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Natural history and mechanisms of COPD

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ABSTRACT

The natural history of COPD is complex, and the disease is best understood as a syndrome resulting from numerous interacting factors throughout the life cycle with smoking being the strongest inciting feature. Unfortunately, diagnosis is often delayed with several longitudinal cohort studies shedding light on the long ‘preclinical’ period of COPD. It is now accepted that individuals presenting with different COPD phenotypes may experience varying natural history of their disease. This includes its inception, early stages and progression to established disease. Several scenarios regarding lung function course are possible, but it may conceptually be helpful to distinguish between individuals with normal maximally attained lung function in their early adulthood who thereafter experience faster than normal FEV₁ decline, and those who may achieve a lower than normal maximally attained lung function. This may be the main mechanism behind COPD in the latter group, as the decline in FEV₁ during their adult life may be normal or only slightly faster than normal. Regardless of the FEV₁ trajectory, continuous smoking is strongly associated with disease progression, development of structural lung disease and poor prognosis. In developing countries, factors such as exposure to biomass and sequelae after tuberculosis may lead to a more airway-centred COPD phenotype than seen in smokers. Mechanistically, COPD is characterized by a combination of structural and inflammatory changes. It is unlikely that all patients share the same individual or combined mechanisms given the heterogeneity of resultant phenotypes. Lung explants, bronchial biopsies and other tissue studies have revealed important features. At the small airway level, progression of COPD is clinically

imperceptible, and the pathological course of the disease is poorly described. Asthmatic features can further add confusion. However, the small airway epithelium is likely to represent a key focus of the disease, combining impaired subepithelial crosstalk and structural/inflammatory changes. Insufficient resolution of inflammatory processes may facilitate these changes. Pathologically, epithelial metaplasia, inversion of the goblet to ciliated cell ratio, enlargement of the submucosal glands and neutrophil and CD8-T-cell infiltration can be detected. Evidence of type 2 inflammation is gaining interest in the light of new therapeutic agents. Alarmin biology is a promising area that may permit control of inflammation and partial reversal of structural changes in COPD. Here, we review the latest work describing the development and progression of COPD with a focus on lung function trajectories, exacerbations and survival. We also review mechanisms focusing on epithelial changes associated with COPD and lack of resolution characterizing the underlying inflammatory processes.

Key words: airway remodelling, chronic obstructive pulmonary disease, epidemiology, epithelium, lung function, natural history.

INTRODUCTION

The term COPD covers a heterogeneous group of patients, who, despite the common presence of post-bronchodilator airflow limitation, often exhibit different clinical, pathoanatomical and physiological characteristics.¹ Mechanistically and pathologically, COPD is characterized by a combination of structural and inflammatory changes that are reflected, in turn, by the natural history of the disease.

The predominant COPD phenotype is a result of numerous interacting factors of which active smoking is the strongest.² On the other hand, not all smokers develop COPD which suggests that other intrinsic or extrinsic factors are important for the development of clinical disease. At the same time, COPD patients show substantial disease heterogeneity, which by far exceeds the two iconic archetypes of ‘the pink puffer’ and ‘the

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blue bloater' suggested by Dornhorst³ and pictured by Netter more than 50 years ago.³ These have been trans-itioned to the concept of individual treatable traits.⁴

It is increasingly evident that individuals presenting with different COPD phenotypes likely differ regarding the natural history of their COPD, with regards to its inception, early stages and disease progression.^{5,6} In addition, the presence of comorbidities in older age including heart disease, osteoporosis, diabetes and lung cancer often affect the course of COPD in the individual patient.⁷ Early COPD diagnosis⁸ has remained challenging due to small impact of early lung function loss and social and psychological factors on the part of the patient as well as physician delay.⁹ A Canadian population-based study estimated that the lifetime risk of developing COPD, defined as either a hospitalization or an ambulatory care visit with COPD diagnosis, is around 28%.¹⁰ Relatively few cases were diagnosed before the age of 50 years and the cumulative incidence curves for both women and men showed there- after a relatively linear trend until 80 years of age. Danish investigators demonstrated that the average patient starting inhaled medication for COPD was 67.8 years old and was treated for 8.2 years until death occurred at the age of 76.0 years.¹¹

Thus, at the first glance, the course of clinical COPD in most patients seems to span relatively few years. Yet, it has for many years been recognized that the natural history of COPD begins with a long preclinical period spanning many decades. At the time point when diagnosis is established and treatment initiated, many patients already have moderate to severe airflow limita- tion. A recent population-based study from the UK characterized established COPD patients and those at the time of their first inhaled maintenance therapy; the latter were 68.9 years of age with a mean forced expira- tory volume in 1 s (FEV₁) of 63% predicted.¹² Several population-based studies have shown that underdiag- nosis of COPD is substantial.¹³ Although undiagnosed individuals with COPD have lesser symptoms,¹⁴ they exhibit an increased risk of adverse respiratory events and mortality.^{15,16} In fact, many experts consider the late diagnosis of COPD as the most important factor responsible for the poor outcome in a substantial proportion of patients and therefore focus on dis- tinguishing between early and mild disease.⁸

Pathological features and mechanisms of COPD are clinically expressed as progressive loss of lung function with airflow obstruction that is irreversible. Loss of lung function primarily arises from small airways disease (SAD), a key feature of COPD.¹⁷ Bronchiolar remodelling (a structural synonym for SAD) is characterized by luminal obstruction due to mucus, peribronchial fibrosis, structural changes in airway epithelium and immune cell involvement. Emphysema, often described as the destruction of alveolar compartmentalization that is another hallmark of COPD, is believed to be related to cigarette smoke (CS) toxicity, extracellular matrix remodelling and protease-antiprotease disequilibrium.

Numerous comprehensive reviews of the natural his- tory of COPD have been published.¹⁸⁻²¹ Most of these focused on the course of disease, particularly as defined by FEV₁ decline, which has been considered as a physiological surrogate of development and progression

of COPD since the seminal study of Fletcher and Peto.²² In fact, the acceptance that an intervention is able to change the natural history of COPD has in most people's mind implied that such treatment would normalize or substantially reduce FEV₁ decline. In the first section of this review, we therefore aim to update previous reviews with recent insight on FEV₁ trajectories, exacerbations, symptoms and comorbidities. By reviewing the mecha- nisms of COPD, we describe COPD-related changes at the airway epithelium, including advances drawn from the fields of pluripotent stem cell research, disease modelling and, finally, the T1/T2 inflammation paradigm at the epithelial level.

NATURAL HISTORY OF COPD

Methodological aspects and historical overview

Most of the longitudinal studies describing the natural history of COPD have been conducted in the Western societies where active tobacco smoking plays an over- whelming role in the development of disease. However, studies from developing countries have documented that exposure to biomass-based fuels, even in the absence of smoking, is associated with the develop- ment of irreversible airflow limitation.^{23,24} Various occupational or environmental exposures can contrib- ute to the development of COPD²⁵ and irreversible air- flow limitation can be observed in never-smoking adults both with and without apparent asthma.²⁶ In the present review, we will mainly focus on the natural his- tory of smoking-related COPD, as there are limited studies of natural history of COPD among non- smokers.

An important limitation of our knowledge is the lack of sufficient study of participants who have been followed from birth into old age and monitored for the development of symptoms and lung function through the life cycle.⁸ Although participants in some childhood cohort studies have now reached 50-60 years of age,²⁷⁻³⁰ longer observation periods are required to identify individuals developing COPD. Similarly, cohorts of individuals at risk for COPD development should be populated and studied to describe the full picture of the natural history of the disease.

Although early clinical studies of COPD patients described the prognosis of the disease during the 1960s,³¹ our understanding of the natural history of COPD has for many years been influenced by the semi- nal study of Fletcher and Peto.²² This study that was conducted in London followed up male postal workers aged 30-60 years for 8 years from 1961 to 1969 and was originally designed to test the so-called 'British hypoth- esis', which postulated that lung function impairment in COPD is caused by recurrent airway infections. The study refuted the British hypothesis and concluded that rather than the infectious chest episodes, continuous smoking itself led to irreversible airflow limitation by steadily accelerating the rate of decline in FEV₁ in sus- ceptible smokers. Importantly, the study showed that stopping smoking resulted in normalization of the FEV₁ decline towards the rates observed in never-smokers of

similar age. These findings were subsequently confirmed in various population studies recruiting both men and women with a broader age span, including the Tucson Study.³² Simultaneously, another hypothesis on the origins of COPD was formulated by Dutch investigators.³³ This hypothesis was thereafter named as ‘the Dutch hypothesis’ and emphasized the interaction between endogenous factors (genes, age and sex) and environmental factors (allergens, infections, air pollution and smoking) both during childhood and later in life, finally resulting in COPD. The Dutch hypothesis enhanced ongoing discussion regarding the inter-relationship between asthma and COPD.³⁴ Nevertheless, there remains controversy on the clinical and biological implications to the nosological labels of asthma and COPD.³⁵

Additional important data come from general population and occupational cohorts initiated in the 1970s–1980s. These studies, previously reviewed by others,³⁶ have generally confirmed many of the prior findings highlighting the deleterious effects of smoking on FEV₁ decline and the beneficial effects of smoking cessation, as finally confirmed by the Lung Health Study.³⁷ In addition, the presence of respiratory symptoms and recurrent pulmonary infections play an important role by priming children and adolescents to increased susceptibility with regards to the development of COPD later in life.^{28,29,38,39}

Preclinical phase of COPD

Both cross-sectional and longitudinal studies have identified numerous factors associated with the risk of being diagnosed with COPD. These factors can be grouped according to the strength of the association, with active smoking remaining the strongest factor, which during the human life course interacts with the genetic constitution of the individual and extrinsic exposures such as respiratory infections and indoor and outdoor air pollution. The latter approach distinguishes between events and exposures taking place while the lung is still developing until it reaches its peak, factors affecting the so-called plateau phase during the third decade of life where the lung function is believed to be relatively stable and finally factors involved in the decline of lung function during adulthood and old age as illustrated in Figure 1.

