Asthma-COPD overlap: current understanding and the utility of experimental models

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Understanding the pathogenesis of asthma-COPD overlap is critical for improving therapeutic approaches. We present current knowledge on asthma-COPD overlap and the requirements for developing an optimal animal model of disease. https://bit.ly/3lsjyvm

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ABSTRACT Pathological features of both asthma and COPD coexist in some patients and this is termed asthma-COPD overlap (ACO). ACO is heterogeneous and patients exhibit various combinations of asthma and COPD features, making it difficult to characterise the underlying pathogenic mechanisms. There are no controlled studies that define effective therapies for ACO, which arises from the lack of international consensus on the definition and diagnostic criteria for ACO, as well as scant in vitro and in vivo data. There remain unmet needs for experimental models of ACO that accurately recapitulate the hallmark features of ACO in patients. The development and interrogation of such models will identify underlying disease-causing mechanisms, as well as enabling the identification of novel therapeutic targets and providing a platform for assessing new ACO therapies. Here, we review the current understanding of the clinical features of ACO and highlight the approaches that are best suited for developing representative experimental models of ACO.

Introduction
Asthma and COPD are prevalent chronic respiratory diseases driven by complex interactions between intrinsic and extrinsic factors that result in a wide spectrum of clinical presentations [1–4]. Despite clear differences in their aetiology and pathophysiology [5, 6], differentiating asthma and COPD at a diagnostic level is often challenging, particularly as analyses of patient spirometry data and inflammatory markers frequently identify obstructive airway disease phenotypes that encompass a mix of asthma and COPD features [7, 8]. Presently, this patient group is referred to as patients with asthma-COPD overlap (ACO) [9, 10] and is estimated to encompass 11.1–61.0% of the 339 million patients with asthma and 4.2–66.0% of the 252 million patients with COPD, worldwide [11, 12]. Individuals with ACO have increased disease severity [13], poorer quality of life [14] and incur higher healthcare costs when compared with patients with asthma or COPD alone [15]. Notably, patients with ACO are excluded from the majority of asthma...
and COPD randomised control trials, which contributes to the lack of efficacy and safety data on effective therapies for patients with ACO. This, in combination with the limited number of specific interventional studies in cohorts of patients with ACO, has resulted in a lack of knowledge of effective treatment strategies.

Here we review ACO as an emerging and distinct category of obstructive airway disease phenotypes and discuss potential avenues for research. We highlight the limitations of using ACO as a diagnostic label and examine alternative approaches for improved therapeutic targeting of specific disease features. Few evidence-based treatments are currently available for patients with ACO due to a shortage of human clinical studies and animal models that can be used to rigorously test the effectiveness of potential therapies. We thereby discuss the requirements for animal models that recapitulate the cardinal features of human ACO and how they may be used to improve the understanding of underlying disease-causing mechanisms, identification of novel therapeutic targets, and development of more effective treatments.

Characteristics of ACO

Chronic airway inflammation is a key component of ACO and is linked to airway remodelling, airflow obstruction, as well as subsequent symptoms of disease including wheezing and dyspnoea that worsen during acute exacerbations [10, 16]. In addition, various cardinal features of mild/moderate allergic asthma (reversible airflow limitation and eosinophilic/type-2 inflammation) and COPD (irreversible airflow limitation and neutrophilic/type-1/-17 inflammation) frequently coalesce in patients with ACO [17]. This is evidenced by findings of overlap clusters in studies that have recruited both asthma and patients with COPD (table 1). Recurring characteristics of the overlap cluster included moderate-to-severe non-fully reversible airflow obstruction, peak flow variability or airway hyperresponsiveness (AHR) and presence of atopy in terms of elevated serum IgE. However, different clusters of patients with ACO demonstrated differences in severity of airflow obstruction, pack-years of smoking, and numbers/percentages of neutrophils and eosinophils in sputum and blood. These phenotypic variations should be validated in larger cohorts to mitigate the effects of sampling techniques, but ACO manifests as a spectrum of clinical and pathophysiological features, which is becoming increasingly apparent given the heterogeneity of both asthma and COPD. This means that patients should not be given homogeneous treatments and that therapies tailored to subgroups/clusters should be prioritised instead. In addition, while certain disease features such as the extent and location of airway remodelling is known to differ between asthma and COPD [26], how they are altered or combined in ACO remain to be explored in depth. Interestingly, in one study where the cluster of patients with ACO was subdivided into patients with ACO-asthma and patients with ACO-COPD based on the initial diagnosis, a higher percentage of patients with ACO-asthma were admitted to emergency departments and intensive care units than patients with ACO-COPD, despite similar spirometry results [25]. This suggests a further stratification of patients who progress from asthma to ACO versus those from COPD to ACO and the differences in their underlying mechanisms is of interest for future studies.

