

REVIEW

Handle with care: targeting neutrophils in chronic obstructive pulmonary disease and severe asthma?

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Clinical and Experimental Allergy

Summary

Neutrophils play an important role in the pathogenesis of airway inflammation in both chronic obstructive pulmonary disease (COPD) and severe asthma. Currently available drugs have only limited effects on neutrophilic airway inflammation, particularly in COPD. Therefore, great efforts are undertaken to address neutrophilic inflammation in chronic respiratory disorders, in particular COPD. This review summarizes the rationale for anti-neutrophilic treatment in COPD and asthma and gives a critical overview of current developments in drug therapy. Moreover, unanswered questions and limitations of clinical trial design and choice of outcome parameters for proof-of-concept studies with novel anti-neutrophilic drugs are discussed as well as potential safety issues.

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Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is a debilitating inflammatory disease of the lungs. Besides infiltration of bronchial mucosa and the alveolar space by CD8⁺ lymphocytes [1], increased presence of neutrophils and macrophages in the airways of affected patients is a hallmark of COPD [2–6]. The disease is usually progressive by nature, and available treatments have failed to influence the annual decline of lung function in affected patients [7–10]. In contrast, smoking cessation has been consistently shown to reduce the rate of decline in pulmonary function in smoker's COPD [11]. Nevertheless, even in ex-smokers there is evidence of ongoing neutrophilic inflammation, once the disease is established [12, 13]. Additionally, as COPD progresses, small airway wall and luminal neutrophilia increases [14]. Finally, the degree of neutrophilic inflammation is also correlated with the clinical severity of COPD [4], and increased airway inflammation is to some extent associated with the frequency of exacerbations because of COPD [15]. In particular, protease–antiprotease imbalance and oxidative stress are believed to play a major role in COPD pathogenesis, disease progression and matrix destruction [16]. Because of their ability to rapidly generate reactive oxygen intermediates and elastolytic enzymes in response to

various stimuli, neutrophils are the major source of oxidative stress and protease-mediated inflammation in COPD. Neutrophils also facilitate increased mucus production in COPD [17], which itself is an independent predictor of COPD mortality [18]. It has been shown that peripheral blood neutrophils from COPD patients display an altered activation status in stable disease or during exacerbations [19–22], show increased activation of inflammatory genes during COPD progression [23], and that airway markers of neutrophilic inflammation increase further during an exacerbation [24]. Moreover, their abundant presence in diseased airways and tissue is associated with inflammatory mediators including chemokines [25–27], elastase [28], metalloproteases [29–31], cytokines [32, 33], nitrosative [34–37] and oxidative [38, 39] stress. These chemokines primarily initiate recruitment of neutrophils to the airways, a process orchestrated by a complex sequence of cellular and signalling events including initial tethering of blood neutrophils, firm adhesion to endothelial cells and subsequent chemotactic migration into airways and alveoli [40, 41]. In particular, IL-8, and leukotriene B₄ (LTB₄) are potent neutrophil chemotactic stimuli involved in the underlying inflammatory process of COPD. In addition, neutrophils secretory products like myeloperoxidase (MPO), hydrogen

peroxide (H₂O₂), elastase or metalloproteases contribute to the pathogenic features of COPD. While MPO or H₂O₂ promote both epithelial and lung parenchymal damage through formation of e.g. toxic hypochlorous acids, activation or increased release of cytokines [42], unopposed elastase facilitates tissue elastolysis, emphysema, and promotes mucus secretion from bronchial glands [17]. Elastase and metalloproteinases, in particular matrix metalloproteinase (MMP)-9, also contribute to airway remodelling associated with fixed airflow limitation in COPD, as well as in long-standing asthma [43].

There is also evidence that neutrophil clearance from the lungs is reduced in COPD [44], and increased airway concentrations of e.g. granulocyte macrophage colony-stimulating factor [32] promote neutrophil survival in COPD lungs.

Interestingly, despite abundance of neutrophils in bronchoalveolar lavage (BAL) samples or induced sputum, some authors [1, 45] – albeit not all [46, 47] – failed to find increased numbers of neutrophils within biopsies of the peripheral airway wall. In contrast, recent studies confirmed a marked neutrophilic infiltration of airway smooth muscles in smokers with COPD, suggesting an important role of neutrophils and – related mediators on smooth muscle thickening and contraction in COPD [48]. However, taken together these data underline that not only the mere presence of neutrophils is important in COPD, but rather the concise location and distribution of these cells within the diseased airways of affected patients. This may have important implications for selection of therapeutic targets as well as the route of delivery (systemic vs. inhalational).

Given the predominant role of neutrophils in COPD (overview in Table 1), several trials have therefore addressed the issue of reducing inflammation in COPD patients, mainly involving randomized-controlled clinical studies with inhaled or oral corticosteroids. Altogether, these studies have generated at least conflicting results. While inhaled corticosteroids are the cornerstone of anti-inflammatory treatment in asthma, their role in the maintenance treatment of COPD is of less importance. With regard to their potential effect on inflammatory surrogate

markers in stable COPD, no [49, 50] or only marginal [51, 52] benefits of high-dose inhaled or oral corticosteroids on inflammatory surrogate markers in COPD have been reported. In long-term clinical trials, high-dose inhaled corticosteroids failed to ameliorate the progressive decline of lung function in moderate-to-severe COPD [7, 9, 10], albeit in selected patients, the use of inhaled corticosteroids was associated with a reduction of severe exacerbations and a slower decline of health status [7]. It has been hypothesized only recently, that the observed relative corticosteroid resistance in COPD may be because of an inactivation of histone deacetylase [53] preventing the switch-off of inflammatory genes. Consequently, current guidelines recommend bronchodilators as first-line agents in COPD [54], whereas corticosteroids are indicated in patients with moderate-to-severe disease and a history of recurrent exacerbations. Therefore, there appears to be an imminent need for novel anti-inflammatory treatment options in COPD.

