Eosinophilic airway inflammation: role in asthma and chronic obstructive pulmonary disease

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***Abstract*:** The chronic lung diseases, asthma and chronic obstructive pulmonary disease (COPD), are common affecting over 500 million people worldwide and causing substantial morbidity and mortality. Asthma is typically associated with Th2-mediated eosinophilic airway inflammation, in contrast to neutrophilic inflammation observed commonly in COPD. However, there is increasing evidence that the eosinophil might play an important role in 10–40% of patients with COPD. Consistently in both asthma and COPD a sputum eosinophilia is associated with a good response to corticosteroid therapy and tailored strategies aimed to normalize sputum eosinophils reduce exacerbation frequency and severity. Advances in our understanding of the multistep paradigm of eosinophil recruitment to the airway, and the consequence of eosinophilic inflammation, has led to the development of new therapies to target these molecular pathways. In this article we discuss the mechanisms of eosinophilic trafficking, the tools to assess eosinophilic airway inflammation in asthma and COPD during stable disease and exacerbations and review current and novel anti-eosinophilic treatments.

Keywords: ACOS, anti-CRTh2, anti-IL5, anti-IL-13, anti-IL4R, asthma, biomarker, COPD, corticosteroid, eosinophil

Introduction

Asthma and chronic obstructive pulmonary disease (COPD) cause considerable morbidity affecting over 500 million people worldwide and managing these conditions consumes substantial healthcare resources exceeding €56 billion per year in the European Union [Burrowes *et al.* 2014]. Both conditions are characterized by airflow obstruction, which is typically variable and reversible in asthma, but fixed in COPD. Similarly the symptoms in asthma are usually intermittent, although persistent in more severe disease, whereas in COPD symptoms occur daily with reduced exercise capacity and respiratory failure occurring as the disease progresses. Both conditions are characterized by exacerbations whereby sufferers have worsening of their symptoms often precipitated by infection and these exacerbations amplify the morbidity and contribute substantially to the mortality of these airway diseases.

Eosinophilic inflammation orchestrated by allergic sensitization and T helper 2 lymphocytes (Th2)-mediated immune response is the hallmark of airway inflammation in asthma, but importantly eosinophilic inflammation can occur independent of allergy and the presence of eosinophilic inflammation is neither necessary nor sufficient for the development of asthma [Eltboli and Brightling, 2013]. Conversely, COPD is typically associated with T helper 1 lymphocytes (Th1)-mediated immunity with a neutrophilic response [Stanescu *et al.* 1996] often in association with bacterial colonization. However, this probably represents only half of COPD patients and in a subgroup of 10–40% eosinophilic airway inflammation is a feature [Brightling *et al.* 2000, 2005; Eltboli *et al.* 2014; Leigh *et al.* 2006; Pizzichini *et al.* 1998; Saetta *et al.* 1994]. Therefore, eosinophilic inflammation cannot be assumed to be present or absent dependent on the airway disease, but needs to be measured as part of the assessment and phenotyping of airways disease. This is critical when considering the potential immunopathogenic role of eosinophilic inflammation in disease; its role as a biomarker to direct current therapies and

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for the future development of eosinophil-directed therapies.

Herein, we shall discuss the mechanisms of eosinophilic trafficking, the tools to assess eosinophil airway inflammation, cross-sectional and longitudinal analysis of eosinophilic airway inflammation in asthma and COPD during stable disease and exacerbations and review anti-eosinophilic treatments including therapies in development.

Eosinophil biology: mechanisms of eosinophilic airway inflammation

Eosinophils are granulocytic leukocytes first discovered by Heinrich Caro in 1874 and described by Paul Ehrlich in 1879 [Kay, 2014]. They constitute 1–4% of circulating white cells and are distinguished phenotypically by their bilobed nuclei and large acidophilic cytoplasmic granules. The pathological role of eosinophils primarily occurs in tissues and therefore a major focus has been to outline the molecular mechanisms involved in selective eosinophil recruitment summarized in Figure 1 [Rosenberg *et al.* 2007; Wardlaw, 1999].

Eosinophils are derived from pluripotent CD34+ progenitor stem cells found in normal bone marrow. This differentiation occurs under the influence of granulocyte-monocyte colony stimulating factor (GM-CSF) and interleukin (IL)-3 in early phases and IL-5 in the latter phases of differentiation [Denburg, 1998]. Mature eosinophils are released from bone marrow into circulation and this is primarily regulated by IL-5. Eosinophils are transformed from a quiescent state to a state of increased hyper responsiveness by priming agents such as these cytokines IL-3, IL-5, GM-CSF [Luijk *et al.* 2005]. The priming process does not fully activate the cell but increases its responsiveness to chemotaxis, degranulation and cytokine production [Fulkerson and Rothenberg, 2013]. As eosinophils flow in the blood stream they roll onto the bronchial vascular endothelium. Infiltration of eosinophils into the airway will not occur unless there is adhesion and transmigration across the bronchial vascular endothelium. This preferential adhesion of eosinophils from the blood stream into various tissues results from specific interactions between integrins on surface of eosinophils and adhesion receptors on the surface of vascular endothelium, which include P-selectin/P-selectin glycoprotein ligand-1 and very late activation antigen/vascular cell adhesion molecule, VCAM-1 ligand [Fukuda *et al.* 1996; Symon *et al.* 1996]. IL-4 and IL-13 have been found to increase VCAM-1 and P-selectin expression [Patel, 1998; Woltmann *et al.* 2000]. Recruitment to the airway is under the control of the chemokines CCL5, 7, 11, 13, 15, 24 and 26 and their cognate receptor CCR3 [Smit and Lukacs, 2006; Ying *et al.* 1997], which play a critical role together with chemoattractant receptor homologous molecule expressed on Th2 cells (CRTh2) and its ligand prostaglandin D2 (PGD2) [Hirai *et al.* 2001; Mutalithas *et al.* 2010]. PGD2 acts both as an eosinophil chemoattractant in its own right, but also augments the effects of the CCR3 chemokines [Mesquita-Santos *et al.* 2006]. Activated mast cells are the predominant source of PGD2 with release via IgE-dependent and independent activation [Lewis *et al.* 1982].