Current definitions and diagnostic criteria

Currently, there is no universally accepted definition of ACO, primarily owing to its heterogeneity. Several national guidelines, as well as the joint document from the Global Initiative for Asthma (GINA) and COPD (GOLD), only provide generalised descriptions of ACO [27–30]. The Spanish Guidelines on the Management of Asthma (GEMA) and COPD (GesEPOC) define ACO as the presence of asthmatic features in conjunction with irreversible airflow obstruction in a smoker or former smoker [31]. While this provides a more selective framework for designing protocols to trial treatments, it excludes individuals whose COPD originates from other environmental pollutants and fails to acknowledge that many smokers do not develop COPD, therefore smoking history alone should not be the only surrogate marker for COPD. Thus, the optimal definition of ACO should balance sufficient specificity in order to be clinically useful, with a broad range of criteria to ensure potentially important groups are not inappropriately excluded. However, and as discussed by others, this may be unachievable as ACO likely encompasses a wide spectrum of phenotypes, e.g. patients with COPD with eosinophilia and partially reversible airflow limitation versus patients with severe asthma with neutrophilia and fixed airflow limitation, or elderly nonsmokers with long-standing asthma and irreversible airflow limitation [32–35]. Therefore, in order to progress our understanding of ACO, and to develop optimal treatment strategies, there is an urgent need for new markers and/or classification criteria that enable additional sub-categorisation of patients with ACO. In the interim, ACO studies may benefit from stratifying patients based on the severity of their airflow limitation, similar to the stages of COPD (GOLD initiative guidelines), as a means of optimising treatment levels and monitoring outcomes.

For diagnosis, GINA/GOLD guidelines recommend a checklist approach to identify concurrent asthma and COPD features [27]. In contrast, GEMA/GesEPOC proposed stepwise approaches requiring an initial
| Subjects                                                                 | Total identified clusters | Overlap cluster | Overlap cluster characteristics                                                                                                                                                                                                 | First author [ref.] |
|--------------------------------------------------------------------------|---------------------------|-----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|
| 27 asthma and 22 COPD patients                                          | 4                         | 5 (10.2)        | Severe airflow obstruction Greatest post-bronchodilator reversibility † Sputum eosinophil % and † serum IgE compared with COPD cluster but lower than asthma cluster                                                                                                                      | WARDLAW [18]       |
| 175 participants: Wellington Respiratory Survey                           | 5                         | 15 (8.6)        | Severe airflow obstruction Greatest post-bronchodilator reversibility and peak flow variability † Pack-years smoking history † Serum IgE and peripheral blood eosinophils and high prevalence of eczema and rhinitis Chronic sputum production † Prevalence of macroscopic emphysema | WEATHERALL [19]    |
| 191 patients ≥60 years of age (predominantly male smokers) with obstructive airway diseases | 3                         | 59 (30.9)       | Intermediate airflow obstruction and emphysema compared with the other two clusters Greatest post-bronchodilator reversibility † Serum IgE                                                                                                                                                     | JI [20]            |
| 389 subjects with symptoms of wheeze and breathlessness in last 12 months | 5                         | 34 (8.7)        | Severe airflow obstruction High post-bronchodilator reversibility and peak flow variability ↓ FENO † Serum IgE High pack-years smoking history † Neutrophil count and ↓ eosinophil count in blood                                                                                             | FINGLETON [21]     |
| 86 patients with severe asthma and 75 patients with moderate-to-severe COPD | 3                         | 47 (27.4)       | ↑ Neutrophil count and ↓ eosinophil count in sputum ↑ Sputum IL-1β, IL-8, IL-10 and TNF-α ↑ Sputum bacterial load in patients with COPD                                                                                                                                               | GHEBRE [22]        |
| 191 patients with doctor-diagnosed asthma or COPD                       | 4                         | 58 (30.4)       | ↑ Blood eosinophil count Low pack-years smoking history High percentage of positive sensitisation to aeroallergens ↑ Post-bronchodilator reversibility compared with COPD clusters Intermediate airflow obstruction compared with asthma and COPD clusters                                      | ROOTMENSEN [23]    |
| 152 patients with asthma and 50 patients with COPD                       | 4                         | 50 (24.8)       | High pack-years smoking history Airflow obstruction intermediate to COPD and asthma clusters High prevalence of atopy and ↑ serum IgE, ↑ peripheral blood eosinophils and ↑ FENO                                                                                           | HIRAI [24]         |
| 877 patients with asthma and 228 patients with COPD: French national Bronchial Obstruction and Asthma Cohort | 3                         | 386 (34.9)      | Worse airflow obstruction than asthma cluster but better compared with COPD cluster ↑ Post-bronchodilator reversibility                                                                                                                                                              | BOURDIN [25]       |