Asthma

Asthma is a chronic inflammatory disease of the lower airway characterized by variable, largely reversible airway obstruction, that is associated with increased bronchial responsiveness and presence of eosinophils in the airways of affected individuals [55]. However, the concept of a central importance of eosinophils in asthmatic inflammation has been challenged by recent studies casting doubt on the role of eosinophils in asthma [56, 57], although the results from these studies have been discussed at least controversially [58]. Nevertheless, focussing on eosinophils and T cells during the last decade has led to underrecognition of the role of neutrophils in asthma and related allergic disorders, e.g. allergic rhinitis (AR). However, the interest in the role of neutrophils in particularly severe asthma has largely increased over the past years [59], and this growing interest is based on a large body of evidence (Table 1). Airway neutrophils increase after bronchial allergen challenge [60–62], and there is also a growing body of evidence supporting increased neutrophilic inflammation in patients with persistent asthma

Table 1. Overview of selected references providing evidence of neutrophil involvement in the pathogenesis of COPD and asthma

Key finding (reference number)	COPD	Asthma
↑ Neutrophil number in sputum	[5, 12, 13, 27, 33]	[60, 64, 65, 68, 200]
↑ Neutrophil number in BAL	[6, 12]	[61–63, 66]
↑ Neutrophil number in biopsies	[14, 47, 48]	[66, 67]
↑ Neutrophil activation	[19–23]	[32, 64, 74–76, 79]
Neutrophilia associated with oxidative stress	[38, 39, 37]	[76, 77, 200]
↑ Neutrophil mediators	[24–27, 102]	[64, 65]
↑ Neutrophil-related proteolysis	[28–31]	[71, 138–140]
Neutrophilia associated with progression, poorer prognosis, or treatment response	[14, 18, 195]	[72, 81]

BAL, bronchoalveolar lavage; COPD, chronic obstructive pulmonary disease.

[63–67]. In particular, severe asthma and asthma exacerbations [68] are characterized by increased sputum neutrophils, although there is still an ongoing debate whether the observed neutrophilia is at least partially a consequence of corticosteroid treatment promoting neutrophil survival [69] and decreasing the relative proportion of eosinophils. Despite this unresolved controversy, inhaled corticosteroids, although highly effective, do not completely suppress airway inflammation in asthmatics, in particular in step 4 (severe persistent) asthma, where some degree of resistance to corticosteroids seems to be prevalent [70–72]. In an allergen challenge model of asthma, it has been demonstrated that the ability of corticosteroids to ameliorate late-phase asthmatic reactions is diminished by increased presence of neutrophils in the airways of affected subjects [73]. Increased secretion of neutrophil-derived MMP-9 in severe asthma is also poorly inhibited by corticosteroids [71]. Further, blood neutrophils from asthmatic patients are activated [74–77] and display an increased secretion of MPO [78], which is in part mediated through activation of IgE receptors [79, 80]. Hence, neutrophils and derived mediators may at least partially explain the persistence of asthmatic inflammation despite effective inhibition of eosinophils.

Interestingly, it was earlier demonstrated by Little et al. [81] that the degree of sputum neutrophilia in asthma inversely correlated with the maximal forced expiratory volume in 1 s (FEV₁) achieved by both bronchodilators and corticosteroids, indicating that sputum neutrophilia in asthma may be a marker of remodelling. Sputum neutrophilia in asthma can be observed as a unique feature in the so-called 'non-eosinophilic' asthma [65, 66], but can also accompany sputum eosinophilia as a secondary phenomenon [82].

Potential targets of anti-neutrophilic treatment

Mechanisms of neutrophil-mediated inflammation

Recruitment of neutrophils to the airways involves a series of regulated events including chemotactic stimuli like IL-8, LTB₄, growth-related oncogen (GRO)- α , transendothelial migration of neutrophils through selectin-mediated tethering, rolling and subsequent adhesion by integrin-dependent mechanisms [40], e.g. [41] binding of neutrophil CD11b/CD18 (Mac-1) to intercellular adhesion molecule (ICAM)-1. At sites of inflammation, neutrophils become activated through multiple stimuli, which leads to release of proteolytic enzymes, cytokines and oxidative stress. In inflamed tissues, clearing of neutrophils is also often delayed by survival-promoting factors, e.g. granulocyte-macrophage colony stimulating factor (GM-CSF) [83].

Although the inflammatory airway diseases discussed in this article represent distinct entities with unique clinical and pathophysiological features, the basic me-

chanisms with regard to neutrophil-mediated inflammation are widely uniform. Thus, the majority of therapeutic approaches discussed herein will more or less have a rationale for both COPD or asthma (Fig. 1).

