

The role of eosinophils and neutrophils in inflammation

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Summary

The eosinophil is well recognized as a central effector cell in the inflamed asthmatic airway. Eosinophils release toxic basic proteins and lipid mediators such as cysteinyl-leukotrienes that cause bronchial epithelial damage and airflow obstruction. Eosinophil-selective cytokines and chemokines including interleukin (IL)-5, eotaxin and RANTES may represent targets for novel asthma therapies. In contrast, the role of the neutrophil in asthma remains relatively obscure. Recent evidence from the ENFUMOSA project and elsewhere suggests that neutrophils not only contribute to acute asthma exacerbations, but also are present in high numbers in the airways of patients with chronic severe asthma. Production by neutrophils of lipid mediators, reactive oxygen intermediates (ROI) and proteases such as elastase, may contribute to airflow obstruction, epithelial damage and remodelling. Leukotriene B₄ and cytokines such as IL-8, granulocyte-macrophage colony stimulating factor (GM-CSF), and tumour necrosis factor (TNF) α chemoattract neutrophils and reduce neutrophil apoptosis, and selective agents directed against these may prevent neutrophil influx and accumulation. Airway neutrophilia remains apparent in severe asthma patients even after treatment with high doses of corticosteroids. *In vitro*, corticosteroids paradoxically enhance neutrophil survival by reducing apoptosis, so corticosteroid therapy may exacerbate neutrophil activity *in vivo*. Both corticosteroids and cytokines may suppress neutrophil apoptosis by upregulating endogenous synthesis of leukotriene (LT)B₄. Specific blockade of LTB₄ synthesis or LTB₄ receptors may induce neutrophil apoptosis and combat the unwanted effects of high-dose steroids on neutrophil survival. Phagocytosis of apoptotic neutrophils stimulates important signals that down-regulate pro-inflammatory cytokine production by macrophages, allowing resolution and repair processes to prevail.

Keywords: eosinophil, neutrophil, leukotrienes, chemokines, cytokines, asthma, apoptosis, airways inflammation

Eosinophils in airways inflammation

The immunopathology of allergic asthma is complex and involves a range of cell types and mediators (Fig. 1). Nevertheless, it is well recognized that eosinophils are important cells in asthma. Eosinophils release basic proteins that are cytotoxic and lipid mediators such as cysteinyl leukotrienes that cause airflow obstruction and bronchial epithelial damage.

Over the last few years, the strategy for looking for new asthma medications has moved away from the final common

effect or mediators towards the command and control pathways. Type 2 T-helper (Th₂) cells and mast cells both contribute eosinophilic cytokines, perhaps producing a secondary release of more specific and more potent chemokines, particularly eotaxin acting at the C-C chemokine receptor 3 (CCR3). This may be the best target for a specific anti-eosinophil drug.

Table 1 lists a range of potential anti-eosinophilic therapies. It includes corticosteroids, which are very effective at reducing eosinophils in the airways. More surprisingly, the leukotriene modifiers reduce eosinophils in blood by about 50%, and recent preliminary data has shown reductions in airway eosinophils of 50–60% after 3–4 weeks treatment with a leukotriene modifier. Future antagonists will need to be small stable molecules that can be given orally to patients.

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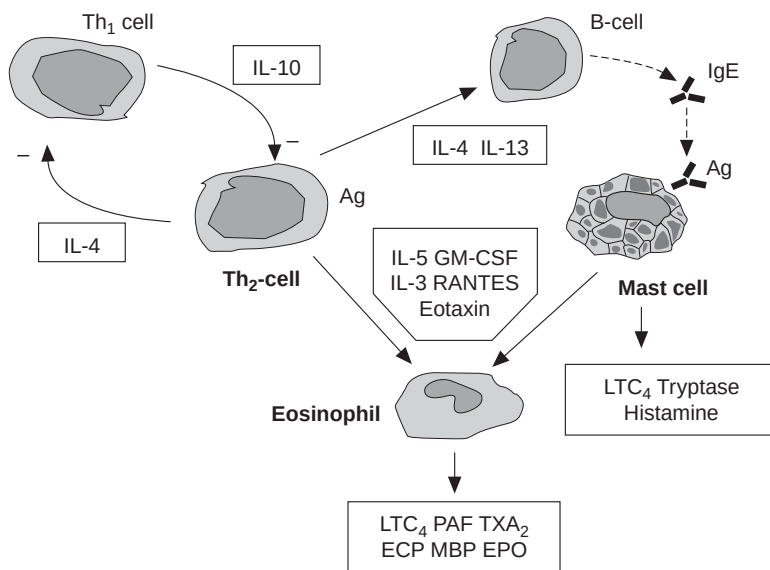


Fig. 1. Immunopathology of allergic asthma.

