

MiniReview

Eosinophil Apoptosis as a Therapeutic Target in Allergic Asthma

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Abstract: Asthma is a chronic inflammatory disease of the airways manifesting in many different phenotypes. Allergic asthma, comprising approximately half of patients with asthma, is characterized by the accumulation of eosinophils into the lungs. Eosinophils release factors that damage the surrounding cells and participate in the maintenance and exacerbation of inflammation. In the absence of any inflammatory survival-prolonging factors, eosinophils die by apoptosis in few days but in inflamed airways, eosinophil survival is thought to be prolonged due to the surrounding pro-inflammatory factors such as IL-5, IL-3 and GM-CSF. Resolution of eosinophilic inflammation is an important goal in the treatment of allergic asthma. Apoptosis is a physiological and non-inflammatory way to eliminate these harmful cells, and development of drugs targeting eosinophil apoptosis is one possible strategy for the therapy of allergic asthma. Importance of this strategy is supported by the fact that promotion of eosinophil apoptosis is a property of many anti-asthmatic agents such as glucocorticoids, the current main anti-inflammatory therapy of asthma, theophylline and leukotriene modifiers. β_2 agonists have been shown to modulate eosinophil longevity by increasing survival. Also, anti-IL-5 antibody mesolizumab has shown efficacy in reducing asthma exacerbations in patients with severe eosinophilic asthma. Many potential future anti-asthmatic agents, such as Siglec-8 activating antibody and novel humanized anti-IL-5 antibody MEDI-563, have the property of inducing eosinophil apoptosis. This MiniReview aims to present eosinophil apoptosis as a therapeutic target in the treatment of allergic asthma. We summarize the effects and mechanisms of current and potential future anti-asthmatic drugs on eosinophil apoptosis and additionally, discuss the potential factors that promote eosinophil longevity in the lungs.

Asthma is a chronic inflammatory disease of the airways and constitutes a major health problem in Western countries. Asthma is a more complex disease than previously thought, manifesting in several different inflammatory phenotypes with different underlying pathogenesis. Even though detailed characterization of these phenotypes is still ongoing, approximately half of patients with asthma seem to belong to a phenotype associated with predominant eosinophilic inflammation, also known as allergic asthma [1].

Traditionally, eosinophils were regarded as end-stage effector cells in asthma, but recent studies have revealed a more diverse role for these inflammatory cells. In addition to their potential to release an array of products that damage epithelium, induce bronchoconstriction, mucus production and vascular permeability, they have been shown to have an important role in Th2 polarization and airway remodelling. Eosinophils themselves produce drivers of Th2 polarization and via functioning as antigen-presenting cells, they are able to stimulate Th-cell activation, proliferation and production of IL-4, IL-5 and IL-13 [2,3]. Evidence exists on the role of eosinophil-derived TGF- β in mediating airway remodelling [2]. Finally, recent data from clinical studies with anti-IL-5

antibody suggest that eosinophils are significant players in asthma exacerbations [4].

Resolution of eosinophilic inflammation is a critical goal for asthma therapeutics, and the promotion of eosinophil cell death is a possible strategy to achieve that. According to the current knowledge, lifespan of airway eosinophils may end up in apoptosis (programmed cell death), cytolysis or alternatively, these cells may be eliminated with secretions after migration into airway lumen (transepithelial elimination or egression) [5]. Also, other forms of cell death exist (e.g. autophagy and netosis) [6], but their occurrence in airway eosinophils remains unknown. Cytolysis is an undesirable form of cell death characterized by cell membrane rupture and release of intracellular contents to the surroundings. This form of cell death ends up in amplification of inflammation. Apoptosis represents an ideal means to deplete eosinophils from the airways. Apoptosis is a non-inflammatory way of cell death characterized by cell shrinkage, chromatin condensation, DNA fragmentation and maintenance of membrane integrity. Apoptotic cells fragment into apoptotic bodies that are engulfed by phagocytes such as macrophages. Apoptosis can be executed by intrinsic (mitochondria-centred) or extrinsic (receptor-mediated) pathways, and the general mechanisms of these pathways are presented in fig. 1. Eosinophil apoptosis may be accelerated by pharmacological agents raising it as an important therapeutic strategy in allergic asthma [7]. Glucocorticoids form