Chemotaxis

Interleukin-8 blocking agents. IL-8 is a CXC chemokine with potent neutrophil chemoattractant activities [41, 84]. IL-8 is consistently associated with neutrophilic inflammation, and sputum, BAL or airway wall neutrophilia is directly correlated with IL-8 concentrations. Epithelial cells and macrophages are believed to be the major source of IL-8 in the airways, and IL-8 is released following a number of stimuli [85]. In COPD, airway concentrations of IL-8 are markedly elevated [25], increase during exacerbations [15], and account for a significant part of total chemotactic activity of airway secretions [86]. Similar observations have been made in non-eosinophilic and severe asthma [64, 65], cystic fibrosis [87], pulmonary fibrosis [88] and obliterative bronchiolitis following lung transplantation [89]. Moreover, IL-8 contributes to inflammation in rheumatoid arthritis [90], and promotes tumour metastasis, particularly in melanoma [91, 92]. Therefore, blocking IL-8 has been considered an attractive therapeutic approach to a variety of disorders for several years. Consequently, humanized monoclonal anti-IL-8 antibodies have been developed [93]. Preclinical data in primates suggest, that application of anti-IL-8 may be safe [94]. To date, a single clinical trial in patients with COPD has been published. In this 3-month study using a monoclonal anti-IL-8 antibody, active treatment reduced dyspnea without changing lung function or exercise capacity [95]. Moreover, no direct markers of airway inflammation were reported in this trial. Nevertheless, given the crucial role of IL-8 in mounting an adequate response to infectious organisms, systemic and enduring blockage of IL-8 remains a potential hazard for the patient and long-term studies need to be undertaken.

An alternative way to reduce biological effects of chemoattractants like IL-8 towards inflammatory cells is to antagonize signalling events by blockage of cell surface receptors. Therefore, CXC-chemokine receptor antagonists have been developed. Such compounds effectively inhibit neutrophil influx and chemotaxis in animal models of neutrophilic inflammation [96, 97], but no data in humans have been reported this far.

Leukotriene B₄ antagonists. Neutrophils are controlled by various chemoattractants and activators, and among these LTB₄ is one of the more potent chemoattractants and activators. After binding to its neutrophil receptor, LTB₄ elicits calcium influx, transmembrane potential changes, degranulation, increased expression of the CD11b/CD18

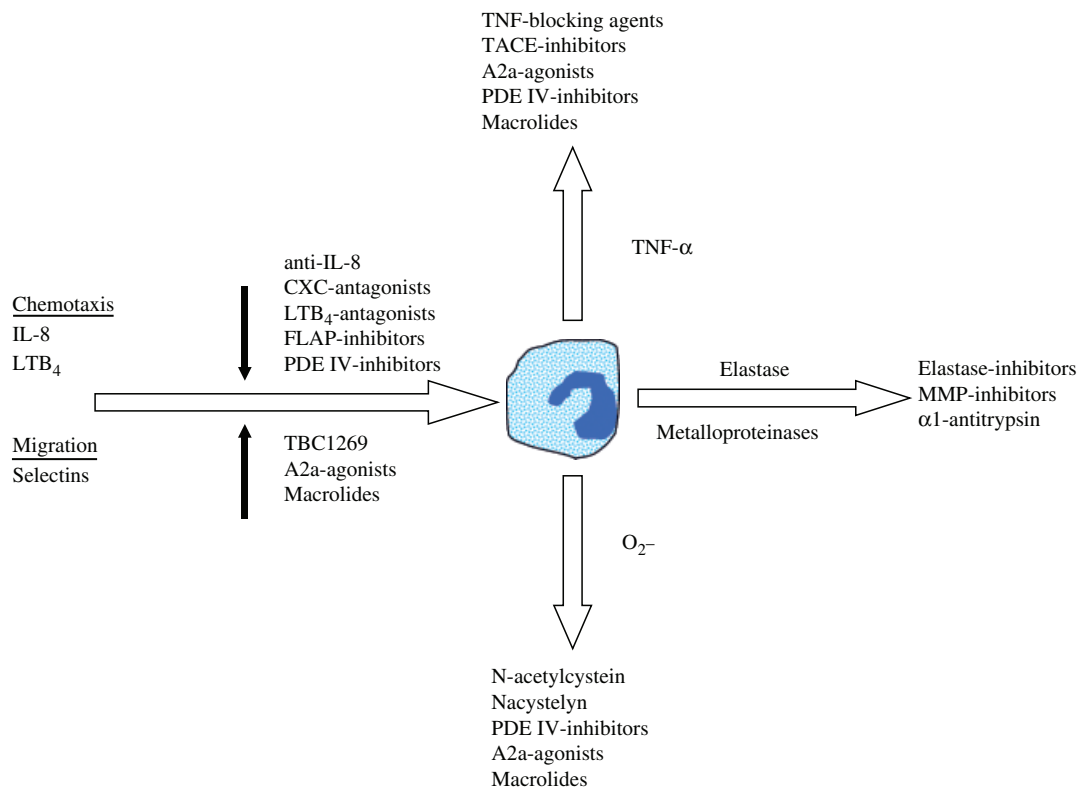


Fig. 1. Neutrophil functions in airway inflammation and potential new therapeutic approaches.

adhesion molecule and, as a result, chemotaxis. Correspondingly, CD11b/CD18 has been found up-regulated in neutrophils of smokers, in parallel to airway obstruction [19]. Intratracheal instillation of LTB₄ induces neutrophil recruitment into the bronchoalveolar space in human subjects [98], and patients with COPD show increased levels of LTB₄ in serum and BAL fluid (BALF) [99, 100]. LTB₄ accounts for a large part of the total neutrophil chemotactic activity of airway secretions obtained from COPD patients [8, 101]. LTB₄ concentrations in exhaled breath condensate further increase during COPD exacerbations and are reduced with treatment [102]. Finally, LTB₄ also inhibits neutrophil apoptosis [103].