Data are presented as n (%), unless otherwise stated. Ig: immunoglobulin; IL: interleukin; TNF-α: tumour necrosis factor-α; FENO: fractional exhaled nitric oxide.
diagnosis of COPD (aged >35 years, history of smoking, and post-bronchodilator forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) <0.70), followed by either a diagnosis of asthma or demonstration of a type-2 inflammatory profile [31, 36]. Alternatively, and as previously reviewed [10], multiple diagnostic proposals have utilised a combination of major and minor criteria that describe essential primary and secondary disease features [37–41]. A recent study incorporated elements of this approach by tracking features such as persistent airflow limitation, 40 years of age or older, a history of asthma during early life, and significant smoke exposure (≥10 pack-years) [40]. Importantly, there remains no global consensus on appropriate diagnostic criteria for ACO, and the implementation of different criteria among epidemiologic studies has produced significant variations in reported statistics for disease prevalence and outcomes [12, 42, 43]. These limitations are intrinsic to the conventional systems of disease labelling, which may be addressed by incorporating elements of the treatable traits strategy to characterise the complexity and heterogeneity of ACO.

**Treatable traits**

The treatable traits concept proposed by *Agusti et al.* [44] promises therapeutic precision using individual pheno/endotypic features, rather than the traditional assignment of disease labels. This multi-dimensional strategy of examining pulmonary, extrapulmonary, and behavioural/psychosocial traits has been trialled in two cross-sectional asthma studies [45, 46], with the results showing that higher numbers of identifiable traits correspond to more severe disease, and that specific traits (such as eosinophilic inflammation) are associated with more frequent exacerbations [45, 46]. While the therapeutic benefits of targeting specific traits remain to be assessed, this label-free approach of delivering precision medicine to heterogeneous obstructive airway diseases may eliminate the need for use of umbrella terms such as ACO [47, 48]. However, full implementation of the treatable traits system in primary and specialised care settings requires the rigorous process of generating a high-quality evidence base. One way forward is to trial the incorporation of elements of the treatable traits approach into the current clinical practise in order to improve diagnostic resolution/sensitivity of existing methods, while retaining indispensable elements of the current system *i.e.* spirometry. There are strong arguments for the continued use of the descriptor ACO in the interim: 1) distinguish patients with overlapping features from classic asthma and patients with COPD; and 2) promote human and animal studies of the overlap to elucidate underlying disease mechanisms and identify the most effective treatment strategies.