Table 1. Potential anti-eosinophilic agents

Corticosteroids
Leukotriene modifiers
IL-5/IL-3/GM-CSF antagonists
Eotaxin/RANTES antagonists
IL-4/IL-13 antagonists
IL-10R agonists
VCAM/VLA-4 antagonists
Anti-IgE agents

Interleukin (IL)-5 and IL-3 antagonists are more likely to reduce activation of eosinophils than to block chemotaxis.

Are neutrophils important in asthma?

Previous studies have shown that neutrophil influx into the airways is associated with non-allergic triggers of asthma such as nitrogen dioxide and ozone [1,2]. In addition, neutrophils are the first cell type to enter the lung following allergen challenge [3–5]. Unlike eosinophils, the influx of neutrophils is transient following allergen challenge. However, challenge with a large bolus dose of allergen in the laboratory probably does not reflect what happens in the airways of an allergic asthmatic exposed to low levels of allergen during the allergen season or perennially. There is no reason why a cell type that is transient during allergen challenge cannot be there long-term in the asthmatic airway. The fact that the neutrophil disappears very quickly following allergen challenge suggests that the rate of apoptosis

may be critical in regulating neutrophil numbers in the airway.

Nocturnal asthma is associated with high levels of neutrophils, which correlate with the severity of nocturnal asthma [6]. Furthermore, in a small number of patients who died of sudden-onset asthma, the predominant cell type in the sputum was the neutrophil not the eosinophil [7]. In addition, neutrophils predominated more frequently than eosinophils in the sputum of patients with acute exacerbations of asthma, mostly associated with respiratory tract infection [8]. High neutrophil counts are also observed in bronchial lavage from patients undergoing acute exacerbations that were not associated with infection [9]. Furthermore, patients with severe oral steroid-dependent chronic persistent asthma consistently have more neutrophils in bronchoalveolar lavage (BAL) and bronchial biopsies than patients with well-controlled mild to moderate persistent asthma [10]. In contrast, there is no difference in the eosinophil counts between these two groups of patients. It is unclear whether this reflects a predisposition to neutrophilia in severe asthmatics or whether it is a consequence of oral steroid use by these patients.

ENFUMOSA study

In the European network for understanding the mechanisms of severe asthma (ENFUMOSA) study [11], patients were stratified into four groups:

Group 1 patients were using high-dose inhaled steroids ($> 1600 \mu\text{g/day}$ beclomethasone dipropionate or equivalent) for at least a year, salmeterol or theophylline for at least a year, and no more than occasional short-courses of oral prednisolone or very low daily doses of prednisolone;

Group 2 patients were the same as group 1, but were also receiving daily doses of oral prednisolone (>5 mg/day);

Group 3 patients did not fit the criteria for groups 1 and 2, but had experienced a near-fatal asthma attack within the previous 5 years;

Group 4 were the control group with chronic persistent mild to moderate asthma that was stable and well-controlled with modest doses of inhaled steroids (<1000 µg/day of BDP).

Compared with the well-controlled asthmatics (group 4), the severe asthma groups (groups 1 and 2) had significantly more females (3.6:1), were more likely to have perennial symptoms, had less allergic rhinitis and a lower history of maternal allergy, and were more likely to have self-reported asthma triggers of exercise and aspirin (but not allergens). A range of inflammatory markers was examined. Compared with group 4 patients, the patients with severe asthma had higher levels of exhaled nitric oxide (particularly in group 2) and higher urinary levels of leukotriene (LT)E₄ and eosinophil protein X, suggesting chronic activation of eosinophils. Unsurprisingly, the severe asthmatics (groups 1 and 2) had increased total leucocytes in the blood compared with the well-controlled asthmatics, and this was entirely accounted for by more neutrophils, especially in group 2 (the oral steroid users). There was no difference between the groups in the number of eosinophils, lymphocytes, monocytes and basophils in the blood. It is often thought that steroids reduce influx of neutrophils into the inflammatory site despite elevated blood counts. However, the sputum of ENFUMOSA patients in groups 1, 2 and 3 had significantly more neutrophils than the well-controlled asthmatics (group 4), while there were no differences from well-controlled patients in the numbers of eosinophils, lymphocytes, macrophages, epithelial cells and metachromatic cells [11]. It is a reasonable hypothesis that persistent airway neutrophilia, either spontaneously or as a consequence of steroid use, may contribute to airway inflammation in severe asthma.