Reduction of LTB₄ can theoretically be achieved by two distinct ways. One way is to block LTB₄ synthesis, e.g. by inhibiting the 5-lipoxygenase pathway. Alternatively, LTB₄ receptor antagonists could prevent biological actions of LTB₄ by blocking LTB₄ receptors on cell surfaces. Zileuton is a 5-lipoxygenase inhibitor approved for the treatment of asthma. Primarily, it was designed to ameliorate eosinophilic inflammation by reducing cys-leukotriene synthesis. However, because of the common pathways of cys-leukotriene and LTB₄ synthesis, recent interest has focussed on the ability of 5-lipoxygenase or 5-lipoxygenase activating protein (FLAP) inhibitors to reduce neutrophilic inflammation. To date, few studies have been published in COPD. In one trial, 2 weeks treatment with the leukotriene synthesis inhibitor BAYx

1005 led to a small reduction of sputum LTB₄ concentrations, but without any clinical benefit [104].

Moreover, at least three LTB₄ receptor antagonist have proceeded into proof-of-concept studies in humans, LTB019A (Novartis, Horsham, UK), LY293111 (Lilly, Indianapolis, IN, USA), and BIIL 284 (Boehringer, Ingelheim, Germany). LY293111 inhibited BALF accumulation of neutrophils following antigen challenge in guinea-pigs [105] and reduced allergen challenge induced airway neutrophilia in mild asthmatics, although the late asthmatic reaction was unaffected [106] (a result strikingly similar to recent studies with eosinophils and anti-IL-5). In COPD, preliminary clinical results this far have been disappointing, showing no effect on neutrophilic inflammation and – related surrogate markers [107]. Hence, the feasibility of this approach remains to be confirmed in future trials.

Migration and rolling

Selectin antagonists. In both COPD and asthma, the inflammatory cascade is mediated by a sequence of signalling events directing effector cells into sites of inflammation. One of the initial events, leucocytes tethering and rolling along endothelial walls of blood vessels is mediated by the selectins, which belong to the family of cell adhesion molecules. The groups of selectins comprise

three members, P-, L-, and E-selectin. Selectin ligands include CD34, P-selectin glycoprotein ligand (PSGL), and sialyl Lewis X, and some of these ligands are up-regulated in chronic airway inflammatory airway diseases [108, 109], while the expression of L-selectin itself is reduced on circulating inflammatory cells during asthma exacerbations [110]. Although relatively well established in asthma, the role of selectins in COPD is as yet less clear [111]. Several animal models have confirmed the role of selectin-dependent pathways in the recruitment of neutrophils to sites of inflammation [112]. Moreover, the use of both selectin antagonists and selectin knockout mice have proved the requirement of selectins to promote infiltration of inflammatory cells in chronic or acute disease models [113–115]. Among currently available antagonists, one small molecule pan-selectin antagonist, TBC1269 (Revotar, Henningsdorf, Germany), has advanced into clinical trials involving patients with asthma and psoriasis. TBC1269 (Bimosiamose = 1,6-Bis [3-(3-carboxymethylphenyl)-4-(2- α -D-mannopyrano-syloxy)-phenyl] hexane) is a synthetic computer designed pan-selectin antagonist targeted against all three identified selectins [116]. Different *in vitro* studies under static and dynamic flow conditions demonstrated that this compound is able to block different types of leucocytes including neutrophils and eosinophils, with a preferential effect on neutrophils *in vitro* [117]. Inhaled TBC1269 potentially inhibited allergic airway responses and neutrophilic inflammation in a sheep model of allergy [112]. In humans, a single intravenous dose of TBC1269 in asthmatic subjects had only a minor effect on sputum eosinophils following allergen challenge, and failed to attenuate allergen-induced late asthmatic reactions [118]. Inhaled TBC1269, however, was effective in a phase II study using an allergen challenge model in mild asthmatics, where it reduced late-phase asthmatic reactions by approximately 50% compared with placebo [119]. This finding is in line with the above-cited animal study, where inhaled TBC1269 was also superior to the systemic route of application. Moreover, in the sheep study, lavage fluid content of mast cell mediators such as histamine and tissue kallikrein were markedly reduced by TBC1269, suggesting a role of selectin blockage in inflammatory cell-mediator release and signal transduction. In airway diseases, the inhaled route of TBC1269 may offer an advantage over systemic delivery in terms of safety issues.