Growth of the normal lung starts in utero and continues during childhood and adolescence until the lung structure and function reaches its peak, which is believed to take place at approximately 25 years of age.^{40,41} The concept of the maximally attained level of lung function has been discussed for some decades and received additional interest after demonstration of significant variability of FEV₁ decline.⁴² A sentinel study in the field combined multiple large cohort studies to demonstrate that approximately 50% of individuals in the general population presenting with airflow limitation at the age of 60 years did not experience an accelerated FEV₁ decline, but developed airflow limitation through a lung function trajectory characterized by FEV₁ decline within the normal range, but starting from a below normal lung function at approximately 35 years of age (Fig. 2).⁴³ Thus, maximally attained lung

function in early adulthood can be considered as a sum of genetic, intrinsic and extrinsic factors associated with lung development, before the onset of the inevitable decline during adult life caused by the ageing process itself and exposures that may damage the function of airways and the alveoli.^{20,44} Thus, all other things being equal, the lower the maximally attained lung function in early adulthood, the higher the risk of ending up with poor lung function in old age and the higher risk of developing clinical disease and of premature death.^{21,45,46}

Genetic factors

Genetic predisposition clearly plays a role in COPD susceptibility with family studies and analyses of unrelated individuals suggesting heritability accounting for approximately 30% of variation in risk.⁴⁷ Alpha-1 antitrypsin (A1AT) deficiency is the best described genetic association with COPD, caused by a single mutation in the A1AT gene (SERPINA1).⁴⁸ Alas, it has been estimated that A1AT deficiency accounts for approximately 1% of COPD.⁴⁹ Other than A1AT deficiency, several well-described genes have been associated with lung function and COPD susceptibility, such as Hedgehog-interacting protein⁵⁰ and family with frequency similarity 13 member A.⁵¹ The CHRNA3/CHRNA5/IREB2 region on chromosome 15q25 has been associated with COPD susceptibility.⁵² Several loci have been linked to COPD phenotypes including emphysema.⁵³

Several groups have combined data from multiple genes to define genetic risk scores. Investigators from the MESA Lung and SPIROMICS cohorts defined a genetic risk score that was associated with lower lung function, increased COPD risk, lower lung density, smaller airway lumen and fewer small airways without effect modification by smoking.⁵⁴ The COPD Gene investigative group has associated 20 genetic loci with various COPD phenotypes.⁵⁵ Data from the UK Biobank were used to create a genetic risk score for COPD susceptibility using approximately six alleles; genes involved in development, elastic fibres and epigenetic regulation were particularly evident.⁵⁶ A separate investigative group completed a Genome-Wide association study (GWAS) of cases and controls from the UK Biobank and the International COPD Genetics Consortium to identify 82 loci associated with COPD or lung function.⁵⁷ These genetic risk loci are associated with quantitative imaging measures and comorbidities, while gene-enrichment analysis confirmed the importance of developmental pathways suggesting factors related to early life.⁵⁷ An approach combining multiple COPD risk alleles appears to define the risk of lung function abnormality.⁵⁸

Lung function trajectories in childhood and adolescence

A recent review has described various lung function trajectories during the first part of life that may reduce the maximally attained lung function in early adulthood.⁴⁴ This review concluded that factors such as the presence of asthmatic features, episodes of airway and

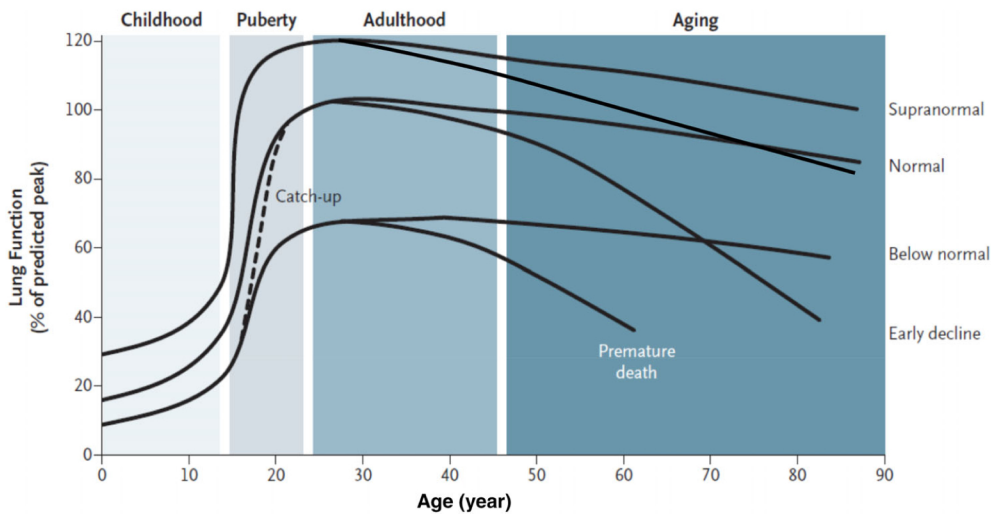


Figure 1 Possible lung function trajectories through life cycle (Note the new trajectory starting from the supranormal one and ending up in the normal one). Reproduced from Agusti and Faner,²⁰ with permission.

Distribution of the individuals in the Four Trajectories

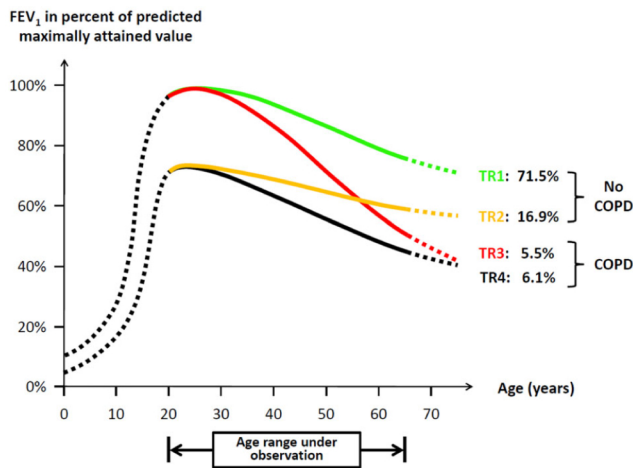


Figure 2 Simplified diagram of forced expiratory volume in 1 s (FEV_1) progression over time and development of COPD according to the two most important trajectories: low maximally attained FEV_1 in early adulthood and normal maximally developed FEV_1 followed by fast decline. —, TR1: normal; —, TR2: small lungs but no COPD; —, TR3: normal initial FEV_1 with rapid decline leading to COPD; —, TR4: small lungs leading to COPD. Reproduced from Lange *et al.*,⁴³ with permission.

lung infections in the first years of life and being born prematurely and/or with low birth weight are of major importance in the Western world.⁴⁴ Outdoor and indoor air pollution and nutritional deficits may also play an important role in developing countries (Table 1).^{44,59,60} An increasing number of recent studies have characterized the growth in lung function in children and adolescents with asthma,^{61,62} whereas other studies with very long follow-up contrasted asthma symptom severity and clinical features in children with asthma followed up to the sixth decade of life.^{29,63,64} These studies suggest that a substantial proportion of individuals with childhood asthma, in particular those with the most severe symptoms, follow below average lung function trajectories which may lead to relatively fixed airflow limitation in adult life.⁶⁵

Interesting questions concern the long-term outcome of low lung function in childhood. First, can this disadvantage in some individuals be compensated by repair processes and/or extra growth during the teenage years, so the maximally attained lung function in early adulthood will end within normal ranges? This is the so-called lung function catch-up. Second, does the presence of lung function impairment in childhood imply a faster than normal lung function decline during adulthood? Studies suggest that several scenarios are possible. Allinson *et al.* examined the interaction between early life exposures during infancy and lung function until age 60–64 years.²⁸ They noted that in never-smokers, the FEV_1 level at the age of 43 years was not significantly related to early life exposures, whereas in ever-smokers these childhood disadvantages associated significantly with clinically important lung function reduction (Fig. 3). This suggests that, at least in some circumstances, the lung can overcome the disadvantages that were present at birth and/or emerged during early childhood, although similar lung function catch-up was not observed in other longitudinal studies, implying that the low FEV_1 may track into adulthood.³⁰ Studies relating early life events to lung function decline in adulthood have also shown diverging results, probably reflecting the heterogeneity of factors related to both lung function growth and decline.⁶⁶ One investigative group suggested that childhood infections were not related to the accelerated lung function decline in adulthood,⁶⁷ whereas a separate group noted that early life disadvantages, including features as parental asthma, parental atopy, childhood asthma, childhood respiratory infections and parental smoking, were associated with both lower lung function and a slightly greater decline in lung function in their early adulthood.⁶⁶

Factors related to progression and an excess decline in FEV_1

The role of smoking

The smoking epidemic, which gained momentum after the invention of the cigarette-rolling machine in 1880, is the main reason for the rise and devastating global consequences of COPD.⁶⁸ Although all but 25–50% of

Table 1 Factors potentially affecting FEV₁ trajectories in childhood and adolescence

| | Low FEV ₁ trajectory | Catch-up |
|--|---|--|
| Host factors | <ul style="list-style-type: none"> • Genetic constitution | |
| Perinatal factors | <ul style="list-style-type: none"> • Prematurity • Low lung function at birth • Maternal smoking • Nutritional deficiencies | <ul style="list-style-type: none"> • Better management of bronchopulmonary dysplasia? • Dietary interventions? |
| Childhood exposures | <ul style="list-style-type: none"> • Respiratory infections • Childhood asthma • Passive smoking • Indoor air pollution including biomass fuels • Outdoor air pollution • Obesity | <ul style="list-style-type: none"> • Relevant treatment of infections and asthma? • Reduction of air pollution • Weight loss |
| Exposures in adolescence and early adulthood | <ul style="list-style-type: none"> • Active smoking • Asthma • Obesity • Passive smoking • Indoor air pollution including biomass fuels • Outdoor air pollution • Occupational exposures | <ul style="list-style-type: none"> • Non-smoking status • Relevant treatment of infections and asthma? • Physical activity and weight loss? • Reduction of air pollution and occupational exposures? |

FEV₁, forced expiratory volume in 1 s.

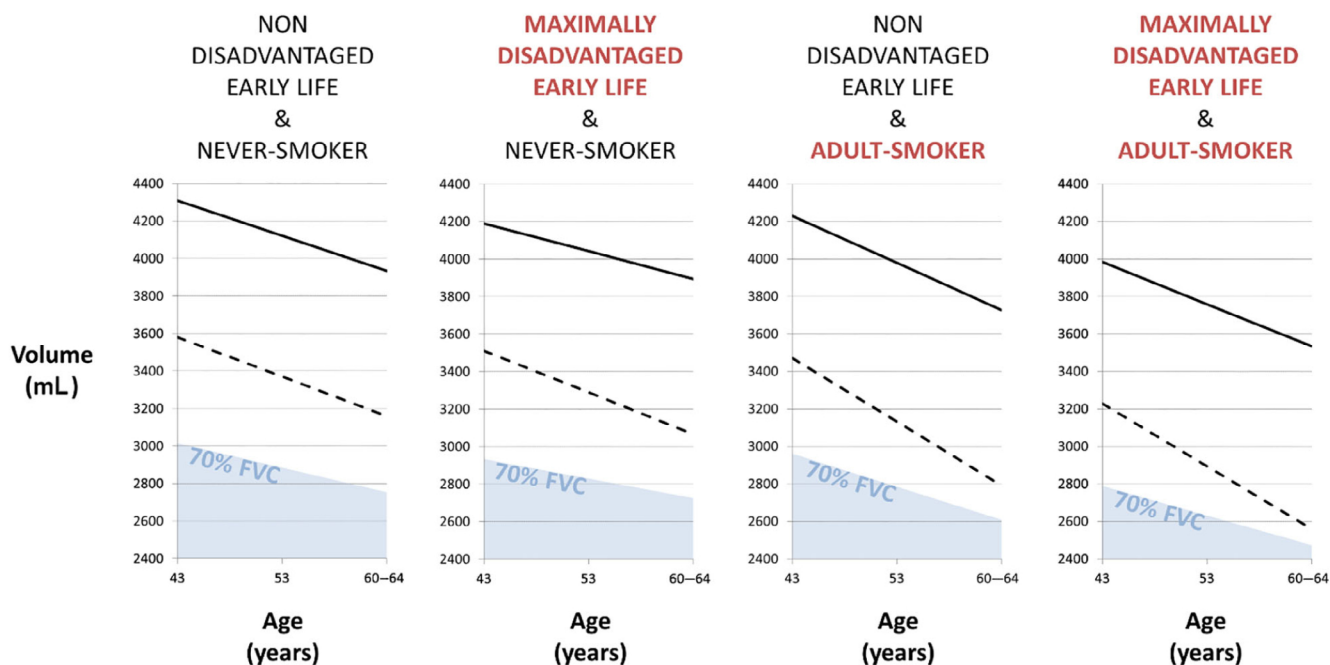


Figure 3 Comparison of estimated pattern of forced expiratory volume in 1 s (FEV₁) (—) decline in relation to forced vital capacity (FVC) (-----) decline between ages 43 and 60–64 years for males of average age 43 years and average birth weight according to adult smoking behaviour and early life disadvantage. Reproduced from Allinson *et al.*,²⁸ with permission.

