthma model, leading to resolution of allergic pulmonary inflammation.[38] Indeed, siglec-8 expression appears restricted to human eosinophils, mast cells and basophils, thereby generating great interest in targeting this molecule as a potential treatment for eosinophil and mast-cell-driven diseases.[39]

Inhaled GC are a first-line therapy for asthma due to their potent anti-inflammatory properties that primarily result in reduced numbers of airway inflammatory cells and their associated mediators. In addition, GC induce apoptosis in peripheral blood eosinophils,[40] as well as in tissue eosinophils resident in nasal polyp tissue sections,[41] suggesting that eosinophil apoptosis induction by GC might be relevant to their anti-inflammatory effects in asthma. The intracellular signaling mechanisms by which GC induce apoptosis in human eosinophils include the involvement of caspases and release of mitochondrial cytochrome C.[42] Studies with eosinophils derived from both healthy and asymptomatic allergic individuals have demonstrated involvement of caspase-3 and -8 in glucocorticoid-induced apoptosis.[43] In contrast, another study reported that the GC dexamethasone induced eosinophil apoptosis that was not associated with specific caspase-3 and -8 activity in eosinophils, compared with spontaneous apoptosis in these cells.[44] Our own findings demonstrate that different caspase pathways are involved in controlling receptor-ligation mediated apoptosis-induction in human eosinophils.[45] While caspases are key regulators of apoptosis in diverse human cells, oxidant-induced mitochondrial injury associated with translocation of the pro-apoptotic protein Bax to the mitochondria has been shown to be pivotal in eosinophil apoptosis. This effect was mediated by GC-induced prolonged activation of c-Jun NH2-terminal kinase that was, in turn, inhibited by GM-CSF.[46] Other factors important in the control of apoptosis and caspase activation in many cellular systems include the Bcl-2 family of proteins.[47] There are several reports demonstrating constitutive expression of Bcl-2,[48,49] or Bax and Bcl-x[50] by human eosinophils, whereas a decrease in Bcl-xL messenger RNA and protein levels was found to be associated with eosinophil apoptosis.[51] The intracellular pathways by which viability-enhancing cytokines act in eosinophils include triggering of PI3K/Akt/ERK signaling, preventing Bax activation through a Pin1-dependent process. NFkB-mediated transcriptional upregulation of anti-apoptotic Bcl-2 family members, as well as inhibitors of apoptosis such as Mcl-1

and c-IAP, are also likely to contribute to eosinophil survival.[28]

Phagocytic Recognition

Though much is spoken of the importance of apoptosis induction in homeostasis, its benefits arise only in conjunction with the efferocytosis of these apoptotic cells by neighboring cells. The clearance of dying cells represents a fundamental process serving multiple functions in the regulation of normal tissue turnover and homeostasis. Failure or inhibition of these clearance mechanisms permits the apoptotic cells to enter into secondary necrosis, which results in injurious perpetuation of the inflammatory response.[52] Indeed, defects in apoptosis and/or subsequent efferocytosis of pro-inflammatory cells are increasingly recognized in conditions as diverse as autoimmunity[53] and chronic inflammatory diseases of the lung.[54] Evidence for the rationale of targeting apoptosis/efferocytosis in respiratory disease is provided by studies that demonstrated defects in recognition of apoptotic cells by alveolar macrophages from patients with COPD[55] or chronic asthma.[56] Our own clinical study[57] and those of others[58,59] provide further "real-world" evidence that apoptosis induction in eosinophils and their subsequent efferocytosis is a rational avenue for the development of novel therapies for asthma.

Various studies in macrophages[60,61] have elucidated the means utilized in the recognition and engulfment of apoptotic cells. These include an uncharacterized lectin-dependent interaction;[62] a charge-sensitive process involving the CD36/vitronectin receptor complex interacting with unknown moieties on apoptotic neutrophils' surfaces via a thrombospondin bridge;[63,64] a stereo specific recognition of phosphatidylserine (PS) that is exposed on the surface of the apoptotic cell after loss of membrane asymmetry,[65,66] redistribution of PS on the phagocyte,[67] macrophage scavenger receptors,[68,69] CD14,[70,71] CD68,[72] ABC1 transporter,[73] Dock180, beta-1-integrins,[74] CD44[75] and opsinization by MFG-E8, C1q, Mannonse binding lectin (MBL) and E6 (reviewed in Elliott and Ravichandran).[76] It is been known for many years that a number of non-professional phagocytes, including dendritic cells, lung fibroblasts, and smooth muscle cells, also have the capacity to recognize and ingest apoptotic cells.[77] Although the bronchial epithelium is generally considered to be the target for cell damage

