Macrophage metabolic reprogramming during chronic lung disease

Patricia P. Ogger and Adam J. Byrne

Airway macrophages (AMs) play key roles in the maintenance of lung immune tolerance. Tissue tailored, highly specialised and strategically positioned, AMs are critical sentinels of lung homeostasis. In the last decade, there has been a revolution in our understanding of how metabolism underlies key macrophage functions. While these initial observations were made during steady state or using in vitro polarised macrophages, recent studies have indicated that during many chronic lung diseases (CLDs), AMs adapt their metabolic profile to fit their local niche. By generating reactive oxygen species (ROS) for pathogen defence, utilising aerobic glycolysis to rapidly generate cytokines, and employing mitochondrial respiration to fuel inflammatory responses, AMs utilise metabolic reprogramming for host defence, although these changes may also support chronic pathology. This review focuses on how metabolic alterations underlie AM phenotype and function during CLDs. Particular emphasis is given to how our new understanding of AM metabolic plasticity may be exploited to develop AM-focused therapies.

Mucosal Immunology (2021) 14:282–295; https://doi.org/10.1038/s41385-020-00356-5

INTRODUCTION
The respiratory mucosa is a unique site, as our airways are continually exposed to particulates, viruses, bacteria, and fungi, which challenge the pulmonary immune system. To maintain pulmonary homeostasis and ensure efficient gas exchange, a complex regulatory system is in place, of which airway macrophages (AMs) are a core component. AMs are the most numerous immune cell type present in healthy lungs, are strategically positioned at the interface of airways and environment, and critical sentinels of barrier immunity. AMs form the first line of defence against inhaled particles, pathogens and antigens. Although inherently suppressive, AMs exhibit significant functional and phenotypical specialisation, allowing efficient responses to environmental signals and rapid alterations in phenotype. Increasing evidence suggests that metabolic alterations provide an additional layer of functional plasticity to AM populations. Activation of macrophages in vitro with a range of inflammatory stimuli, induce profound metabolic adaptations, such as the switch from oxidative phosphorylation (OXPHOS) to glycolysis in oxygen-sufficient conditions, similar to the “Warburg effect” seen in some cancers. It is now clear that how macrophages utilise energy dictates immune responses, and that manipulating cellular metabolism can alter the inflammatory response. However in vivo, the unique oxygen rich environment of the airways coupled with specific local nutrient availabilities, shapes AM phenotype and function. Indeed, many recent studies have indicated that in chronic lung diseases (CLDs), such as asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), idiopathic pulmonary fibrosis (IPF), and during infection such as with Mycobacterium Tuberculosis (Mtbc), there are significant alterations in AM metabolic processes and that targeting these pathways could represent an exciting therapeutic approach. This review focuses on how metabolic adaptations underlie AM phenotype and function during CLDs. Particular emphasis is given to how our new understanding of AM metabolic plasticity may be exploited to develop AM-focused therapies.

Airway macrophages: guardians of the lung environment
To maintain gas exchange, it is critical that AMs sustain a naturally hyporesponsive state whilst also reviewing the ability to rapidly mount effective inflammatory responses. This balance is achieved through complex AM-airway epithelial cell (AEC) interactions via cell surface-expressed receptors and secreted products. AMs express transforming growth factor beta receptor (TGF-βR), interleukin (IL)-10 receptor (IL-10R), CD200 receptor (CD200R) and signal regulatory protein-α, key components mediating AM-AEC crosstalk and in turn, regulating AM activation. For example, AM-AEC contact decreases AM phagocytosis and cytokine production in a TGF-β-dependent manner. Conversely, loss of the integrin αvβ6 such as through loss of contact of AMs with AEC upon toll-like receptor (TLR) activation leads to initiation of the AM pro-inflammatory phenotype and inflammatory response.