A number of neutrophil chemotactic factors may be responsible for attracting neutrophils to the airway (Table 2). The most potent of these is LTB₄ [12]. The C-X-C chemokines IL-8, GROα, and epithelial-derived neutrophil activating peptide 78 (ENA-78) all produce chemotaxis of neutrophils via a single receptor, the C-X-C R2,

which may be a good target for blocking neutrophil chemoattractants.

Neutrophil products

Neutrophils can produce a wide range of products including lipids (LTB₄, PAF, TXA₂, LTA₄), cytokines (IL-1β, IL-6, IL-8, TNFα, TGFβ), proteases (elastase, collagenase, MMP-9), microbicidal products (lactoferrin, MPO, lysozyme), reactive oxygen intermediates (superoxide, H₂O₂, OH⁻) and nitric oxide. Elastase in particular has an extensive range of actions. It breaks down not only elastin, but also collagen III, promotes the formation of collagen IV, proteolyses surfactant proteins, and activates matrix metalloproteases. On the epithelium, elastase enhances mucus secretion and reduces ciliary beat frequency; these two effects are reduced by leukotriene synthesis inhibitors suggesting secondary release of leukotrienes from the epithelium. Elastase may also release IL-8 and possibly other cytokines from the airway epithelium. Elastase damages the endothelium by increasing permeability and promoting apoptosis, and activates mast cells and eosinophils (Fig. 2).

Leukotriene B₄

Neutrophils produce large amounts of LTB₄ and also secrete its precursor LTA₄. When the neutrophil is in apposition to airway structural cells, this LTA₄ may be converted either into LTB₄ or into LTC₄, a very potent bronchoconstrictor [13].

LTB₄ is a potent chemoattractant for neutrophils, eosinophils, monocytes and fibroblasts. The LTB₄ receptor (BLT) which has recently been cloned appears to be identical to the human chemoattractant receptor-like receptor1 [14]. The BLT receptor has homology with both C-C chemokine receptors and C-X-C chemokine receptors, which may explain its wide spectrum of chemoattractant activity. LTB₄ also activates nuclear factor (NF)-κB [15], promotes the synthesis of IL-5, IL-6 and IL-8 [16], and enhances IgE synthesis in B cells [17].

Instillation of LTB₄ into human airways leads to BAL neutrophilia 4 h later [6]. LTB₄ is not a bronchoconstrictor and has no effect on FEV₁, sGaw or bronchial responsiveness in either healthy subjects or patients with asthma [18]. However, following inhalation of LTB₄ in healthy subjects, there is a rapid blood neutropenia, with counts dropping to <20% of baseline levels at 5 min. This is followed by a rebound blood neutrophilia at 30 min. Curiously, in mild allergic asthmatics, these blood neutrophil responses to LTB₄ are diminished [18], possibly reflecting neutrophil desensitization due to chronic exposure to LTB₄ in asthmatic airways. Following allergen challenge, neutrophils and eosinophils infiltrating the airway are desensitized to LTB₄

Table 2. Neutrophil chemotactic factors

Eicosanoids	LTB ₄ , PAF
C-X-C chemokines	IL-8, GROα, ENA-78
CC chemokines	MIP-1α
Cytokines	GM-CSF, IL-6, PDGF, ET-1
Bacterial peptides	FMLP
Complement	C5a