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the basis of modern anti-inflammatory asthma therapy, and inhaled glucocorticoids are the mainstay of treatment of patients with chronic asthma. The main mechanism of action of glucocorticoids is the modulation of genes involved in inflammation by either down- or up-regulating their expression [8]. In addition, glucocorticoids have been shown to accelerate eosinophil apoptosis [9,10], which is considered as an additional beneficial mechanism of action for glucocorticoids [11]. Interestingly, glucocorticoids are efficient especially in the treatment of eosinophilic phenotype of asthma and even a short-treatment trial with inhaled fluticasone led to a dramatic reduction in the number of eosinophils in these patients [12]. Successful phagocytic clearance is critical for a beneficial outcome of apoptosis because non-ingested apoptotic eosinophils undergo inflammation-aggravating secondary necrosis and cytolysis. In addition to macrophages, also small airway epithelial cells have been shown to function as phagocytes in the lung environment [13]. Phagocytosis is also a process that can be enhanced by pharmacological agents (e.g. glucocorticoids),

thereby promoting anti-inflammatory clearance of apoptotic cells [14].

This MiniReview concentrates in presenting eosinophil apoptosis as a therapeutic strategy in the treatment of asthma. We aim to discuss the factors that prolong eosinophil longevity in the lungs and to summarize the effects and mechanisms of current and potential future anti-asthmatic drugs on eosinophil apoptosis.

What Keeps Eosinophils Alive in the Airways?

Enhanced eosinophil survival is considered critical for the accumulation of eosinophils in the lungs of asthmatics. Apoptosis of both blood and tissue eosinophils was delayed in patients with asthma when compared to apoptotic rate in healthy individuals [15,16]. Eosinophil survival-prolonging cytokines GM-CSF, IL-5 and IL-3 are considered to be strong contributing factors towards initiation and maintenance of eosinophilic airway inflammation in asthma [11,17–19]. However, plenty of

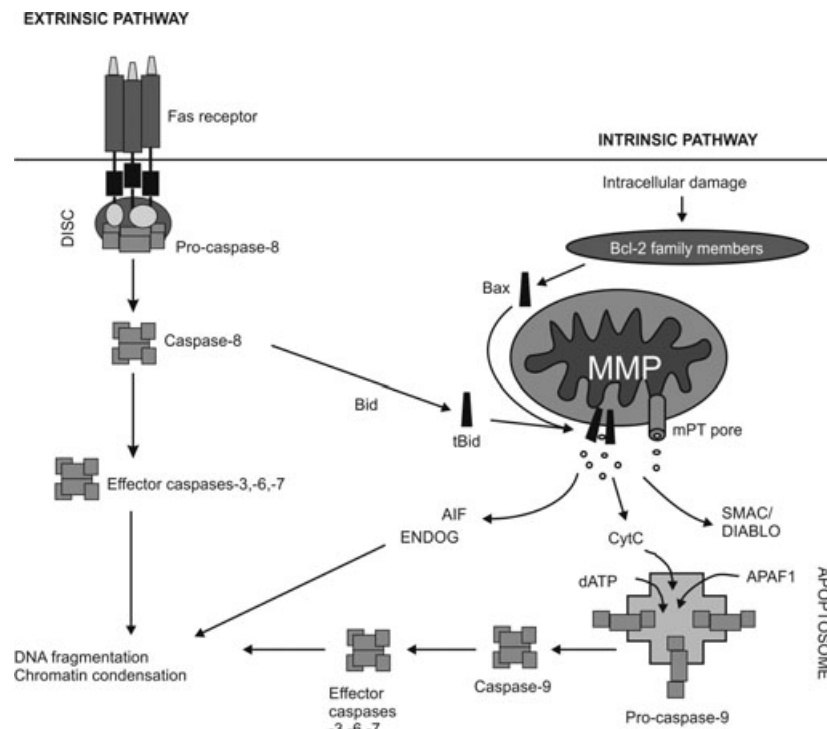


Fig. 1. Pathways of extrinsic and intrinsic apoptosis. Extrinsic apoptosis is initiated by ligation of death receptor such as Fas leading to the formation of death-inducing signalling complex (DISC) and activation of caspases. Caspases execute apoptosis by degrading cellular components. Sometimes an additional mitochondrial loop is required for caspase activation in extrinsic apoptosis. Intrinsic pathway can be initiated by several intracellular stress conditions such as DNA damage, oxidative stress or cytosolic Ca^{2+} overload, which are sensed by the members of B-cell lymphoma (Bcl)-2 family. Typically, both pro- and anti-apoptotic mechanisms are involved because the cells are struggling to survive at the stressful situation. Mitochondrial membrane permeabilization (MMP) is a central event in intrinsic apoptosis, considered as the point of no return. It can be mediated by pore-forming activity of Bcl-2 family members to the mitochondrial outer membrane (e.g. Bax, Bid, detailed role is beyond this Mini-Review) or mitochondrial permeability transition (mPT). During mPT, a channel is formed at the junction of mitochondrial membranes where solutes and molecules up to 1.5 kDa can freely pass. Mitochondrial membrane permeabilization via either of these mechanisms typically results in loss of mitochondrial membrane potential, halted mitochondrial ATP synthesis and release of pro-apoptotic proteins [e.g. cytochrome c (CytC), apoptosis-inducing factor (AIF), second mitochondria-derived activator of caspases/direct IAP-binding protein with low pI (SMAC-DIABLO)] from the mitochondrial intermembrane space to the cytosol. These proteins participate in the formation of apoptosome, a platform to activate caspases. A caspase-independent form of intrinsic apoptosis also exists and when the caspases are inhibited, the execution phase is often mediated by AIF and endonuclease G (ENDOG). Abbreviations: tBid, truncated Bid; dATP, 2'-deoxy adenosine triphosphate; APAF1, apoptotic protease-activating factor 1.