AMs are characterised by a distinct cellular phenotype. Human AMs highly express the lectin-binding transmembrane glycoprotein CD68, the adhesion molecule CD169, the integrin CD11c and mannose receptor CD206. In mice, expression of the CD68+CD206+CD11c+CD11b+ cell surface phenotype is conserved at steady state, while murine AMs also express the Mer tyrosine kinase (MerTK), sialic acid dependent adhesion molecule SiglecF, hormone receptor F4/8, glycoprotein CD64 and the CD200 receptor. Recent work in mice has indicated that many tissue resident macrophages, including those in the airways, are foetally derived and self-maintain locally with minimal contribution from circulating monocytes, during steady state conditions. During murine prenatal development, foetal liver or yolk sac macrophages are the major contributing pool to AM...
During pulmonary inflammation, circulating monocytes do not adaptability to environmental exposures results in remarkable AM plasticity and airway niche combined with distinct ontological origins, age and human lung transplant patients to investigate the origins of AMs in the resident AMs (TR-AM) which are prenatally derived and monocyte-contain at least two ontologically distinct AM populations, tissue derived into AM-like cells18,25. Thus, post-injury murine airways derived macrophages (BMDMs) are categorized as M1 or M2 monocyte derived macrophages (MDMs) or murine bone marrow macrophages, respectively. Seminal studies have demonstrated that pro-wound healing M2 (IL-4 stimulated) macrophages in vitro rely on fatty acid oxidation (FAO), an intact tricarboxylic acid cycle (TCA) cycle, high rates of OXPHOS and increased expression of arginase 1 (Arg1), which catalyses the production of ornithine from arginine as precursor for collagen to facilitate wound healing35,37. Conversely, pro-inflammatory M1-macrophages rely on glycolysis and breaks in the TCA cycle lead to accumulation of metabolites, many of which have signalling functions such as citrate, succinate, fumarate and α-ketoglutarate38,39. However, although useful in defining the range of potential macrophage responses, in vitro derived cells do not recapitulate the core aspects of AM phenotypes which are shaped by the local niche11. As AMs are highly adapted to the unique environment of the airway lumen, it is perhaps unsurprising that the metabolic state of AMs is also distinct. Glucose concentrations in the alveolar lumen are less than 10% of blood glucose concentrations and AMs exhibit extremely low levels of glycolysis40; in stark contrast to BMDMs, AMs do not undergo glycolytic reprogramming in response to LPS40. Consequently, AMs readily engage OXPHOS and highly express the peroxisome proliferator-activated receptor gamma (PPARγ)41, which regulates lipid accumulation and promotes FAO to sustain OXPHOS.

AMs also play a major role in the catabolism of pulmonary surfactant, a monolayer composed mainly of phospholipid-based lipids, phospholipids and cholesterol which lines the alveoli, lowers surface tension and prevents alveolar collapse during expiration42. Mice lacking GM-CSF and thus the AM compartment, develop pulmonary alveolar proteinosis (PAP), an inflammatory lung syndrome caused by the defective clearance of surfactant42,43. In humans, mutations in genes encoding for GM-CSF receptors, result in hereditary PAP as a result of progressive alveolar surfactant accumulation44–49. AM phenotype and behaviour are influenced by surfactant exposure, which has major implications for AM-mediated immune responses in pulmonary tissue. There are four principle surfactant proteins (SP-A-D) and SP-A and SP-D have been shown to directly influence AM functions such as cell migration, phagocytosis and activation phenotypes45. Both SP-A and SP-D bind carbohydrates, lipids, and nucleic acids and initiate phagocytosis of inhaled pathogens and apoptotic cells46. Furthermore, SP-A blocks the binding of TLR ligands to TLR2, TLR4 and TLR co-receptors and furthermore inhibits complement activation47,48.

Whilst the alveoli are covered with a monolayer of surfactant, a thin layer of mucus produced by goblet cells and ciliated epithelial cells protects the airways; Mucous serves as a barrier and facilitates clearance of microorganisms and pollutants. A major component of mucus is mucin glycoproteins, which may be categorized as polymerizing, nonpolymerizing and cell-surface associated. Of the cell-surface associated mucins, MUC1 is expressed in AMs and contributes to the resolution of inflammation by decreasing phagocytic potential and pro-inflammatory cytokine production51. The polymerizing mucins include MUC5AC and MUC5B; in particular, MUC5B deficiency has been linked to particle accumulation in the lung, mucus obstruction and impaired macrophage phagocytosis52. Pro-inflammatory macrophages induce MUC5B expression to aid mucociliary clearance53. Furthermore, a single nucleotide polymorphism in the MUC5B promoter has been strongly associated with the risk of developing IPF, highlighting the importance of mucins for the pulmonary environment54.