**Biomarkers**

Biomarkers are a key component of the treatable traits concept, and are increasingly utilised in assessing treatment options as well as in the diagnosis and monitoring of obstructive airway diseases [44, 49]. Biomarkers including fractional exhaled nitric oxide [38, 41], eosinophil counts in peripheral blood [39–41] or sputum [37, 38, 41] and serum IgE levels [37–39, 41] have been investigated across different cohorts of patients with ACO. Blood eosinophil counts have been identified as a predictor of response to inhaled corticosteroid (ICS) treatment in COPD [50–52]. Consequently, the 2019 GOLD guidelines recommend ICS treatment for patients with COPD with blood eosinophil count ≥300 cells·µL⁻¹ (and consider ICS treatment for 100–300 cells·µL⁻¹); however, blood eosinophil count data needs to take into account exacerbation severity and frequency for accurate interpretation [6]. Recently, *Shibai et al.* [53] reported that serum periostin, associated with type-2 eosinophilic inflammation, is significantly elevated in ACO-asthma, whereas YKL-40, a chitinase-like protein involved in airway inflammation and remodelling [54, 55], is significantly elevated in patients with ACO-COPD [56]. However, conflicting data for the expression of periostin [57, 58] or YKL-40 [59] in patients with ACO necessitates further studies to clarify their sensitivity/reliability as biomarkers. Finally, increased sputum levels of neutrophil gelatinase-associated lipocalin has shown promise as an ACO-specific marker [60–62]. Taken together, there remains a strong need for interventional studies that rigorously examine the diagnostic and/or therapeutic potential of *bona fide* ACO biomarkers. Such studies should also address current limitations relating to the lack of homogenous treatments in existing ACO studies that may cloud the interpretation and evaluation of biomarkers.

**Endotypes**

Endotypes refer to disease subtypes with specific mechanisms implicated in pathogenesis [63] and are considered to be amenable to precision medicine approaches [44]. This review does not exhaustively evaluate all known endotypes of obstructive airway diseases, but rather highlights areas that are suitable for investigation in future ACO studies. Type-2 responses (*i.e.* IL-4/-5/-13) and eosinophilic inflammation are widely regarded as a critical mechanism underlying certain asthma endotypes [63, 64] and can be suppressed with targeted therapy [65]. A comprehensive panel of candidate genes that define potential asthma endotypes can also be assessed in ACO studies [66]. Interestingly, upregulated expression of asthma-associated type-2 inflammatory genes have been identified in subgroups of patients with COPD and are linked to more severe disease (reviewed in [67]). In addition to alpha-1 antitrypsin deficiency, an
established endotype of COPD, recent evidence identifies eosinophilic COPD as a new disease endotype marked by high sputum, blood or bronchoalveolar lavage fluid (BALF) eosinophil counts and responsiveness to ICS therapy [68]. As the field of endotyping continues to mature, the advent of high-throughput “-omics” technologies (epigenomics, genomics, transcriptomics, proteomics, metabolomics) [69, 70] and their application to respiratory diseases will undoubtedly further improve the resolution of endotyping and clarify the utility of other potential targets including type-17 factors, neutrophilic inflammation [71–75] and the microbiome [76–80]. One promising example from the European Unbiased Biomarkers for the Prediction of Respiratory Diseases Outcomes (U-BIOPRED) cohort has already utilised sputum proteomics and transcriptomics to reproducibly cluster asthma patients and similar strategies can be implemented in future studies of ACO [81].

Risk factors for ACO

The aetiology of ACO most likely involves the interplay between various host and environmental factors, commencing from as early as fetal development and continuing into adulthood [82]. It is likely that ACO development requires innate predisposition including atopy, genetic factors evidenced by family history and newly identified genetic markers of risk (COPDGene studies) and exposure to risk factors, including rhinitis and respiratory infections, as well as exposure to cigarette smoke (CS) or air pollution [10, 82–86] for both asthma and COPD. Airway diseases that occur during early life or as a result of prenatal insults (including maternal smoking, poor nutrition), when lung development and immature pulmonary immunity are vulnerable to external assaults, can lead to impaired lung function in adulthood [87, 88]. Additionally, some patients with asthma may acquire an ACO phenotype by developing irreversible airflow obstruction possibly through progressive airway structural changes and remodelling [89–92]. Likewise, ageing is associated with reduced FEV1/FVC ratio and can contribute to fixed airflow obstruction in elderly asthmatics [93, 94]. Elucidating the genetic determinants of ACO may illuminate disease mechanisms and aid in the early diagnosis of at-risk patients. A genome-wide association study (GWAS) of non-Hispanic White and African American subjects failed to find significant differences in single nucleotide polymorphisms between patients with ACO and patients with COPD [95], and previous GWASs have yet to identify significant overlaps between asthma and COPD loci [96, 97]. Nevertheless, expression of disease-associated genes from the airway epithelium of patients with asthma are enriched among COPD cohorts, and subgroups of patients with COPD also exhibit increased type-2 responses [67]. Additionally, candidate gene studies have identified genes such as ADRB2, GSTM1, GSTP1, IL13, TGFB1 and TNF to be common between asthma and COPD [98], however, their roles in ACO remain to be elucidated.