and loss by eosinophil-derived mediators, we characterized the capacity for normal and small airway human bronchial epithelial cells to specifically recognize and phagocytose apoptotic, but not freshly isolated eosinophils.[78] Recognition and phagocytosis of apoptotic eosinophils was a specific event under the control of integrin, lectin and phosphatidylserine membrane receptors. Importantly, we also demonstrated that the GC dexamethasone increased both the percentage of airway epithelial cells (AEC) engulfing apoptotic eosinophils and, in particular, the number of apoptotic eosinophils ingested by each epithelial cell.[79] These findings add a new dimension to the anti-inflammatory effects of GC. We also demonstrated that actin rearrangement is involved in the efferocytosis of apoptotic eosinophils by AEC, and that the phagocytic capacity of cytokine-stimulated small and large AEC was approximately half that of human monocyte-derived macrophages. Intriguingly, the AEC did not phagocytose apoptotic neutrophils,[80] and in screening epithelial cell lines derived from alveolar (A549), mammary (ZR-75-1) and colon (HT-29) tissues, this selective efferocytosis of apoptotic eosinophils was consistent. Indeed, this preferential uptake of eosinophils was similarly observed in our monocyte-derived-macrophages, which exhibited consistently higher phagocytic uptake of apoptotic eosinophils compared with uptake of apoptotic neutrophils. Given the extent of the lung epithelium and that LPS-dependent phagocytosis of apoptotic cells by alveolar macrophages is greatly impaired in patients with chronic asthma,[54] bronchial epithelial cells may prove to be vital in the clearance of apoptotic eosinophils.

Targeting Apoptosis in Asthma

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 GC resistance. IL-12 induces apoptosis in human eosinophils, likely explaining its ability to decrease tissue eosinophilia in murine models of allergic inflammation.[81] Furthermore, the level of IL-12 mRNA expression in the airways of asthmatic subjects is significantly lower than that in non-asthmatic controls, and levels significantly increased following treatment with GC.[82] IL-12 would, therefore, appear to be a

good candidate for the treatment of asthma. However, while a clinical study demonstrated that treatment of asthmatic patients with rhIL-12 reduced sputum and blood eosinophil numbers, no significant effect was observed on either AHR or the late-asthmatic response to an inhaled antigen challenge.[83] The bronchodilator theophylline is widely used in asthma therapy and has also been shown to trigger apoptosis in human eosinophils, an effect that may contribute to its anti-inflammatory properties.[84] Thus, there is interest in the development of more specific phosphodiesterase type 4 (PDE4) inhibitors with enhanced bronchodilator and anti-inflammatory effects. However, side effects such as nausea and vomiting have been problematic for some agents in this class.[85]

Statins reduce cholesterol levels by inhibiting the first enzyme in the cholesterol pathway, namely 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, and have established effectiveness in the treatment of atherosclerotic disease. They also exhibit anti-inflammatory properties, most likely by inhibiting the prenylation of signaling molecules. In other words, they are no longer anchored to the cell membrane, with subsequent down-regulation of gene expression, thereby reducing cytokine or chemokine levels and adhesion molecule expression.[86] Furthermore, the mevalonate metabolic pathway is reversibly inhibited by statins and is known to be integral to cell membrane lipid raft formation, which is an essential process for immune system autocrine signaling, MHC expression, and inflammatory cell interactions, including antigen presentation.[87] Statins also induce apoptosis in a variety of cell types in vitro, including rheumatoid synovial cells, smooth muscle cells and cardiac monocytes thereby contributing to their pleiotropic benefits. Statin-induced apoptosis appears to be executed via alteration in isoprenoid synthesis with resultant tyrosine phosphorylation, causing a rise in cytosolic calcium that in turn activates calpain. The latter decreases the Bcl-2/Bax ratio, leading to mitochondrial cytochrome c release and activation of caspase-9, followed by activation of caspase-3.[88] In vitro, clinically-relevant concnetrations of fluvastatin and lovastatin inhibited eosinophil adhesion to ICAM-1 under physiological shear stress conditions.[89] These immunomodulatory effects may be of potential benefit in asthma; in particular, their potential effect upon eosinophil function and survival are of interest