smokers develop COPD, smoking is the strongest risk factor for COPD and may affect the risk in several ways throughout the life cycle. Maternal smoking during pregnancy can result in low birth weight and premature birth, which is a strong risk factor for underdeveloped lungs at birth.⁶⁹ Maternal smoking during pregnancy has also been associated with low lung function in both

childhood and adulthood, although the latter association seems to be stronger in adults who have been smokers than in never-smokers.^{70,71} Exposure to passive smoking during the first years of life can impair normal lung growth and promote respiratory infections^{29,72} and is associated with higher risk of COPD in adulthood.⁷³ In vulnerable schoolchildren, like Pi heterozygotes for

A1AT, the detrimental effect of environmental tobacco smoke on lung function is even more pronounced than in PiM homozygotes.⁷⁴

Starting smoking at a young age can impair normal lung development and reduce the duration of (and even abolish) the plateau phase resulting in an earlier onset of the decline in lung function.^{40,75-77} In a recent analysis of the Copenhagen General Population Study, the prevalence of early COPD (defined as FEV₁/forced vital capacity (FVC) < lower limit of normal (LLN) in participants younger than 50 years with at least 10 pack-years of tobacco consumption) was 15%.⁷⁸ Active smoking in adulthood may overwhelm the influence of other factors. On a population level, there is a strong relationship between cumulative tobacco exposure and FEV₁ impairment⁷⁹ and persistent smoking is longitudinally very strongly related to the development of COPD.⁸⁰

Smoking cessation leads to reduction of the intensity of lung symptoms and to a small improvement in FEV₁ followed by normalization of subsequent FEV₁ decline.⁸¹ These observations from population or occupational cohorts were supported by the interventional Lung Health Study, which enrolled individuals with mild COPD.³⁷ However, based on mechanistic, clinical and population studies, there has been an ongoing discussion as to what extent factors such as old age and presence of established disease modify the beneficial effects of smoking cessation. Recent analyses of data from six US population-based cohorts show that former smokers also had a slightly higher adjusted decline in FEV₁ than never-smokers, suggesting that all levels of smoking exposure are likely to be associated with lasting and progressive lung damage.⁸²

Biomass-related COPD

In 2009, Salvi and Barnes suggested, based on questionnaire and spirometry measurement from several epidemiologic surveys, that 25–45% of patients with COPD have never smoked.⁸³ Importantly, they highlighted the fact that use of biomass fuel for cooking is an important risk factor in low-income countries, where occupational exposures and sequelae after pulmonary tuberculosis may also play an important role.⁸⁴ The natural history of biomass-induced COPD is not well described and most information comes from cross-sectional studies. A newly published systematic review and meta-analysis has summarized results from 35 such studies comprising more than 73 000 individuals²³ and concluded that exposure to biomass was associated with 2.5–3 times higher risk of both airflow limitation and chronic bronchitis. An elegant recent study noted that biomass-induced COPD, compared to that in cigarette smokers, was generally seen in younger subjects with more equal male–female distribution, affecting predominantly small airways with less emphysema, higher rate of bronchodilator reversibility and hyper-responsiveness, as well as slower rate of decline in lung function.²⁴ A longitudinal study in Mexico followed up adult patients (~90% female) for 15 years and documented that individuals with biomass-associated COPD have a lower FEV₁ but a less pronounced decline in FEV₁ compared to smoking-

associated COPD.⁸⁵ At present, there are no available data from longitudinal studies on how often biomass-induced COPD leads to respiratory failure and death.

Non-smoking COPD in the Western society

Population studies with spirometry measurements show that a substantial proportion of adults with airflow limitation in the Western world are never-smokers. In a study from Northern Sweden, the prevalence of FEV₁/FVC < LLN was 3%, with a prevalence of GOLD stage >2 of 1.3%.⁸⁶ In this study, occupational exposure to dusts, gas and fumes was significantly associated with increased risk of COPD. In a study of the general population in Copenhagen, Denmark, where exposure to occupational pollutants is less than that in the industrial areas of Northern Sweden, approximately 22% of cases of airflow limitation in individuals without asthma were observed among never-smokers.⁸⁷ In this study, never-smokers with COPD had more respiratory symptoms than individuals without COPD and had an increased risk of COPD-related and pneumonia-related hospital admissions, but not an increased risk of death. Contrary to what is seen in smoking-related COPD, the never-smoking individuals did not experience a higher risk of myocardial infarction, ischaemic heart disease or heart failure, suggesting that smoking itself rather than the presence of airflow limitation is a major risk factor for these comorbidities.⁸⁷ In this cohort, never-smokers with COPD did not differ from never-smokers without COPD with regards to exposures to occupational smoking, passive smoking and history of respiratory infections; additional analyses have shown that the majority of them did not have asthma suggesting that other mechanisms were responsible for their airflow limitation.²⁶

Natural history of COPD associated with A1AT deficiency

The natural history of lung disease in individuals with severe A1AT depends strongly on the presence of active smoking. During 1972–1974, all 200 000 Swedish newborn infants were screened for A1AT deficiency and follow-up studies of this cohort have shed light on the natural history of lung disease in individuals with A1AT deficiency. At age 35–40 years, no significant differences were found in lung function between the never-smoking A1AT-deficient and control subjects, whereas PiZZ ever-smokers showed signs of hyperinflation and emphysema, lower post-bronchodilator FEV₁ and poorer median activity score according to the St George's Respiratory Questionnaire (SGRQ) than the PiZZ never-smokers.⁸⁸⁻⁹⁰ In clinical practice, A1AT deficiency is usually diagnosed late (average age of diagnosis was 46 years in the National Heart, Lung, and Blood Institute registry) at the time when both FEV₁ and diffusion capacity were approximating 50% of predicted values.⁹¹ Thus, the progression is faster than in the usual context of smoking-related COPD. Although the decline in FEV₁ did not differ significantly between never- and ex-smokers in an UK study, there was substantial inter-individual variability and the

progression of emphysema assessed by lung density in computed tomography (CT) scans appeared to continue even when FEV₁ remains stable.⁹² Recent work from a large Spanish A1AT registry confirmed great inter-individual variability regarding lung function decline, but reported values comparable to usually observed in COPD.⁹³ Once clinical lung disease has developed survival is poor, whereas it seems that never-smokers with A1AT-deficiency need not have an increased mortality.⁹⁴

Early radiological predictors of COPD progression

CT imaging, particularly quantitative assessment, has revolutionized the assessment of lung structure.⁶ Anatomical tissue validation has confirmed that areas of low lung density reflect reduced alveolar surface area as a measure of emphysema.⁹⁵ This is quite instructive as CT metrics of emphysema relate to symptoms and exacerbations,^{96–98} as well as mortality.⁹⁹ In fact, the simple visual presence of emphysema has been associated with more rapid FEV₁ decline.⁴² An additional technique, parametric response mapping (PRM), pairs inspiratory and expiratory chest CT scans to quantify regional changes in lung density thereby distinguishing regions of ‘normal’ lung from ‘functional SAD’ and ‘emphysema’.¹⁰⁰ Importantly, tissue validation confirms an excellent correlation between small airway pathology and the PRM^{fSAD} metric.¹⁰¹

COPDGene investigators have confirmed that baseline PRM^{fSAD} associates with FEV₁ decline, even among at-risk current and former smokers without airflow limitation (Table 2).¹⁰² A separate investigative group has associated total airway count with an accelerated loss of FEV₁.¹⁰³ Another group has recently demonstrated that the ‘Jacobian determinant’, a measure of local lung expansion and contraction of normal voxels located within 2 mm of emphysematous voxels, was associated with FEV₁ decline.¹⁰⁴ Longitudinal measures of CT-defined lung structural abnormality are being increasingly reported. Accelerated loss of lung function and decreasing lung density are well established in A1AT deficiency.¹⁰⁵ The RAPID study, a 24-month, multicentre, double-blind, randomized, placebo-controlled study of alpha-1 proteinase inhibitor augmentation confirmed that the annual rate of lung density loss was significantly less in patients receiving active therapy compared to those receiving placebo.¹⁰⁶

Limited analyses among COPD patients with GOLD 1–2 spirometric severity suggest that areas of small airway abnormality (PRM^{fSAD}) convert to emphysema (PRM^{emph}) over time.¹⁰⁷

COPDGene investigators also recently demonstrated increasing measures of emphysema and air trapping over 5 years in smokers, with FEV₁ accounting for less than 10% of emphysema progression and less than 50% of air trapping progression.¹⁰⁸ The complexity of progressive imaging changes was also suggested by a separate group who described varying patterns of tissue to airway or vice versa using a subset of the same COPDGene patients; importantly, individuals with early imaging changes were 2.5 times more likely to meet COPD physiological criteria over time.¹⁰⁹

Exacerbations and bronchitis in the natural history of COPD

Exacerbations play an important role in the natural history of COPD by impairing quality of life and contributing significantly to the burden of COPD, both in the individual patient and in the society.¹¹⁰ It is recognized that these events may have multiple causes and are likely to have differing phenotypes and endotypes, including the role of viral or bacterial infections.¹¹¹ The concepts regarding the role of exacerbations for the natural history of COPD have varied throughout the years and have been connected to the presence of chronic bronchitis,²² suggesting its importance to lung function decline, exacerbations and mortality,¹¹² with more recent work suggesting that mucus hypersecretion in both young and middle-aged smokers may represent an early developmental phase of COPD.^{27,113} Recent work in individuals with more advanced airflow obstruction than those studied by Fletcher and Peto has strongly suggested that exacerbations are associated with faster FEV₁ decline, although whether this is the cause or consequence is still controversial.¹¹⁴

Patients with concomitant asthma and COPD

A substantial proportion of patients show characteristics of both asthma and COPD, although the nosology of this overlap remains controversial.^{35,115} The natural history of patients with overlapping features remains unclear, although studies generally agree that frequent exacerbations are characteristic for this group.^{116,117} The Copenhagen City Heart Study followed up lung

Table 2 Association between PRM^{emph} and PRM^{fSAD} with FEV₁ mL/year as a function of GOLD spirometric grade (estimate, 95% CI, P-value)¹⁰²

| | PRM ^{fSAD} | PRM ^{emph} |
|-------------------------------------|--|--|
| GOLD 0 (n = 751) | | |
| Parameter estimate per 5% (mL/year) | –2.2 (95% CI: –4.2 to –0.1; P = 0.04) | 5.5 (95% CI: –8.0 to 19.1; P = 0.42) |
| GOLD 1–4 (n = 757) | | |
| Parameter estimate per 5% (mL/year) | –4.5 (95% CI: –6.3 to –2.6; P < 0.001) | –3.5 (95% CI: –5.6 to –1.4; P = 0.001) |

Reproduced from Bhatt *et al.*,¹⁰² with permission.

