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Airway inflammation in COPD- progress to precision medicine

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Abstract:	Chronic obstructive pulmonary disease (COPD) is a significant cause of morbidity and mortality worldwide and its prevalence is increasing. Airway inflammation is a consistent feature of COPD and is implicated in the pathogenesis and progression of COPD, but anti-inflammatory therapy is not first line treatment. This inflammation has many guises and phenotyping this heterogeneity has revealed different patterns. Neutrophil-associated COPD with activation of the inflammasome, T1 and T17 immunity is the most common phenotype with eosinophil-associated T2-mediated immunity in a minority and autoimmunity observed in more severe disease. Biomarkers have enabled targeted anti-inflammatory strategies and revealed that corticosteroids are most effective in those with evidence of eosinophilic inflammation. Whereas in contrast to severe asthma response to anti-IL5 biologics in COPD has been disappointing with smaller benefits for the same intensity of eosinophilic inflammation questioning its role in COPD. Biological therapies beyond T2-mediated inflammation have not demonstrated benefit and in some cases increased risk of infection suggesting that neutrophilic inflammation and inflammasome activation might be largely driven by bacterial colonisation and dysbiosis. Herein we shall describe current and future biomarker approaches to assess inflammation in COPD and how this might reveal tractable approaches to precision medicine and unmask important host-environment interactions leading to airway inflammation.

Airway inflammation in COPD- progress to precision medicine

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Summary

Chronic obstructive pulmonary disease (COPD) is a significant cause of morbidity and mortality worldwide and its prevalence is increasing. Airway inflammation is a consistent feature of COPD and is implicated in the pathogenesis and progression of COPD, but anti-inflammatory therapy is not first line treatment. This inflammation has many guises and phenotyping this heterogeneity has revealed different patterns. Neutrophil-associated COPD with activation of the inflammasome, T1 and T17 immunity is the most common phenotype with eosinophil-associated T2-mediated immunity in a minority and autoimmunity observed in more severe disease. Biomarkers have enabled targeted anti-inflammatory strategies and revealed that corticosteroids are most effective in those with evidence of eosinophilic inflammation. Whereas in contrast to severe asthma response to anti-IL5 biologics in COPD has been disappointing with smaller benefits for the same intensity of eosinophilic inflammation questioning its role in COPD. Biological therapies beyond T2-mediated inflammation have not demonstrated benefit and in some cases increased risk of infection suggesting that neutrophilic inflammation and inflammasome activation might be largely driven by bacterial colonisation and dysbiosis. Herein we shall describe current and future biomarker approaches to assess inflammation in COPD and how this might reveal tractable approaches to precision medicine and unmask important host-environment interactions leading to airway inflammation.

Key words: COPD, ACOS, eosinophil, neutrophil, macrophage, inflammasome, biologics, eosinophil, interleukin (IL)5, benralizumab, mepolizumab, tumour necrosis factor (TNF)α, IL6, IL8, IL1β, IL33, IL13, IL17, anti-IgE antibody, thymic stromal lymphopoietin (TSLP), prostaglandin D_2 receptor type 2 (DP2)

Take home message

Airway inflammation drives COPD, but corticosteroids only work in those with eosinophilic inflammation. There is a need to better understand the patterns of inflammation, the reason for its persistence and the opportunities for new treatments.

Introduction

Chronic obstructive pulmonary disease (COPD) is a common disease of chronic lung inflammation that results in persistent symptoms and fixed airflow obstruction [1]. This is caused by an inflammatory response following inhalation of cigarette smoke or other noxious external particles such as air pollution and biomass fuel [1]. Airway and systemic inflammation in COPD is related to disease progression and mortality [1-2]. Current diagnostic criteria do not capture the heterogeneity of COPD in terms of the complex pathological changes occurring within lung, the different airway inflammatory patterns or the airway microbial ecology. Airway inflammation is a consistent feature of COPD and is present in both the large and small airways [1, 3-6]. The airway inflammation can persist after smoking cessation and is likely a consequence of altered immunity [6] and changes in the airway microenvironment [8-10].

Despite the long-standing recognition that airways inflammation is a key driver of COPD progression and exacerbations, first-line treatment strategies are aimed at symptomatic treatment of bronchoconstriction in the form of bronchodilators, rather than anti-inflammatory therapy [1]. In this review we shall describe the heterogeneity of airway inflammation in COPD, current and future biomarker approaches to dissect this heterogeneity and redefine COPD using multi-dimensional phenotyping and how this might reveal tractable approaches to precision medicine and provide important insights into the host-environment interactions.

Multi-dimensional COPD phenotyping providing insights into pathophysiology

COPD is a consequence of complex host-environment interactions that occur over time summarised in **Figure 1**. Smoking, and other pollutants, pathogens and allergens insult the lung promoting airway inflammation and damage in a susceptible host as a consequence of

genetic predisposition and altered immunity [6, 10-12]. This in turn leads to irreversible damage resulting in fixed airflow obstruction and the consequent typical symptoms of COPD.

Approaches to phenotyping airway inflammation and damage in COPD

Insights into airway inflammation and damage to the airways have been derived from lung specimens obtained from surgical resection and at post mortem. Importantly *in vivo* measures of airway and systemic inflammation have been characterised longitudinally, at exacerbations and in response to therapies through invasive sampling of the airway by bronchoscopy (large airway brush and biopsy and smaller airways by bronchoalveolar lavage), non-invasive sputum sampling (mostly large airways) which is safe even in severe COPD [13], breath analysis (large and small airways), lung imaging (large airways directly and small airways indirectly) and beyond the lung by assessing upper airway samples and systemically using blood and urine [5, 14] (**Figure 2**).

Neutrophil-associated airway inflammation

The inflammatory response in COPD involves both innate and adaptive immunity with neutrophilic inflammation the commonest inflammatory phenotype in COPD. Following exposure to cigarette smoke, other pollutants, and oxidants there is airway damage [15] leading to release of pro-inflammatory mediators and damage-associated molecular patterns (DAMPs) such as interleukin (IL)-33 and thymic stromal lymphopoietin (TSLP) [15]. The distribution of the IL-33 receptor ST2 is altered in response to cigarette smoke with down-regulation in innate type-2 innate lymphoid cells with up-regulation by macrophages leading to a triggering of an IL-33-dependent exaggerated pro-inflammatory cascade [16]. As a consequence of airway damage the altered barrier function predisposes the airway to infection and bacterial dysbiosis which together with pollutants drive switching of ILC2 cells towards ILC1 cells further

amplifying the type-1 inflammatory cascade [17]. In COPD there is an increase in the phyla proteobacteria and the emergence of *H. Influenzae* predominance such that the ratio of gammaproteobacteria to firmicutes (γP:F) increase [7-9, 18]. These pathogens themselves promote an inflammatory response via activation of pathogen-associated molecular patterns (PAMPs) and further amplification of the airway inflammation with the intensity of airway inflammation related to the abundance of *H. Influenzae* [19, 20]. In this scenario, epithelial cells are activated and are involved in the release of inflammatory mediators, such as tumor necrosis factor (TNF), IL-1β, IL-6 and IL-8. Macrophages are recruited with further release or pro-inflammatory cytokines and activation of the NLRP3 inflammasome with caspase-1-dependent release of pro-inflammatory IL-1-like cytokines IL-1α, IL-1β, IL-33 and IL-18 [6, 15]. Activation of the inflammasome can lead to persistence of an inflammatory response by triggering an auto-inflammatory response with intrinsic production of pro-inflammatory mediators independent of exogenous stimuli [6]. Interestingly activation of type 1 responses are more closely related to COPD severity than inflammasome activation and thus autoimmunity can occur across disease severity [21]. Neutrophils are recruited as the predominant cells with consequent release of proteases and airway damage as well as activation of innate lymphoid type 3 cells (ILC3). The adaptive immune response is also involved with polarization and subsequent recruitment of CD4+ Th1 and Th17 cells, which produce IFN-γ and IL-17A and IL-17F [6, 15, 22] respectively with a later predominance of CD8+ T-cells. In concert or independent of the auto-inflammatory response there is an auto-immune response which can also promote persistence of inflammation [6]. In more severe disease there is an accumulation of B cells particularly in the smaller airways which together with T cells and follicular dendritic cells comprise aggregates organised into tertiary lymphoid follicles [23]. These lymphoid follicles support the priming and clonal expansion of T and B-cells with an increase proportion of IgA+ B-cells perhaps in response to increased persistent airway infection

or auto-antigens [24, 25]. The cytokine network in neutrophil-associated COPD is summarised in **Figure 3A**.