Neutrophil activation and mediator release

Adenosine A2a receptor agonists. Human neutrophils express the adenosine A2a receptor, and agonists at this receptor inhibit a variety of cell functions, including release of reactive oxygen species, elastase and toxic granula proteins like MPO, and neutrophil adherence to

vasculature [120]. Therefore, adenosine A2a agonists are considered a potential treatment for asthma and COPD. There are at least two compounds, that have been tested in animal models and early human trials (CGS21680, Novartis, and GW328267, GlaxoSmithKline, Stevenage, UK). CGS21680 potentially inhibited inflammation and granulocyte recruitment in a rat allergen challenge model [121]. A small clinical trial using GW328267 in mild asthmatics in an allergen challenge model has been recently communicated, but results have been disappointing this far [122]. Therefore, it is as yet unclear whether such compounds will be effective treatments for asthma or COPD.

Elastase and metalloprotease inhibitors. Neutrophil granula contain a variety of proteases that are physiologically secreted during anti-microbial defense. Of these, neutrophil elastase is believed to be a key player in the pathogenesis of pulmonary emphysema in COPD patients. Increased amounts of unopposed elastase activity have been consistently found in stable COPD patients [123, 124] or during exacerbations, and particularly deficient elastase neutralization by endogenous inhibitors, mainly α -1-antitrypsin, is associated with neutrophilic inflammation and the severity of emphysematous changes [125, 126]. Moreover, instillation of elastase into rat or guinea-pig trachea leads to extensive pulmonary inflammation and emphysematous destruction of alveolar spaces [127]. In contrast, elastase-deficient mice fail to develop emphysematous changes following exposure to cigarette smoke [128]. Besides α -1-antitrypsin substitution, which is approved for the treatment of COPD and emphysema associated with homozygous α -1-antitrypsin deficiency, several specific elastase inhibitors have been developed for the treatment of COPD, cystic fibrosis or acute lung injury [129]. However, most synthetic elastase inhibitors were associated with limiting side-effects or failed to demonstrate clinical efficacy so far [130]. Alternatively, inhaled recombinant α -1-antitrypsin may be a feasible approach also in COPD unrelated to α -1-deficiency [131].

Besides neutrophil elastase, MMPs are crucial for homeostasis and turnover of the extracellular matrix in health and disease [132]. Airway inflammatory cells, in particular neutrophils, are a major source of MMPs [133, 134]. Of all known classes of MMPs, MMP-9 is the one that has been studied to the greatest extent in inflammatory airway diseases. Beside its role in tumour invasion and metastasis [135], MMP-9 also contributes to airway remodelling and subepithelial fibrosis in chronic asthma [136, 137]. Airway MMP-9 is also increased after allergen challenge [138] or during asthma exacerbations [139, 140]. Increased secretion of MMP-9 by alveolar macrophages has also been demonstrated in smokers and COPD patients. Finally, increased sputum concentrations of MMP-9 have also been observed in COPD patients [30] and lung transplant recipients with chronic rejection

[141]. MMP-9 expression and activation is controlled by a variety of mediators, e.g. cytokines like TNF- α [142–144]. MMP activity is physiologically controlled by a class of protein inhibitors called the tissue inhibitors of metalloproteinases (TIMP). MMP-9 and its counterpart, TIMP-1, have been detected in serum [145] and sputum [140] samples of asthmatics, and their concentration and molar MMP-9:TIMP-1 ratio correlated with clinical parameters and lung function.

Recently, selective inhibitors of MMP have been developed, primarily aiming at the prevention of tumour metastasis. Of these, marimastat has progressed into advanced stages of clinical trials. However, data from cancer trials are at least equivocal [146], and no clinical studies so far have been reported in non-malignant, chronic inflammatory disorders. In a small study involving an allergen challenge model in atopic subjects, 3 weeks treatment with marimastat had only a marginal effect on allergen-induced hyper-responsiveness, without influencing sputum cells, exhaled nitric oxide or FEV₁ [147].

Tumour necrosis factor- α blocking agents. TNF- α blocking strategies include humanized monoclonal antibodies against TNF or soluble TNF receptors. All of these compounds have been approved for the treatment of either severe rheumatoid arthritis, ankylosing spondylitis, Crohn's disease or psoriasis-associated arthritis [148]. TNF is also associated with the inflammatory process in severe asthma, AR, and COPD. In the latter, TNF has the potential to promote irreversible airway remodelling and emphysema through activation and secretion of metalloproteinases, e.g. MMP-9 [29]. TNF is increased in BALF from chronic smokers compared with non-smokers [149]. Cigarette smoke also promotes the release of TNF by alveolar macrophages in COPD [150]. In Taiwanese, a TNF polymorphism resulting in increased TNF production was associated with the occurrence of chronic bronchitis and COPD [151]. Finally, elevated plasma levels of TNF are associated with a variety of systemic effects of COPD, and poorer prognosis [152]. In asthma, elevated sputum levels of TNF were found in severe patients [64]. Thus, TNF-blocking strategies are considered a potential new treatment for COPD and severe asthma. However, the mere fact of elevated TNF in COPD and asthma does not necessarily mean that it is deleterious or even causing the disease. Moreover, a growing number of case reports on patients with severe infections or lymphoma during anti-TNF therapy raised concerns about the long-term safety of this approach [153, 154]. Particularly in COPD, where bacterial colonization or even infection is present in a large number of cases, risks of TNF blockage need to be carefully weighed against potential benefits. The role of TNF in rheumatoid arthritis is much more established than in COPD. Moreover, unlike pain or joint swelling in rheumatoid arthritis, which shows relatively rapid response to

treatment, particularly COPD does not offer any outcome parameter to evaluate possible beneficial effects of anti-TNF treatment in the short term. Therefore, negative results from a recent pilot study did not come surprising [155]. Finally, one should also consider the substantial cost of this treatment.