FEV₁, forced expiratory volume in 1 s; GOLD, Global Initiative for Obstructive Lung Disease; PRM, parametric response mapping; PRM^{emph}, PRM of emphysema; PRM^{fSAD}, PRM of functional small airway disease.

function trajectories, exacerbations and survival in individuals with asthma-COPD overlap with early-onset (<40 years of age) asthma, and asthma-COPD overlap with late-onset (>40 years of age) asthma.¹¹⁸ Individuals with late-onset asthma experienced FEV₁ decline at a faster rate than in those with early-onset asthma and an increased risk of hospital admissions and reduced life expectancy, which was even more pronounced than in those with COPD without asthma. The better survival of those with COPD with early-onset asthma is keeping with the findings of Burrows *et al.* reported decades earlier.¹¹⁹ Altogether, these findings suggest that COPD evolving from early-onset asthma is a more benign condition than smoking-induced COPD that is complicated by the development of late-onset asthmatic features.

Prognosis of more advanced COPD

In addition to the severity of airflow limitation, a number of different patient characteristics have a negative influence on the prognosis, including the severity of dyspnoea, presence of emphysema, cor pulmonale, frequent exacerbations, presence of comorbidities, low body mass index and low walking distance but also extrinsic factors like poor socio-economic status.^{42,120,121} Continuous fast decline in FEV₁ in elderly patients has been linked to an increased risk of hospital admissions and death.¹²² Recent analyses suggest that the trajectory leading to the development of COPD also has prognostic information. In the Copenhagen City Heart Study, COPD that developed through normal maximally attained FEV₁ trajectory in early adulthood followed by an accelerated FEV₁ decline was associated with an increased risk of both respiratory disease mortality and all-cause mortality compared with COPD that developed through low maximally attained FEV₁ trajectory (Fig. 4).¹²³ Numerous cohorts of established COPD patients have demonstrated very heterogenous disease progression.^{124,125}

In conclusion, several FEV₁ trajectories are seen in individuals with COPD. In the Western world, regardless of the FEV₁ trajectory, continuous smoking is the main factor associated with disease progression, development of emphysema and poor prognosis, whereas the course of COPD in never-smokers is much more benign. In developing countries, factors such as exposure to biomass and sequelae after tuberculosis may lead to a type of COPD with more airway pathology and less emphysema than we see in smokers.

MECHANISMS OF COPD

Insights at the epithelial level

COPD-related changes are found in the airway epithelium and are key to appreciating recent developments in our understanding of disease mechanisms operative in COPD (Table 3). Also, at the epithelial level, important advances have occurred in the fields of pluripotent stem cell research, disease modelling and T1/T2 inflammation paradigms (Fig. 5).

Goblet cells

In healthy subjects, goblet cells are present in human trachea/bronchi and submucosal glands, but normally absent or only sparsely present in the smaller airway generations. However, goblet cells have been found in larger numbers in the small airways of smokers.¹²⁶ In human lung samples, goblet cell metaplasia (GCM) occupied 33% on average of the distal airway epithelia in COPD versus <5% in normal lungs.¹²⁷

Epidermal growth factor receptor (EGFR) immunoreactivity is enriched in regions of increased GCM. Cigarette smoking can promote GCM independent of inflammation, by activating EGFR signalling in airway basal cells (BC).¹²⁸ A number of proteins have been shown to act downstream from inflammatory or EGFR signalling, including SAM Pointed Domain Containing ETS Transcription Factor (SPDEF) or forkhead box protein A2 (FOXA2), with airway epithelial expression of both proteins increased in COPD.¹²⁹ FOXA2 is a transcription factor that represses goblet cell differentiation in the airway epithelium, and aberrant methylation patterns have recently been observed in the FOXA2 promoter in COPD epithelium.¹³⁰ FOXA2 expression is depleted in human COPD airways overexpressing mucin.¹³¹

Interleukin (IL)-13, a pro-inflammatory cytokine, promotes mucus cell hyperplasia and mucin 5 (MUC5) AC expression¹³² in an SPDEF-dependent manner. IL-17 is associated with neutrophilic airway inflammation in severe asthma/COPD exacerbations and can promote GCM via Notch2-dependent signalling in airway BC.¹³³

Rao *et al.* demonstrated that a subset of COPD airway epithelial clones can differentiate into GCM characterized by p63+ BC and SCGB1A1, MUC5AC and MUC5B markers.¹²⁷ This observation concurs with several studies, suggesting that mucus metaplasia arises from metaplastic p63+ BC clones. The authors finely phenotyped a new subset of transient receptor potential (TRP) Ca²⁺ channel 6 (TRPC6+) BC more specifically involved in GCM. In the terminal bronchioles from end-stage COPD patients, TRPC6+ BC represented >50% of all p63+ BC, while less than 2% were found in healthy controls.

Basal cells

BC are assumed to be progenitor cells within the airway epithelium. In COPD, they exhibit a pathological differentiation process, leading to both metaplasia and hyperplasia. Differentiation is driven mainly by EGFR and Notch signalling pathways. CS has a major impact on BC phenotypes,¹³⁴ partly due to epigenetic modifications.¹³⁵ BC numbers are increased in the COPD airway epithelium, but their regeneration capacity is largely decreased.¹³⁶ CS¹³⁷ and EGF/amphiregulin-EGFR signalling play a role in reprogramming BC, with EGF inducing squamous metaplasia.^{128,138}

EGF/EGFR pathway hyperexpression has been found to mediate BC differentiation patterns in the small airways of COPD patients. More specifically, a proximal differentiation pattern arises instead of a distal one, with an accompanying switch in 60% of RNA sequencing (RNAseq) transcripts.¹³⁹ In another study, certain

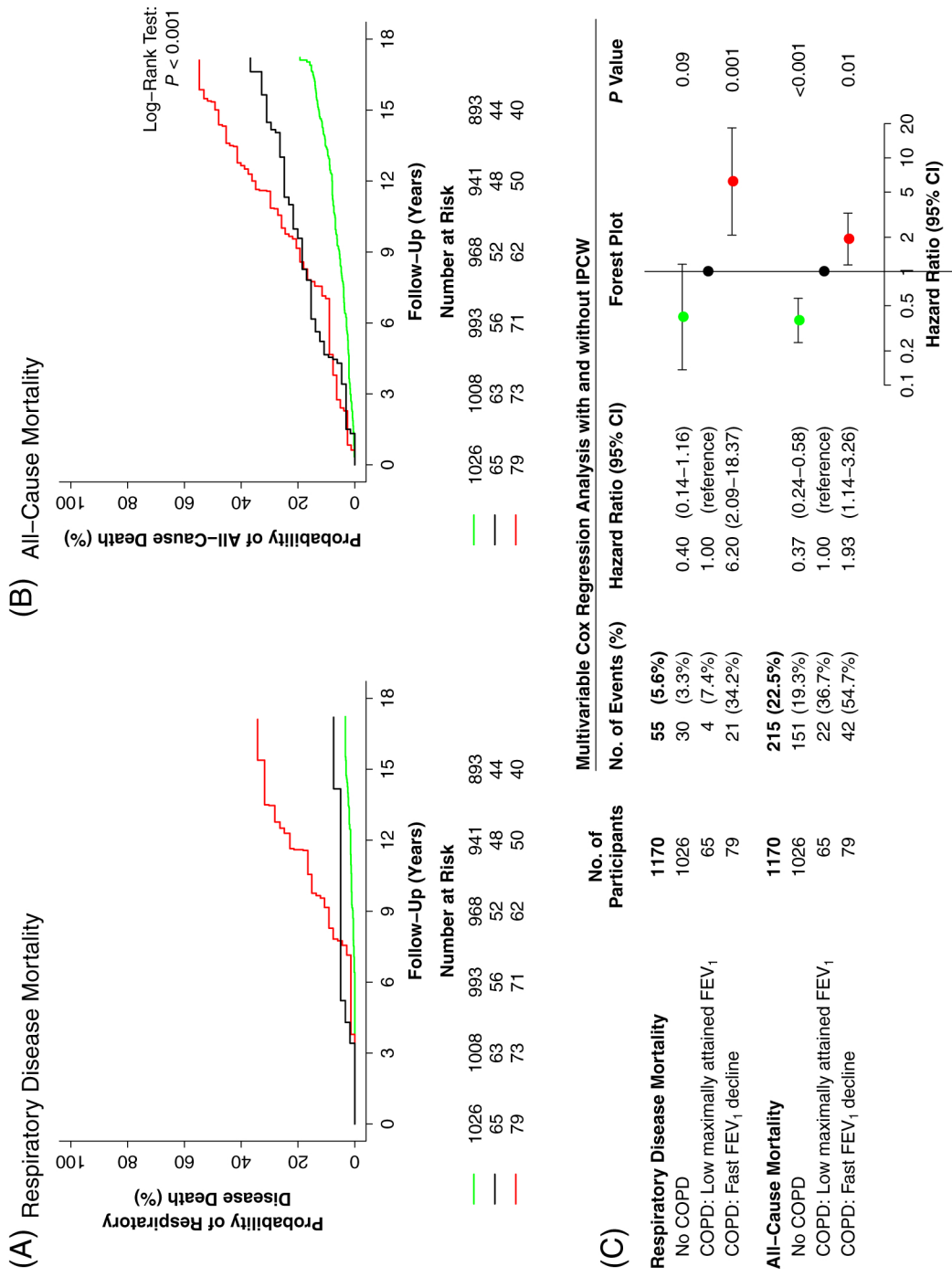


Figure 4 Respiratory disease mortality and all-cause mortality according to normal lung function trajectory and two trajectories leading to COPD: normal maximally attained forced expiratory volume in 1 s (FEV₁) in early adulthood and subnormal maximally attained FEV₁ in early adulthood. (A,B) Inverse Kaplan-Meier curves for respiratory disease mortality and all-cause mortality (—, no COPD; —, COPD: low maximally attained FEV₁; —, COPD: fast FEV₁ decline). (C) Cox proportional hazards regression. Reproduced from Marott *et al.*,¹²³ with permission.