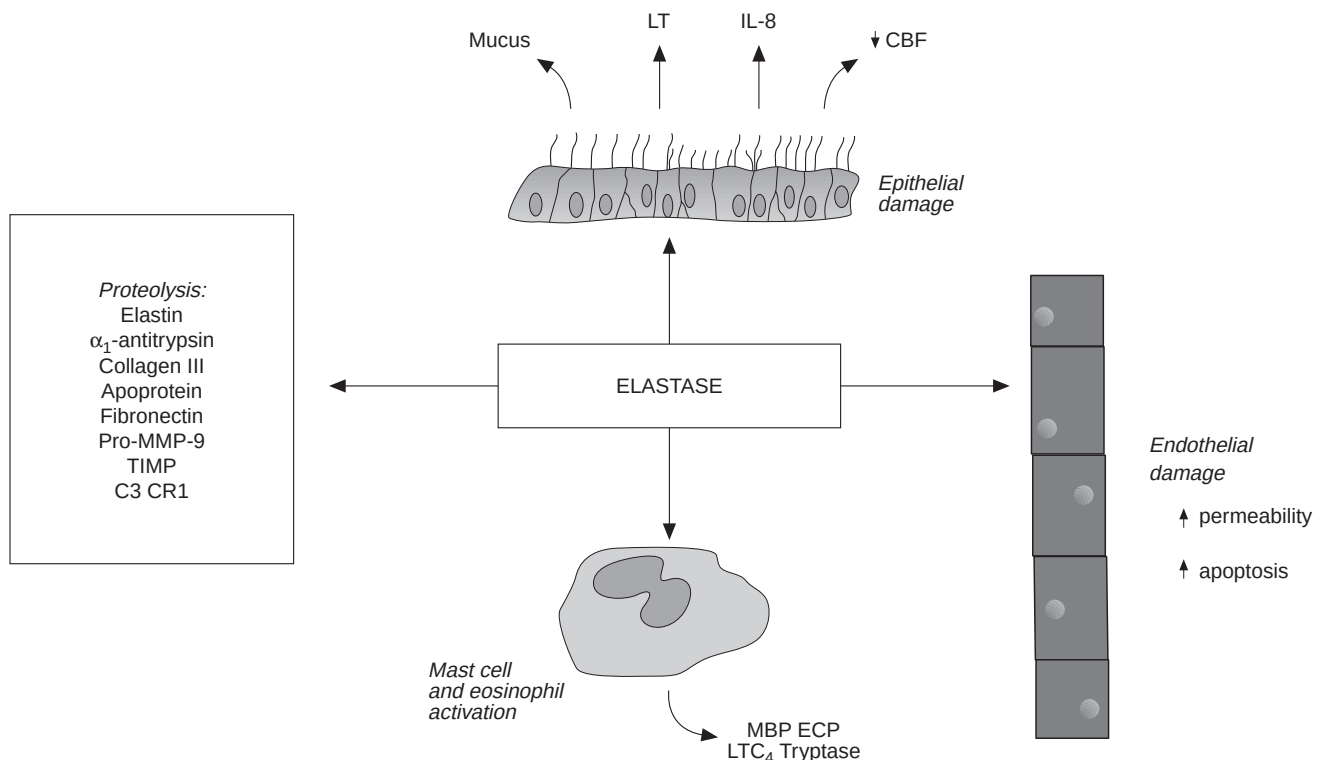


Fig. 2. Neutrophil elastase.

[19,20], suggesting that they have been exposed to endogenous LTB₄.

Only one study of a specific LTB₄ antagonist (LY293111) in asthma has been reported [21]. As expected, treatment with LY293111 for 7 days had no effect on the early and late-phase airway responses to allergen or bronchial hyper-responsiveness. However, it reduced BAL neutrophil counts by 50–70% and also reduced BAL fluid levels of myeloperoxidase, LTB₄, LTC₄ and IL-8. LTB₄ may thus be the predominant chemoattractant for neutrophils during allergic responses in the asthmatic airway.

Is airway neutrophilia a consequence of corticosteroid therapy?

Acute, macrophage-induced neutrophil-dependent inflammation depends on production of a range of neutrophil-priming cytokines and chemoattractants (Fig. 3). The normal fate of the neutrophil is to undergo apoptosis and then phagocytosis by macrophages. The process of neutrophil engulfment changes the macrophage phenotype from its pro-inflammatory production of cytokines to a different profile of PGE₂ and TGFβ release, associated with resolution and repair [22,23]. Whereas steroids enhance eosinophil apoptosis, they paradoxically prolong neutrophil survival

by reducing their apoptosis [23]. By reducing the turnover of apoptotic neutrophils, corticosteroids may sustain the airway macrophage in its acute inflammatory phenotype and prevent their transformation to a phenotype that promotes resolution of inflammation and structural repair.

Novel therapeutic targets may include specific agents that promote neutrophil apoptosis. Recent work suggests a critical role of LTB₄ in mediating neutrophil survival *in vitro*, and the LTB₄ antagonist SB210148 promoted neutrophil apoptosis survival [24]. This compound also blocked the survival enhancing effects of cytokines. Both corticosteroids and cytokines such as TNFα and IL-8 may promote neutrophil survival by upregulating endogenous synthesis of LTB₄ [24,25]. Specific LTB₄ antagonists may block neutrophil migration and promote neutrophil apoptosis, thus combating the unwanted effects of steroids on neutrophil survival.