Eosinophil-associated airway inflammation

Even though neutrophil-associated COPD is the most common inflammatory phenotype, consistent with the heterogeneity of the disease 10-40% of COPD patients demonstrate increased eosinophilic inflammation in the sputum and or blood [5, 26, 27] with increased T2-transcriptome signatures [28]. The broad range in prevalence is in part due to differences in patient populations but also due to different cut-offs applied in sputum (>2 or >3% eosinophils) or blood (2% or >250, 300, 400 eosinophils/μL). Increased eosinophilic inflammation in peripheral blood and sputum samples in COPD, like asthma, is associated with a greater future risk of severe exacerbations [29, 30].The aetiology of eosinophilic inflammation in COPD is uncertain. As with neutrophil-associated COPD eosinophilic COPD is also likely to be a combination of innate and adaptive immunity summarised in **Figure 3B**. These pathways are well-described for asthma [5, 30]. Following allergic sensitisation and T-cell polarisation TH2 cells produce interleukin (IL)-4, IL-5, and IL-13. IL-5 is an obligate cytokine for the survival and maturation of eosinophils, and IL-4 and IL-13 promote IgE production from B cells and have direct effects upon structural cells. Recruitment of eosinophils to the lung mucosa is mediated via production of predominantly epithelium-derived CCR3 chemokines and other eosinophil chemoattractants, such as mast cell-derived prostaglandin (PG)D2. PGD2 amplifies T2 immunity via activation of PGD2 type 2 receptors (DP2 or CRTH2). Total IgE is elevated in eosinophilic COPD even though atopy is not increased. Whether this reflects a hitherto undescribed allergen is unclear. Eosinophilic inflammation can also occur via activation of ILC2 cells, which produce IL-5 and IL-13 in response to PGD2 and the epithelial-derived 'alarmins' IL-33, IL-25, and TSLP released after epithelial damage by pollutants and microbes.

Additional contributions might be from macrophage-derived IL33 released following inflammasome activation. Whether these innate and acquired T2-mediated immune mechanisms occur in COPD, the predominance of one over another is more important in COPD versus asthma or whether there are alternative mechanisms driving eosinophilic inflammation in COPD remains unclear.

Biological clustering to dissect heterogeneity of airways inflammation

These eosinophilic versus neutrophilic associated inflammatory profiles represent extreme phenotypes. However they are consistently reproducible and demonstrate phenotype stability [20, 26]. The neutrophil and eosinophil-associated phenotypes also exhibit distinct microbial ecology with γP:F predominance in the neutrophilic phenotype [8, 9, 31]. However, to describe extremes can be an over-simplification of a complex underlying biology. To validate these phenotypes and to further inform the understanding of the heterogeneity of COPD in stable state unbiased statistical approaches such as cluster analysis have been applied to large clinical and biological datasets [18, 32, 33]. Interestingly these have underscored the importance of eosinophilic airway inflammation in asthma, COPD and the asthma-COPD overlap syndrome (ACOS) [32, 34]. Combined data from asthma and COPD revealed three biological clusters [32]. 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. 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 colonisation with an increased γP:F ratio in the former and increased CCL13 in the latter possibly explaining the observed airway inflammation differences seen between these clusters (**Figure 4A**).

Using a similar unbiased cluster analysis approach for COPD exacerbations four biological clusters were identified and these validated the *a priori* aetiological groups: 'Pro-inflammatory' bacterial-associated, 'Th1' viral-associated, 'Th2' eosinophilic-associated and a fourth group that were termed 'pauci-inflammatory' as this was associated with limited changes in the inflammatory profile (**Figure 4B**) [33]. Disease severity was not different between these biological clusters and the biomarkers were associated with their respective potential aetiologies. In the pro-inflammatory bacterial-associated group the strongest discriminating inflammatory mediator was sputum IL-1β with increased γP:F consistent with bacterial dysbiosis. The blood eosinophil count was the best predictor of sputum eosinophilic inflammation (>3% eosinophils) at the time of the exacerbation in this study although the correlations are typically weaker in stable disease [35] Interestingly Bafadhel *et al* found that patients experienced more bacterial exacerbations if their stable sputum samples contained more bacteria and high γP:F and more eosinophilic exacerbations if eosinophilic inflammation was present in the stable state suggesting that the exacerbation event was an amplification of the underlying phenotype [33]. Thus these biomarkers in addition to directing therapy during the exacerbation event might identify subgroups to target therapy in stable state with the aim of reducing future risk. The exception to this was a viral infection representing a new event and a new inflammatory profile with increased blood and sputum concentrations of the interferon-inducible chemokines CXCL10 and CXCL11.

Airway damage and remodelling- emphysema and small airway obliteration

Airway inflammation in COPD contributes to airway damage, remodelling, loss of small airways and emphysema (tissue damage with permanent dilatation distal to the terminal bronchiole). Chronic airflow obstruction is due to a combination of emphysema and small airway obliteration. Small airways are the major site of airway obstruction in COPD [48]. This

small airways obliteration is due to a combination of remodelling and accumulation of inflammatory exudates within the airway lumen, both of which increase with disease severity [36, 37]. Remodelling changes observed in COPD include disruption and loss of epithelial cilia, squamous metaplasia of the respiratory epithelium, goblet cell hyperplasia and mucous gland enlargement, bronchiolar smooth muscle hypertrophy, airway wall fibrosis and inflammatory cell infiltration [36, 37].

Computed tomography (CT) and micro CT has demonstrated a reduction in the luminal area of terminal bronchioles in COPD, but also substantial loss of terminal airways [38-40]. This is consistent with the view that the inflammation and remodelling of the small airways largely as a consequence of inflammation leads to destruction of the terminal followed by respiratory bronchioles to form centrilobular lesions. This in turn can result in destruction of entire lung lobules which coalesce to form bullous emphysema. Thus narrowing and consequent disappearance of small conducting airways can explain the increased peripheral airway resistance reported in COPD prior to the development of emphysema [38-40]. The distribution of emphysema can be centrilobular or panacinar. It is uncertain if these represent a spectrum with panacinar a consequence of centrilobular emphysema or if they represent distinct conditions. Panacinar is observed in individuals with alpha-1-anti-trypsin deficiency perhaps suggesting this form of emphysema might be largely a consequence of the imbalance between protease and anti-protease activity whereas centrilobular is largely due to loss of and remodelling of small airways caused by persistent airway inflammation. Quantitative CT has demonstrated that gas trapping due to small airway disease moreover than emphysema is related to lung function impairment [41, 42]. These mechanisms of small airway obliteration and emphysema are important when considering anti-inflammatory therapy as only the

remaining inflamed airways can be targeted in contrast to the airways and alveoli that are already destroyed in patients with COPD.

Airway inflammation in COPD- progress to precision medicine

Increasing knowledge of disease pathology and inflammatory phenotypes will inform our understanding of COPD and enable phenotype-specific clinical management beyond the first line bronchodilator therapy for COPD.

Eosinophilic COPD- corticosteroids

Corticosteroids have been used in the treatment of COPD for more than 40 years with moderate overall benefit in terms of improvement in lung function, health status, 6 minute walk distance and exacerbation frequency [1]. More recently a differential response in patients has been seen based on eosinophil count. An elevated sputum eosinophil count is associated with a greater response to both inhaled and oral corticosteroids in stable disease [43, 44], whilst blood eosinophil count can be used to predict response to corticosteroid response in stable [45, 46] and acute COPD [47] and titration of corticosteroids directed by sputum eosinophil counts reduces hospital admissions [48]. Importantly most of these studies have recruited COPD subjects with frequent exacerbations and thus whether findings can be generalised to all COPD subjects is uncertain. Additionally it is unclear if the clinical benefits, such as lung function and health status, with corticosteroids are independent of the reduction of exacerbations. In contrast non-T2 pathways such as IL-17 activation as determined by the epithelial IL-17A response transcriptome signature are associated with a decreased response to corticosteroids [49]. Whether the benefit from corticosteroids in COPD associated with eosinophilic inflammtion is restricted to its effects upon the eosinophil or due to other broader anti-inflammatory effects is uncertain. GOLD now includes the blood eosinophil count as a

biomarker to direct the use of ICS in COPD patients with frequent exacerbations [1]. Benefits in response to roflumilast are also possibly due to attenuation of eosinophilic inflammation [50].

Eosinophilic COPD- T2 targeted therapies

Evidence for targeting T2-mediated inflammation using biologics has revolutionised clinical practice in severe asthma [30, 51]. As described above significant eosinophilic inflammation does exist in COPD, albeit in a smaller proportion of patients than in asthma. However, the findings from the phase 2 and 3 trials of T2-directed therapies for COPD summarised in **Table 1** have been disappointing compared to asthma [52].