evidence suggests that also other life-supporting factors exist. This is supported by the results showing that delayed apoptosis of blood and tissue eosinophils was only partially prevented by anti-GM-CSF, anti-IL-5 and anti-IL-3 antibodies, added together or separately [15,16]. Additionally, treatment with anti-IL-5 antibody only partially depleted eosinophils from airways in patients with asthma [20]. Some other candidates have been presented to further explain the enhanced survival of eosinophils in asthma. Recently, thymic stromal lymphopoietin (TSLP) and IL-33, that are expressed e.g. by damaged airway epithelium, were shown to enhance eosinophil survival [21,22]. Leptin is a cytokine that is mainly produced by adipocytes of white adipose tissue. Its main function is related to inhibition of appetite. Leptin was shown to be an eosinophil survival-prolonging factor even though it is unclear whether the survival-prolonging concentrations of leptin may be reached *in vivo* [23]. In addition, viability of eosinophils was reported to be increased with decreased pH, at least down to pH 6, which may have clinical relevance because acidosis is common in the asthmatic airways. This effect was shown to be mediated via acid-sensing receptor GPR65 and increased cyclic adenosine monophosphate (cAMP) [24]. Recently, lower airways were found to contain a mean of 2000 bacterial genomes per cm² surface. Instead, bronchi of asthmatic individuals have been reported to contain bacterial genomes up to 20,000/cm² [25]. Bacterial DNA, but not mammalian DNA, prolongs eosinophil survival via TLR9-mediated pathway providing a possible mechanism how bacterial DNA might contribute to eosinophilic inflammation [26,27]. The effects of survival-prolonging cytokines and some other factors on eosinophil apoptosis have been described in detail elsewhere [11,28,29]. Figure 2 summarizes the potential eosinophil survival-prolonging factors that may be present in the airways and contribute to eosinophilia. It remains to be determined which factors in addition to GM-CSF, IL-3 and IL-5 are clinically relevant for the prolongation of eosinophil survival and amplification and maintenance of eosinophilic inflammation.

Current Anti-Asthmatic Drugs and Eosinophil Apoptosis

Glucocorticoids.

Glucocorticoids were shown to accelerate spontaneous eosinophil apoptosis and to overcome the survival-increasing effect

of low concentration of IL-5, IL-3 or GM-CSF [10,30,31]. Glucocorticoids enhanced spontaneous apoptosis by 1.5–2.1-fold *in vitro*, and the order of potency was fluticasone propionate (EC₅₀ 3.7 nM) $\approx$ budesonide (EC₅₀ 5.0 nM) > beclomethasone (EC₅₀ 51 nM) > dexamethasone (EC₅₀ 303 nM) [10]. These effects occurred at therapeutically relevant drug concentrations [10]. The pro-apoptotic effect of glucocorticoids is not unique for eosinophils because glucocorticoids also promote apoptosis of certain T-cell subsets [32]. Instead, neutrophil apoptosis is inhibited by glucocorticoids [9,31,33] which is consistent with the evidence showing that glucocorticoid resistance is mostly associated with neutrophilic inflammation in asthma [34].