Eosinophils have four specific basic proteins that include major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO) and eosinophil derived neurotoxin (EDN), which are stored in secondary granules. ECP has been found to attach to cell membrane altering permeability as well as increasing production of reactive oxygen species [Navarro *et al.* 2008]. MBP was also found to cause alveolar epithelium cell lysis allowing for penetration of inhaled antigen [Ayars *et al.* 1985]. In combination these granules are cytotoxic and disrupt the protective pulmonary epithelial barrier allowing further inflammatory responses to occur [Frigas *et al.* 1991]. Eosinophils have also been characterized by the ability to store preformed mediators as well as synthesize and secrete a number of pro-inflammatory cytokines, chemokines and growth factors including IL-2, IL-3, IL-4, IL-5, IL-10, IL-12, IL-13, IL-16, IL-25, tumour necrosis factor, transforming growth factor (TGF) α/β, CCL5, CCL11, 13 facilitating their key role as immunoregulators [Davoine and Lacy, 2014; Moqbel *et al.* 1994].

Thus, although eosinophils have an immunomodulatory role they are recruited into the airway orchestrated by other cells and can be considered largely a final important effector cell.

Phenotyping the heterogeneity of eosinophilic airway inflammation

How can you measure eosinophilic inflammation?

Eosinophilic airway inflammation can be measured in the airway noninvasively by sputum analysis

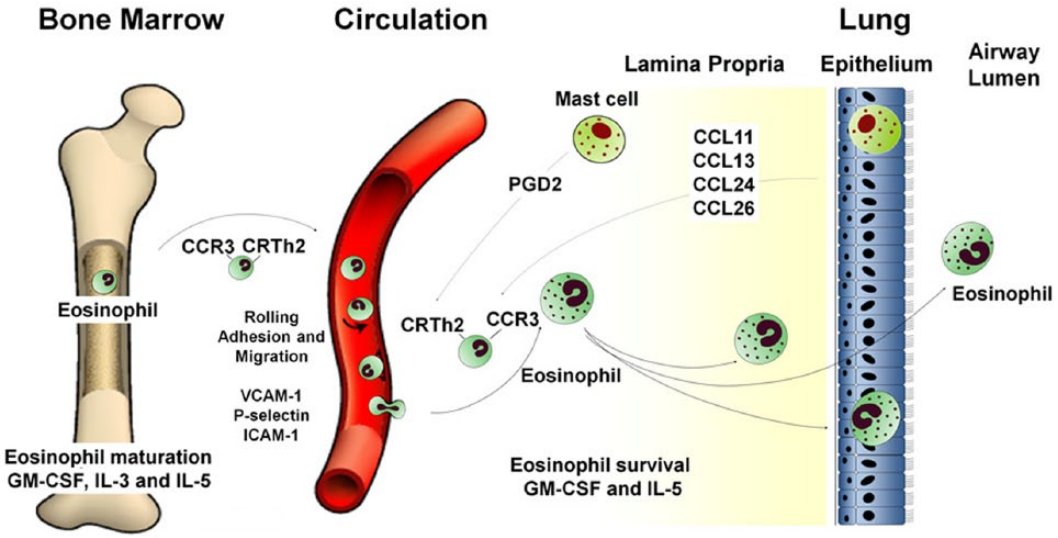

Figure 1. Eosinophil trafficking from the bone marrow to the airway.

and invasively by bronchoscopic sampling. Bronchoscopy enables investigators to sample airway inflammation directly in the airway wall by bronchial biopsy, in the large airway lumen by proximal bronchial wash and in the smaller distal airways with bronchoalveolar lavage. Bronchoscopic sampling is invasive costly, time consuming and requires the need of experienced investigators. The relationship between sputum and bronchoscopic sampling has been investigated in several studies and although they generally report correlations between compartments these correlations are weak [Keatings *et al.* 1997; Maestrelli *et al.* 1995; Rutgers *et al.* 2000]. The use of sputum cytology to explore airway inflammation has been in existence since the first descriptions by Ernst Leyden and Jean Charcot of Charcot-Leyden crystals [Sakula, 1986] and was used clinically in the 1950s by Dr Morrow-Brown [Brown, 1958] and then optimized in the 1990s by Freddy Hargreaves and colleagues [Pavord *et al.* 1997]. Normal sputum eosinophil count ranges in large populations have been defined. A blood eosinophil count has been used as a surrogate for eosinophilic airway inflammation and to determine the intensity of eosinophilic inflammation in the blood compartment [Bafadhel *et al.* 2012; Wagener *et al.* 2015]. Studies have shown a moderate correlation between blood eosinophil count and sputum eosinophil count ($r=0.6$; $p<0.0001$) [Schleich *et al.* 2013] ($r=0.59$; $p<0.001$) [Wagener *et al.* 2015]. Using the (ROC) curves the diagnostic accuracy of a blood eosinophil count was 0.85 [95% confidence interval (CI) 0.78–0.93] [Bafadhel *et al.* 2011; Wagener *et al.* 2015] to detect a sputum eosinophilia $\geqslant 3\%$. Blood eosinophils can be assessed in most hospitals worldwide and thus presents a biomarker that has advantages due to its simplicity and availability. The relationship between blood and sputum eosinophils is complicated in obesity. Recent evidence demonstrates that in obese asthmatics the relationship is distinct with strong associations between blood and tissue eosinophilia, but a consistently reduced sputum eosinophilia suggesting altered eosinophil trafficking in obesity [Desai *et al.* 2013].