Table 3 Histological, cellular and molecular modifications occurring in the small airways of COPD patients

| Histological phenotype | Cellular modifications | Molecular mediators |
|---|---|--|
| BC hyperplasia/metaplasia More than a unique layer of BC above the basal membrane Squamous cell metaplasia Loss of pseudo-stratification 25% of airways in COPD Peribronchiolar fibrosis | Increase in the number of BC Aberrant BC differentiation into squamous cell involucrin+, KRT13/14 CC loss ECM remodelling Senescence of bronchial fibroblasts Secretory phenotype of fibroblast into myofibroblast vimentin+ Squamous cell metaplasia Epithelial phenotype changes Immune cells | CS: EGFR and Notch signalling Epigenetic reprogramming CXCL8 BC secretion driving neutrophilic inflammation EGFR pathway IL-1 β -derived squamous cell metaplasia \rightarrow activation of integrin α V- β 8 in fibroblasts \rightarrow TGF- β activation in fibroblast Epithelial production of TGF- β 3, GDF15, acting on fibroblasts TGF- β R2 Epithelial growth factors: PDGF-b, TGF, CTGF CS (cadmium) IL-17 from Th17 cells, ILC2, IL-12, dendritic cells EGFR pathway |
| Loss of proximal to distal patterning | Proximal airway epithelium identity in small airways Loss of CC and CCSP secretion Loss of ciliated cells, structural and functional abnormalities | |
| Goblet cell hyperplasia/metaplasia Mucus plugging | Proximal submucosal glands and distal airway secretory cells increase MUC5B plugging Neuronal stimulation of submucosal glands Secretory differentiation fate from metaplastic basal clone cells Immune cells | SPDEF, IL-13-inducing SPDEF, FOXA3, EGFR/amphiregulin activation (through CSE) FOXA2 loss Smad signalling inhibition induced by IL-13/IL-17A IL-17 axis \rightarrow Notch2 activation in BC |
| Epithelial to mesenchymal phenotype of airway epithelium | Mesenchymal signature (vimentin), EMT transcription factors, loss of highly specialized epithelial proteins (E-cadherin, tight junctions) | EGFR signalling TGF- β pathway |
| Emphysema | Apoptosis, cigarette toxicity Protease/anti-protease unbalance in bronchiolar and alveolar epithelium Senescence, exhaustion of AT2 progenitors Immune cells | CS-mediated toxicity Telomere attrition Auto-immunity C1q mediated Th2 inflammation |
| Small airway smooth muscle Mass increase Vascular remodelling Area increase | Hypertrophy, hyperplasia? Increase of proliferation rate Morphological changes of the mucosal micro-vessels, neoangiogenesis? Endothelial activation, leads to immune cells recruitment | NOX4 protein Immune cells VEGF, TGF- β from epithelial cells FGF-2/FGF2-R axis from submucosal glands Epithelial mediators and IL-13 induced V-CAM1 on endothelium |

AT2, alveolar type 2; BC, basal cell; CC, club cell; CCSP, club cell secretory protein; CS, cigarette smoke; CSE, cigarette smoke extract; CTGF, connective tissue growth factor; CXCL8, C-X-C motif-chemokine ligand 8; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EMT, epithelial to mesenchymal transition; FGF-2/FGF2-R, basic fibroblast growth factor 2/receptor; FOXA2/3, forkhead box A2/3; GDF15, growth and differentiation factor 15; IL, interleukin; ILC2, type 2 innate lymphoid cells; KRT13/14, keratin 13/14; MUC5B, mucin 5B; NOX4, NADPH oxidase 4; PDGF-b, platelet-derived growth factor-beta; SPDEF, SAM Pointed Domain Containing ETS Transcription Factor; TGF- β , transforming growth factor β ; TGF- β R2, transforming growth factor receptor β 2; Th17, T-helper type 17; V-CAM1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

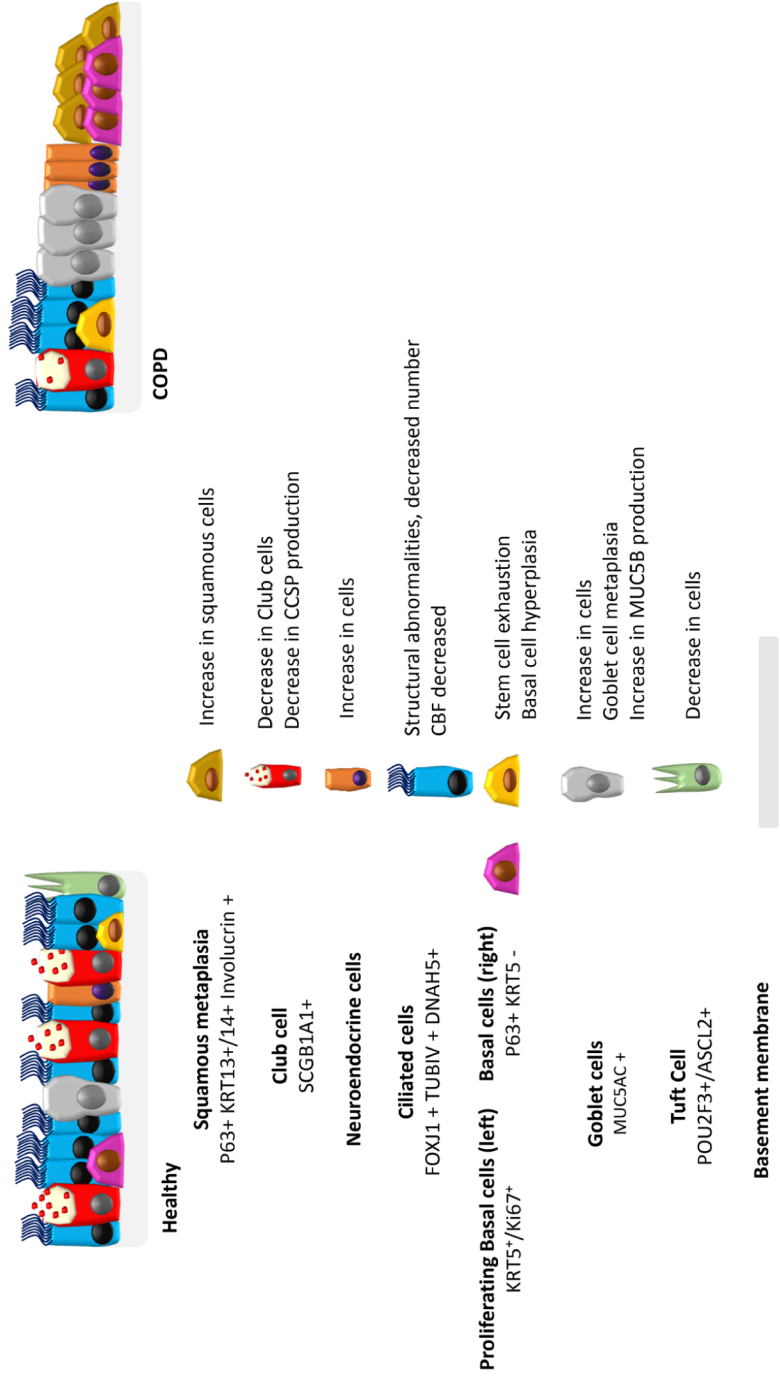


Figure 5 Insight at the cellular level: healthy and COPD airway epithelium. Healthy condition. Ciliated cells represent the most frequent cells; club cells (CC) are also found and secrete club cell secretory protein (CCSP) and play a progenitor role in small airways. The other secretory cell, goblet cells (or mucus cells), are found in smaller proportions and do not exist in the small airway epithelium. Basal cells (BC) are considered as progenitors in the proximal airways and give rise to CC, ciliated cells and also rarer epithelial subtypes such as neuroendocrine cells and tuft cells. COPD. Reprogramming of BC leads to aberrant differentiation with distal to proximal pattern switching in small airways. Proliferating BC are more frequently found in COPD airways, leading to exhaustion of the progenitors. A subclone of BC is able to differentiate in a squamous manner, leading to metaplasia characterized by squamous BC that lose contact with the basal membrane. Squamous cell numbers increase proportionally with the intensity of cumulative smoke exposure in large airways and are able to produce interleukin 8 (IL-8). They are characterized by specific markers such as keratins 13/14, p63 transcription factor and involucrin. Goblet cells are largely increased in both large and small COPD airways. Two regions are described in COPD airway epithelium: (i) the first is described as 'morphologically normal' and characterized by a decrease in SCGB1A1+ MUC5B+/MUC5AC- cells, an increase in MUC5AC+/MUC5B- cells and no change in MUC5B+ MUC5AC+, and (ii) regions described as goblet cell hyperplasia (GCH) with high MUC5AC+ cell density. They produce large amounts of mucin 5B (MUC5B) that contribute to mucus plugs in small airways. CC progenitors are reduced in airways, as well as CCSP secretion, promoting inflammation. Multiciliated cell (MCC) numbers are decreased, and the remaining MCC have impaired functions, such as decreases in cilia beating frequency (CBF), shorter cilia or otherwise abnormal cilia. Rare cell subtypes, such as neuroendocrine cells, are increased and organized into neuroepithelial bodies (NEB), which are highly innervated structures. Tuft cells, however, are largely decreased in COPD.

COPD airway epithelial clone subtypes gave rise to squamous cell metaplasia marked by immature p63 cells and expression of Krt10 and involucrin markers.¹²⁷ These BC also expressed C-X-C motif-chemokine ligand 8 (CXCL8), thereby driving neutrophilic inflammation. The authors demonstrated that squamous metaplasia occupied more than 25% of COPD airway epithelia compared to <1% in normal lungs.

Club cells

Small airways contain specific bronchiolar progenitor cells: the club cells (CC), which are self-renewing, give rise to ciliated cells, and help repair the bronchiolar epithelium.¹⁴⁰ CC numbers are decreased in the small airways of COPD patients.^{141,142}

Ontogeny studies of CC in murine/human small airways demonstrated that they can be derived from a 'suprabasal' cell type (p63-, KRT5+ and SCGB1A1-) that give rise to CC (SGB1A1+/KTR5-MUC5AC-), but spanning a continuous spectrum of cell types, including KRT5+/SCGB1A1+ cells.¹⁴³ This intermediate stage was discovered by single-cell RNAseq analysis. However, a small subset of SCGB1A1+ KRT5- also exist early in cell culture models, suggesting a second population of CC progenitors that do not arise from BC.¹⁴³

Club cell secretory protein (CCSP or SCGB1A1) is the primary protein secreted by CC and has anti-inflammatory/immune functions. Both plasma and airway CCSP protein levels are decreased in COPD,¹⁴⁴ correlating with disease severity and accelerated FEV₁ decline.¹⁴⁵ COPD patients carrying the CCSP G38A polymorphism had lower serum/sputum CCSP levels and a more severe clinical phenotype.¹⁴⁶

In vitro epithelial club cell 10-kDa protein (CC10) gene transduction may inhibit IL-1 β -induced IL-8 expression and nuclear factor κ B (NF- κ B) activation.¹⁴⁷ CCSP may also directly neutralize IL-8, therefore damping neutrophil chemotaxis.¹⁴⁸ Club cell secretory protein-16 (CC16) (-/-) mice are more susceptible to CS effects, with induction of a COPD phenotype characterized by greater emphysema, airway remodelling, increased airway MUC5AC expression, alveolar cell apoptosis and inflammation.¹⁴² Delivering CC16 protein to the airways of CC16-/- mice induced airway immunostaining for CC16 and rescued the development of COPD-like lung lesions¹⁴² via a yet-to-be elucidated mechanism. Intra-tracheal recombinant CC16 supplementation has anti-inflammatory effects in chronic CS murine COPD models.^{142,149} In vitro recombinant CC16 reduced mucus metaplasia induced by CS in airway epithelial cells from COPD patients.^{150,151}

Ciliated cells

Mature multiciliated cells (MCC) arise from FOXJ1 progenitors. Large airway epithelium in COPD, in contrast to controls, has fewer ciliated cells. CS exposure significantly reduced the number of ciliated cells.^{152,153} Ciliary beating frequency was found to be decreased by 25% in nasal cilia from COPD patients.¹⁵⁴ Cilia length is decreased in smokers versus controls, and smokers with COPD had shorter cilia length compared to both groups.¹⁵⁵ Smokers with COPD also had an increased

percentage of abnormal cilia compared to both the healthy smokers and non-smokers.^{156,157}

Deuterosomal cells and deuterosomal mucus cells

Single-cell RNAseq has recently revealed lineage hierarchies in the airway epithelium. Data analysis revealed a specific group of cells, called deuterosomal cells, which are intermediate between secretory and MCC, express FOXJ1 and specific markers such as DEUP1, without expressing the mature proteins found in MCC.¹⁵⁸ A second cell subtype has also been discovered by Ruiz García *et al.*,¹⁵⁸ who identified 'hybrid' cells expressing both MCC and goblet cell markers that give rise to MCC. The role of these cells and their abundance in COPD patient airways has not been elucidated yet.