Glucocorticoids seem to initiate intrinsic, mitochondrial pathway of apoptosis in eosinophils. The mechanism has been reported to involve glucocorticoid receptor, mitochondrial injury induced by reactive oxygen species (ROS), translocation of pro-apoptotic Bax to mitochondria, early and late activation of c-Jun N-terminal kinase (JNK), diminished production of X-linked inhibitor of apoptosis protein (XIAP), a known inhibitor of JNK, and reduced protein levels of Mcl-1L [10,35,36]. Interestingly, glucocorticoids show divergent effect on the levels of anti-apoptotic Bcl-2 member Mcl-1L in eosinophils and neutrophils, which may partially account for the opposite effects of glucocorticoids on apoptosis in these cells. In neutrophils, levels of Mcl-1 were retained in response to glucocorticoid treatment, whereas in eosinophils, levels of Mcl-1L declined over time, and the reduction induced by glucocorticoids was accelerated when compared to that of untreated eosinophils [36]. Furthermore, signalling in glucocorticoid-induced eosinophil apoptosis closely resembles that mediating nitric oxide (NO)-induced apoptosis in eosinophils, where ROS, mitochondrial injury (mitochondrial permeability transition) and early- and late-JNK activation were also evident. This study [37] showed that early-JNK activation was a consequence of transient mitochondrial permeability transition, and these events were not related to apoptosis but represented likely a stress response. Eventually, eosinophils underwent apoptosis and the mechanism involved ROS, late activation of JNK and permanent mitochondrial permeability transition [37]. Oxidative stress may be the common factor initiating these chains of events, even though the mechanisms leading to ROS

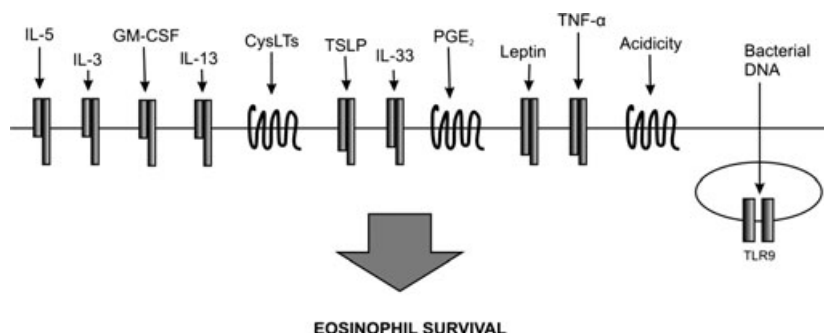


Fig. 2. Possible eosinophil survival-prolonging factors in the inflamed airways. IL, interleukin; GM-CSF, granulocyte macrophage-colony-stimulating factor; CysLTs, cysteinyl leukotrienes; TSLP, thymic stromal lymphopoietin; PGE₂, prostaglandin E₂; TNF, tumour necrosis factor; TLR, Toll-like receptor.

generation may differ between NO and glucocorticoids. Eventually, both compounds seemed to induce intrinsic pathway of apoptosis.

Regulation of gene expression is generally considered as the main mechanism of action of glucocorticoids [8]. Attempts were made to evaluate whether modulation of apoptosis in eosinophils by glucocorticoids is linked to transrepression or transactivation by using the so-called dissociated steroids, developed to have fewer transactivation effects. However, a known dissociated steroid (RU24858) was not a dissociated steroid in human eosinophils [38]. Altogether, no data exist on whether the apoptosis-inducing effect of glucocorticoids is related to transrepression or transactivation or generally, to the regulation of gene expression.

Evidence exists on the clinical significance of glucocorticoid-induced eosinophil apoptosis. An increased number of apoptotic eosinophils have been found from lung samples of steroid-treated asthmatics when compared to steroid-untreated asthmatics [7,39]. Also, the ratio of apoptotic eosinophils/total eosinophils inversely correlated with the severity of asthma [40,41].

In addition to the eosinophil apoptosis-promoting effect, glucocorticoids have been reported to enhance the capacity of monocyte-derived macrophages and lung epithelial cells to phagocytose apoptotic eosinophils [42,43]. Phagocytosis occurs via many receptor-mediated mechanisms that are not yet well understood. The glucocorticoid-induced up-regulation of phagocytic action was dependent on glucocorticoid receptor and new protein synthesis in monocyte-derived macrophages [42]. Annexin I is a glucocorticoid-inducible protein that was involved in mediating the prophagocytic effect of glucocorticoids on macrophages and lung epithelial cells [14,44,45].

β_2 -adrenoceptor agonists.

β_2 -adrenoceptor agonists are used as bronchodilators in the treatment of asthma. However, β_2 receptors are also expressed in some inflammatory cells, and their activation may modulate inflammation [8]. Long-acting β_2 agonist salmeterol and short-acting β_2 agonists salbutamol and fenoterol were shown to inhibit eosinophil apoptosis by 10–20% [15,46]. The anti-apoptotic effect of salbutamol in eosinophils was mediated via a pathway involving cyclic AMP and protein kinase A [15,46]. Salmeterol monotherapy in patients with asthma has been associated with increased risk of death [47], and the inhibitory effect of β_2 agonists on eosinophil apoptosis and thus aggravated eosinophilic inflammation may be one mechanism for this problem with the long-acting β_2 agonists in the absence of glucocorticoids.