In addition to low glucose and a lipid rich environment, the airways also have a unique distribution of amino acids and central carbon metabolites. Surowiec et al. showed that whilst several glucogenic and ketogenic amino acids were present in the bronchial wash, only alanine is present in BAL55 (Fig. 2). In addition, the central airways contained key glycolytic and OXPHOS metabolites such as fructose, glucose-6-phosphate, fumarate and malate as well as oxidised glutathione (GSSG, indicating oxidative stress, Fig. 2); interestingly, these could not be detected...
in the periphery, suggesting either minimal secretion, high utilization or as a result of anatomical location (i.e. close proximity to nutrient rich pulmonary capillaries)\(^57\). Recently the lung microbiome has gained attention as a factor which modifies the pulmonary environment and directs immune responses by producing short chain fatty acids (SCFA). Whilst the airways and alveoli are colonised mainly by proteobacteria, bacteroidetes and firmicutes\(^58,59\), the nasal mucosa additionally hosts actinobacteria\(^60,61\) (Fig. 2). Proteobacteria, bacteroidetes and firmicutes produce large amounts of SCFA, including acetate, propionate and butyrate, which influence barrier function by regulating epithelial tight junctions\(^62\) and anti-inflammatory immune responses\(^63\). Recent advances in understanding the pulmonary microbiome during homeostasis and CLDs are described in detail elsewhere\(^58,59,64\). Thus, at homeostasis AMs are exposed to a unique environment, with minimal glucose availability and a distinct distribution of nutrients and SCFA, which depend on anatomical location (Fig. 2). However, despite the profound influence that local substrate availabilities may exert on macrophage development, activation and function, this is an understudied area. New knowledge, which further defines especially human AM substrate dependencies at homeostasis is required in order to fully understand how local metabolic perturbations during CLDs may contribute to pathology. This is particularly relevant as already slight changes in nutrient availability during inflammation, such as succinate or citrate, can alter macrophage phenotypes through stabilization of Hif1\(\alpha\), post-translational modification of proteins and production of NO and ROS\(^65\), thereby contributing to a pathological development.

AM metabolism during CLDs

Chronic lung diseases affect a significant proportion of the world’s population, killing more than 100,000 people in the UK alone, each year\(^66\). Persistent inflammation, impaired repair processes and pulmonary remodelling are cardinal features of CLDs\(^67–69\). There are multiple overlaps in environmental exposures driving CLDs, such as smoking, pollution and environmental exposures; viral infection can also exacerbate symptoms in each disease\(^2,70,71\). Interestingly, AM metabolic adaptation may play a central role in dictating pathology during CLDs and present novel therapeutic opportunities (Fig. 3).

Asthma. Asthma is a heterogeneous disease of the airways characterized by airway remodelling, mucus production, airway hyperresponsiveness (AHR), and inflammation\(^72\). Although most patients have good control with standard medication, a proportion experience life-threatening, severe disease\(^72\). In vitro, macrophages respond to type-2 cytokines such as IL-4 that drive an ‘alternative’ M2 activation phenotype, linked to wound repair and type-2 pathology\(^74,75\). Manipulation of AM phenotype via genetic deletion of the transcription factor interferon regulatory factor 5 (Irf5), a master regulator of macrophage activation\(^74\), promoted pulmonary remodelling. AHR
and mucus secretion in mice, in an IL-13 dependent manner. Indeed, a recent study has shown that both CD206+ AM (activated by IL-4 and IL-13) and pro-inflammatory Irf5+ AM are increased in asthmatic patients, highlighting the plasticity of macrophages and heterogeneity of human asthma. Roles of AMs during antigen induced airway inflammation include phagocytosis of apoptotic cells and eosinophils as well as triggering anti-inflammatory pathways to regulate airway hyper responsiveness, mucus secretion and matrix deposition. In severe asthma however, this protective function is impaired, resulting in the loss of phagocytic ability and anti-inflammatory programme, which can contribute to airway remodelling. Thus, AMs are uniquely involved in responses to allergen and type-2 inflammation, and aberrant AM-phenotypes can directly influence respiratory pathology.

Numerous lines of evidence suggest that metabolic stress leading to the production of reactive oxygen species (ROS) plays a role in asthma. Increased ROS have been detected in AMs of asthmatic patients, and contributes to lung injury and pro-inflammatory tumour necrosis factor-alpha (TNF-α) and IL-1β secretion by macrophages. Furthermore, heme-oxygenase-1 (HO-1), which mediates ROS production in response to chemical and physical agents, is increased in AMs in asthmatics. In addition to these pro-inflammatory characteristics, AMs show key features of a more anti-inflammatory phenotype in studies using ovalbumin to model allergic asthma. Using this model, Al-Khami et al. show that expression of carnitine palmitoyltransferase (CPT) is increased in AMs, shunting fatty acids into the mitochondria, as well as increased gene expression of FAO related genes.