Interestingly, sputum cytology also reveals information about longer term eosinophil exposure beyond the simple sputum eosinophil differential cell count. The apoptosis and subsequent removal of dead cells by phagocytes (efferocytosis) is a critical mechanism for the noninflammatory clearance of granulocytes including eosinophils. Macrophage red hue due to eosin staining within sputum macrophages reflects eosinophil efferocytosis and can be used as a biomarker of eosinophil load and its clearance [Eltboli *et al.* 2014; Kulkarni *et al.* 2010].

Other biomarkers that are associated with eosinophilic airway inflammation include fractional exhaled nitric oxide (FeNO) or Th2-activation marker, e.g. serum periostin [Berry *et al.* 2005]. Breath analysis is an attractive alternative as a near-patient test. Nitric oxide is produced by various cells including airway epithelial cells and

inflammatory cells under the action of inducible NO synthase enzyme which converts L-arginine to L-citrulline. FeNO levels are correlated with peripheral blood eosinophils [Zietkowski *et al.* 2006] and sputum eosinophils particularly in mild-to-moderate disease whereas the relationship is more complex in severe disease. FeNO in severe asthma was poorly related to blood eosinophils as highlighted in a national registry audit in refractory asthma, which showed that though blood eosinophils levels decreased, FeNO levels paradoxically increased in response to oral corticosteroids [Sweeney *et al.* 2012]. Novel 'breathomic' approaches also have promise to identify different inflammatory phenotypes.

Periostin is a matricellular protein secreted basolaterally from epithelial cells in response to various stimuli including IL-13 and its levels are highly correlated with Th2 signature [Woodruff *et al.* 2007]. Two major studies have highlighted apparently conflicting views on the correlation of periostin with eosinophilia. An observational study found periostin to be a superior predictor of eosinophil inflammation in the airway [Jia *et al.* 2012] while a more recent a cross-sectional study [Wagener *et al.* 2015] showed a strong correlation of sputum eosinophils with blood eosinophils, but failed to demonstrate a relationship between serum periostin and eosinophils in sputum. The most likely explanation for these apparent differences are that former study investigated the sensitivity and specificity of periostin to identify an airway eosinophilia using a composite score rather than specifically comparing the relationship between blood periostin and a sputum eosinophil count as performed in the later study. This suggests that blood periostin might provide additional value as a biomarker of Th2 inflammation, but is not directly comparable with either blood or sputum eosinophilia. Periostin may be more useful as a marker to assess specific IL-13 activity and responses to anti IL-13 therapy [Corren *et al.* 2011]. IL-13 induces epithelial-derived periostin directly and is associated with upregulated FENO expression, whereas the relationship between airway eosinophilic inflammation and IL-13 is more complex and indirect such as via upregulation of epithelial-derived chemoattractants. These variations in biomarker measurements in various studies further highlights the heterogeneity and complexity of airway inflammation and the possible need of measuring a number of biomarkers in conjunction with one another to accurately try and determine a phenotype.

How common is eosinophilic inflammation in asthma and COPD and is it a stable phenotype?

In asthma up to 80% of corticosteroid-naïve patients and 50% of corticosteroid treated patients have a sputum eosinophilia [Douwes *et al.* 2002; Eltboli and Brightling, 2013]. Short-term repeatability of a sputum eosinophilia is very good [Boorsma *et al.* 2007; Pizzichini *et al.* 1996]. However, there is emerging evidence that in some patients their inflammatory profile is stable whereas in others it is highly variable. McGrath and colleagues in a population of 995 asthma patients reported that persistent eosinophilic inflammation was less frequent than subjects with an intermittent eosinophilia, 22% *versus* 31% [McGrath *et al.* 2012]. Interestingly Newby and colleagues [Newby *et al.* 2014a] have demonstrated that in severe asthma those subjects with intermittent eosinophilia had increased rate of lung function decline compared with those without eosinophilic inflammation or persistent eosinophilic inflammation.

Further insights into how common eosinophilic inflammation is in asthma can be derived from corticosteroid withdrawal studies as emergence of eosinophilic inflammation following treatment withdrawal in apparently noneosinophilic disease is informative. In a 1-year follow-up study of asthmatic patients in whom treatment was stepped-down in the absence of a sputum eosinophilia, persistent noneosinophilia was observed in 12.5% of patients [Kulkarni *et al.* 2010]. Interestingly, the macrophage red hue predicts the emergence of eosinophilic inflammation in treatment withdrawal with continued corticosteroid step-down only possible in those subjects without evidence of a sputum eosinophilia nor elevated macrophage red hue [Kulkarni *et al.* 2010]. This suggests that in severe asthma the absence of an underlying eosinophilic inflammation is probably uncommon.

In COPD, although neutrophilic inflammation is commonly reported, a sputum eosinophilia is present in 10–40% of patients with COPD [Brightling *et al.* 2000; Leigh *et al.* 2006; Pizzichini *et al.* 1998; Saetta *et al.* 1994] Similarly to asthma the repeatability of eosinophilic inflammation is very good, but again there are three groups with either persistent, variable or absent eosinophilic inflammation. In the ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate End points) cohort repeated blood eosinophil counts were available in 1483 subjects over 3 years

[Singh *et al.* 2014]. Using a cutoff for blood eosinophils of 2%, 37% of subjects had persistently elevated eosinophils, 49% were intermittent and the remainder were noneosinophilic. The persistently eosinophilic group were older, predominantly male with milder disease and fewer symptoms. Emphysema progression was greater in the non-eosinophilic group [Singh *et al.* 2014] and in contrast to severe asthma there were no differences in lung function decline between groups.