In the first anti-IL5R biologic (benralizumab) trial in COPD while a reduction in eosinophilic inflammation was observed, the primary outcome annual rate of acute exacerbations was not met, which included all patients with COPD, irrespective of baseline eosinophil count [53]. Importantly the sample size was small to study exacerbations and was underpowered to observe small effects. Secondary outcomes showed an improvement in FEV_1 in those receiving benralizumab but no difference was observed in health status. In a pre-specified post hoc analysis improvements in exacerbation frequency, lung function and health status were related to the intensity of baseline blood and sputum eosinophil count. In the yet to be fully reported phase 3 trials of benralizumab in COPD the primary outcome of exacerbations in those with increased blood eosinophil count (≥220 cells/μL) was also not met [54]. In a small single centre study mepolizumab reduced sputum eosinophil count, but did not improve lung function or health status [55]. In two phase 3 trials of mepolizumab in COPD (METREX and METREO) there were small reductions in moderate or severe exacerbations in the eosinophilic sub-group (≥150 cells/μL), which was statistically significant in the METREX (18% reduction) but not

in METREO [56]. In a *post hoc* analysis there was no reduction in exacerbation events treated with antibiotics alone in those receiving mepolizumab versus placebo but the reduction in exacerbations treated with oral corticosteroids with or without antibiotics was ~35% in those with blood eosinophil counts >300 eosinophils/μL. No improvements in lung function and health status in those receiving mepolizumab versus placebo were observed.

Importantly, both the mepolizumab and benralizumab studies suggest that the effect size is smaller than that seen in severe asthma (**Figure 5**) although, like asthma, the magnitude of benefit is directly related to the intensity of eosinophilic inflammation [57]. The sub-population of COPD patients most likely to respond to anti-IL-5(R) therapy remains unclear, although it is most likely those with a greater disease burden and higher degree of eosinophilic inflammation. Importantly in those with a low blood eosinophil count there was a suggestion of a poorer outcome following treatment with ant-IL5(R) which was not observed in asthma. Whether this reflects a role for the eosinophil in host defence in COPD or the importance of IL-5 in IgA B cell differentiation [58] as a possible reason for this adverse effect in the low eosinophil group and an attenuated response in those with the same degree of eosinophilic inflammation as asthma or because the eosinophil is less important in COPD needs to be further explored. However, a small *post hoc* study of the effects of benralizumab upon the airway microbiome from samples obtained in the phase 2a study suggest that benralizumab does not have an adverse effect on the bacterial load or composition [59].

Other T2-directed therapies have been tested in COPD or are ongoing. GATA 3 inhibition reduces the sputum eosinophil count in COPD but like ant-IL5 did not affect clinical endpoints [60]. A single trial of an anti-IL-13 (Lebrikizumab) has been tested in COPD. The full result of the study is yet to be published but the press release reported that COPD exacerbations were

not reduced in those receiving lebrikizumab versus placebo (NCT02546700). In phase 3 studies for asthma, anti-IL-13 [51] failed to meet their primary outcome for reduction in exacerbations, whereas in contrast anti-IL4Rα substantially reduced exacerbations. Whether anti-IL4Rα has efficacy in COPD is currently being tested. The role of the DAMPs thymic stromal lymphopoietin (TSLP) and IL33 are also being tested in COPD. DP2 antagonism in COPD reduced the intensity of eosinophilic inflammation [61]. Whether DP2 antagonists are beneficial in a subgroup of COPD patients with underlying eosinophilic inflammation requires future studies.

Specific pro-inflammatory and pro-neutrophilic cytokines and chemokines in COPD

While the main inflammatory pathway in COPD is neutrophilic in nature, studies targeting neutrophilic inflammation have been disappointing to date (**Table 2**). The chemokine CXCL8 (IL-8) is known to attract and activate neutrophils during an inflammatory response via the CXC chemokine receptor 1 (CXCR1) and CXCR2. In a small study a monoclonal antibody targeting IL-8 in COPD showed improved dyspnoea measured using the transitional dyspnoea index [62]. Anti-CXCR2 demonstrated small improvements in lung function particularly in those who were current smokers but did reduce exacerbations and led to increased infection rates in longer-term follow-up [63, 64]. Anti-TNF (infliximab) in COPD showed no improvements in health status, lung function, symptoms nor exacerbation frequency [65-67]. Importantly, increased adverse events were noted in those receiving infliximab, including cancer and pneumonia [67]. Targeting IL-17 with biological therapy has also been ineffective in COPD [68]. The inflammasome has been targeted with two independent anti-IL-1R1 biologics [69, 70]. In both trials there were neither benefits nor increased adverse events in those COPD subjects that received the biologic versus placebo.

Thus targeting neutrophilic inflammation, the inflammasome, TNF and IL17 have been ineffective in COPD and in some cases have increased risk of infection. This suggests that intrinsic activation of these pathways driving an auto-inflammatory process is probably less important than their activation secondary to persistent airway colonisation and infection. It remains a possibility that targeting auto-immunity with B-cell targeted biologics could be beneficial in COPD. However, it is more likely that targeting bacterial dysbiosis in stable state and infection at exacerbation events will be more efficacious and will consequently impact upon airway inflammation. Indeed benefits with long-term anti-microbials such as azithromycin might exert their effects largely upon the airway ecology and then ameliorate airway inflammation rather than having substantial direct anti-inflammatory effects [71, 72].

Future Directions

Our current understanding of the role of different inflammatory phenotypes in COPD demonstrate that the identification of eosinophilic COPD has value in directing the use of corticosteroids in COPD. This fits with the concept of a 'treatable trait' [73]. This suggests that in some COPD sufferers targeting T2-immunity beyond corticosteroids might have value. However as described above it is not straightforward to extrapolate findings in asthma to COPD and the response to T2-targeted therapies is likely to be different and will need to be carefully tested for each mechanism. Notwithstanding this limitation it would seem likely that this approach will uncover further effective therapies for eosinophilic COPD patients. The impact on the airway ecology and potential risk of promoting airway infection as observed with non-T2 targeted anti-inflammatory therapies needs to be carefully studied. However eosinophilic associated inflammation remains a minority of patients with COPD, meaning therapies to target other pathways are a priority. Targeting neutrophilic and inflammasome mediated inflammation in COPD does not seem to be an attractive strategy and more attention should be

focussed upon trying to normalise the airway ecology either through novel anti-microbials or alternative strategies such as vaccines and phage therapy [74, 75].

The multi-dimensional phenotyping strategy also suggests that the impact of the airway inflammation might have led to airway and alveoli loss which is then not amenable to anti-inflammatory therapy. This suggests that again in contrast to asthma the degree to which the COPD is reversible in response to anti-inflammatory therapy in established disease is limited. This will require a paradigm shift in identifying disease early and having biomarkers that are predictive of high risk of progression in order to intervene early and change the natural history of the disease. This would be similar to approaches for inflammatory joint diseases and other chronic inflammatory conditions. Genome-wide association studies have revealed multiple genes that are associated with lung function and implicated some genes involved in tissue repair and immunity. Together these genes have formed a genetic risk score for COPD. This risk score needs to be extended to identify genetic risk of disease progression or under-development of full lung function with altered lung function trajectories [76] and increased likelihood of response to treatment. To date the clinical impact of COPD genetic studies has been limited. However, the genetic risk score together with early disease biomarkers of changes in small airway disease such as oscillometry and imaging which have been extensively validated in the asthma study ATLANTIS [77] could identify at risk groups. The longitudinal study of airway inflammation and airway ecology in these at risk groups with ‘early’ COPD [78] would help to define mechanism for disease onset and progression such as whether changes in bacterial dysbiosis trigger inflammation and airway damage or a consequence of these features. Improved adoption of current biomarkers into clinical practice and the development of new simple, safe, repeatable and preferably near-patient biomarkers will provide insights of the inflammatory profile in the patient and their airway microenvironment. This will mean that the

tests could be done serially to help with clinical decision making in stable state but also predict exacerbation events [79] prior to their onset. Breathomics is a particularly attractive approach with early findings suggesting this could be applied to measure airway and systemic inflammation as well as microbial dysbiosis with pathogen- and inflammatory profile-specific breath signatures beginning to be described [80]. Urine biomarkers of systemic inflammation are more distant from the lung but with the development of home monitoring strategies for multiple inflammatory mediators coupled to artificial intelligence algorithms to provide risk stratification of future events could become part of clinical care [81].

Conclusion

In conclusion, airway inflammation is a consistent feature of COPD and is implicated in the pathogenesis and progression of COPD. Inflammation in COPD is heterogeneous underscoring the need for a precision medicine approach [82]. Corticosteroids are most effective in those with eosinophilic inflammation. Anti-IL5 biologics have been disappointing in COPD versus asthma suggesting that the role of the eosinophil is different in COPD. However, the response to corticosteroids and partial response to anti-IL5 in this group does suggest that it is a tractable phenotype and further studies of mechanism and alternative interventions are warranted. Therapies targeting neutrophilic inflammation and the inflammasome have been ineffective and in some cases increased risk of infection suggesting that their activation might be a consequence of bacterial colonisation and dysbiosis. Underscoring the need to focus on bacterial dysbiosis as a target to then secondarily attenuate airway inflammation. Therefore to realise anti-inflammatory precision medicine in COPD we need to stop chasing rainbows and improve the characterisation of the disease to reflect the complexity of the multi-dimensional mechanisms driving COPD in individual patients.