As an alternative approach, dual TNF- α convertase enzyme (TACE) and metalloprotease inhibitors are currently developed [156]. Clinical data, however, have so far not been reported.

Phosphodiesterase-IV inhibitors. During recent years, selective phosphodiesterase (PDE)-IV inhibitors have been developed for the treatment of COPD and asthma [157, 158]. In comparison with theophylline, a non-selective inhibitor of PDE, these novel compounds are believed to offer the advantage of increased anti-inflammatory efficacy by selectively blocking the PDE type IV predominantly found in inflammatory cells, including generation of reactive oxygen intermediates and chemotaxis by neutrophils and eosinophils [159–161]. In COPD, it was demonstrated that PDE-IV inhibition directly inhibited cellular effector functions of airway inflammatory cells [162]. PDE-IV inhibition also reduced LPS stimulated TNF- α release of blood cells *ex vivo* [163]. As with neutrophil elastase inhibitors, several orally active PDE-IV inhibitors have been developed for the treatment of respiratory diseases. However, the clinical development of most compounds has been suspended (e.g. CDP-840, Merck/CellTech, Whitehouse Station, NJ, USA; D-4418, Schering-Plough, Kenilworth, NJ, USA) because of an unfavourable safety profile or failure to meet clinical efficacy criteria (reviewed in detail by Souness et al. [164]). Presently, cilomilast (GlaxoSmithKline) and roflumilast (Altana Pharma, Konstanz, Germany) are the only compounds in advanced development stages. In humans, both cilomilast and roflumilast have been effective in clinical trials involving patients with rhinitis [165], mild asthma, or moderate-to-severe COPD [163, 166, 167]. Despite promising early results, it remains to be proven, whether the observed clinical effects owed to a potential bronchodilator or true anti-inflammatory effect of PDE-IV inhibitors [168]. In a biopsy study in COPD patients, cilomilast led to a minor decrease of tissue neutrophils and CD8 lymphocytes [169]. However, the clinical significance of these effects is as yet unclear, and carefully designed clinical trials need to confirm the intermediate and long-term anti-inflammatory effects of this class of compounds in COPD. In 2003, the US Food and Drug Administration declined to recommend approval of cilomilast for the maintenance treatment of COPD because of only marginal benefits and unresolved safety issues including gastrointestinal haemorrhage (<http://www.fda.gov/ohrms/dockets/ac/03/briefing/3976b1.htm>). This decision has largely renewed scepticism about the

therapeutic potential of PDE-IV inhibitors, and complete results from the roflumilast pivotal trials are eagerly awaited. As potential side-effects of PDE-IV inhibitors still remain a major concern, inhaled delivery of PDE-IV inhibitors to the lung would be an attractive alternative. One such compound, AWD 12-281, is currently evaluated in human trials [170]. If the inhalational route demonstrates comparable efficacy, it can be easily anticipated that inhaled PDE-IV inhibitors will be the most promising approach in the near future.

Macrolides. Macrolides are generally used as antibiotics to treat a variety of infectious diseases. Besides their antibiotic activity, macrolides possess several anti-inflammatory effects not related to the eradication of microorganisms from inflamed tissues, best demonstrated in patients with diffuse panbronchiolitis [171]. *In vitro*, macrolides inhibit recruitment of neutrophils through reduction of adhesion molecule expression; ameliorate the generation of reactive oxygen species, and release of pro-inflammatory cytokines, e.g. TNF [172]. Thus, macrolides have been considered a potential anti-inflammatory treatment in asthma and COPD. In asthma, several clinical trials with troleandomycin, roxithromycin or clarithromycin were undertaken showing small changes in symptoms or surrogate markers of inflammation [173, 174], but without overall improvements in lung function [173–176]. However, most of the larger trials were conducted in asthmatics showing evidence of chlamydia or mycoplasma infection, hence it remains debatable, whether the observed effects were because of the anti-inflammatory or antibiotic potency of the drugs [177]. In COPD, a large, unblinded long-term trial in Japanese patients demonstrated that low-dose erythromycin reduced the frequency of exacerbations and common colds [178]. With regard to inflammatory markers, results are equivocal. Two studies using clarithromycin have been recently reported. While one study showed a significant reduction in sputum total cells, IL-8 and TNF [179], the other failed to detect any effect of macrolide treatment on sputum inflammatory parameters including sputum neutrophils [180]. Nevertheless, the favourable safety profile and low costs of agents like roxithromycin warrant the conduction of large-scale clinical trials in COPD and asthma.