To further inform the understanding of the heterogeneity of airway disease unbiased statistical approaches such as cluster analysis have been applied to large clinical datasets [Amelink *et al.* 2013; Haldar *et al.* 2008; Newby *et al.* 2014b; Wardlaw *et al.* 2005]. Interestingly, these have underscored the importance of eosinophilic airway inflammation in asthma and COPD and perhaps most importantly in asthma-COPD overlap syndrome (ACOS). For example, Ghebre and colleagues combined data from asthma and COPD and identified three clusters [Ghebre *et al.* 2015]. Cluster 1 consisted of asthma subjects with increase IL-5, IL-13 and CCL26 mediators and eosinophil predominance. Cluster 2 consisted of an overlap between asthma and COPD with neutrophil predominance with 11% of patients with asthma having sputum eosinophilia. Cluster 3 consisted mainly of COPD patients with a mixed granulocytic airway inflammation. The differences seen between neutrophilic COPD in cluster 2 and eosinophilic COPD in cluster 3 included the presence of increased bacterial colonization in the former and increased CCL13 in the latter possibly explaining the observed airway inflammation differences seen between these clusters. Importantly few studies have investigated cluster stability and predictably due to the variability of eosinophilic inflammation in some patients as described above cluster membership can change overtime. This further highlights the importance of considering the dynamics of eosinophilic inflammation within an individual to appreciate the relationship of this inflammatory phenotype with natural history of disease and response to therapy.

Is eosinophilic inflammation related to disordered airway physiology and remodelling?

Eosinophil-derived granule proteins and pro-inflammatory mediators promote persistent inflammation, which has been associated with increased exacerbations and lung function decline [Green *et al.* 2002b; Jatakanon *et al.* 2000; Siva *et al.* 2007]. However, the relationship between eosinophilic inflammation and airflow obstruction and airway hyper-responsiveness is weak exemplified by the presence of eosinophilic inflammation in eosinophilic bronchitis without asthma [Gibson *et al.* 1989, 1995]. Indeed comparisons with this disease group have revealed the important role of mast cell localisation to the airway smooth muscle bundle in driving disordered airway physiology [Brightling *et al.* 2002]. Eosinophils also contribute to airway remodelling [Flood-Page *et al.* 2003; Kay *et al.* 2004]. Airway remodelling is a consequence of increased extracellular matrix deposition, increased subepithelial mesenchymal cells and most importantly increased airway smooth muscle mass, which is the major determinant of airflow obstruction. Eosinophil-derived TGF-β activates fibroblast release of matrix proteins and although increased airway smooth muscle mass was eosinophil dependent in a mouse model of asthma [Humbles *et al.* 2004] whether the eosinophil is necessary for this critical feature of remodelling in human disease is questionable as evidenced by intervention studies described later. Whereas in contrast, reticular basement membrane thickening is associated with eosinophilic inflammation in asthma, COPD and nonasthmatic eosinophilic bronchitis [Brightling *et al.* 2003; Eltboli *et al.* 2015; Siddiqui *et al.* 2008].

Is eosinophilic inflammation a feature of asthma and COPD exacerbations?

The definition of asthma exacerbations vary according to literature source and asthma severity. In summary asthma exacerbations are defined as a decrease in peak flow with worsening of symptoms requiring the use of reliever treatment and or systemic corticosteroids [Reddel *et al.* 2009; see also http://www.ginasthma.org]. Severe asthma exacerbations require 3 or more days of high-dose oral corticosteroids. GOLD (see http://www.goldcopd.org) have defined a COPD exacerbation as 'an acute event characterised by worsening of the patients' respiratory symptoms that is beyond normal day-to-day variations and leads to a change in medication'. The differences in exacerbation definitions for asthma and COPD poses limitations in comparisons between the diseases. However, both asthma and COPD exacerbations are commonly associated with identification of pathogens usually viral although also bacterial particularly in COPD. However, pathogens are not identified at

exacerbations in 20–50% of cases [Bafadhel *et al.* 2011; Sethi and Murphy, 2008] either due to insensitivity of assays or as a consequence of a lack of a role of new infections in some exacerbations. Therefore, airway inflammation at exacerbations might represent a consequence of an acute environmental insult on an intrinsically unstable state reflecting an emergent phenomenon in a complex system [Brightling, 2013].

Eosinophilic airway inflammation is present in exacerbations of asthma and COPD, particularly in those subjects with eosinophilic inflammation in stable state. A high baseline sputum eosinophil count is a predictor of loss of control of asthma and an increased number of subsequent exacerbations [Jatakanon *et al.* 2000]. Treatment strategies aiming to maintain sputum eosinophil counts <2–3% is associated with a significant decrease in the number of exacerbations as compared with treatments focused on clinical history or spirometry [Brightling *et al.* 2000; Green *et al.* 2002a; Jayaram *et al.* 2006]. In COPD sputum eosinophilia has been seen in 10–40% of patients at baseline [Brightling *et al.* 2000; Pizzichini *et al.* 1998; Singh *et al.* 2014] with higher eosinophilic inflammation seen at exacerbation states compared to stable states [Saetta *et al.* 1994]. In a study aimed at phenotyping COPD exacerbations and identifying biomarkers a sputum eosinophilia was identified in 28% of exacerbations [Bafadhel *et al.* 2011]. The combination of sputum eosinophil count and macrophage red hue was used to categorize COPD subjects into four groups according to high and low sputum eosinophils (cutoff ⩾3%) and high and low percentage macrophage red hue area (cutoff >6%) [Eltboli *et al.* 2014]. The group with high eosinophil count, but low red hue had the greatest fall in lung function during an exacerbation and also increased exacerbation frequency. In this group macrophage efferocytosis is defective contributing to persistent eosinophilic inflammation, which in turn is likely to impact upon the severity of the exacerbation event.

Is the future risk of an adverse outcome or response of an outcome to an intervention the same for given eosinophil cutoffs?

Studies have not consistently used the same sputum or blood eosinophil cutoff. This has led to the question of what cutoff should be applied in clinical trials and clinical practice. Importantly, different clinical outcomes have different relationships with eosinophilic inflammation at a single point in time, longitudinally in stable disease, at an exacerbation and in response to therapy. For example, in the study of benralizumab in COPD described below beneficial effects were observed in terms of lung function, health status and exacerbations at different baseline blood and sputum eosinophil counts [Brightling *et al.* 2014]. Thus, the application of different cutoffs to direct therapies and monitor disease are likely to vary depending on the intervention of interest together with consideration of the potential health economic benefits for these interventions at different cutoffs. For this reason although normal ranges are established a single cutoff to direct all therapy is unlikely.