Another functional pathway that is altered in asthmatic AMs and links to the underlying metabolic phenotype is the eicosanoid pathway, which is induced by Th2 cytokines IL-4 and IL-13. Eicosanoids, including prostaglandins and leukotrienes, are produced from the poly-unsaturated fatty acid arachidonic acid, which is released during asthma. Increased production of the eicosanoid 5-HETE and leukotrienes B4 (LTB4) and E4 (LTE4) has been detected in AMs from asthmatic patients stimulated ex vivo. This contributed to bronchial constriction and pro-inflammatory phenotype and failure to generate the anti-inflammatory eicosanoids 15-HETE and prostaglandin E2 (PGE2), which is associated with reduced AM phagocytosis. LTE4 has been shown to cause AHR in subjects with aspirin-induced asthma and can be produced in AMs by γ-glutamyl transpeptidase. IL-13 furthermore induces Arg1, which may further contribute to the asthmatic phenotype via metabolism of collagen precursors ornithine and proline and can thereby contribute to extracellular matrix deposition.

Several of these observed alterations have been targeted therapeutically, attempting to rewire AM phenotype. These include the eicosanoid pathway, ROS, glycolysis and FAO. Administration of the anti-inflammatory eicosanoid 15-HETE inhibited leukotriene synthesis and reduced AHR in asthmatics. Ex vivo, the corticosteroid dexamethasone decreased levels of thromboxane B2 and LTB4 in macrophages and asthmatic AMs, while prednisone decreased LTB4 production in AMs. Treatment with the antioxidant AD4 improved AHR and airway inflammation by decreasing ROS in the OVA-sensitised mouse model of allergic airway disease (AAD). Inhibiting glycolysis with 2-DG, Zhao et al. show altered AMs phenotype ameliorated AAD, while Al-Khami et al. reported improvements in AHR after treatment with FAO inhibitor etomoxir.

Together, these studies indicate that there is significant disruption of AM metabolism during asthma and AAD, notably via dysfunction in eicosanoid, glycolysis and fatty acid pathways. In
order to evaluate candidate therapies, it is crucial that studies utilise relevant preclinical models and ex vivo patient samples to understand disease. Models which more closely recapitulate the complex immune response to allergens, are more likely to reveal viable targets for intervention; in particular the ovalbumin model of AAD, which requires an adjuvant and a sensitisation phase, is a poor murine model of asthma. Furthermore, our new understanding of asthma heterogeneity has allowed the development of biologics which target “type-2 high” asthma; delineation of how metabolic changes underlie distinct asthma phenotypes could lead to new treatments for other phenotypes, such as neutrophilic and paucigranulocytic asthma.

COPD. COPD is the 5th leading cause of death in high income countries, affecting over 200 million people worldwide. COPD is a heterogeneous disease, characterised by destruction of the parenchyma and emphysema, narrowing of the airways, remodelling and chronic inflammation driven by chronic exposure to cigarette smoke and particular matter. AM numbers are increased in COPD BAL and contribute to COPD pathology through numerous pathways. During COPD, AMs are found in areas of lung destruction and produce pro-inflammatory cytokines, chemokines and matrix metalloproteases (MMPs) with elastolytic properties. At the same time, tissue inhibitor of metalloproteases (TIMP)-1 is decreased in AMs in COPD and furthermore, decreased phagocytic capacity and impaired bacterial killing have been described in COPD-AMs. COPD show increased sequestering of iron, which can contribute to new treatments for other phenotypes, such as neutrophilic and paucigranulocytic asthma.

Recent studies have suggested that increased ROS production is a key feature compared to healthy controls, COPD-AMs secrete increased levels of mitochondrial ROS (mtROS)10, superoxide and hydrogen peroxide, whilst glutamyl cysteine ligase for GSH synthesis is downregulated. Cigarette smoking also alters iron homeostasis and AMs in COPD show increased sequestering of iron, which can furthermore contribute to ROS production. Bevley et al. showed recently that increased generation of mtROS in COPD AMs results in impaired bacterial clearance. This study also reported a decrease in the mitochondrial membrane potential, which has recently been linked to AM exposure to particulate matter. This may explain the impaired phagocytic capacity of AMs in COPD as decreased mitochondrial membrane potential results in energy failure in the cell, proton leakage and increased mtROS. AMs from healthy smokers, non-smokers and COPD patients. While all groups had similar baseline glycolysis rates, there was a decrease in coupling efficiency, maximal respiration and spare respiratory capacity in COPD-AMs, whilst proton leak was significantly increased. In addition, expression of genes related to glutathione metabolism, mitochondrial transport, pyruvate metabolism, TCA cycle and electron transport chain were altered in smokers and COPD patients, compared to non-smoking healthy controls.