Leukotriene modifiers.

Cysteinyl leukotrienes (CysLTs) LTC₄, LTD₄ and LTE₄ are produced in inflammatory cells such as granulocytes, mast cells and monocytes from arachidonic acid via activity of 5-lipoxygenase (5-LO), 5-lipoxygenase-activating protein (FLAP) and leukotriene synthases. CysLTs function by inducing bronchoconstriction and increasing mucus production and

vascular permeability in asthmatic airways. CysLT receptor antagonists are used as add-on medication in moderate to severe asthma and function by inhibiting actions of CysLTs [48]. CysLT receptor antagonists as well as inhibitors of 5-LO and FLAP have been shown to enhance the rate of apoptosis in unstimulated and GM-CSF-stimulated eosinophils *in vitro* [49]. They also reduced sputum and blood eosinophilia in asthmatics [48] suggesting that their pro-apoptotic effects may have clinical relevance.

Theophylline.

Theophylline has been used in the treatment of asthma since the late 1930s, before introduction of glucocorticoids and β_2 agonists. Firstly, its efficacy was thought to be based on its bronchodilatory action, but more recently, it has been shown to possess also many anti-inflammatory mechanisms of actions. It was thought to suppress inflammatory cell function by inhibiting phosphodiesterases (PDE) non-selectively, leading to increased cyclic AMP. However, the clinical relevancy of this mechanism of action is unclear because the effect is modest at therapeutic concentrations of theophylline. Antagonism of adenosine receptors and activation of HDAC2 leading to suppressed inflammatory gene transcription has been suggested as its other anti-inflammatory mechanisms of actions [50]. Theophylline has been shown to accelerate eosinophil apoptosis in the absence and presence of eosinophil survival-prolonging cytokines [51]. Because other cAMP-elevating agents (such as β_2 agonists) reduce eosinophil apoptosis in contrast to theophylline, inhibition of PDE does not most likely mediate pro-apoptotic action of theophylline. However, theophylline suppressed levels of anti-apoptotic protein Bcl-2 in eosinophils [52]. Theophylline reduced airway eosinophilia and levels of eosinophilic cationic protein in patients with asthma suggesting that the pro-apoptotic action of theophylline may contribute to its clinical efficacy [53].

Potential Targets for Drug Development

Siglec-F/Siglec-8.

Sialic acid-binding immunoglobulin-like lectins (Siglecs) are membrane receptors mainly expressed by the haematopoietic cells. They are activated by specific sialylated glycans and artificial specific antibodies. Activation of siglecs promotes cell–cell interactions and regulates cell functions. Siglec-8 is selectively expressed by human eosinophils, basophils and mast cells, while siglec-F is considered as the functional murine analogue of siglec-8. Restricted expression makes these receptors as attractive therapeutic targets in asthma. Siglec-8 ligation induced human eosinophil apoptosis *in vitro*, and similarly, siglec-F ligation promoted eosinophil apoptosis *in vivo* and decreased eosinophil levels in allergen-challenged mice. Siglec-F-deficient mice showed increased tissue eosinophilia, suggesting a physiological role for these receptors in limiting eosinophilia. In resting eosinophils, siglec-8-induced apoptosis was dependent on ROS and caspases. Interestingly, eosinophils activated by IL-5 or IL-33 were more susceptible to die by

siglec-8. However, the cell death resembled regulated necrosis instead of apoptosis and involved production of ROS and activation of MEK/ERK pathway [54]. Function of siglec-8 in basophils is completely unknown, but in human mast cells, ligation of siglec-8 inhibited FcR1-dependent release of histamine and PGD₂ but did not induce apoptosis [55]. Altogether, ligation of siglec-8 has many beneficial therapeutic properties in both eosinophils and mast cells and is a promising candidate for the treatment of allergic asthma and other eosinophil- and mast cell-related disorders.

IL-5 receptor alpha.

IL-5 is critical for eosinophil maturation, survival, trafficking and activation. So far, antibodies developed for IL-5-neutralization (mesolizumab) have resulted in only partial removal of airway eosinophils [20]. To more efficiently deplete airway eosinophils, a novel humanized anti-IL-5 receptor α monoclonal antibody (mAb) (MEDI-563) was developed, where oligosaccharide core of IgG1 was afucosylated. Removal of the fucose from IgG1 augmented interaction of MEDI-563 with Fc γ RIIIa, enhancing antibody-dependent cell-mediated toxicity. Indeed, MEDI-563 significantly enhanced eosinophil and basophil apoptosis *in vitro* when compared to the fucosylated form of the antibody that did not increase eosinophil apoptosis above background level [56]. In an early clinical study, MEDI-563 depleted peripheral blood eosinophils from patients with mild asthma, decreased ECP and had an acceptable side effect profile [57]. Further clinical trials are awaited with interest.