Anti-oxidants. Oxidative stress results from the increased presence and activation of airway neutrophils, which generate vast amounts of reactive oxygen intermediates as part of their innate anti-bacterial defense. Several cellular and non-cellular defense mechanisms protect resident cells from injury because of oxidative stress. However, in chronic inflammatory conditions, there is ample evidence that endogenous defense mechanisms are insufficient to counteract oxidative injury, and several attempts have been undertaken to restore oxidant/anti-

oxidant imbalance in such diseases. Oxidative stress has been demonstrated in allergic asthma and rhinitis [181], obliterative bronchiolitis [182, 183], and COPD [184]. In the latter, oxidative injury seems to be of particular importance, as cigarette smoke itself contains vast amounts of oxidants, which may directly initiate the chronic and persistent inflammatory process in COPD. Moreover, oxidative stress further increases during COPD exacerbations [38, 102]. Hence, anti-oxidants are considered an anti-inflammatory treatment in COPD. Augmentation of pulmonary glutathione, a major lung anti-oxidant, has been evaluated through direct inhalation, or supplementation with glutathione precursors, i.e. n-acetylcysteine (NAC) [185, 186]. NAC, a cheap over-the-counter drug in most countries with a favourable safety profile, reduces the frequency of exacerbations in chronic bronchitis [187–189]. However, results from carefully designed controlled clinical trials have shown only marginal benefits of long-term NAC treatment on lung function in mild-to-moderate COPD without any effect on the longitudinal decline in lung function [190]. In a subgroup of patients not receiving inhaled corticosteroids, NAC led to a small reduction in COPD exacerbations. The effects of NAC in COPD do not seem to be related to the mucolytic properties of the compound, as other mucolytics e.g. ambroxol failed to reduce the rate of exacerbation in COPD [191]. Future developments targeting redox balance in airway diseases include the stable thiol mucolytic nacystelyn, which possesses anti-oxidant activity *in vitro* and *in vivo* [192, 193]. Nacystelyn has been studied in patients with cystic fibrosis, where it decreased mucous viscosity [194]. However, benefits because of anti-oxidant properties of nacystelyn remain to be confirmed in human trials.

Implications for clinical trials. Assessing the clinical efficacy of anti-neutrophilic therapies in chronic inflammatory airways diseases is at least cumbersome. Given the assumption, that even significant reductions of airway neutrophilia will not immediately transfer into easily measurable improvements of physiologic parameters, e.g. lung function, the choice of primary outcome variables in clinical trials and proof-of-concept studies becomes a critical issue. Unlike in other neutrophil-dominated disorders like obliterative bronchiolitis following lung transplantation or even cystic fibrosis, where survival time or progression-free survival time are primary outcome measures of choice, these variables are of less importance in asthma or COPD. Apparently, when evaluating the potential clinical benefits of anti-neutrophil therapies in severe asthma or COPD, the rate of exacerbations and decline of lung function can be considered as appropriate outcomes.

In COPD, there is a significant correlation between airway neutrophilia [46] or neutrophilic smooth muscle

infiltration [48] and FEV₁. Given the predominantly irreversible nature of COPD, this does not necessarily implicate that a reduction of neutrophils will also transfer into an improvement of lung function. Hence, other clinical outcome parameters need to be included. Stanescu et al. [195] have correlated airway neutrophilia with accelerated decline of lung function over time in COPD patients. In their study, an excess sputum neutrophilia of ~30% resulted in at least +10 mL difference in the annual loss of FEV₁ compared with patients having lower neutrophil levels. It seems therefore reasonable that an efficacious drug treating neutrophilic inflammation in COPD should also reduce the decline of lung function in the long term.

In both asthma and COPD, the association of airway inflammation and exacerbations is generally accepted, in as much as exacerbation rates are regarded a meaningful outcome parameter in trials with anti-inflammatory agents in either disease. However, at least in COPD, during an acute exacerbation no differences in the number of sputum neutrophils were observed when compared with stable disease in a cohort of patients [15], and elevated sputum neutrophils did not predict the frequency of exacerbations in these patients. Similar observations were made by Gompertz et al. [124], who concluded that patients with frequent and infrequent COPD exacerbation were indistinguishable by their inflammatory pattern. Hence, a consistent association of excess sputum neutrophilia and COPD exacerbation frequency remains to be established. With these data in mind, it is difficult to speculate about the degree of neutrophil reduction any novel therapeutic approach should minimally achieve to reinforce confidence that such reduction will translate into any clinically meaningful response.

Nevertheless, the pronounced airway neutrophilia, e.g. in COPD and its accessibility by non-invasive methods like sputum induction also offers several advantages. We and others have shown, that sputum neutrophils are highly reproducible in stable COPD, even over prolonged periods of time [33, 196]. With this high reproducibility as background, it is feasible to design primarily mechanistic clinical trials with sputum neutrophilia as primary outcome parameter. Based on own data on sputum variability in COPD, and further taking into account published values [49, 50], it would be possible to demonstrate a 25% reduction of sputum neutrophils with 80% power by a crossover trial involving only 15–20 patients [197].

Despite this, considerations on power calculation also lead to the question, what extent of neutrophil reduction is expected to result in a clinical meaningful improvement? The easiest way to answer this question would be to state that sputum neutrophils should return to 'normal' values of healthy, non-smoking volunteers reported in the literature, which vary between 5% and 37% of all non-squamous cells [30, 198–200]. Unfortunately, more distinguished answers to that question are nearly entirely

speculative and not adequately supported by data. Moreover, a recent study by Thomas et al. [201] revealed sputum neutrophils counts in elderly healthy subjects to be as high as 60–70%. If these data are reconfirmed, many published studies on sputum cell counts indeed will have to be reinterpreted. Further, the desired extent of neutrophils reduction will also vary with the chosen clinical outcome parameter, i.e. symptoms, airflow limitation, exacerbation rates or decline of lung function.