Alveolar epithelium

The alveolar sacs of the distal lung are lined by two major epithelial subtypes: the alveolar type 1 (AT1) and type 2 (AT2) cells. Alveolar rupture or distortion results in emphysema. CS causes chronic inflammation, oxidative stress and apoptosis of epithelial cells and contributes to emphysematous processes. However, the diverse mechanisms by which the lung fails to repair the CS-induced damage remains unclear. Oxidative stress is thought to be one of the most important mechanisms involved.¹⁵⁹ CS is also known to enhance the degradation of key proteins involved in DNA integrity.¹⁶⁰

AT2 cells are considered to be the stem cells of the alveolar compartments and are responsible for repairing/maintaining lung tissues. Certain AT2 subsets can dysfunction through telomeric attrition, leading to emphysema.^{161,162} Further mechanisms purportedly leading to emphysema include a loss of protease-antiprotease balance in the alveolar compartment. Such a serine protease imbalance has been described in the small airways of COPD patients.¹⁶³ Finally, auto-immune processes resulting in emphysema have also been hypothesized, where loss of complement (C1q) on antigen-presenting cells (APC) leads to the expansion of autoreactive T-helper lymphocytes.¹⁶⁴

Lessons from iPSC biology

In 2006, Takahashi and Yamanaka¹⁶⁵ demonstrated that the transient expression of four transcription factors (OCT3/4, SOX2, KLF4 and C-Myc) in mouse somatic cells using murine retroviruses could reprogram these somatic cells into 'induced pluripotent stem cells' (iPSC). The first human iPSC-derived cell line was obtained in 2007.¹⁶⁶ iPSC are characterized by an infinite proliferation capacity and the ability to differentiate into all cell types from the three germ layers. They share the same characteristics and behaviour as embryonic stem cells.¹⁶⁷ Currently, somatic human cell reprogramming can be performed with small amounts of blood with development of the first iPSC colonies at around 1 month.

iPSC present a unique means of elucidating the biological mechanisms behind COPD and a drug screening tool in the domain of personalized medicine.

iPSC: Modelling the paediatric roots of COPD

Growing evidence supports the impact of early-life events on the occurrence of chronic airway diseases such as asthma and COPD. Toxic exposures in utero (mainly represented by CS), prematurity, childhood respiratory infections and probably genetic background can lead to the occurrence of early-onset and severe phenotypes. This trajectory represents >50% of adulthood COPD, suggesting that COPD may be a developmental lung disease.⁴³

Human iPSC offer a unique opportunity to model this trajectory by not only recapitulating the physiological lung development in vitro for each patient, but also providing a model to evaluate diverse environmental factors as well.¹⁶⁸

In the laboratory, iPSC differentiation into airway epithelium is based on our current knowledge of human and animal lung development and the process mimics specific developmental stages. iPSC are cultured in specific media with specific growth factors (activin A, CHIR99021, SB-noggin and Y-27632) and such timing so as to imitate signals occurring during physiological embryonic development, resulting in iPSC-derived airway epithelial cells. The most important and difficult stage is the generation of progenitors characterized by NKX2.1 factor transcription expression, also known as Thyroid Transcription Factor-1 (TTF-1). These lung progenitors are then differentiated into airway epithelial cells based on physiological signals that drive lung development. The sequence of steps used

for the latter are protocolized in different publications.^{169,170} However, such protocols are heterogeneous and vary with the part of the airway studied (e.g. proximal versus distal airways and alveolus¹⁷¹).

iPSC as a new tool in the field of cell therapy

Cell therapy based on iPSC represents a new potential tool in the field of regenerative medicine. Crucially, iPSC can be specifically developed for a given patient, thus circumventing the cellular rejection observed in allografted tissues and the risk-laden, chronic immunosuppression that is often required. The therapeutic effects of iPSC-derived macrophages in a humanized disease model of hereditary pulmonary alveolar proteinosis in mice have recently been evaluated. Macrophages derived from human iPSC were transplanted in an intra-tracheal manner. Two months after engraftment, iPSC cells displayed the expected morphology/functionality of human alveolar macrophages. The cell therapy led to a significant reduction in alveolar proteinosis.¹⁷²

From bench to bedside: iPSC for the development of new drugs

iPSC represent a tool for drug screening, already used in the field of genetic nervous system diseases.¹⁷³ They can be maintained and produced in an unlimited manner, and genetically manipulated for monogenic disease repair. Their proliferation and longevity make iPSC a key model for screening chemical libraries not only for specific diseases, but also for individual patients in the context of personalized medicine (Fig. 6).

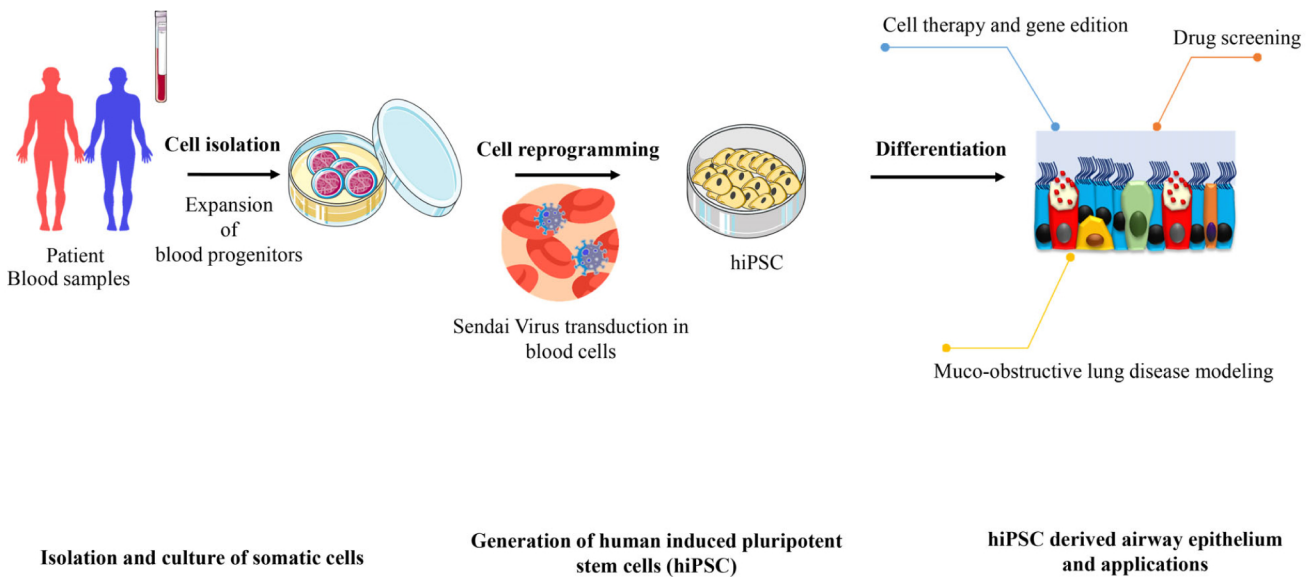


Figure 6 Human induced pluripotent stem cells (hiPSC) in chronic airway diseases. Somatic cell reprogramming (e.g. isolation of circulating progenitors from blood sample) by a non-integrative method (Sendai virus) allows the generation of hiPSC. hiPSC provide a novel means of modelling chronic airway disease by mimicking normal lung development in sequential steps. hiPSC can therefore be differentiated into proximal, distal or alveolar airway epithelium, depending on the cytokine combination used. The large amount of induced pluripotent stem cells (iPSC) in culture that can be maintained virtually indefinitely represents a key tool in drug screening. Drug screening on airway epithelium-derived iPSC from individual patients enables a personalized therapeutic development strategy. Autologous transplantation of airway epithelium or progenitors derived from iPSC can be performed for the patient whose iPSC line has been generated.

Defective resolving mechanisms may govern the T1/T2 paradigm at the airway epithelial level

Type 2 immunity in COPD

We will focus on airway epithelial-derived inflammatory mediators and other mediators that can impact airway epithelial cells (Fig. 7).

Alarmins. Alarmins are molecules released from cells that either receive inflammatory signalling or respond directly to a 'danger' that can damage the cells (e.g. an allergen or virus). Alarmins are released from airway epithelial cells and include IL-33, IL-25 and thymic stromal lymphopoietin (TSLP).

Interleukin-5. IL-5 plays a key role in eosinophilic inflammation. It promotes the differentiation of eosinophils from precursors in the bone marrow and prolongs the survival of eosinophils in the airways. IL-5 also activates eosinophils that then acquire their in situ functions. Eosinophilic inflammation can also occur via the activation of type 2 innate lymphoid cells (ILC2), which produce IL-5 and IL-13 in response to epithelial-derived alarmins.

Data concerning the correlation between blood and lung/bronchial tissue eosinophil infiltration in COPD are contradictory,^{174,175} with at best a poor correlation between blood biomarkers and airway inflammation. Targeting eosinophilic inflammation has been successful in managing severe eosinophilic asthma, but data from COPD studies have not provided encouraging results.¹⁷⁶

Interleukin-4 and -13. IL-4 and IL-13 can promote eosinophilic inflammation through the release of chemoattractants such as CC-chemokine ligand 26 (CCL26) from airway epithelial cells. They stimulate mucus hypersecretion, airway fibrosis and remodelling in asthma.

IL-4 and IL-13 signal through a common receptor, IL-4R α , expressed by airway epithelial cells. Overexpression of either in murine airways induces inflammation, sub-epithelial fibrosis and mucus cell metaplasia.¹⁷⁷ Transgenic IL-13 pulmonary expression in an adult murine model results in a COPD phenotype with inflammation, mucus metaplasia and emphysema.¹⁷⁸ However, therapies targeting IL-4/IL-13 pathways failed to show any improvement in COPD patients.

Thymic stromal lymphopoietin. TSLP is a member of the IL-7 family (produced by epithelial cells) that activates dendritic cells (DCs) to drive the differentiation of naïve lymphocytes into Th2 cells. TSLP also acts on ILC2 as a chemoattractive signal, leading to Th2 cytokine (e.g. IL-4/IL-13) production. TSLP is highly expressed in the airway epithelial cells of patients with severe asthma and COPD and affects eosinophil function.¹⁷⁹ Viruses can induce overproduction of TSLP in COPD epithelial cells,¹⁸⁰ highlighting a further role for TSLP in COPD exacerbations.

TSLP and IL-17A levels were higher in induced sputum supernatants from COPD patients, with TSLP synthesized in an IL-17A-dependent manner in the airway

epithelium. The anticholinergic drug tiotropium significantly decreased airway epithelium TSLP mRNA after exposure to rhIL-17A or COPD-induced sputum supernatants.¹⁸¹ TSLP production in COPD can also be induced by T-helper type 1 (Th1) cytokines, suggesting the existence of a TSLP-mediated negative feedback loop that can limit the magnitude of the inflammatory response to injury.¹⁸² These data suggest that TSLP blocking may impact Th2 and non-Th2 COPD endotypes. A clinical trial is ongoing (ClinicalTrials.gov: NCT04039113).