Histone deacetylases.

Histone deacetylases (HDAC) function by removing acetyl groups from lysine residues of histones and other target proteins. In histones, this modification enables tight compaction of DNA around them leading to inhibited expression of genes. Inhibition of HDAC, in contrast, leads to loosening of DNA around histones and increased expression of genes [58]. In addition to the role of HDAC in the modulation of gene expression, they are involved in DNA repair, proliferation and apoptosis. HDAC inhibitors have pro-apoptotic and anti-proliferative effects in malignant cells and thereby are candidate drugs for the treatment of cancer, especially haematological malignancies. Normal cells are typically resistant to apoptosis induced by HDAC inhibitors. However, HDAC inhibitors (trichostatin A, apicidin) have been shown to accelerate apoptosis of both eosinophils and neutrophils, in the absence and presence of survival-prolonging cytokines IL-5 and GM-CSF [59]. This suggests that HDAC inhibitors may also have anti-inflammatory properties. Pro-apoptotic effects of HDAC inhibitors may be mediated by modification of non-histone target proteins rather than by modification of histones. This is suggested by the finding that even though HDAC inhibitors increased acetylation of histones from both melanocytes and melanoma cells, only melanoma cells underwent apoptosis [60]. The pro-apoptotic effect of TSA on eosinophils was mediated by caspases and JNK but not by the acetylation of NF- κ B or PI3K pathway. However, other non-histone target

proteins may still be involved in mediating the pro-apoptotic effect. HDAC inhibitors have shown contradictory effects in animal models of asthma, especially regarding eosinophilic inflammation. TSA has been reported to inhibit metacholine-induced airway hyper-responsiveness, but the number of eosinophils in bronchoalveolar lavage fluid was either reduced or remained unchanged [61,62]. One disadvantage of HDAC inhibitors may be impairment of glucocorticoid action because induction of HDAC2 seems to be an important anti-inflammatory mechanism for glucocorticoids [58].

Cyclin-dependent kinases.

Cyclin-dependent kinases (CDK) are key enzymes involved in cell cycle via phosphorylation of transcription factors and tumour suppressor proteins. Inhibitors of CDK promote apoptosis and inhibit proliferation of rapidly dividing cells such as cancer cells and are under clinical investigation as anticancer agents. One would expect that inhibitors of CDK have no effect on terminally differentiated cells such as granulocytes that have completed the cell cycle and retain in the G₀ phase for the remaining lifespan. Archetypal CDK inhibitor R-roscovitine as well as AT7519, a selective inhibitor for CDKs 1, 2, 4, 5 and 9, have been reported to enhance spontaneous eosinophil apoptosis as well as to induce apoptosis in the presence of IL-5 *in vitro* [63,64]. They both induced a rapid apoptosis when compared to that induced by other common factors (Fas, glucocorticoids). Cell death profile induced by AT7519 was more beneficial with lesser extent of necrosis when compared to that of R-roscovitine [63,64]. Eosinophils expressed CDK-1, CDK-2, CDK-5, CDK-7 and CDK-9 but whether the pro-apoptotic effects of these compounds are directly related to inhibition of CDKs remains uncertain [64]. Eosinophil apoptosis induced by CDK inhibitors was dependent on caspases and was associated with a loss of anti-apoptotic Bcl-2 family member Mcl-1L. AT7519 was very potent in reducing pleural cavity eosinophilia in ovalbumin-challenged mice. This compound also enhanced the number of macrophages with ingested apoptotic bodies, suggesting that clearance of apoptotic eosinophils occurred *in vivo*. CDK inhibitors also increased apoptosis of neutrophils, but AT7519 did not affect apoptosis of non-granulocytes. However, *in vivo* AT7519 reduced number of other inflammatory cells as well, which may be secondary to reduced eosinophilia [63]. R-roscovitine was ineffective in reducing BAL or airway tissue eosinophilia possibly due to solubility issues and inadequate concentration achieved *in vivo* [64]. Both of these drugs are in clinical investigation as anticancer therapeutic agents, but it remains to be determined whether they proceed into clinical trials for the treatment of eosinophilic disorders.

Mitogen-activated protein kinase p38.