Conclusions

Both eosinophilia and neutrophilia in the airway are associated with severe asthma. It is unclear whether neutrophilia occurs as a primary pathological process or is secondary to high doses of corticosteroids used in those patients. Potential new targets for therapy include antagonists of neutrophil

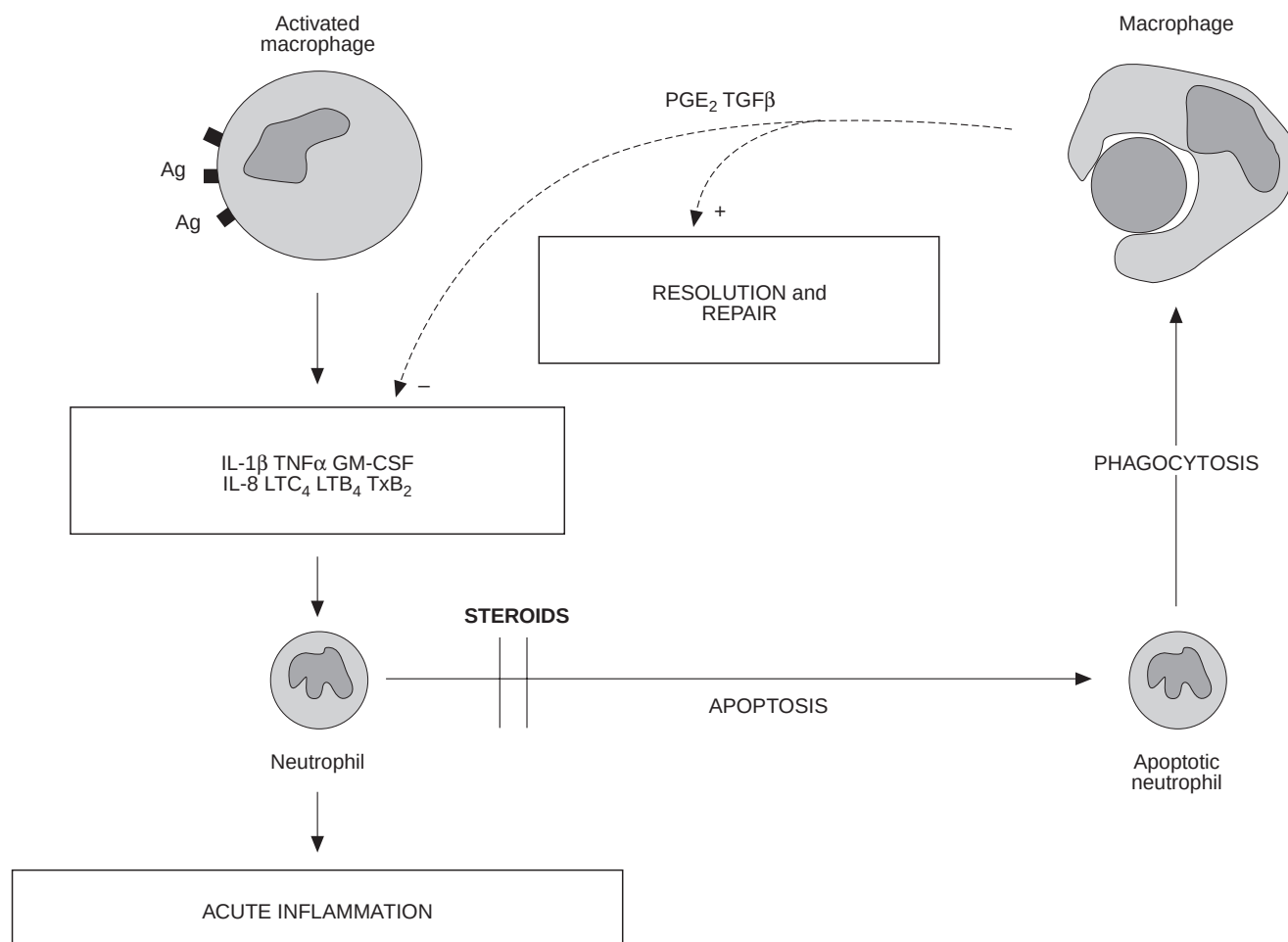


Fig. 3. Neutrophil engulfment inhibits macrophage activity. Drawn from data in [23].

chemoattractants, including LTB_4 , GM-CSF, and IL-8, and blockade of neutrophil products such as elastase. LTB_4 may have a central role in enhancing neutrophil survival in response to cytokines and corticosteroids. LTB_4 antagonists may specifically promote neutrophil apoptosis in the airway leading to resolution and repair.

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