Other metabolic alterations in COPD AMs include increased expression of inducible nitric oxide synthase (iNOS) contributing to increased levels of nitric oxide (NO) and increased levels of the adenine receptor A2BR, suggesting increased adenosine metabolism, which might be linked to the increased levels of Hif1α in COPD AMs. While excessive ROS production through oxidant burden and iron accumulation has been identified as an important regulator of AM phenotype in COPD, it has only recently been linked to mitochondrial dysfunction and metabolic reprogramming. It would be interesting to follow up on these transcriptomic and metabolic alterations to understand their underlying disease driving role and to identify ways to rewire AM metabolism.

As corticosteroids have been found to be particularly ineffective in COPD, more specific pathways involved in AM function and metabolism have been investigated recently, such as the ROS pathway and iron accumulation. A study by Harvey et al. showed that treatment with sulforaphane in COPD AMs ex vivo improved bacterial clearance by activating the antioxidant and anti-inflammatory NRF2 pathway, while Cloonan et al. found that treatment with an iron chelator or a low iron diet protected mice from cigarette smoke induced COPD. Furthermore, procysteine, a precursor of GSH, increased AM effector cytokines in a mouse model of COPD.

Overall, COPD is marked by distinct iron sequestration, ROS, NO production and energetic dysfunction in AMs; further delineation of how mitochondrial phenotype links to inflammatory processes and pathology in COPD will allow the identification of molecular targets for modulating mitochondria during the disease.

Cystic fibrosis. Cystic fibrosis (CF) is caused by mutation of the CF transmembrane conductance regulator (CFTR), a chloride channel, which regulates fluid homoeostasis in mucosal surfaces. In the lung, CFTR mutation and subsequent loss of function results in a reduced aqueous film covering the epithelium and mucus thickening, leading to impaired mucociliary clearance and frequent bacterial infection. CF is furthermore characterised by hyper-inflammation of the lungs, airway obstruction, structural damage and progressive reduction of lung function. During recurring airway inflammation, large numbers of neutrophils, macrophages and T lymphocytes infiltrate the lungs and secrete pro-inflammatory cytokines, while anti-inflammatory IL-10 is reduced. Although AM numbers are increased in CF patients, pathogen clearance is attenuated, leading to colonisation of the airways and chronic inflammation. Meyer et al. report a more pro-inflammatory phenotype of AMs in a murine model of CF, even in the absence of infection, and MDMs differentiated from CF patients show an increased inflammatory profile. While others have shown that monocytes from CF patients had an impairment in activation upon IL-13 stimulation, CF-AM phenotype can be heterogeneous, depending on infection status and local environment. While AMs from P. Aeruginosa infected CF patients showed increased expression of mannose receptor CD206 and augmented arginase activity, in CF sputum AMs a decrease in expression of CD206 and scavenger receptor MARCO was detected. Furthermore, AMs are involved in the structural damage in CF airways by secreting serine- and metalloproteases, which subsequently degrade connective tissue components. The lower volume of airway surface liquid in CF airways activates AMs to increase their release of MMP12, resulting in the cleavage of elastin and degradation of the airway and parenchyma.

In CF airways, GSH is depleted, while levels of iron, transferrin, haem and haemoglobin are increased, resulting in high oxidative stress and ROS production. ROS in turn can induce TGF-β1 which has recently been shown to be increased in CF-BAL and AMs and inhibits CFTR biogenesis and cellular trafficking to the surface of epithelial cells, while also contributing to airway remodelling by recruitment and differentiation of myofibroblasts. However, during infection with bacteria from the Burkholderia family, both MDMs and AMs from CF patients showed reduced superoxide production as well as decreased phosphorylation of NADPH oxidase (NOX) components p47phox and p40phox, suggesting an inherent deficit in CF-AMs generating oxidative bursts for pathogen defence.

P. Aeruginosa is one of the most common pathogens to cause recurrent pulmonary infection in CF patients and exploits the host to maintain infection by inducing production of the TCA cycle metabolite itaconate in AMs. Itaconate exerts antimicrobial properties via inhibition of bacterial isocitrate lyase in the glyoxylate shunt and to evade this mechanism P. Aeruginosa has developed a way to use itaconate as an energy source. Similarly succinate, which is secreted in high levels during CF and...
especially during bacterial infection\textsuperscript{144}, can be utilised by \textit{P. Aeruginosa} and \textit{S. Aureus} as a substrate to generate oxidative stress.