Box 1: Key Points

- COPD results from an abnormal inflammatory response which is highly heterogeneous in nature
- Eosinophilic COPD is responsive to corticosteroids and identifies those most likely to respond to T2 targeted biological therapy
- Treatments to target neutrophilic inflammation have failed to show efficacy
- Neutrophilic inflammation is likely to be a response to changes in microbial ecology

Table 1. Randomised placebo-controlled trials of anti-T2 therapies in COPD

Drug/target; dose and duration; subject number	Primary outcome	Secondary outcome
Benralizumab; anti-IL-5R 100mg every 4 weeks (3 doses) then every 8 weeks (5 doses), 56 week N= 82 [53]	↔ Moderate-to-severe exacerbations	↑ FEV1 in intervention group ↔ health status ↓ Blood and sputum eosinophils
Benralizumab (TERRANOVA); anti-IL5R (NCT02155660) 10, 30 or 100mg every 4 weeks (3 doses) then 8 weekly, 48 weeks; N=2255 [54]	↔ Exacerbations	Not yet reported
Benralizumab (GALATHEA); anti-IL5R (NCT02138916) 30 or 100mg every 4 weeks (3 doses) then 8 weekly, 48 weeks; N=1656 [54]	↔ Exacerbations	Not yet reported
Mepolizumab; anti-IL-5 (NCT01463644) 750mg/month, for 6 months N= 18 [55]	↓ Sputum eosinophils	↓ Blood eosinophil ↔ FEV1, CAT, CRQ, exacerbations
Mepolizumab; anti-IL-5 (METREX) (NCT02105961) 100mg or 300mg every 4 weeks, 52 weeks N= 1070 [56]	↓ Exacerbations in pre-specified (n= 462) eosinophilic group	↑Time to first exacerbation ↔ FEV1, SGRQ, CAT
Mepolizumab; anti-IL-5 (METREO) (NCT02105948) 100mg or 300mg every 4 weeks, 52 weeks N= 674 [56]	↔ Exacerbations	↔ Time to first exacerbation ↔ FEV1, SGRQ, CAT
Anti-GATA3 Inhaled 10 mg SB010 BID 28 days, N=23 [60]	Feasibility study	↓ Sputum eosinophil ↔ FEV1, FENO, symptoms

Table 2. Randomised placebo-controlled trials of anti-neutrophil, TNF and inflammasome targeted therapies in COPD

Drug/target; dose and duration; subject number	Primary outcome	Secondary outcome
Anti-IL8; IL-8 (NCT00035828) 800mg loading dose, 400mg/month for 3 months, 5 month follow-up N= 109 [62]	↓ Severity of dyspnoea as measured by transition dyspnoea index	↔ Health status, lung function, 6-min walk test, rescue use of albuterol
Anti-CXCR2 50mg BD OR 80mg BD, 4 weeks [63]	Safety and tolerability	↓ Blood neutrophil counts
AntiCXCR2 Dose 10mg, 30mg or 50mg, 6 months [64]	↑ FEV1 at 6 months	↔Time to first exacerbation ↓absolute and percent sputum neutrophil counts ↔SGRQ score ↑Rate of respiratory infection
Infliximab; anti-TNF (NCT00244192) 5mg/kg, for 8 weeks N= 22 [65]	↔ Sputum inflammatory cells	↔ FEV1, SGRQ
Etanercept; anti-TNF (NCT 00789997) 50mg, for 90 days N= 81 [66]	↔ FEV1 over 14 days from exacerbation onset	↔ 90 day treatment failure, dyspnoea, health status
Infliximab; TNF (NCT00056264) 3mg/kg or 5mg/kg, 44 weeks. N= 157 [67]	↔ CRQ	↔ FEV1, 6 mins walk test, TDI ↑ Malignancy, pneumonia
CNTO 6785(61); anti-IL-17 (NCT01966549) 6mg/kg every two weeks for 4 weeks then every 4 weeks for remaining 8 weeks N= 186 [68]	↔ pre-BD % predicted FEV1	↔ Post-BD % predicted FEV1 ↔ SGRQ-C ↔ frequency of AECOPD ↔ weekly usage of rescue medication
MEDI 8968; anti-IL-1 (NCT01448850) 300 mg every 4 weeks, 52 weeks N= 160 [69]	↔ Moderate-to-severe exacerbations	↔ SGRQ-C
Canakinumab/ IL-1 (NCT00581945) 1x 1mg/kg, 2x 3mg/kg, , 42x 6mg/kg, 45 weeks [70]	Changes form baseline in FEV1, FVC No statistical analysis provided for changes in FEV1, FVC from baseline	Serious adverse events No statistical analysis provided

Figure Legends

Figure 1. COPD is a heterogeneous complex disease as a consequence of complex host-environment interactions due to multiple environmental exposures over time the host's underlying susceptibility and various host responses at the protein-to-cell and tissue-to-organ scales leading onto the clinical presentation of daily symptoms and exacerbations.

Figure 2. Sampling approaches to study inflammation in COPD illustrating how these approaches in concert provide insights into the host airway and systemic inflammatory response and the local airway ecology

Figure 3. Cytokine networks in a) Neutrophil-associatedinflammasome mediated COPD and b) eosinophil-associated T2-mediated COPD illustrating the immunological responses to the multiple environmental stimuli.

Figure 4. a) Biological cluster analysis of COPD exacerbations derived from multiplex of sputum mediators revealing 4 clusters: T2-mediated eosinophilic inflammation, T1-mediated viral associated, Inflammasome mediated bacteria associated neutrophil associated and pauci-inflammatory without evidence of increased airway inflammation. Ellipsoid size is reflective of the number of patients in each cluster. b) Principal component analysis of biological clusters derived from subjects with asthma and COPD illustrating that the viral, bacterial and eosinophilic clusters are present in asthma and COPD exacerbations with different proportions represented in each cluster for each disease.

Figure 5. Forest-plot of the effect of mepolizumab versus placebo in severe asthma derived from the MENSA trial and in COPD from the METREX and METREO trials illustrating the greater reduction in exacerbations in asthma versus COPD for the same blood eosinophil counts

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Airway inflammation in COPD- progress to precision medicine

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Summary

Chronic obstructive pulmonary disease (COPD) is a significant cause of morbidity and mortality worldwide and its prevalence is increasing. Airway inflammation is a consistent feature of COPD and is implicated in the pathogenesis and progression of COPD, but anti-inflammatory therapy is not first line treatment. This inflammation has many guises and phenotyping this heterogeneity has revealed different patterns. Neutrophil-associated COPD with activation of the inflammasome, T1 and T17 immunity is the most common phenotype with eosinophil-associated T2-mediated immunity in a minority and autoimmunity observed in more severe disease. Biomarkers have enabled targeted anti-inflammatory strategies and revealed that corticosteroids are most effective in those with evidence of eosinophilic inflammation. Whereas in contrast to severe asthma response to anti-IL5 biologics in COPD has been disappointing with smaller benefits for the same intensity of eosinophilic inflammation questioning its role in COPD. Biological therapies beyond T2-mediated inflammation have not demonstrated benefit and in some cases increased risk of infection suggesting that neutrophilic inflammation and inflammasome activation might be largely driven by bacterial colonisation and dysbiosis. Herein we shall describe current and future biomarker approaches to assess inflammation in COPD and how this might reveal tractable approaches to precision medicine and unmask important host-environment interactions leading to airway inflammation.

Key words: COPD, ACOS, eosinophil, neutrophil, macrophage, inflammasome, biologics, eosinophil, interleukin (IL)5, benralizumab, mepolizumab, tumour necrosis factor (TNF)α, IL6, IL8, IL1β, IL33, IL13, IL17, anti-IgE antibody, thymic stromal lymphopoietin (TSLP), prostaglandin D_2 receptor type 2 (DP2)

Take home message

Airway inflammation drives COPD, but corticosteroids only work in those with eosinophilic inflammation. There is a need to better understand the patterns of inflammation, the reason for its persistence and the opportunities for new treatments.

Introduction

Chronic obstructive pulmonary disease (COPD) is a common disease of chronic lung inflammation that results in persistent symptoms and fixed airflow obstruction [1]. This is caused by an inflammatory response following inhalation of cigarette smoke or other noxious external particles such as air pollution and biomass fuel [1]. Airway and systemic inflammation in COPD is related to disease progression and mortality [1-24]. Current diagnostic criteria do not capture the heterogeneity of COPD in terms of the complex pathological changes occurring within lung, the different airway inflammatory patterns or the airway microbial ecology. Airway inflammation is a consistent feature of COPD and is present in both the large and small airways [1, 3-65-8]. The airway inflammation can persist after smoking cessation and is likely a consequence of altered immunity [68] and changes in the airway microenvironment [8-109-14].