Mitogen-activated protein kinases (MAPK) are serine/threonine kinases mainly activated by proinflammatory cytokines, growth factors and environmental stress. MAPK family consists of p38, JNK and ERK family members. MAPK phosphorylate transcription factors resulting in transcription of

genes involved in apoptosis, survival, proliferation and differentiation [65]. Enhanced expression of p38 is evident in alveolar macrophages of patients with severe asthma and p38 may be involved in mediating corticosteroid insensitivity of severe asthma and COPD. MAPK p38 is also involved in migration of neutrophils and eosinophils [66]. Furthermore, basal activity of MAPK p38 has been demonstrated to be important for eosinophil survival and p38 inhibitors enhanced spontaneous eosinophil apoptosis [67]. In mouse models of asthma, inhibition of p38 prevented allergen-induced pulmonary eosinophilia, mucus hyper-secretion and airway hyper-responsiveness, which may be partly explained by its effects on eosinophil apoptosis [66]. The focus of interest for p38 inhibitors is in the treatment of COPD, but it remains to be seen whether p38 inhibitors undergo clinical trials for their potency in the treatment of severe asthma.

NF- κ B.

NF- κ B is a transcription factor expressed in numerous inflammatory cells. NF- κ B pathway is activated by stress and many endogenous and exogenous stimuli via action of I κ B kinase, and it modulates expression of pro-inflammatory cytokines, chemokines, inflammatory enzymes, adhesion molecules and receptors involved in immune response. NF- κ B activation is enhanced in asthmatic tissue, and the transcription factor has a central role in the pathogenesis of asthma [68]. Constitutive NF- κ B activity is critical for eosinophil survival and inhibition of NF- κ B results in dramatic increase in eosinophil apoptosis [26,69]. Glucocorticoids repress function of NF- κ B, which is one of its most important mechanisms of actions. However, glucocorticoids have many other target proteins resulting in adverse effects and glucocorticoid resistance comprises another concern in their use. Therefore, the development of specific inhibitors of NF- κ B would be beneficial. Numerous different types of inhibitors for NF- κ B [NF- κ B decoy oligonucleotides, small molecule inhibitors of I κ B kinase- β , proteasome inhibitors, small interfering RNA (siRNA)] exist. NF- κ B inhibitors reduced the number of eosinophils and other inflammatory cells

in the airways as well as levels of pro-inflammatory cytokines in animal models of asthma [68]. Inhibitors have not yet been reported to have been tested to treat human asthma.

PI3K.

Members of PI3K family (γ and δ) are activated in response to cytokines, chemokines, growth factors and pathogenic components and are important mediators of many leucocyte functions. Inhibition of PI3K has shown promising results in experimental models of allergic airway inflammation. In these models, non-selective inhibitors (LY294002 and wortmannin) and PI3K δ -selective inhibitor (IC87114) reduced levels of Th2 cytokines and attenuated tissue eosinophilia, airway hyper-responsiveness and airway mucus production [70]. In eosinophils, PI3K has been shown to mediate cell survival because inhibition of PI3K prevented IL-5-induced eosinophil survival [71,72]. Also, a role for PI3K in eosinophil migration has been reported [70]. In antigen-challenged and antigen-sensitized mice, systemic treatment with PI3K inhibitors (LY294002 and wortmannin), before antigen challenge, prevented recruitment of eosinophils into pleural cavity. However, accumulation of eosinophils into pleural cavity was also effectively resolved when mice were treated with PI3K inhibitor 24 hr after antigen challenge. This was associated with inhibition of Akt phosphorylation (a downstream target of PI3K) and with increased number of apoptotic cells, suggesting that induction of eosinophil apoptosis played a significant role in the clearance of eosinophilic inflammation [72]. However, use of non-selective PI3K inhibitors may cause toxic effects in long-term treatment and selective targeting of PI3K γ and δ likely provides a safer approach for asthma therapy.

Other targets and potential compounds.

Nitric oxide (NO) has a complex role in asthma. The exhaled NO correlates with eosinophilic airway inflammation [73], but NO has also shown anti-eosinophilic properties [74,75]. Inhibitors of inducible nitric oxide synthase (iNOS) showed no