Interleukin-33. IL-33 is a member of the IL-1 family that mainly drives type 2 inflammation.¹⁸³ In response to inflammation/damage, IL-33 binds its transmembrane receptor suppression of tumourigenicity 2 (ST2), which is expressed by both structural and immune cell types such as ILC2, eosinophils or macrophages. Recent studies have demonstrated that IL-33 can induce haematopoietic progenitor cell differentiation into eosinophils in an IL-5-dependent manner.¹⁸⁴

In COPD, epithelial damage signals (via CS, viral infection or oxidative stress) enhance the expression and release of IL-33 in human airway epithelial cells.¹⁸⁵ Conversely, the inflammatory effects of CS are reduced by anti-IL-33 antibodies.¹⁸⁶ In another study, mice were infected with influenza virus after CS exposure, leading to IL-33 release from epithelial cells and increased viral-induced responses.¹⁸⁷ CS exposure highlighted an IL-33-driven Th1 inflammatory response whose mechanisms remain unclear. The effect does not seem to be ILC2-dependent as CS decreased ST2 expression on ILC2 but enhanced ST2 on other cells, such as natural killer (NK) cells and macrophages.¹⁸⁸

In COPD airway epithelium and endothelium, IL-33 expression is increased at the mRNA and protein levels compared to non-COPD subjects.^{189,190} In another study, IL-33 was increased in plasma from COPD patients and was correlated with eosinophil counts and chronic bronchitis.¹⁹¹ IL-33 is localized in the nucleus of certain basal epithelial progenitor cells in patients with COPD. Interestingly, IL-33 immunostaining was most intense in BC present in regions of airway epithelial hyperplasia and mucus cell remodelling.¹⁸⁹ The suggested role of IL-33 in goblet metaplasia has been shown in an air-liquid interface (ALI) culture model. IL-13 induced ST2 expression on the apical surface of goblet cells. Under IL-13 culture conditions, IL-33 stimulated apical IL-8 release from goblet cells and promoted mucus metaplasia.¹⁹² In COPD, IL-33 responses may switch from a localized, rather short-term, well-controlled danger signal to an uncontrolled, amplified, long-term signal, leading to lung structural destruction. IL-33 blocking is therefore a promising therapeutic avenue for COPD patients and antibodies are now in clinical development.

Non-type 2 immunity in COPD

Non-type 2-driven inflammation is usually insensitive to corticosteroids. Epithelial cells are activated by CS and other inhaled irritants to produce inflammatory mediators, including tumour necrosis factor (TNF)- α , IL-1 β , IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-8.¹⁹³ Epithelial cells

in the small airways express transforming growth factor- β (TGF- β), which is implicated in small airway fibrosis.

T-helper type 1 axis. Neutrophils are the predominant cells in Th1 inflammation. They release several proteases purportedly involved in lung structural

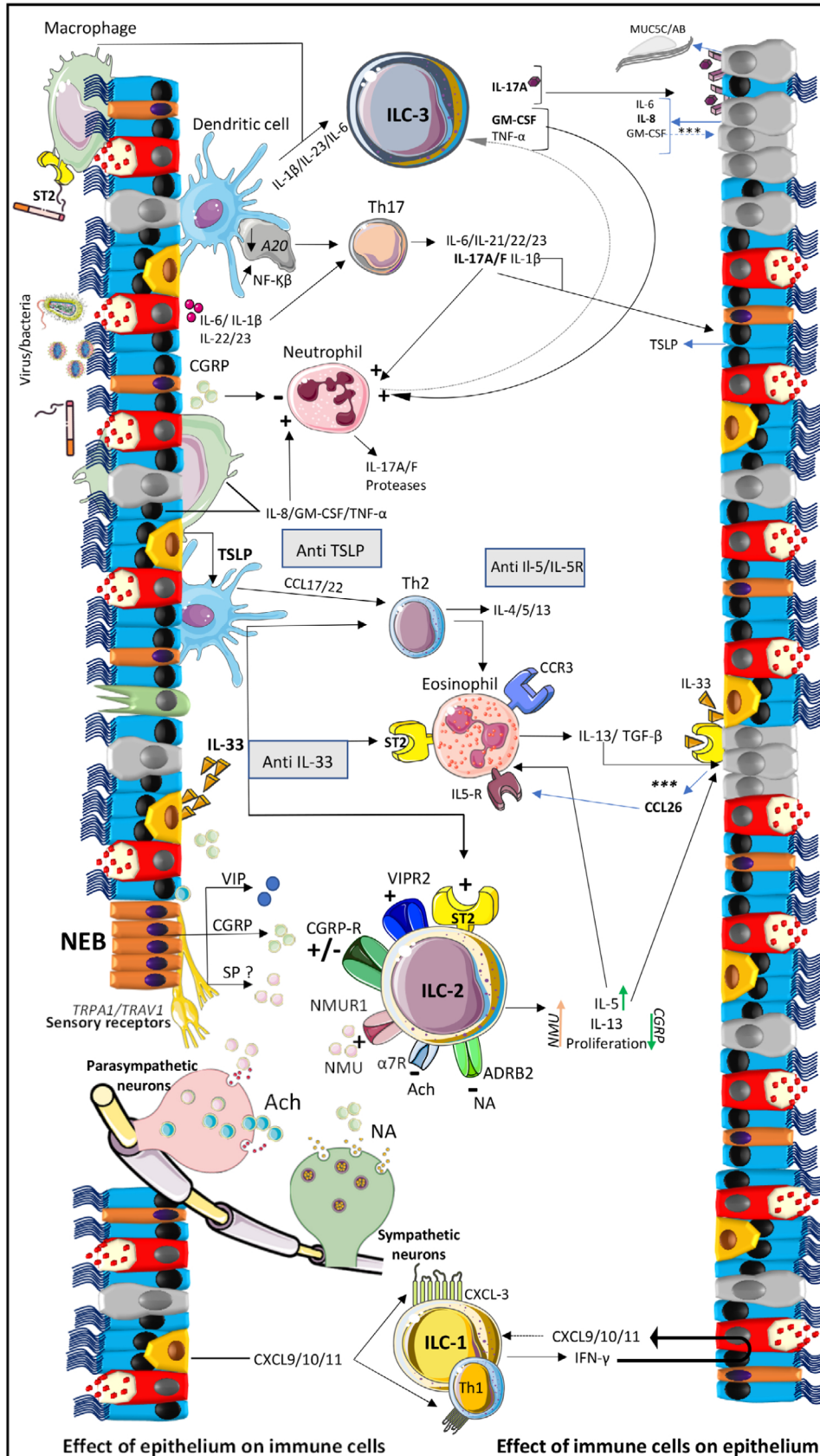


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damage. Several cytokines are thought to drive neutrophilic inflammation, such as IL-8, TNF- α , IL-17 and IL-23. Human bronchial epithelial cells also express IL-17 and IL-22/IL-22R. IL-8 is secreted by macrophages, T cells, epithelial cells and neutrophils, and is chemotactic for neutrophils via CXC-chemokine receptor 2 (CXCR2).

Non-Th2 adaptive immune responses in COPD also involved recruitment of peripheral blood and bronchoalveolar lavage CD4+ Th1 cells which produce interferon- γ (IFN- γ).¹⁸⁴ Damaged tissues release TGF- β , IL-33 and IL-1 β from epithelial cells.¹⁹⁵ IL-1 β and TNF levels are increased in severe asthma/COPD and are associated with macrophage activation and neutrophilic inflammation.

Characterization of a Th1 phenotype patients is difficult because studies show a high variability in sputum neutrophil counts and data concerning Th1/Th17 inflammatory mediators in blood of COPD patients are not consistent. Although targeting neutrophilic inflammation appears to be a promising avenue, clinical trials in COPD so far provide no evidence of related reductions in exacerbation rate.

T-helper type 17 axis. Non-Th2 immunity also involves the recently described CD4+ T-helper type 17 (Th17) axis. Th17 differentiation occurs under APC priming in the presence of IL-6/IL-1 β ,¹⁹⁶ whereas their maintenance requires IL-23. Th17 cells classically

produce IL-17A, IL-17F, IL-21, IL-22 and IL-23 in the proximal airways in response to mucosal infections.

Activation of IL-17 and IL-22 receptors on airway epithelial cells induces secretion of neutrophil chemoattractant factors. In patients with COPD, sputum IL-17A and IL-22 levels are increased, and IL-17A positively correlates with sputum neutrophils.¹⁹⁷ However, other studies present conflicting data concerning Th17 cytokine levels in COPD airways. In most studies, blood IL-17A concentrations in COPD are higher compared to healthy smokers. IL-17 can also induce IL-6 production by airway epithelium, both of which induce mucins (MUC5B and MUC5AC) in human, mouse or monkey airway epithelium cultures.¹⁹⁸

In steady-state COPD small airways, the number of IL-22 and IL-23 immunoreactive epithelial cells¹⁹⁹ and IL-17A inflammatory cells are increased²⁰⁰ versus control groups. Both IL-17 and IL-22 can induce airway-derived antimicrobial peptides such as β -defensins and S100 proteins.¹⁹⁵ ILC3, NK cells are also able to produce Th17 cytokines²⁰¹ and neutrophils can activate ILC3. One human study showed that a subset of ILC3 was notably increased in lung tissue from COPD patients, with increased IL-17/IL-22 production by ILC3.²⁰²

A20. TNF- α -induced protein 3 (TNFAIP3, also known as 'A20') has recently been described as a negative regulator of NF- κ B activation in myeloid cells and