Therapies that modulate eosinophilic inflammation

The importance of eosinophilic inflammation in asthma and COPD and its role in different states of diseases is perhaps best informed by response to therapies. We describe here current and emerging therapies targeting eosinophilic inflammation and consider their overall clinical effusiveness; their impact upon eosinophilic inflammation and consequent new insights into the relationship between changes in eosinophilic inflammation and clinical outcomes.

Corticosteroids

Inhaled and systemic corticosteroids are the mainstay anti-inflammatory therapy for asthma and COPD in stable disease and at exacerbations as reflected in their central position in international guidelines supported by extensive meta-analyses and Cochrane reviews of multiple randomized controlled trials [Edwards *et al.* 2010; Loymans *et al.* 2014; Yang *et al.* 2012]. The mechanism of action of corticosteroids has been studied extensively, but remains incompletely understood. In brief, corticosteroids diffuse across the eosinophil cell membrane and enter the cell cytoplasm where they bind with glucocorticoid receptors causing exposure of nuclear localization signals [Heitzer *et al.* 2007]. This results in rapid transport of the glucocorticoid receptor complex into the nucleus which then binds to DNA altering the transcription of target genes which code for pro-inflammatory proteins thereby inhibiting the synthesis of cytokines such as IL-3, IL-5 and GM-CSF, promoting eosinophil apoptosis.

Inhaled and oral corticosteroids decrease eosinophilic bronchial mucosal inflammation [Djukanović

et al. 1992] and induced sputum differential eosinophil count [Brightling *et al.* 2000; Jatakanon *et al.* 2000; Pizzichini *et al.* 1998]. Importantly, the presence of sputum eosinophilia (>3%) in asthma and COPD [Gibson *et al.* 1995; Green *et al.* 2002a,b] and the Th2 gene signature [Woodruff *et al.* 2009] has shown to be a good predictor of the response to corticosteroid treatment in stable disease. These observations have been extended to acute exacerbations of COPD with the blood eosinophil count predicting a good response to oral prednisolone [Bafadhel *et al.* 2012]. Studies further showed that treatment aimed to maintain eosinophil counts <2–3% decreased the number of exacerbations and hospital admissions in asthma and COPD. Interestingly in the study by Green and colleagues the macrophage red hue was further studied and identified that in those subjects with increased red hue but normal eosinophil count that corticosteroid withdrawal was associated with onset of poor control and increased exacerbation risk whereas only in those subjects without evidence of either sputum eosinophils nor increased macrophage red hue could corticosteroids be successfully withdrawn [Kulkarni *et al.* 2010].

Leukotriene receptor antagonists

Leukotrienes are potent lipid mediators derived from arachidonic acid metabolism and released predominately by mast cells and eosinophils. They are potent bronchoconstrictors, causing airway smooth muscle contraction and mucus hyper-secretion [Hallstrand and Henderson, 2010]. Leukotriene receptor antagonists decrease eosinophilic airway inflammation in blood and sputum in asthma [Pizzichini *et al.* 1999], albeit to a lesser extent than inhaled corticosteroids [Jayaram *et al.* 2005]. In moderate-to-severe disease an effect of leukotriene receptor antagonists upon eosinophilic airway inflammation is minimal or absent [Green *et al.* 2006; Pavord *et al.* 2007]. In a study where montelukast was used as an add on to a steroid treatment regime as compared to arm where steroid dose was doubled both arms showed improvement in forced expiratory volume in 1 second (FEV_1) but there was no difference in sputum eosinophilic inflammation with either arms [Barnes *et al.* 2007]. In COPD small studies suggest limited clinical benefit and to date no studies have reported effects upon eosinophilic airway inflammation [Chauhan and Ducharme, 2012; Ducharme *et al.* 2011].

Theophylline

Theophylline is indicated as third line with most studies showing an equivalent effect and few studies showing a very minimal benefit as compared to doubling steroid regimens or adding a long acting beta agonist (LABA) [Lim *et al.* 2000; Subramanian *et al.* 2015; ZuWallack *et al.* 2001]. Theophylline is commonly prescribed for asthma and COPD as add on treatment in poorly controlled disease [Barnes, 2006; see also http://www.ginasthma.org and http://www.goldcopd.org]. It has both bronchodilator and anti-inflammatory actions with the latter possibly due to its effects upon the activity of a key corticosteroid-associated corepressor protein, histone deacetylase (HDAC)2. Histone acetylation and deacetylation is key in regulating expression of inflammatory genes [Barnes *et al.* 2005]. Theophylline is an activator of HDAC2 even at low therapeutic levels and enhances the action of corticosteroids [Ito *et al.* 2006]. In asthma theophylline caused significant reduction in the number of eosinophils in bronchial biopsies, bronchoalveolar lavage (BAL) and induced sputum [Lim *et al.* 2001] as compared with placebo. Low-dose theophylline when used in combination with inhaled corticosteroids significantly decreased sputum eosinophils counts in COPD patients as compared to theophylline alone [Ford *et al.* 2010].

Immunomodulators

Immunomodulators such as methotrexate and cyclosporin have steroid-sparing effects, but also predictable side-effects that have limited their value in severe asthma. To date only one study has specifically investigated the effect of methotrexate upon peripheral blood eosinophil count and found no difference between the placebo or treatment arms [Erzurum *et al.* 1991]. These therapies have not been tested in COPD.

Anti-IgE omalizumab

Omalizumab is the only biological therapy approved for the treatment of asthma. It is a humanized monoclonal anti-IgE antibody. A recent Cochrane review of 25 omalizumab trials in moderate-to-severe asthma showed effectiveness in decreasing exacerbations and hospitalizations as compared with placebo with increased potential to withhold inhaled corticosteroids in patients on anti-IgE treatment as compared with placebo [Normansell *et al.* 2014]. The effects of omalizumab upon eosinophilic inflammation

have been studied in a single multicentre study in which omalizumab reduced the sputum, epithelial and sub mucosal eosinophil count [Djukanović *et al.* 2004] and high blood eosinophils, serum periostin or FeNO prior to treatment initiation predict favourable responses to omalizumab [Hanania *et al.* 2013].