Current treatments

The 2017 GINA/GOLD ACO guidelines recommend ICS as the first-line pharmacological intervention for the asthma component of ACO [9]. ICS reduce serum IgE and sputum eosinophils, and improves lung function and patient-reported symptom scores in some patients with ACO [21, 99]. One study showed that ICS treatment of patients with ACO in a COPD cohort had no effect on annual FEV1 decline, exacerbation rates or mortality [100]; however, the study design did not include eosinophil counts before/after ICS treatment and therefore may not have captured truly ICS-sensitive groups. Bronchodilators, namely long-acting β2 agonists (LABAs) and muscarinic antagonists (LAMAs), are mainstay treatments for COPD, and are often prescribed in conjunction with ICS to patients retrospectively identified to have ACO [101, 102]. Improved outcomes in terms of FEV1 were reported in patients with ACO versus patients with COPD following 3 months of ICS/LABA treatment [103]. In >250 000 patients with ACO, treatments with ICS/LABA and, interestingly, LAMA monotherapy, were both effective in reducing exacerbation rates [104]. However, there is a need for prospective studies that compare the responses and elucidate the predictors of responses of patients with ACO to combinations of ICS/LABA, ICS/LAMA, and ICS/LABA/LAMA therapies in order to improve current treatment regimens and inform future clinical guidelines.

Acute exacerbations of ACO are managed based on established guidelines for asthma and COPD, which include treatment with short-acting β2-agonists and systemic corticosteroids if symptomatic relief cannot be achieved with the former [105]. Omalizumab, a monoclonal antibody targeting free IgE, significantly improved spirometry outcomes in a small cohort of patients with ACO (n=10) and reduced both eosinophilic (pre-omalizumab 10.36±4.25% to post-omalizumab 2.50±1.38%) and neutrophilic (pre-omalizumab 38.79±14.6% to 29.65±9.7%) airway inflammation [106]. Other prospective therapies including immunomodulatory macrolide antibiotics [107], cytokine inhibitors targeting IL-5, -13, -17 and -25, and blockers of inflammatory pathways have been reviewed elsewhere [10, 108]. While evidence supporting the use of these compounds in patients with ACO are yet to be demonstrated through clinical trials, it is imperative in the meantime that appropriate models/tools are developed to establish the feasibility and validity of the abovementioned therapeutics.
Animal models

Complementary in vivo experiments maximise the translatability of findings from cell lines or primary human tissues and assist in the development of new treatments for patients. In vivo animal models are able to accommodate both local and systemic interactions of multifaceted diseases including ACO and thus are essential for: 1) investigating the complex interplay between different molecular pathways; and 2) designing and testing preventative strategies and drug treatments. So far, asthma and COPD have been widely modelled in numerous mammal species including rats, mice, guinea pigs, dogs and monkeys [109, 110]. Of these, mouse models have been critical for our understanding of disease-causing processes, identification of potential therapeutic targets and the development of novel biologics (e.g. anti-IL-5, anti-IL-13, anti-PD-1, anti-PD-L1) [65, 73, 111, 112]. While larger animals can accommodate human-comparable spirometry and drug-delivery procedures [113], smaller animals (e.g. mice) are advantageous in terms of lower costs for procurement, housing and rearing, as well as the abundance of deficient and transgenic strains and immunological tools, thereby making them a valuable starting point for modelling ACO.

BALB/c and C57BL/6 mice strains have intrinsic type-2- and type-1-biased immune responses, respectively [114–116]. BALB/c mice are more commonly used for allergic asthma models [117–121] due to their robust type-2 responses with increased expressions of IL-4, -5, and -13 [122]. In several asthma studies comparing BALB/c and C57BL/6 strains, levels of serum IgE were generally higher in BALB/c mice but eosinophil levels in BALF were comparable and sometimes higher in C57BL/6 mice [122–125]. Furthermore, there is a propensity for eosinophils to localise around the airways in BALB/c mice and be more evenly distributed in the lungs of C57BL/6 mice [125, 126]. Despite these differences, long-term CS exposure (experimental COPD) has repeatedly produced comparable features in both strains in terms of recapitulating the hallmarks of human COPD. These include increased cell infiltrates in the lung parenchyma, tissue remodelling, emphysematous destruction and impaired lung function [127–133].