Despite the long-standing recognition that airways inflammation is a key driver of COPD progression and exacerbations, first-line treatment strategies are aimed at symptomatic treatment of bronchoconstriction in the form of bronchodilators, rather than anti-inflammatory therapy [1]. In this review we shall describe the heterogeneity of airway inflammation in COPD, current and future biomarker approaches to dissect this heterogeneity and redefine COPD using multi-dimensional phenotyping and how this might reveal tractable approaches to precision medicine and provide important insights into the host-environment interactions.

Multi-dimensional COPD phenotyping providing insights into pathophysiology

COPD is a consequence of complex host-environment interactions that occur over time summarised in **Figure 1**. Smoking, and other pollutants, pathogens and allergens insult the lung promoting airway inflammation and damage in a susceptible host as a consequence of

genetic predisposition and altered immunity [6, 10-128, 15-17]. This in turn leads to irreversible damage resulting in fixed airflow obstruction and the consequent typical symptoms of COPD.

Approaches to phenotyping airway inflammation and damage in COPD

Insights into airway inflammation and damage to the airways have been derived from lung specimens obtained from surgical resection and at post mortem. Importantly *in vivo* measures of airway and systemic inflammation have been characterised longitudinally, at exacerbations and in response to therapies through invasive sampling of the airway by bronchoscopy (large airway brush and biopsy and smaller airways by bronchoalveolar lavage), non-invasive sputum sampling (mostly large airways) which is safe even in severe COPD [138], breath analysis (large and small airways), lung imaging (large airways directly and small airways indirectly) and beyond the lung by assessing upper airway samples and systemically using blood and urine [5, 147, 19] (**Figure 2**).

Neutrophil-associated airway inflammation

The inflammatory response in COPD involves both innate and adaptive immunity with neutrophilic inflammation the commonest inflammatory phenotype in COPD. Following exposure to cigarette smoke, other pollutants, and oxidants there is airway damage [1520] leading to release of pro-inflammatory mediators and damage-associated molecular patterns (DAMPs) such as interleukin (IL)-33 and thymic stromal lymphopoietin (TSLP) [1520-24]. The distribution of the IL-33 receptor ST2 is altered in response to cigarette smoke with down-regulation in innate type-2 innate lymphoid cells with up-regulation by macrophages leading to a triggering of an IL-33-dependent exaggerated pro-inflammatory cascade [1625]. As a consequence of airway damage the altered barrier function predisposes the airway to infection

and bacterial dysbiosis which together with pollutants drive switching of ILC2 cells towards ILC1 cells further amplifying the type-1 inflammatory cascade [17~~26~~]. In COPD there is an increase in the phyla proteobacteria and the emergence of *H. Influenzae* predominance such that the ratio of gammaproteobacteria to firmicutes (γP:F) increase [7-9~~-13~~, 18~~27, 28~~]. These pathogens themselves promote an inflammatory response via activation of pathogen-associated molecular patterns (PAMPs) and further amplification of the airway inflammation with the intensity of airway inflammation related to the abundance of *H. Influenzae* [19, 20~~29,30~~]. In this scenario, epithelial cells are activated and are involved in the release of inflammatory mediators, such as tumor necrosis factor (TNF), IL-1β, IL-6 and IL-8. Macrophages are recruited with further release or pro-inflammatory cytokines and activation of the NLRP3 inflammasome with caspase-1-dependent release of pro-inflammatory IL-1-like cytokines IL-1α, IL-1β, IL-33 and IL-18 [6, 15~~8, 20~~]. Activation of the inflammasome can lead to persistence of an inflammatory response by triggering an auto-inflammatory response with intrinsic production of pro-inflammatory mediators independent of exogenous stimuli [6~~8~~]. Interestingly activation of type 1 responses are more closely related to COPD severity than inflammasome activation and thus autoimmunity can occur across disease severity [2~~3~~1]. Neutrophils are recruited as the predominant cells with consequent release of proteases and airway damage as well as activation of innate lymphoid type 3 cells (ILC3). The adaptive immune response is also involved with polarization and subsequent recruitment of CD4+ Th1 and Th17 cells, which produce IFN-γ and IL-17A and IL-17F [6, 15, 22~~8, 20, 32, 33~~] respectively with a later predominance of CD8+ T-cells. In concert or independent of the auto-inflammatory response there is an auto-immune response which can also promote persistence of inflammation [6~~8~~, ~~34-36~~]. In more severe disease there is an accumulation of B cells particularly in the smaller airways which together with T cells and follicular dendritic cells comprise aggregates organised into tertiary lymphoid follicles [23]. These lymphoid follicles

support the priming and clonal expansion of T and B-cells with an increase proportion of IgA+ B-cells perhaps in response to increased persistent airway infection or auto-antigens [24, 2537]. The cytokine network in neutrophil-associated COPD is summarised in **Figure 3A**.

Eosinophil-associated airway inflammation

Even though neutrophil-associated COPD is the most common inflammatory phenotype, consistent with the heterogeneity of the disease 10-40% of COPD patients demonstrate increased eosinophilic inflammation in the sputum and or blood [5, 26, 277, 38, 39] with increased T2-transcriptome signatures [2840]. The broad range in prevalence is in part due to differences in patient populations but also due to different cut-offs applied in sputum (>2 or >3% eosinophils) or blood (2% or >250, 300, 400 eosinophils/μL). Increased eosinophilic inflammation in peripheral blood and sputum samples in COPD, like asthma, is associated with a greater future risk of severe exacerbations [29, 3039, 41].The aetiology of eosinophilic inflammation in COPD is uncertain. As with neutrophil-associated COPD eosinophilic COPD is also likely to be a combination of innate and adaptive immunity summarised in **Figure 3B**. These pathways are well-described for asthma [5, 307, 42]. Following allergic sensitisation and T-cell polarisation TH2 cells produce interleukin (IL)-4, IL-5, and IL-13. IL-5 is an obligate cytokine for the survival and maturation of eosinophils, and IL-4 and IL-13 promote IgE production from B cells and have direct effects upon structural cells. Recruitment of eosinophils to the lung mucosa is mediated via production of predominantly epithelium-derived CCR3 chemokines and other eosinophil chemoattractants, such as mast cell-derived prostaglandin (PG)D2. PGD2 amplifies T2 immunity via activation of PGD2 type 2 receptors (DP2 or CRTH2). Total IgE is elevated in eosinophilic COPD even though atopy is not increased. Whether this reflects a hitherto undescribed allergen is unclear. Eosinophilic inflammation can also occur via activation of ILC2 cells, which produce IL-5 and IL-13 in

response to PGD2 and the epithelial-derived 'alarmins' IL-33, IL-25, and TSLP released after epithelial damage by pollutants and microbes. Additional contributions might be from macrophage-derived IL33 released following inflammasome activation. Whether these innate and acquired T2-mediated immune mechanisms occur in COPD, the predominance of one over another is more important in COPD versus asthma or whether there are alternative mechanisms driving eosinophilic inflammation in COPD remains unclear.

Biological clustering to dissect heterogeneity of airways inflammation

These eosinophilic versus neutrophilic associated inflammatory profiles represent extreme phenotypes. However they are consistently reproducible and demonstrate phenotype stability [20, 2630, 38]. The neutrophil and eosinophil-associated phenotypes also exhibit distinct microbial ecology with γP:F predominance in the neutrophilic phenotype [8, 9, 3112, 13, 43]. However, to describe extremes can be an over-simplification of a complex underlying biology. To validate these phenotypes and to further inform the understanding of the heterogeneity of COPD in stable state unbiased statistical approaches such as cluster analysis have been applied to large clinical and biological datasets [18, 32, 3328, 44, 45]. Interestingly these have underscored the importance of eosinophilic airway inflammation in asthma, COPD and the asthma-COPD overlap syndrome (ACOS) [32, 3444, 46]. Combined data from asthma and COPD revealed three biological clusters [3244]. 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. 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 colonisation with an increased γP:F ratio in the former and

increased CCL13 in the latter possibly explaining the observed airway inflammation differences seen between these clusters (**Figure 4A**).