Therapies in clinical development

There is tremendous interest in targeting eosinophilic/Th2-mediated inflammation and this has led to the development of both biological and small molecule therapies. Clinical trials in this space published since 2010 are summarized in Table 1.

Anti-IL-5/anti-IL-5R

As described above, IL-5 has a pivotal role in the differentiation and maturation of eosinophils in bone marrow and survival in tissue. Monoclonal antibody therapies are in late phase clinical development neutralising IL-5 or blocking IL-5Rα.

Mepolizumab is a humanized monoclonal antibody against free IL-5 [Desai and Brightling, 2009; Haldar *et al.* 2009]. Earlier trials confirmed that anti-IL-5 monoclonal antibody therapy attenuated markedly blood, sputum and, to a lesser extent, bronchial mucosal eosinophils, but failed to demonstrate significant benefits in clinical endpoints [Flood-Page *et al.* 2007; Leckie *et al.* 2000]. Although the clinical outcomes from these studies were disappointing together they suggested a possible effect upon exacerbation frequency. This gave encouragement to undertake a phase IIa study in severe asthmatics with evidence of eosinophilic inflammation and frequent exacerbations, which demonstrated a reduction in exacerbation frequency together with reductions in eosinophilic inflammation [Haldar *et al.* 2009; Pavord *et al.* 2012]. This observation was replicated in phase IIb and III studies confirming that mepolizumab reduced severe asthma exacerbations, together with benefits in health status, a small increase in lung function as well as a safe glucocorticosteroids sparing effects [Bel *et al.* 2014; Ortega *et al.* 2014]. Benefits were associated with the blood eosinophil count and previous exacerbation frequency. Following cessation of mepolizumab therapy benefits are lost within 6 months after treatment withdrawal [Haldar *et al.* 2014].

Reslizumab is another humanized monoclonal antibody, which neutralises circulating IL-5, showed in phase II studies improvements in lung function and a decrease in both sputum and blood eosinophil counts [Castro *et al.* 2011]. Improvements in asthma control were associated with high baseline blood eosinophil counts. Two duplicate phase III trials confirmed reduction in asthma exacerbations; improvements in health status and asthma control [Castro *et al.* 2015].

Benralizumab is a human afucosylated monoclonal antibody that targets IL-5Rα expressed by eosinophils and basophils and following receptor blockade initiates antibody-dependent cell-mediated cytotoxicity [Ghazi *et al.* 2012]. A small phase I double-blind placebo-controlled trial showed that benralizumab decreased airway mucosal and sputum eosinophils in addition to a completely suppressing blood and bone marrow eosinophils and their precursors when compared with placebo [Laviolette *et al.* 2013]. Benralizumab showed a significant reduction in exacerbation rates, lung function and asthma control in severe asthmatics with high baseline blood eosinophil count [Castro *et al.* 2014]. Although targeting the IL-5Rα is likely to have more profound effects upon eosinophilic inflammation than IL-5 neutralization whether this translates into differential clinical effects remains unclear.

In the first study of a biologic therapy in COPD, benralizumab improved lung function, but did not decrease exacerbation rates, although there was a trend to reduction in exacerbations and improvement in health status in those COPD subjects with higher baseline blood and sputum eosinophil counts [Brightling *et al.* 2014]. These encouraging findings have supported progression to phase III studies.

Anti-IL-13 and IL-4Rα

The Th2-derived cytokines IL-4 and IL-13 have multiple effects upon inflammatory and structural cells. IL-4 and IL-13 activate epithelial cells to release important eosinophil chemoattractants, promote IgE class switching and IL-13 has direct effects upon airway smooth muscle augmenting hyper-contractility. IL-13 binds with IL-13Rα1 with a low affinity and then with IL-4Rα to form a high affinity cytokine binding heterodimer. [Brightling *et al.* 2010]. Therefore monoclonal antibody therapies directed towards IL-4Rα inhibit the function of IL-4 in concert with IL-13 via IL-13RI. However, IL-13 neutralization has no effect upon IL-4, but inhibits the activation of

Table 1. Phase II and III parallel double-blind placebo-controlled randomized controlled trials of anti-Th2 cytokine/chemokine therapies since 2010.

	Duration	Intervention	Subjects	Primary outcomes	Secondary outcomes
Corren *et al.* [2011]	6 months	Anti-IL-13 Lebrikizumab	Severe asthma, $n=219$	↑FEV_1% predicted Greater in periostin^{High}	↔ symptoms Trend in ↓exacerbation rate
Hanania [2014]	12 weeks	Anti-IL-13 Lebrikizumab	Severe asthma $n=463$	↓ exacerbation rate Greater in periostin^{High}	↑FEV_1% predicted Greater in periostin^{High}
Piper *et al.* [2013]	12 weeks	Tralokinumab Anti-IL-13	Moderate-to-severe asthma, $n=194$	↔ ACQ	↑ FEV_1 ↓ β2 agonist use
Brightling *et al.* [2014]	52 weeks	Tralokinumab Anti-IL-13	Severe asthma $n=452$	Trend in ↓exacerbation rate in periostin^{High} DPP4^{High}	↑ FEV_1 Improved symptom scores in DPP4^{High}
Wenzel *et al.* [2013]	12 weeks	Dupilumab Anti IL4Rα	Moderate to severe asthma, $n=104$	↓ exacerbation rate	↑ FEV_1 Improved symptom scores
Wenzel *et al.* [2014]	12 weeks	Dupilumab Anti IL4Rα	Moderate to severe asthma $n=104$	↑ ACQ	-
Pavord *et al.* [2012]	12 months	Mepolizumab Anti-IL-5	Severe asthma, $n=621$	↓ exacerbation rate ↓ blood, sputum eosinophils	Small ↓ FEV_1 Small ↑ symptom scores
Bel *et al.* [2014]	20 weeks	Mepolizumab Anti-IL-5	Severe asthma, $n=135$	↓ in glucocorticoid daily dose	↓ exacerbation rate
Ortega *et al.* [2014]	32 weeks	Mepolizumab Anti-IL-5	Severe asthma, $n=576$	↓ exacerbation rate	↑ FEV_1 Improved symptom scores
Castro *et al.* [2014]	12 months	Benralizumab Anti-IL-5Rα	Moderate to severe asthma $n=609$	↓ exacerbation rate.	↑ FEV_1 Improved ACQ
Brightling *et al.* [2014]	48 weeks	Benralizumab Anti-IL-5Rα	Moderate to severe COPD $n=101$	↔ exacerbation rate	↑ FEV_1 Trend in ↓ exacerbation rate in ↑ blood and sputum eosinophil group
Castro *et al.* [2011]	12 weeks	Reslizumab Anti-IL5	Moderate to severe asthma $n=106$	Improved ACQ	↓ blood, sputum eosinophils Trend in ↓ exacerbation rate
Castro *et al.* [2015]	12 months	Reslizumab Anti-IL5	Moderate to severe asthma $n=953$	↓ exacerbation rate	↑ FEV_1 Improved symptom scores