An approach to modelling the coexistence of asthma and COPD features is to induce both diseases in the same animal. This requires careful consideration of aetiological agents, as well as the order, route and timeframe of exposures. Based on protocols that have separately modelled asthma and COPD (reviewed in [109, 134–136]), experimental allergic asthma is frequently induced using a combination of systemic sensitisation with ovalbumin (OVA) in the presence of an adjuvant (typically aluminium hydroxide) in order to elicit robust type-2 responses, which is followed by inhalation challenge with OVA to recruit the cells to the airways [115, 137, 138], or with environmental allergens such as house dust mite (HDM) antigen (lung local sensitisation and challenge) that induce respiratory disease directly through the airways [139] and result in airway remodelling [120, 140]. Common experimental models for COPD include acute or chronic exposure to CS, particulates, ozone, elastase, and lipopolysaccharide (LPS) [128–130, 133, 141]. Emphysema, a hallmark of COPD characterised by destruction of alveolar walls and airspace enlargement [142], has also been established in strains of transgenic mice as well as models with spontaneous mutations including the Tight Skin (impaired neonatal alveolar septation leading to emphysema) and Klotho mice (airspace enlargement and exaggerated ageing phenotype leading to emphysema-like changes) [143]. To recapitulate acute exacerbations [112], asthma models commonly use higher levels of the sensitising agent during challenge or through secondary respiratory infections and exposure to pollutants [144–146]. By comparison, COPD exacerbations have been modelled using intranasal administrations of LPS or viral and bacterial infections [129, 131, 134]. Therefore, careful consideration of the agents and sequence of exposures employed must be adopted for developing a model of ACO.

Additionally, gene–environment interactions are key contributors to the complexity of both asthma [147] and COPD [148] and are therefore likely important in ACO. Currently, robust gene signatures or GWAS studies of well-defined human ACO cohorts are yet to be obtained; however, genetic susceptibility is likely to contribute to the development of disease. This is critical in determining whether gene/environment interactions are important in the development and progression of ACO. However, interrogation of experimental models will contribute to answering these questions, and we propose that the sequence and duration of exposures and the strain of mice used will be important in translating experimental findings to different cohorts of patients.

Animal studies of ACO

Published animal studies on ACO are scarce, and only one study has explicitly investigated ACO using a murine model [149]. In this study, surfactant protein-D gene deletion in C57BL/6 (SP-D−/−) mice resulted in spontaneous development of emphysema and increased static lung compliance. Subsequent sensitisation and challenge with OVA in these mice significantly increased the expression of the mucin-encoding gene Muc5AC and induced goblet cell hyperplasia. Lower eosinophil counts and IL-5 and -13 levels were also observed in OVA-challenged SP-D−/− mice compared with wild-type mice, which was partially attributed to the antigen-binding properties of SP-D. Notably, increased AHR was observed in allergic SP-D−/− mice.
compared with wild-type mice at a ventilation rate of 120 breaths per min, but not at 100 breaths per min. This frequency-dependent AHR was postulated by the authors to be a mechanism that may distinguish ACO from asthma and COPD. In this study, pathological features of COPD were induced via gene knockout and presented soon after birth, future studies may instead consider inducing disease at a later stage in life to better mirror the course of COPD in people. Additionally, the use of HDM antigen, a prevalent trigger of human allergies, may be given prior to the onset of COPD features in order to confirm the OVA results as well as model the early-onset nature of asthma typically observed in patients (figure 1).