Using a similar unbiased cluster analysis approach for COPD exacerbations four biological clusters were identified and these validated the *a priori* aetiological groups: 'Pro-inflammatory' bacterial-associated, 'Th1' viral-associated, 'Th2' eosinophilic-associated and a fourth group that were termed 'pauci-inflammatory' as this was associated with limited changes in the inflammatory profile (**Figure 4B**) [4335]. Disease severity was not different between these biological clusters and the biomarkers were associated with their respective potential aetiologies. In the pro-inflammatory bacterial-associated group the strongest discriminating inflammatory mediator was sputum IL-1β with increased γP:F consistent with bacterial dysbiosis. The blood eosinophil count was the best predictor of sputum eosinophilic inflammation (>3% eosinophils) at the time of the exacerbation in this study although the correlations are typically weaker in stable disease [3547] Interestingly Bafadhel *et al* found that patients experienced more bacterial exacerbations if their stable sputum samples contained more bacteria and high γP:F and more eosinophilic exacerbations if eosinophilic inflammation was present in the stable state suggesting that the exacerbation event was an amplification of the underlying phenotype [3345]. Thus these biomarkers in addition to directing therapy during the exacerbation event might identify subgroups to target therapy in stable state with the aim of reducing future risk. The exception to this was a viral infection representing a new event and a new inflammatory profile with increased blood and sputum concentrations of the interferon-inducible chemokines CXCL10 and CXCL11.

Airway damage and remodelling- emphysema and small airway obliteration

Airway inflammation in COPD contributes to airway damage, remodelling, loss of small airways and emphysema (tissue damage with permanent dilatation distal to the terminal bronchiole). Chronic airflow obstruction is due to a combination of emphysema and small airway obliteration. Small airways are the major site of airway obstruction in COPD [48]. This small airways obliteration is due to a combination of remodelling and accumulation of inflammatory exudates within the airway lumen, both of which increase with disease severity [36, 3748, 49]. Remodelling changes observed in COPD include disruption and loss of epithelial cilia, squamous metaplasia of the respiratory epithelium, goblet cell hyperplasia and mucous gland enlargement, bronchiolar smooth muscle hypertrophy, airway wall fibrosis and inflammatory cell infiltration [36, 3748, 49].

Computed tomography (CT) and micro CT has demonstrated a reduction in the luminal area of terminal bronchioles in COPD, but also substantial loss of terminal airways [38-4050-52]. This is consistent with the view that the inflammation and remodelling of the small airways largely as a consequence of inflammation leads to destruction of the terminal followed by respiratory bronchioles to form centrilobular lesions. This in turn can result in destruction of entire lung lobules which coalesce to form bullous emphysema. Thus narrowing and consequent disappearance of small conducting airways can explain the increased peripheral airway resistance reported in COPD prior to the development of emphysema [38-4050-52]. The distribution of emphysema can be centrilobular or panacinar. It is uncertain if these represent a spectrum with panacinar a consequence of centrilobular emphysema or if they represent distinct conditions. Panacinar is observed in individuals with alpha-1-anti-trypsin deficiency perhaps suggesting this form of emphysema might be largely a consequence of the imbalance between protease and anti-protease activity whereas centrilobular is largely due to loss of and remodelling of small airways caused by persistent airway inflammation.

Quantitative CT has demonstrated that gas trapping due to small airway disease moreover than emphysema is related to lung function impairment [41, 42~~53~~]. These mechanisms of small airway obliteration and emphysema are important when considering anti-inflammatory therapy as only the remaining inflamed airways can be targeted in contrast to the airways and alveoli that are already destroyed in patients with COPD.

Airway inflammation in COPD- progress to precision medicine

Increasing knowledge of disease pathology and inflammatory phenotypes will inform our understanding of COPD and enable phenotype-specific clinical management beyond the first line bronchodilator therapy for COPD.

Eosinophilic COPD- corticosteroids

Corticosteroids have been used in the treatment of COPD for more than 40 years with moderate overall benefit in terms of improvement in lung function, health status, 6 minute walk distance and exacerbation frequency [1]. More recently a differential response in patients has been seen based on eosinophil count. An elevated sputum eosinophil count is associated with a greater response to both inhaled and oral corticosteroids in stable disease [43, 44~~54-56~~], whilst blood eosinophil count can be used to predict response to corticosteroid response in stable [45, 46~~57-59~~] and acute COPD [47~~60-62~~] and titration of corticosteroids directed by sputum eosinophil counts reduces hospital admissions [48~~63~~]. Importantly most of these studies have recruited COPD subjects with frequent exacerbations and thus whether findings can be generalised to all COPD subjects is uncertain. Additionally it is unclear if the clinical benefits, such as lung function and health status, with corticosteroids are independent of the reduction of exacerbations. In contrast non-T2 pathways such as IL-17 activation as determined by the epithelial IL-17A response transcriptome signature are associated with a decreased response to

corticosteroids [49~~64~~]. Whether the benefit from corticosteroids in COPD associated with eosinophilic inflammtion is restricted to its effects upon the eosinophil or due to other broader anti-inflammatory effects is uncertain. GOLD now includes the blood eosinophil count as a biomarker to direct the use of ICS in COPD patients with frequent exacerbations [1]. Benefits in response to roflumilast are also possibly due to attenuation of eosinophilic inflammation [50~~65~~].

Eosinophilic COPD- T2 targeted therapies

Evidence for targeting T2-mediated inflammation using biologics has revolutionised clinical practice in severe asthma [30, 51~~42, 66~~]. As described above significant eosinophilic inflammation does exist in COPD, albeit in a smaller proportion of patients than in asthma. However, the findings from the phase 2 and 3 trials of T2-directed therapies for COPD summarised in **Table 1** have been disappointing compared to asthma [~~42,~~ 52~~66, 67~~].

In the first anti-IL5R biologic (benralizumab) trial in COPD while a reduction in eosinophilic inflammation was observed, the primary outcome annual rate of acute exacerbations was not met, which included all patients with COPD, irrespective of baseline eosinophil count [53~~68~~]. Importantly the sample size was small to study exacerbations and was underpowered to observe small effects. Secondary outcomes showed an improvement in FEV_1 in those receiving benralizumab but no difference was observed in health status. In a pre-specified post hoc analysis improvements in exacerbation frequency, lung function and health status were related to the intensity of baseline blood and sputum eosinophil count. In the yet to be fully reported phase 3 trials of benralizumab in COPD the primary outcome of exacerbations in those with increased blood eosinophil count (≥220 cells/μL) was also not met [54~~69~~]. In a small single centre study mepolizumab reduced sputum eosinophil count, but did not improve lung function

or health status [55~~70~~68]. In two phase 3 trials of mepolizumab in COPD (METREX and METREO) there were small reductions in moderate or severe exacerbations in the eosinophilic sub-group (≥150 cells/μL), which was statistically significant in the METREX (18% reduction) but not in METREO [56~~7~~1]. In a *post hoc* analysis there was no reduction in exacerbation events treated with antibiotics alone in those receiving mepolizumab versus placebo but the reduction in exacerbations treated with oral corticosteroids with or without antibiotics was ~35% in those with blood eosinophil counts >300 eosinophils/μL. No improvements in lung function and health status in those receiving mepolizumab versus placebo were observed.

Importantly, both the mepolizumab and benralizumab studies suggest that the effect size is smaller than that seen in severe asthma (**Figure 5**) although, like asthma, the magnitude of benefit is directly related to the intensity of eosinophilic inflammation [57~~72~~]. The sub-population of COPD patients most likely to respond to anti-IL-5(R) therapy remains unclear, although it is most likely those with a greater disease burden and higher degree of eosinophilic inflammation. Importantly in those with a low blood eosinophil count there was a suggestion of a poorer outcome following treatment with ant-IL5(R) which was not observed in asthma. Whether this reflects a role for the eosinophil in host defence in COPD or the importance of IL-5 in IgA B cell differentiation [58~~73~~] as a possible reason for this adverse effect in the low eosinophil group and an attenuated response in those with the same degree of eosinophilic inflammation as asthma or because the eosinophil is less important in COPD needs to be further explored. However, a small *post hoc* study of the effects of benralizumab upon the airway microbiome from samples obtained in the phase 2a study suggest that benralizumab does not have an adverse effect on the bacterial load or composition [59~~74~~].

Other T2-directed therapies have been tested in COPD or are ongoing. GATA 3 inhibition reduces the sputum eosinophil count in COPD but like ant-IL5 did not affect clinical endpoints [60~~75~~]. A single trial of an anti-IL-13 (Lebrikizumab) has been tested in COPD. The full result of the study is yet to be published but the press release reported that COPD exacerbations were not reduced in those receiving lebrikizumab versus placebo (NCT02546700). In phase 3 studies for asthma, anti-IL-13 [51~~66~~] failed to meet their primary outcome for reduction in exacerbations, whereas in contrast anti-IL4Rα substantially reduced exacerbations. Whether anti-IL4Rα has efficacy in COPD is currently being tested. The role of the DAMPs thymic stromal lymphopoietin (TSLP) and IL33 are also being tested in COPD. DP2 antagonism in COPD reduced the intensity of eosinophilic inflammation [61~~76~~]. Whether DP2 antagonists are beneficial in a subgroup of COPD patients with underlying eosinophilic inflammation requires future studies.