(Continued)

Table 1. (Continued)

	Duration	Intervention	Subjects	Primary outcomes	Secondary outcomes
Corren *et al.* [2014]	16 weeks	Reslizumab Anti-IL5	Moderate to severe asthma, *n* = 492	↑ FEV_1 greater in patients with blood eosinophil > 400/μl	↑ ACQ and ↓ rescue medication use in patients with blood eosinophils > 400/μl
Barnes *et al.* [2012]	28 days	OC000459 CRTh2 antagonist	Moderate asthma *n* = 107	↑ FEV_1 Improved symptom scores	Improved AQLQ score
Gonem *et al.* [2014]	12 weeks	QAW039 Dp2/CRTh2 Antagonist	Severe asthma *n* = 61	↓ in sputum eosinophils	Improved AQLQ score ↑ post bronchodilator FEV1
Molfino *et al.* [2013]	6 weeks	KB002 Anti-GM-CSF	Severe asthma *n* = 24	↑ FEV_1 ↓ in sputum eosinophils	↔ symptom scores
Wenzel *et al.* [2014]	4 weeks	ARRY 502 DP2 antagonist	Mild Asthma *n* = 184	↑ FEV_1	↑ ACQ ↑ symptom free days
Hall *et al.* [2012]	Six weeks	BI-671800 DP2 antagonist	Asthma *n* = 243	↑ FEV_1	↑ ACQ
Sutherland *et al.* [2012]	4 weeks	BI-671800 DP2 antagonist	Asthma *n* = 388	↔ FEV_1	↓ ACQ
Krug *et al.* [2012]	2 weeks	BI-671800 DP2 antagonist	Allergic rhinitis *n* = 146	↓ nasal symptoms, ↓ nasal eosinophils, ↓ eosinophil activation	↓ IL-4 ↓ eotaxin levels
Fitzgerald *et al.* [2013]	14–28 days	ADC-3680 DP2 antagonist	Asthma (*n* = 24) Healthy (*n* = 88)	Blocked PGD2 mediated eosinophil activation by >90%	-
Pettipher *et al.* 2014	12 weeks	OC000459 DP2 antagonist	Asthma *n* = 482	↑ FEV_1 and ↑ PEF in eosinophilic subgroup	↑ AQLQ ↑ ACQ ↓ Exacerbation rate
Busse *et al.* [2013]	12 weeks	AMG 853 D2 antagonist	Asthma *n* = 79	↔ ACQ	↔ FEV_1 ↔ symptom scores ↔ exacerbation
KaloBios [2014]	24 weeks	KB003 Anti-GM-CSF	Severe Asthma *n* = 160	↔ FEV_1 ↑FEV_1 in eosinophilic subgroup	↔ ACQ ↔ exacerbations
Gaureauv *et al.* [2011]	9 weeks	TPI ASM8 Anti-IL-3,-4,GM-CSF, CCR3	Mild –Moderate Asthma *n* = 14	↓ sputum eosinophil count ↓sputum ECP	Safe at all doses tested
Greiff *et al.* [2010]	10 weeks	AZD3778 CCR3 antagonist	Seasonal allergic rhinitis *n* = 38	↓ eosinophil	↓ allergic rhinitis symptoms
Neighbour *et al.* [2014]	22 days	GW76694 CCR3 antagonist	Asthma *n* = 60	↔ eosinophils ↔ eosinophil progenitor cell count	↔ FEV_1 ↑ airway hyper responsiveness ↑ ACQ

Abbreviations: AQLQ, asthma quality of life questionnaire; ACQ, asthma control questionnaire; COPD, chronic obstructive pulmonary disease; ECP, eosinophil cationic protein; FEV_1, forced expiratory volume in 1 second; GM-CSF, granulocyte-monocyte colony stimulating factor; IL, interleukin.

IL-13RI and exerts effects upon IL-13RII which are antibody dependent. Inhibition of IL-13RII activation occurs with tralokinumab but not lebrikizumab [Ultsch *et al.* 2013]. Increased IL-13 expression is a consistent feature of asthma in peripheral blood, sputum, BAL and bronchial biopsies. IL-13 concentrations in sputum have been seen to be related to asthma control [Saha *et al.* 2008].

Phase II trials have shown consistent positive results for anti-IL-13 therapy [Corren *et al.* 2011; Hanania, 2014; Piper *et al.* 2013] with improvement in lung function. This effect was more pronounced in patients who had higher levels of pretreatment periostin, dipepityl-pepitidase-4 or evidence of increased sputum IL-13 [She *et al.* 2014]. Similarly in those with evidence of activation of the IL-13 pathway had lower exacerbation rates in response to anti-IL13 therapy.