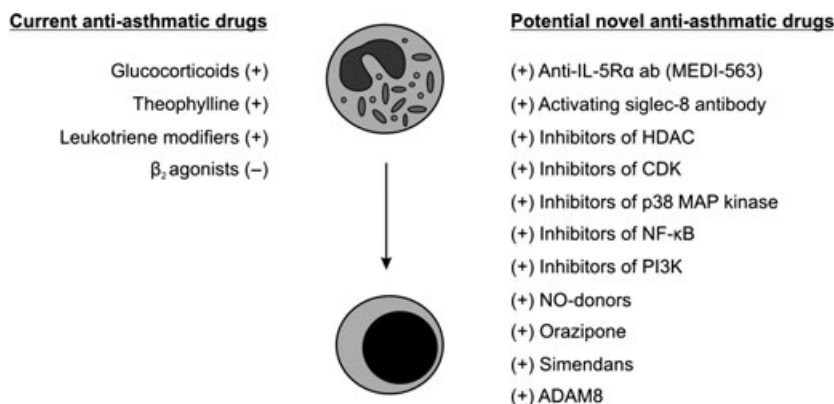


Fig. 3. Current and potential future anti-asthmatic drugs modulating eosinophil apoptosis. Signs (+) or (-) indicate whether the drug induces or inhibits eosinophil apoptosis, respectively. Anti-IL-5R α ab, anti-interleukin-5 receptor α antibody; HDAC, histone deacetylase; CDK, cyclin-dependent kinase; NF- κ B, nuclear factor- κ B; NO, nitric oxide; ADAM8, a disintegrin and metalloproteinase 8; PI3K, phosphatidylinositol-3 kinase; p38 MAP kinase, p38 mitogen-activated protein kinase.

efficacy on allergen challenge in patients with asthma [76]. The suitability of NO donors in the treatment of asthma is also under investigation because of their antibronchoconstrictive and possible anti-inflammatory effects [77]. NO donors (SNAP, S-nitroso-L-cysteine, NOC-18) were shown to induce eosinophil apoptosis in the presence and absence of IL-5 or GM-CSF and the mechanism of SNAP-induced apoptosis involved ROS, JNK, caspases and mitochondrial permeability transition [37,78–80]. However, because the effects of NO are complex during airway inflammation, the therapeutic potential of NO donors in human asthma may be limited.

Orazipone (OR-1384; 3-[[[4-methylsulfonyl]phenyl]methylene]-2,4-pentandione) and its derivatives OR-1958 (3-(3-chloro-4-methanesulfonyl-benzylidene)-pentane-2,4-dione) and OR-2370 (3-(4-chloro-3-nitro-benzylidene)-pentane-2,4-dione) are prototype compounds with unique mechanism of action. They form reversible conjugates with thiol groups (-SH) of proteins and glutathione [81]. Levosimendan is a pharmacological agent used in the treatment of decompensated heart failure. It acts by sensitizing troponin C to calcium, thereby increasing effects of calcium on myofilaments leading to improved contraction. It also opens ATP-sensitive potassium channels causing vasodilation by this mechanism [82]. Levosimendan and orazipone have both previously shown anti-inflammatory effects [83,84]. Levosimendan and its enantiomer dextrosimendan as well as orazipone, and its derivatives enhanced spontaneous eosinophil apoptosis and reversed IL-5-induced eosinophil survival. In contrast to glucocorticoids, their apoptosis-promoting effects occurred also at high IL-5 concentrations, thus being potentially better than glucocorticoids in this respect. Their mechanism of action seems to involve JNK and caspases. Interestingly, the apoptosis-inducing effect of simendans appears not to be related to their known mechanisms of actions, namely sensitization of troponin to calcium or opening of ATP-sensitive potassium channels. These candidate drugs were also shown to reduce lung eosinophilia in animal models of asthma [81,82]. Orazipone and its derivatives and simendans are thus potential novel drug candidates to treat eosinophilic conditions such as asthma.

A disintegrin and metalloproteinase ADAM8 is expressed in the surface of nearly all leucocytes. ADAM8-deficient mice showed enhanced allergic airway inflammation and airway hyper-responsiveness when compared to their wild-type counterparts. ADAM8 expression was increased in the airways of patients with asthma due to its increased expression in granulocytes. ADAM8 was found to promote intrinsic apoptosis of eosinophils from mice [85]. Thereby, ADAM8 seems to have anti-eosinophilic role in asthma and therapies that enhance levels of ADAM8 could be beneficial in the treatment of asthma. All the currently used anti-asthmatic drugs as well as potential anti-asthmatic agents and targets with eosinophil apoptosis-promoting activity are summarized in fig. 3.

Conclusions and Future Perspectives

As the knowledge has increased, it has become apparent that asthma is a heterogenous disease with many overlapping

phenotypes. In the future, focus may be on developing drugs for specific phenotypes of asthma. Eosinophils are abundantly present in most phenotypes of asthma and contribute to the maintenance and exacerbations of the disease. Development of drugs targeting eosinophil apoptosis is one strategy to treat asthma phenotype with predominant eosinophilic inflammation. Promotion of eosinophil apoptosis is a property of many current anti-inflammatory drugs used in the treatment of asthma (glucocorticoids, theophylline and leukotriene modifiers) supporting the importance of this mechanism of action.

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