Specific pro-inflammatory and pro-neutrophilic cytokines and chemokines in COPD

While the main inflammatory pathway in COPD is neutrophilic in nature, studies targeting neutrophilic inflammation have been disappointing to date (**Table 2**). The chemokine CXCL8 (IL-8) is known to attract and activate neutrophils during an inflammatory response via the CXC chemokine receptor 1 (CXCR1) and CXCR2. In a small study a monoclonal antibody targeting IL-8 in COPD showed improved dyspnoea measured using the transitional dyspnoea index [62~~77~~]. Anti-CXCR2 demonstrated small improvements in lung function particularly in those who were current smokers but did reduce exacerbations and led to increased infection rates in longer-term follow-up [63, 64~~78, 79~~]. Anti-TNF (infliximab) in COPD showed no improvements in health status, lung function, symptoms nor exacerbation frequency [65-67~~80-82~~]. Importantly, increased adverse events were noted in those receiving infliximab, including cancer and pneumonia [67~~82~~]. Targeting IL-17 with biological therapy has also been

ineffective in COPD [683]. The inflammasome has been targeted with two independent anti-IL-1R1 biologics [69, 7084, 85]. In both trials there were neither benefits nor increased adverse events in those COPD subjects that received the biologic versus placebo.

Thus targeting neutrophilic inflammation, the inflammasome, TNF and IL17 have been ineffective in COPD and in some cases have increased risk of infection. This suggests that intrinsic activation of these pathways driving an auto-inflammatory process is probably less important than their activation secondary to persistent airway colonisation and infection. It remains a possibility that targeting auto-immunity with B-cell targeted biologics could be beneficial in COPD. However, it is more likely that targeting bacterial dysbiosis in stable state and infection at exacerbation events will be more efficacious and will consequently impact upon airway inflammation. Indeed benefits with long-term anti-microbials such as azithromycin might exert their effects largely upon the airway ecology and then ameliorate airway inflammation rather than having substantial direct anti-inflammatory effects [71, 7286, 87].

Future Directions

Our current understanding of the role of different inflammatory phenotypes in COPD demonstrate that the identification of eosinophilic COPD has value in directing the use of corticosteroids in COPD. This fits with the concept of a 'treatable trait' [7388]. This suggests that in some COPD sufferers targeting T2-immunity beyond corticosteroids might have value. However as described above it is not straightforward to extrapolate findings in asthma to COPD and the response to T2-targeted therapies is likely to be different and will need to be carefully tested for each mechanism. Notwithstanding this limitation it would seem likely that this approach will uncover further effective therapies for eosinophilic COPD patients. The impact

on the airway ecology and potential risk of promoting airway infection as observed with non-T2 targeted anti-inflammatory therapies needs to be carefully studied. However eosinophilic associated inflammation remains a minority of patients with COPD, meaning therapies to target other pathways are a priority. Targeting neutrophilic and inflammasome mediated inflammation in COPD does not seem to be an attractive strategy and more attention should be focussed upon trying to normalise the airway ecology either through novel anti-microbials or alternative strategies such as vaccines and phage therapy [74, 7589, 90].

The multi-dimensional phenotyping strategy also suggests that the impact of the airway inflammation might have led to airway and alveoli loss which is then not amenable to anti-inflammatory therapy. This suggests that again in contrast to asthma the degree to which the COPD is reversible in response to anti-inflammatory therapy in established disease is limited. This will require a paradigm shift in identifying disease early and having biomarkers that are predictive of high risk of progression in order to intervene early and change the natural history of the disease. This would be similar to approaches for inflammatory joint diseases and other chronic inflammatory conditions. Genome-wide association studies have revealed multiple genes that are associated with lung function and implicated some genes involved in tissue repair and immunity. Together these genes have formed a genetic risk score for COPD. This risk score needs to be extended to identify genetic risk of disease progression or under-development of full lung function with altered lung function trajectories [7691, 92] and increased likelihood of response to treatment. To date the clinical impact of COPD genetic studies has been limited. However, the genetic risk score together with early disease biomarkers of changes in small airway disease such as oscillometry and imaging which have been extensively validated in the asthma study ATLANTIS [7793] could identify at risk groups. The longitudinal study of airway inflammation- and airway ecology in these at risk groups with 'early' COPD [78] would

help to define mechanism for disease onset and progression such as whether changes in bacterial dysbiosis trigger inflammation and airway damage or a ~~a~~ consequence of these features~~.~~. Improved adoption of current biomarkers into clinical practice and the development of new simple, safe, repeatable and preferably near-patient biomarkers will provide insights of the inflammatory profile in the patient and their airway microenvironment. This will mean that the tests could be done serially to help with clinical decision making in stable state but also predict exacerbation events [79] prior to their onset. Breathomics is a particularly attractive approach with early findings suggesting this could be applied to measure airway and systemic inflammation as well as microbial dysbiosis with pathogen- and inflammatory profile-specific breath signatures beginning to be described [80~~94-96~~]. Urine biomarkers of systemic inflammation are more distant from the lung but with the development of home monitoring strategies for multiple inflammatory mediators coupled to artificial intelligence algorithms to provide risk stratification of future events could become part of clinical care [81~~97, 98~~].

Conclusion

In conclusion, airway inflammation is a consistent feature of COPD and is implicated in the pathogenesis and progression of COPD. Inflammation in COPD is heterogeneous underscoring the need for a precision medicine approach [82]. Corticosteroids are most effective in those with eosinophilic inflammation. Anti-IL5 biologics have been disappointing in COPD versus asthma suggesting that the role of the eosinophil is different in COPD. However, the response to corticosteroids and partial response to anti-IL5 in this group does suggest that it is a tractable phenotype and further studies of mechanism and alternative interventions are warranted. Therapies targeting neutrophilic inflammation and the inflammasome have been ineffective and in some cases increased risk of infection suggesting that their activation might be a consequence of bacterial colonisation and dysbiosis. Underscoring the need to focus on

bacterial dysbiosis as a target to then secondarily attenuate airway inflammation. Therefore to realise anti-inflammatory precision medicine in COPD we need to stop chasing rainbows and improve the characterisation of the disease to reflect the complexity of the multi-dimensional mechanisms driving COPD in individual patients.

Box 1: Key Points

- COPD results from an abnormal inflammatory response which is highly heterogeneous in nature
- Eosinophilic COPD is responsive to corticosteroids and identifies those most likely to respond to T2 targeted biological therapy
- Treatments to target neutrophilic inflammation have failed to show efficacy
- Neutrophilic inflammation is likely to be a response to changes in microbial ecology

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Table 1. Randomised placebo-controlled trials of anti-T2 therapies in COPD

Drug/target; dose and duration; subject number	Primary outcome	Secondary outcome
Benralizumab; anti-IL-5R 100mg every 4 weeks (3 doses) then every 8 weeks (5 doses), 56 week N= 82 [53~~68~~]	↔ Moderate-to-severe exacerbations	↑ FEV1 in intervention group ↔ health status ↓ Blood and sputum eosinophils
Benralizumab (TERRANOVA); anti-IL5R (NCT02155660) 10, 30 or 100mg every 4 weeks (3 doses) then 8 weekly, 48 weeks; N=2255 [54~~69~~]	↔ Exacerbations	Not yet reported
Benralizumab (GALATHEA); anti-IL5R (NCT02138916) 30 or 100mg every 4 weeks (3 doses) then 8 weekly, 48 weeks; N=1656 [54~~69~~]	↔ Exacerbations	Not yet reported
Mepolizumab; anti-IL-5 (NCT01463644) 750mg/month, for 6 months N= 18 [55~~70~~]	↓ Sputum eosinophils	↓ Blood eosinophil ↔ FEV1, CAT, CRQ, exacerbations
Mepolizumab; anti-IL-5 (METREX) (NCT02105961) 100mg or 300mg every 4 weeks, 52 weeks N= 1070 [56~~71~~]	↓ Exacerbations in pre-specified (n= 462) eosinophilic group	↑Time to first exacerbation ↔ FEV1, SGRQ, CAT
Mepolizumab; anti-IL-5 (METREO) (NCT02105948) 100mg or 300mg every 4 weeks, 52 weeks N= 674 [56~~71~~]	↔ Exacerbations	↔ Time to first exacerbation ↔ FEV1, SGRQ, CAT
Anti-GATA3 Inhaled 10 mg SB010 BID 28 days, N=23 [60~~75~~]	Feasibility study	↓ Sputum eosinophil ↔ FEV1, FENO, symptoms

Table 2. Randomised placebo-controlled trials of anti-neutrophil, TNF and inflammasome targeted therapies in COPD