Anti-IL4Rα, dupilumab, also improves lung function and asthma control and in an inhaled corticosteroid withdrawal study there was significant reduction in the exacerbation rates in those subjects that received dupilumab *versus* placebo [Teper *et al.* 2014; Wenzel *et al.* 2013].

In the anti-IL-13 and anti-IL-4Rα trials the blood eosinophil count increased possibly due to its indirect effects upon generation of eosinophil chemokines or directly due to the effect of IL-13 upon eosinophil endothelial adhesion as described above. Interestingly whether anti-IL-4/13 approaches reduce sputum or bronchial mucosal eosinophilia is unknown.

Anti-CRTh2

Prostaglandin D2 (PGD2) is a product of arachidonic acid metabolism and is a major prostanoid found within the airways of asthmatics immediately following an airway challenge. PGD2 is known to have chemokinetic and chemotactic effects on eosinophils and Th2 lymphocytes acting via DP2/CRTh2 receptor [Hirai *et al.* 2001; Nagata *et al.* 1999]. This interaction plays a key role in airway hyper-responsiveness, IgE and cytokine production [Pettipher and Whittaker, 2012]. An *in vitro* study using a potent CRTH2 antagonist was the first to show decrease in PGD2-mediated human eosinophil migration [Sugimoto *et al.* 2003]. Since this discovery a variety of DP2/CRTH2 antagonists including ARRY 502, BI-671800, QAW-039, OC000459 have been studied in phase II trials. BI-671800 showed better improvement in FEV_1 when compared with montelukast [Hall *et al.* 2012], but did not show much difference in effects on FEV_1 when compared with an inhaled steroid treatment. Phase II trials investigating the DP2 antagonists OC000459 and QAW 039 DP2, respectively, showed a reduction in sputum eosinophil counts and improved symptoms in mild [Barnes *et al.* 2012] and severe asthmatics [Gonem *et al.* 2014]. These effects were also demonstrated with BI-671800 in seasonal allergic rhinitis [Krug *et al.* 2012]. A more recent phase II trial continued to show benefits of oral CRTH2 treatment on FEV1 and symptom scores [Wenzel *et al.* 2014].

Anti-GM-CSF

GM-CSF causes the differentiation proliferation and survival of monocytes, macrophages and granulocytes including eosinophils and neutrophils. The development of the anti-GM-CSF IgG1 antibody MT203 showed decrease in eosinophil activation and survival [Krinner *et al.* 2007]. The safety and efficacy of other anti-GMCSF therapies are in early clinical development and to date whether it impacts upon eosinophilic inflammation is uncertain. Phase I and II trials using anti-GM-CSF treatments KB002 [Bardin *et al.* 2013] and KB003 [KaloBios, 2014] have been done in the hope of finding a treatment to target atopic and non-atopic asthma. These anti-GM-CSF therapies have shown no improvements compared to placebo in overall study populations, but a statistically significant improvement in FEV1 and decrease in sputum eosinophil count was demonstrated in the eosinophilic subgroups (blood eosinophil $\geqslant$300 cells/μl).There was no effect on symptom scores or exacerbations. Whether there will be further development and phase III trials of these agents to establish their effects is uncertain at present.

Anti-CCR3

The CCR3 chemokine receptor is expressed on the surface of eosinophils and is activated by chemokines Eotaxin 1 (CCL11)/Eotaxin 2 (CCL 24) and Eotaxin 3(CCL26). Eotaxin 1(CCL11) has been shown to be actively involved in eosinophil recruitment and in survival along with IL-5. Airway epithelial cells and airway smooth muscle cells express CCR3 and also produce cytokines and fibroblast growth factor. Recent studies using

CCR3 antagonists including GSK766994, GSK 766904,GW824575, DPC 168 and QAP 642 [Pease and Horuk, 2014] in asthma or in allergic rhinitis have not demonstrated significant effects upon eosinophilic airway inflammation and have questioned the importance of CCR3 in eosinophil recruitment *versus* other mechanisms such as CRTh2 [Neighbour *et al.* 2014].

Conclusion

Current treatments and the emergence of new therapies targeting eosinophilic inflammation have informed our understanding of the relationship between eosinophilic inflammation and different characteristics of asthma and COPD. Identification of eosinophilic and Th2-meduiated inflammation predicts a good response to corticosteroid, anti-IgE, anti-IL5, anti-IL5Rα, and anti-13 therapies. Eosinophilic inflammation has a clear role in asthma exacerbations and its inhibition by anti-IL5 improves lung function, symptoms and health status related to the intensity of eosinophilic inflammation and possibly with greater effects upon lung function following IL-5Rα blockade rather than IL-5 neutralization. In COPD, anti-IL-5 improved lung function in eosinophilic COPD, but the role of eosinophils in COPD exacerbations remains uncertain. Anti-IL-13 has consistent effects upon lung function in asthma, but the benefits in exacerbations have to date been less marked than with anti-IL-5 and current late phase studies in selected populations will be critical in determining the role of IL-13 in asthma exacerbations. CCR3 antagonism has been disappointing whereas anti-CRTh2 is emerging as a potentially important target that impacts inflammation, lung function and symptoms with effects upon exacerbations to be determined. The potential role of therapies targeting IL-4/13 and CRTh2 in eosinophilic COPD is unknown.

In the forthcoming 5 years the mechanistic role of the eosinophil in asthma and COPD, the proportion of the asthma and COPD patients in whom the eosinophil is critical and the magnitude of clinical efficacy of anti-eosinophil therapies will become clear. During this time attention will need to focus on the aspects of disease that are independent of eosinophilic inflammation and in those where the eosinophil plays a key role then there is a need to understand the relative merits of each therapy in an individual and how can we apply biomarkers to predict long term outcomes. Addressing this challenge will both further inform our understanding of the role of the eosinophil in asthma and COPD and sharpen our focus on the need for precision medicine.

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