Figure 7 Type 2 and non-type 2 inflammation in COPD airways. Top of the figure: T-helper type 17 (Th17) axis. Alveolar macrophages present defective immune functions, such as decreased phagocytosis. They express suppression of tumourigenicity 2 (ST2) receptors and can be modulated by cigarette smoke (CS). Dendritic cells (DC) have a decreased expression in A20 protein, leading to up-regulation of nuclear factor κ B (NF- κ B) inflammation. Under specific conditions, airway epithelial cells, alveolar macrophages and DC produce interleukin (IL)-1 β , IL-23, IL-22 and IL-6 that polarize innate lymphoid cells (ILC) into the ILC-3 subtype. Activated ILC-3 produce Th17 cytokines (IL-17A, granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumour necrosis factor (TNF)- α). IL-17A effects are driven by IL-17A/IL-17R receptors expressed by goblet cells. IL-17A, GM-CSF and IL-6 induce goblet cell hyperplasia and production of mucins. Goblet cells amplify the signal by secreting these IL. Epithelial inflammatory mediators and DC produce Th17 cytokines such as IL-6/IL-1 β and IL-22/23 that will drive differentiation of naïve T-lymphocyte cells into Th17 cells. Activated Th17 cells thereby secrete pro-Th17 mediators, acting on ILC-3, epithelium and neutrophils. Th17 cytokines are able to promote thymic stromal lymphopoietin (TSLP) production, an alarmin considered to be pro-Th2 that probably has more complex effects in COPD. Pathogens and CS can activate airway epithelial cells to produce inflammatory mediators, such as TNF, IL-8 and GM-CSF, which attract neutrophils. Th17 cells are also a major source of neutrophil activation. Neutrophils produce IL-17A/F and proteases purported to strongly contribute to emphysema. They can also activate ILC-3. In contrast, epithelium-derived calcitonin gene-related peptide (CGRP) can dampen neutrophil activity. The middle part of the figure represents Th2 inflammation. Th2 cytokines (IL-5 and IL-13) act on goblet cells to induce goblet cell hyperplasia and mucus production. The epithelial alarmin TSLP activates DC that releases chemokines (CC-chemokine ligands 17 and 22; CCL17, CCL22). These chemokines recruit Th2 lymphocytes and ILC2. Th2 cell activation and ILC2 induce the secretion of IL-4, IL-5 and IL-13. IL-4 and IL-13 share a common receptor (IL-4 receptor subunit- α (IL-4R α)) and IL-5 acts on IL-5R α expressed on eosinophils. Activated eosinophils secrete IL-13 and transforming growth factor- β (TGF- β)-inducing epithelial cells to release eosinophil-attracting chemokines, such as CCL26. TSLP and IL-33 epithelial alarmins contribute to Th2 inflammation through Th2 and ILC-2 activation. IL-33 acts through its specific receptor ST2. Neuroinflammation from airway nerves or epithelial mediators highly modulates ILC behaviour, mainly described in Th2 airway inflammation. neuroepithelial bodies (NEB) produce neuropeptides such as vasoactive intestinal peptide (VIP), substance P (SP) and CGRP. Transient receptor potential vanilloid 1 (TRPV1) is expressed in mostly all the C-fibres and these sensory nerves are also able to produce these neuropeptides. VIP activates ILC-2, whereas CGRP has a dual effect depending on the inflammatory context. SP effects have not been described for airway ILC2. ILC2 were found to express high levels of the receptor for neuropeptide neuromedin U (NMU), NMUR1. NMU is mainly produced from parasympathetic nerves. When NMU binds its receptor expressed on ILC2, the interaction induces increases in ILC2 proliferation and activation. By contrast, ILC2 are inhibited by catecholaminergic neurons that secrete norepinephrine/noradrenaline which bind to β 2-adrenoreceptor (ADRB2) (β 2AR) on ILC2, thus damping ILC2 responses. Acetylcholine (ACh) release from parasympathetic neurons and possibly non-neuronal cells can also inhibit ILC2 activity via α 7 nicotinic ACh receptor (α 7nAChR), leading to decreased production of IL-5 and IL-13. The main therapeutic anti-Th2 antibodies already tested or currently ongoing trials (IL-33 blocking) in COPD are represented. Bottom of the figure: T-helper type 1 (Th1) axis. Airway epithelial cells are able to produce pro-Th1 chemokines (C-X-C motif-chemokine ligand 8, 9, 10 and 11; CXCL9, CXCL10 and CXCL11) that induce naïve lymphocyte T-cell differentiation into Th1 cells. Chemokines also drive ILC differentiation into ILC1. Both Th1 and ILC1 produce interferon- γ (IFN- γ), which induces epithelial cells to release pro-Th1 cytokines in a feedback loop.

DC. Conditional deletion of the TNFAIP3 gene in either mouse myeloid cells or DC leads to Th17 cell differentiation and increased Th17 cytokines.²⁰³ A20 is reduced in the airway epithelial cells of a murine model of virus-induced COPD exacerbation.²⁰⁴

Neuronal regulation of immunity in lungs

Lung innervation and neurogenic inflammation

The lungs are innervated by the efferent parasympathetic and sympathetic fibres, and sensory components of the peripheral nervous system. Afferent peripheral sensory nerve lung innervation largely originates from vagal afferents. These afferent neurons can be subdivided into A- and C-fibres, and the cough receptors.

A-nerve terminals are associated with airway smooth muscle cells and neuroepithelial bodies (NEB) within the airway epithelium. C-fibres can be activated by physical, chemical and thermal stimuli, such as bradykinin and CS. Transient receptor potential vanilloid 1 (TRPV1) is expressed in almost all C-fibres. A subset of C-fibre neurons express neuropeptides, such as the tachykinin substance P (SP) and calcitonin gene-related peptide (CGRP). Release of neuropeptides upon stimulation of peripheral nerve endings in the airways induces 'neurogenic inflammation'. TRPV1 neurons secrete CGRP, which inhibits neutrophil recruitment and surveillance in a murine bacterial lung infection model.²⁰⁵

The parasympathetic nervous system interacts with immune cells through the action of acetylcholine (ACh) on muscarinic receptors. ACh is a neurotransmitter released from the nerve terminals of postganglionic parasympathetic neuronal and non-neuronal cells, including airway epithelial cells. COPD is associated with increased muscarinic 1 and 3 receptor (M1R and M3R) expression in airways, probably through prolonged ACh release. M3R on structural cells in the lung may also play a pro-inflammatory role. The genetic ablation of M3R prevents neutrophilic airway inflammation in response to CS exposure.²⁰⁶ In COPD, strong evidence supporting a pro-inflammatory role for ACh is limited.

ACh may have anti-inflammatory effects via the $\alpha 7$ nicotinic ACh receptor ($\alpha 7nAChR$). In mice, $\alpha 7nAChR$ are expressed in the distal lung epithelium, especially in CC, AT2 cells and alveolar macrophages. Genetic deletion of the receptor in mice decreased CC and AT2 transcripts, Muc5b distal airway plugs and peribronchiolar fibrosis.²⁰⁷

The sympathetic nervous system also modulates immune cells via noradrenaline-mediated activation of $\beta 2$ -adrenoreceptors ($\beta 2AR$) on ILC and other immune cells. $\beta 2AR$ induces an asthmatic phenotype in mice.²⁰⁸

Neural regulation of innate lymphoid cells

Recent work has shown that neurons closely interact with ILC. ILC2 were found to express high levels of neuromedin U (NMU) receptors (NMUR1). NMU is mainly produced from parasympathetic nerves (cholinergic neurons). When NMU binds its receptor on ILC2 during allergen challenge models, the interaction

provokes ILC2 proliferation and activation. Activated ILC2 lead to Th2 cytokine (IL-5 and IL-13) production, which further induce goblet cell hyperplasia and mucus production.²⁰⁹ Furthermore, vasoactive intestinal peptide (VIP) can activate ILC2²¹⁰ and Th2 cells.²¹¹

Pulmonary neuroendocrine cells (PNEC) also modulate ILC2 functions through CGRP receptors.²¹² The differential effects of CGRP on ILC2 appear tissue-specific (e.g. differing between lung vs gut). *in vitro* analysis demonstrated that CGRP suppresses IL-33-mediated activation of ILC2, especially IL-13 production and cell proliferation, but promoted IL-5 production.²¹³ PNEC are organized into highly innervated clusters called NEB. In mice, NEB reside near airway branch points and colocalize with ILC2.²¹⁴ Of note, PNEC are more abundant in airways from patients with COPD relative to healthy donors.²¹⁵

By contrast, ILC2 are inhibited by catecholaminergic neurons (sympathetic nervous system). These neurons secrete molecules (such as norepinephrine/noradrenaline) that bind to $\beta 2AR$ (ADRB2) on ILC2, thus damping ILC2 responses.²¹⁶ ACh release from parasympathetic neurons and possibly non-neuronal cells can also inhibit ILC2 activity via $\alpha 7nAChR$, leading to decreased production of IL-5 and IL-13.²¹⁷

SUMMARY

It is well established that individuals presenting with different phenotypes under the diagnostic label of COPD may differ regarding the natural history of their COPD, with regards to its inception, early stages and progression of established disease. In some individuals, lower than normal maximally attained lung function in their early adulthood may be the main mechanism behind COPD, in others accelerating decline in FEV₁ during the adult years plays a major role, whereas some individuals may experience both. Regardless of the FEV₁ trajectory, continuous smoking is very strongly associated with disease progression, development of emphysema and poor prognosis. In the Western world, the course of COPD in never-smokers is much more benign. In developing countries, factors such as exposure to biomass and sequelae after tuberculosis may lead to a type of COPD with more airway pathology and less emphysema than we see in smokers. New advances in imaging and biomarkers together with longer observation periods in childhood cohorts will contribute to a better understanding of development and progression of COPD in forthcoming years.

Mechanistically, aberrant repair processes in COPD are likely related to changes in airway cell populations (both progenitors and differentiated). There is a growing corpus of evidence supporting aberrant expression of epigenetic markers in COPD (such as non-coding RNA expression, histone changes and DNA methylation) that may help explain these changes. Progenitor reprogramming, in particular, may be partially explained by epigenetic modifications due to early-life exposures that can be inherited.²¹⁸ Currently, the only curative therapeutic strategy in severe, end-stage COPD is lung transplantation.^{219,220} To move beyond this

impasse, efforts should focus on the development of diverse strategies for restoring lung homeostasis. Potential future treatments include retinoids and growth factors such as fibroblast growth factor-2 (FGF-2) or hepatic growth factor (HGF); intratracheal/nasal administration of the latter improves emphysema in animal models.^{221,222} Administration of mesenchymal stem cells for therapeutic purposes has been tested in animals and humans with COPD, and although safe, failed to provide any benefit to patients. Autologous, iPSC-derived, bronchial progenitors now represent a promising alternative source of cell therapy. Moreover, proof-of-concept has recently been achieved in humans through autologous iPSC-derived retinal cells in macular degeneration.²²³ Epigenetic rewriting also represents a unique opportunity to restore altered gene expression induced by environmental exposure. The therapeutic window, duration and route of administration remain crucial outstanding issues. Finally, ethical frameworks to support and sustain these novel treatment initiatives remain to be established.

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Abbreviations: $\alpha 7$ nAChR, $\alpha 7$ nicotinic ACh receptor; $\beta 2$ AR, $\beta 2$ -adrenoreceptor; A1AT, alpha-1 antitrypsin; ACh, acetylcholine; APC, antigen-presenting cell; AT2, alveolar type 2; BC, basal cell; CC, club cell; CC16, club cell secretory protein-16; CCL, CC-chemokine ligand; CCSP, club cell secretory protein; CGRP, calcitonin gene-related peptide; COPD, chronic obstructive pulmonary disease; CS, cigarette smoke; CT, computed tomography; CXCL, C-X-C motif-chemokine ligand; DC, dendritic cell; EGF, epidermal growth factor; EGFR, EGF receptor; FEV₁, forced expiratory volume in 1 s; FGF-2, fibroblast growth factor-2; FOXA2, forkhead box A2; FVC, forced vital capacity; GCM, goblet cell metaplasia; GM-CSF, granulocyte-macrophage colony-stimulating factor; GOLD, Global Initiative for Obstructive Lung Disease; hiPSC, human iPSC; IFN- γ , interferon- γ ; ILC, innate lymphoid cell; iPSC, induced pluripotent stem cell; LLN, lower limit of normal; M3R, muscarinic 3 receptor; MCC, multiciliated cell; MUC5, mucin 5; NEB,

neuroepithelial body; NF- κ B, nuclear factor κ B; NK, natural killer; NMU, neuromedin U; NMUR1, neuromedin U receptor; PNEC, pulmonary neuroendocrine cell; PRM, parametric response mapping; RNAseq, RNA sequencing; SAD, small airways disease; SP, substance P; SPDEF, SAM Pointed Domain Containing ETS Transcription Factor; ST2, suppression of tumourigenicity 2; TGF- β , transforming growth factor β ; Th, T-helper type; TNFAIP3, TNF- α -induced protein 3; TRPC6+, transient receptor potential Ca²⁺ channel 6; TRPV1, transient receptor potential vanilloid 1; TSLP, thymic stromal lymphopoietin; VIP, vasoactive intestinal peptide

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