suggesting that statins may prove effective anti-inflammatory treatments for asthma.[90] Administration of simvastatin to sensitised mice immediately before OVA challenge attenuated the inflammatory airway response together with a concomitant reduction in pro-inflammatory cytokine levels, eosinophil numbers and bronchoalveolar lavage IL-5 levels.[91] These observations, coupled with the finding that statins directly inhibit T-lymphocyte differentiation and function,[92] led the authors to suggest that modification of the T-helper subset had resulted in reduction in airway eosinophilia. However, another interpretation is that a direct action of simvastatin on airway eosinophil apoptisis and efferocytosis by alveolar macrophages could have contributed to the reduction in airway eosinophilia. Furthermore, simvastatin also inhibited allergic asthmatic symptoms and numbers of macrophages, neutrophils, and eosinophils in bronchoalveolar lavage fluid in a mouse model of asthma.[93] Although these studies in murine asthma models suggest that statins may be of benefit to patients with asthma, this can only be confirmed by well-conducted randomized-clinical trials.[94] To date, several clinical studies have examined the utility of statins in asthma patients, but their results are not consistent. Indeed, a recent systematic literature review of the subject reported that while statins may reduce airway inflammation in asthmatics, this does not translate into significant positive effects on symptoms including improvements in lung function.[95] Further clinical trials may establish statins as effective co-therapies in asthma and establish whether they might benefit patients with more chronic severe disease that may be resistant to treatment with corticosteroids, or in more targeted patient populations, such as obese asthmatics.[96]

Bronchoconstriction in asthma is commonly relieved with β_2-agonists such as salbutamol, fenoterol, formoterol or salmeterol. However, their over-use is associated with detrimental effects,[97] some of which may be related to the observation that clinically relevant concentrations of salbutamol reduced eosinophil apoptosis via the cannonical $\beta(2)$-receptor-adenylyl cyclase-cAMP-protein kinase A pathway.[98]

We have known for many years that IL-5 plays a crucial role in the development and release of eosinophils from the bone marrow, their enhanced adhesion to endothelial cells lining the post-capillary venules,

and their activation, secretion and prolonged survival through apoptosis inhibition in the tissues.[99,100] IL-5 was therefore identified as a promising target to prevent or blunt eosinophil-mediated inflammation in patients with asthma and other eosinophil-related conditions, leading to the development of humanized anti-IL-5 mAb such as mepolizumab and reslizumab or benralizumab, a mAb against human IL-5Rα.[101] Early clinical trials with the anti-IL-5 biologic mepolizumab in patients with mild to severe asthma reported significant reductions in blood and sputum eosinophil numbers, but clinical outcomes such as improvements in lung function were disappointing, most likely because subjects were recruited on the basis of clinical and physiological characteristics that did not specify the presence of eosinophilic airway inflammation.[101] More recently a number of studies have demonstrated that mepolizumab treatment of patients who manifested eosinophilic asthma not only reduced eosinophil numbers in the blood and sputum, but also resulted in a significant reduction in asthma exacerbations.[102–104] Benralizumab is a novel, humanized afucosylated IgG1κ mAb, indicated in the potential treatment of asthma and COPD. It binds to a distinct epitope within the extracellular domain of recombinant human IL-5Rα. At the time of publication, benralizumab was undergoing phase II clinical trials in both the specified indications (ClinicalTrials.gov identifiers: NCT01238861 and NCT01227278). Other anti-IL-5 mAb act by neutralizing the effects of IL-5. Benralizumab, however, targets the effector cells, mainly eosinophils and basophils, many of whose functions are driven by IL-5. Afucosylation is associated with enhanced antibody-dependent cell cytotoxicity, and benralizumab was found to induce apoptosis in eosinophils and basophils. Tissue eosinophils resident in bronchial biopsies of patients with mild atopic asthma exhibited intense immune positivity for benralizumab in contrast to resident mast cells, which were negative.[105] These findings indicate that benralizumab binds human lung tissue-resident eosinophils expressing IL-5Rα, and could delete these cells through apoptosis-induction, thereby acting as a potential asthma therapeutic.[106] Indeed, a phase-1 study in subjects with mild asthma demonstrated that intravenous benralizumab (0.3–3.0 mg/kg) rapidly induced near-total depletion of peripheral blood eosinophils while exhibiting an adequate safety profile and dose-proportional pharmacokinetics.[107]

Conclusion

Our knowledge concerning the mechanisms controlling eosinophil apoptosis and their subsequent phagocytic disposal is now considerable. GC and some novel therapies with potential utility in asthma such as statins or benralizumab appear to have an eosinophil apoptosis-inducing dimension to their effects. However, the development of effective and safe therapies for asthma patients aimed solely at apoptosis induction and/or clearance of tissue eosinophils is currently a theoretical rather than practical possibility. Other mechanisms, such as transepithelial elimination of eosinophils, may also provide novel targets for the safe removal of this important pro-inflammatory cell.[108]

Author Contributions

GMW wrote, revised and approved the final version of the manuscript. The author received no assistance in the preparation of this manuscript.

Competing Interests

The author has not served as a consultant or had any other financial or conflicting interest in association with any of the therapies discussed in this article. The author discloses no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the author has provided signed confirmation of compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review.

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