Drug/target; dose and duration; subject number	Primary outcome	Secondary outcome
Anti-IL8; IL-8 (NCT00035828) 800mg loading dose, 400mg/month for 3 months, 5 month follow-up N= 109 [62~~77~~]	↓ Severity of dyspnoea as measured by transition dyspnoea index	↔ Health status, lung function, 6-min walk test, rescue use of albuterol
Anti-CXCR2 50mg BD OR 80mg BD, 4 weeks [63~~78~~]	Safety and tolerability	↓ Blood neutrophil counts
AntiCXCR2 Dose 10mg, 30mg or 50mg, 6 months [64~~79~~]	↑ FEV1 at 6 months	↔Time to first exacerbation ↓absolute and percent sputum neutrophil counts ↔SGRQ score ↑Rate of respiratory infection
Infliximab; anti-TNF (NCT00244192) 5mg/kg, for 8 weeks N= 22 [65~~80~~]	↔ Sputum inflammatory cells	↔ FEV1, SGRQ
Etanercept; anti-TNF (NCT 00789997) 50mg, for 90 days N= 81 [66~~81~~]	↔ FEV1 over 14 days from exacerbation onset	↔ 90 day treatment failure, dyspnoea, health status
Infliximab; TNF (NCT00056264) 3mg/kg or 5mg/kg, 44 weeks. N= 157 [67~~82~~]	↔ CRQ	↔ FEV1, 6 mins walk test, TDI ↑ Malignancy, pneumonia
CNTO 6785(61); anti-IL-17 (NCT01966549) 6mg/kg every two weeks for 4 weeks then every 4 weeks for remaining 8 weeks N= 186 [68~~83~~]	↔ pre-BD % predicted FEV1	↔ Post-BD % predicted FEV1 ↔ SGRQ-C ↔ frequency of AECOPD ↔ weekly usage of rescue medication
MEDI 8968; anti-IL-1 (NCT01448850) 300 mg every 4 weeks, 52 weeks N= 160 [69~~84~~]	↔ Moderate-to-severe exacerbations	↔ SGRQ-C
Canakinumab/ IL-1 (NCT00581945) 1x 1mg/kg, 2x 3mg/kg, , 42x 6mg/kg, 45 weeks [70~~85~~]	Changes form baseline in FEV1, FVC No statistical analysis provided for changes in FEV1, FVC from baseline	Serious adverse events No statistical analysis provided

Figure Legends

Figure 1. COPD is a heterogeneous complex disease as a consequence of complex host-environment interactions due to multiple environmental exposures over time the host's underlying susceptibility and various host responses at the protein-to-cell and tissue-to-organ scales leading onto the clinical presentation of daily symptoms and exacerbations.

Figure 2. Sampling approaches to study inflammation in COPD illustrating how these approaches in concert provide insights into the host airway and systemic inflammatory response and the local airway ecology

Figure 3. Cytokine networks in a) Neutrophil-associatedinflammasome mediated COPD and b) eosinophil-associated T2-mediated COPD illustrating the immunological responses to the multiple environmental stimuli.

Figure 4. a) Biological cluster analysis of COPD exacerbations derived from multiplex of sputum mediators revealing 4 clusters: T2-mediated eosinophilic inflammation, T1-mediated viral associated, Inflammasome mediated bacteria associated neutrophil associated and pauci-inflammatory without evidence of increased airway inflammation. Ellipsoid size is reflective of the number of patients in each cluster. b) Principal component analysis of biological clusters derived from subjects with asthma and COPD illustrating that the viral, bacterial and eosinophilic clusters are present in asthma and COPD exacerbations with different proportions represented in each cluster for each disease.

Figure 5. Forest-plot of the effect of mepolizumab versus placebo in severe asthma derived from the MENSA trial and in COPD from the METREX and METREO trials illustrating the greater reduction in exacerbations in asthma versus COPD for the same blood eosinophil counts

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Figure 1

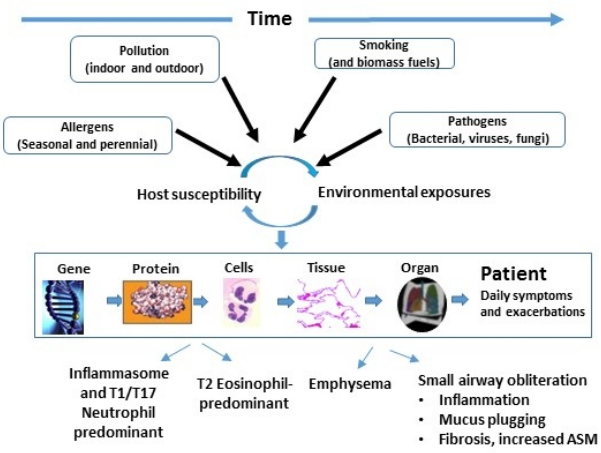

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Figure 2

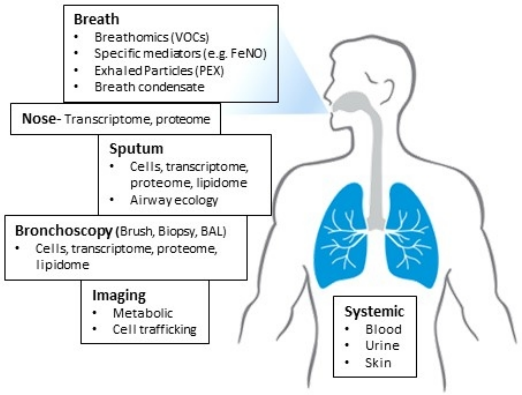

Figure 2

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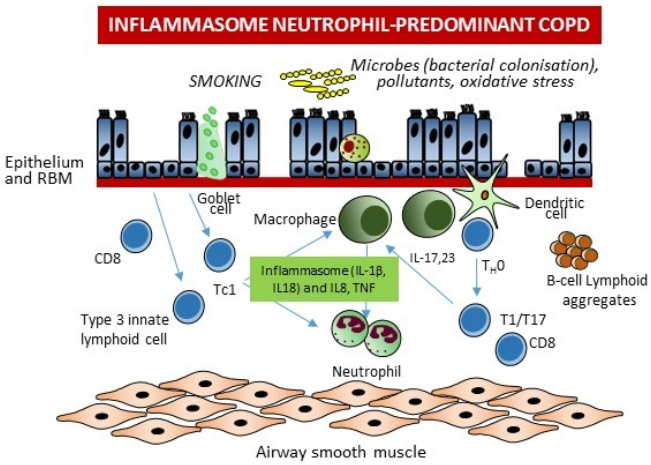
Figure 3A



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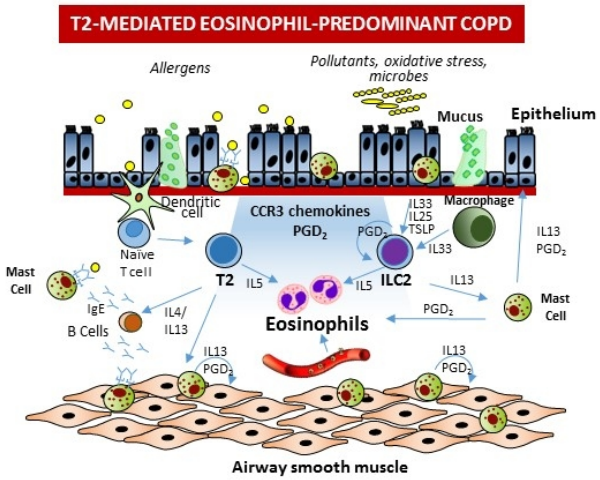

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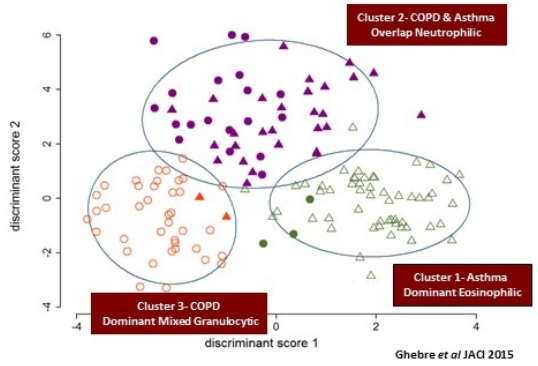

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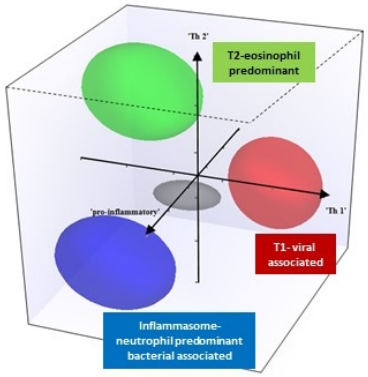

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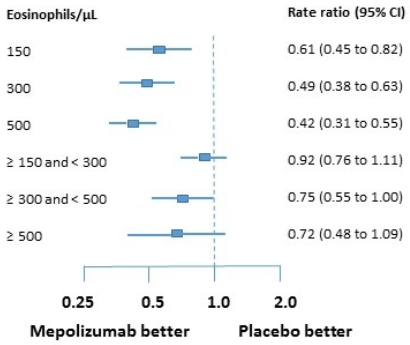
Figure 5



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