In contrast, during early development phase mechanistic proof-of-concepts trials, it appears desirable to use human models allowing for a more timely evaluation of potential anti-neutrophil activity of novel compounds. In asthma, allergen challenge studies at least offer a model to study dynamic shifts in airway neutrophils, although this model needs to be validated with regard to allergen induced neutrophilia in humans, particularly the relation of neutrophilia and changes in FEV₁. This is of even greater importance, as inhibition of allergen-induced neutrophilia did not affect late asthmatic reaction in humans [106]. In addition to allergen challenge, LPS-, platelet activating-factor- [202] or endotoxin-induced airway neutrophilia are somehow established models both in healthy and asthmatic subjects [203]. However, it appears at least doubtful that such a model will be feasible in patients with significant physiological impairment, e.g. severe asthmatics or COPD patients. In addition, even with a reliable model at hand, the natural setting in most of the diseases discussed would be different, as novel therapies would have to reduce an established neutrophilia, in contrast to ameliorating a stimulated influx of airway neutrophils by challenge procedures, thus limiting the applicability of data derived from such mechanistic trials to long-term efficacy.

A variety of neutrophil-derived mediators and surrogate markers of inflammation have also been studied as secondary outcome measures in COPD or asthma trials [49, 50, 104, 107, 204, 205]. However, to our best knowledge, there is no study that supports the – at least 'clinical' – value of determining soluble sputum or BAL mediators in COPD or asthma in addition to simple cell counts. With the single exception of the above-cited study by Bhowmik et al. [15], who have found that sputum IL-6 and IL-8 somewhat predicted the frequency of exacerbations in COPD more accurately than simple cell counts, the value of soluble mediators in COPD or asthma remains to be established. Therefore, the use of sputum surrogate markers as primary or secondary outcome parameters in clinical trials cannot be recommended by now. Similarly, there are no conclusive data indicating, whether other surrogate markers of neutrophilic inflammation would be useful in clinical trials or proof-of concept studies.

Potential hazard of therapies targeting neutrophils in chronic airway diseases. Much effort has been

undertaken during recent years to address neutrophilic inflammation as part of the underlying pathologic process in chronic inflammatory airway diseases. These efforts, particularly the identification of potential targets for drug therapy, and consecutive development of specific inhibitors, have largely increased our knowledge and understanding of the diseases discussed in this review. Balancing between clinical efficacy and safety, however, appears to be a hazardous game. On the one hand, selective treatments targeting only presumably 'pivotal' molecules may be safe, but ineffective, on the other hand, blunting the whole process of neutrophil-mediated defense mechanisms by broad approaches may have deleterious consequences to the individual. Lessons from knockout mice [206, 207] indicate that suppression of neutrophil-related defense mechanisms predisposes to severe systemic infections. Moreover, clinical experience with TNF-blocking agents in arthritis clearly mounts a caveat against the widespread and uncritical use of these agents in chronic airway diseases, particularly when the role of chronic infections in COPD is as yet not fully understood. The recently reported series of fatal central nervous systems infections occurring under systemic treatment with an $\alpha 4$ -integrin blocker [208] should reinforce researchers and clinicians to remain in constant awareness of potential risks of any 'broad-spectrum' anti-neutrophilic therapies.

To date, open questions by far outweigh the amount of possible answers to key issues in targeting neutrophils. As most biological systems, the process of neutrophil recruitment and activation is largely redundant, and targeting single molecules or mediators will probably fail. Systemic delivery of novel drugs may put the individual at high risk of generalized infections, without guaranteeing clinical efficacy. In a small observational study, we have previously demonstrated, that neutrophil-depleting chemotherapy does not affect airway inflammation in COPD patients with concomitant cancer, at least in short term [209]. Therefore, initial disappointing results from clinical trials with oral compounds did not come unexpected. However, the possibility to directly deliver drugs to the pulmonary compartment by inhalation may enhance the therapeutic window of novel approaches by increasing local activity and minimizing systemic effects, and thus represent the approach of first choice when developing anti-neutrophil directed drugs for airway diseases.

Conclusion

In summary, there is as yet no convincing evidence that selected therapies have the potential to efficiently control neutrophilic inflammation in severe asthma or COPD. Of the presented novel approaches, the PDE-IV inhibitors are at most advanced stages of clinical development. However, full results from long-term trials are eagerly awaited

to confirm initial promising results in COPD, with particular regard to exacerbations, decline of lung function, and safety. Hopefully, additional therapies will emerge that effectively target the multiple facets of neutrophil-mediated tissue injury in chronic inflammatory airway diseases. Given the wealth of scientific publications and novel approaches to inflammatory airway diseases, it is within the nature of any review to remain fragmentary. There are several other new or 'emerging' treatments for airway diseases, and many of them also target at neutrophils or neutrophil-related inflammation, including MAP kinase inhibitors, cytokines, monoclonal antibodies or mucoactive agents. For most of these approaches, however, data in humans or even animal models of the disease have not been reported, hence our intention to clearly focus on therapies with a somewhat broader body of evidence stemming from human trials. It is clear, however, that for any of these approaches, the right choice of outcome variables as well as an appropriate clinical trial design remain key issues.

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