Considerations for future ACO animal models

It has been shown that ACO features, including increased neutrophilic and/or eosinophilic inflammation [150] and irreversible airflow obstruction [151], can be found in patients with severe asthma. Steroid resistance, which contributes to mortality and morbidity in patients with severe asthma, is also induced by CS [152] in a substantial proportion of patients with ACO that currently smoke. These similarities have led to the proposal that ACO represents a form of severe asthma and models of severe asthma could potentially be adapted for studies on ACO [153, 154]. Although caution must be taken to differentiate asthma with fixed airway obstruction from ACO in animal models. Current existing murine models of severe, steroid-resistant asthma [155–157] often involve sensitisation with OVA or HDM followed by the administration of IFN-γ and/or LPS [158] or infections with bacterial (e.g. *Chlamydia* and *Haemophilus*) [159–162] or viral (e.g. influenza or respiratory syncytial virus) [163] agents. These protocols induce steroid resistance via multiple mechanisms including IL-27 and IFN-γ, microRNA responses, miR-21/PTEN/P13K/HDAC2 axis and inflammasome-mediated pathways, all of which can be targeted with inhibitors or attenuated using macrolides [111, 112, 158, 160–162, 164]. Depending on the experimental method, the models demonstrate steroid resistance of key disease features including airway inflammation and AHR, as well as varying degrees of neutrophilic and/or eosinophilic inflammation. Similar observations have been made in HDM-sensitised mice with *Atg5* deletion in CD11c+ cells (with impaired autophagy) [165]. Crucially, these experimental models are reminiscent of several phenotypes of ACO that have been described [108, 166] and provide the impetus to adapt severe asthma models as a viable approach to the study of ACO.

However, a history of cigarette smoking is a key driving factor in the development of persistent airflow limitation in individuals ≥40 with early-life asthma [40]. Thus, incorporating CS exposure into models of experimental ACO is highly appropriate. Studies have investigated mice exposed to both CS and allergens such as OVA, HDM, LPS or cockroach antigen, either concurrently or sequentially (table 2). Acute exposures to allergens and CS promoted increased levels of type-2 cytokines and inflammatory cells in BALF, with the exception of one study where addition of CS exposure for the last 2 weeks of a 5 week HDM model attenuated lung eosinophil levels [168]. Chronic CS exposure (10 weeks) robustly increased neutrophil numbers in the airways [172] and reproduced the pathology of neutrophilic airway inflammation in asthmatic smokers [173]. In addition, histone deacetylase 2 (HDAC2), which is required to mediate the anti-inflammatory effects of glucocorticoids, was found in one model to be reduced by CS exposure and partially restored (to levels of asthma alone) following treatment with the macrolide roxithromycin [172]. Whether HDAC pathways are altered in patients with ACO should therefore be assessed in future studies along with potential of macrolide therapy as an effective therapeutic option for ACO.

Until now, chronic exposures to OVA or HDM have been widely used to study the long-term effects of airway inflammation and remodelling observed in clinical asthma [120, 136, 140, 174]. Therefore, combining the most clinically relevant and representative components from both experimental asthma and CS-induced COPD models will likely generate the most human-relevant models of ACO. One such model developed by MELGERT *et al.* [175] employed male C57BL/6J mice exposed to OVA for 6 months with...
concurrent CS exposure commencing from the third month. While significant type-2 inflammation was observed in the OVA alone group and OVA plus CS group, no significant differences were found between the two groups. This was likely due to insufficient CS exposure as the authors noted that CS-exposed mice failed to develop emphysematous features [175]. Nevertheless, we consider that a model (as proposed in figure 1) that incorporates chronic exposures to HDM through local sensitisation and challenge, and CS staged in a manner that reflects the early- and late-onset nature of human asthma and COPD, respectively, would be best suited as an investigative tool for studies of ACO.

| Strain          | Experimental method                                                                 | Key features of model                                                                 | First author                                                                 |
|-----------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|------------------------------------------------------------------------------|
| BALB/c WT       | 3 weeks of CS [5 days·week⁻¹, 5 cigarettes for 7 min, 4 times·day⁻¹] concurrent with OVA challenge via aerosol for 7 min, 4 times·day⁻¹ | ↑ serum IgE and IgG1<br>↑ eosinophils, lymphocytes and dendritic cells in BALF<br>↑ dendritic cells, goblet cell hyperplasia, CD4+ and CD8+ T-cells in lung tissue<br>↑ CCL17 and IFN-γ in BALF<br>↑ IL-5 in lymph node cell cultures | MOERLOOSE [167] |
| BALB/c WT       | 3 weeks of HDM intranasally [5 days·week⁻¹] followed by 2 weeks of concurrent HDM+CS [5 days·week⁻¹, 12 cigarettes for 50 min, 1 or 2 times·day⁻¹] | ↓ total cell count, mononuclear cells, neutrophils in BALF<br>↓ eosinophils in BALF and lung tissue<br>↓ B-cells and serum IgE<br>↓ VCAM-1 and eotaxin-1<br>No significant differences in airway resistance | BOTELHO [168] |
| C57BL/6 WT and CD44 deficient | 3 weeks of CS [5 days·week⁻¹, 5 cigarettes 3 times·day⁻¹] with HDM intranasally once per week at the end of CS exposure | No significant differences in AHR<br>↑ CD4+ and CD8+ T-cells, and hyaluronic acid in BALF<br>↑ goblet cell metaplasia<br>↑ IL-4, IL-5, IL-13 in lymph node cell culture<br>CD44 deletion resulted in:<br>o ↓ number of eosinophils, dendritic cells, neutrophils, CD4+ and CD8+ T-cells in BALF<br>o ↓ IL-4, IL-5 and IL-13 in lymph node cell culture<br>o ↓ goblet cell metaplasia | KUMAR [169] |
| BALB/c WT       | Triple OVA, cockroach antigen, and HDM intraperitoneally on days 0, 14, and 21 followed by 6 weeks of allergen challenge concurrent with CS [5 days·week⁻¹, 4 cigarettes·day⁻¹] | ↑ total cell count, eosinophils, macrophages, neutrophils, lymphocytes, IL-4 and IL-5; however, all except neutrophils were higher with allergen alone<br>↑ IL-10, IL-12, IL-1β, and CXCL1 in BALF<br>↓ compliance versus control following methacholine challenge, no increase in resistance observed | TILP [170] |
| C57BL/6J WT     | LPS intranasally at days 0 and 14 followed by 10 weeks of CS [6 days·week⁻¹, 9 cigarettes for 2 h, 2 times·day⁻¹] | Impaired lung function<br>↑ total cells counts in BALF (↑ neutrophils, lymphocytes and macrophages)<br>↑ IL-6 and CXCL1 in BALF<br>Goblet cell hyperplasia<br>Bronchial wall thickening | SHU [171] |
| BALB/c WT       | OVA intraperitoneally on days 0 and 14. OVA aerosol administered daily from days 24 to 41 in conjunction to CS [15 cigarettes for 40 min, 2 times·day⁻¹] | ↑ total cell count and % of neutrophils in BALF<br>↑ AHR<br>↑ serum TNF-α and CXCL1<br>↓ HDAC2 but ↑ phosphorylated Akt in lung sections measured using integrated optical density | XIA [172] |

WT: wild-type; OVA: ovalbumin; Ig: immunoglobulin; BALF: bronchoalveolar lavage fluid; IFN: interferon; IL: interleukin; HDM: house dust mite; VCAM-1: vascular cell adhesion molecule-1; TNF: tumour necrosis factor; CXCL1: C-X-C motif chemokine ligand 1; LPS: lipopolysaccharide; AHR: airway hyperresponsiveness; HDAC2: histone deacetylase 2; Akt: protein-kinase B.
Conclusions

ACO has emerged as a significant clinical entity whereby the coexistence of asthma and COPD features increases the disease burden and challenges current diagnostic and therapeutic strategies. Elucidating the underlying mechanisms is challenging because of its complexity and heterogeneity, but may be achieved through alternative approaches such as defining and targeting treatable traits. However, it is essential in the meantime to develop animal models that best reflect the natural progression and pathophysiology of human ACO to improve the current understanding of key disease mechanisms and identify better diagnostic markers as well as targeted treatments. State-of-the-art technologies including “omics”-based technologies will be invaluable in comprehensively characterising the important immune pathways, cell-to-cell interactions and structural and functional changes that occur in the lung during the development and progression of ACO. This will also provide insights into the driving factors of ACO compared with asthma or COPD alone. For these reasons, we anticipate that such models will expedite mechanistic discoveries and be relevant pre-clinical testing platforms for future investigations into the development of more effective and better-targeted therapies.

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