Changes in lipid metabolism are a hallmark of CF and increased FAO, lipid turnover in cell membranes and eicosanoid production in AMs have been reported. Furthermore, sterol regulatory-element binding protein (SREBP), a regulator of lipid homeostasis, has been linked to CFTR loss of function\textsuperscript{124}. This results in altered plasma and tissue fatty acid profiles, and while levels of the omega-3 fatty acid docosahexaenoic acid (DHA) were unchanged in AMs upon loss of CFTR\textsuperscript{145}, ex vivo treatment with DHA decreased TNF-α levels\textsuperscript{146}. Furthermore, in CF AMs the anti-inflammatory lipidoxin A₄ (LXA₄), which is synthesised from the fatty acid arachidonic acid, is reduced and the LXA₄/LTB₄ ratio in CF BAL is decreased\textsuperscript{147}, while the fatty acid metabolite resolvin D1 (RvD1) has been suggested as a biomarker\textsuperscript{148}.

Increased energy demand by AMs in CF, either by manipulation through bacterial pathogens or to fight sustained infection, results in increased utilization of all available metabolic pathways. Recently, Lara-Reyna et al. reported increased glycolysis, mitochondrial function, and production of TNF-α in CF macrophages is due to an alteration in the serine/threonine-protein kinase/endoribonuclease IRE1α pathway and this supports exacerbated inflammation\textsuperscript{149}. While this study used PBMCs and monocyte-derived M1 macrophages from CF patients, it would be important to detect such a mechanism in AMs and to target this pathway specifically.

Several of the above described pathways have been identified as potential drug targets in CF, however yet there are no treatments targeting AMs. Delivery of GSH to the lower respiratory tract improves the antioxidant barrier of CF epithelium\textsuperscript{150}, while treatment with cysteamine and restoration of MicroRNA 17 (MiR17) and MiR20 expression improves disease by restoring autophagy\textsuperscript{151,152}. Several studies administered omega-3 fatty acids (DHA/EPA) to CF patients\textsuperscript{153-156} although only one trial reported improved FEV₁ after 8 months treatment with DHA\textsuperscript{157}. Treatment with DHA in a murine model of CF decreased liver fibrosis\textsuperscript{158}, while the fatty acid metabolite resolvin D1 (RvD1) has been suggested as a biomarker\textsuperscript{159}.

In conclusion, AM metabolic phenotype during CF is marked by increased energy expenditure to support exacerbated inflammation and is readily exploited by bacterial pathogens, leaving AMs deficient of oxidative burst capability during infection. It will be important to clarify the role of fatty acids in CF and furthermore, to target metabolic changes in AMs such as increased glycolysis, OXPHOS and FAO specifically to rewire AM phenotype and prevent exploitation through bacterial pathogens.

\textit{Idiopathic pulmonary fibrosis (IPF)}. IPF is a chronic interstitial lung disease characterised by excessive extracellular matrix deposition in the lung parenchyma and has a particularly poor prognosis\textsuperscript{159}. Repetitive alveolar injury in genetically susceptible individuals causes activation of mesenchymal cells, recruitment of fibroblasts and differentiation into myofibroblasts to replace damaged alveolar epithelial cells and provide a matrix for wound healing and tissue repair\textsuperscript{160}. During IPF, the wound healing process is dysregulated, leading to fibrotic plaque formation and excessive build-up of extracellular matrix, resulting in impaired gas exchange. AMs have been identified as key contributors to the dysregulated wound healing process, by secreting large amounts of ROS and TGF-β\textsuperscript{161}. Furthermore, AMs can shape the extracellular matrix by secreting factors contributing to the matrix (proline, collagen) and breaking down the matrix (plasmin, MMPs)\textsuperscript{162-164}.

Several changes to the central carbon metabolism pathways have been identified recently in AMs of IPF patients, including dysmorphic mitochondria\textsuperscript{165}. In murine models of pulmonary fibrosis, increased glucose consumption, glycolysis and enhanced expression of key glycolytic mediators was detected\textsuperscript{166}, while in IPF AMs, expression of the pulmonary glucose transporter GLUT1 was increased\textsuperscript{157}, which enabled augmented glucose uptake\textsuperscript{166}. The increased glucose uptake via GLUT1 can furthermore sustain NADPH production in the pentose phosphate pathway and TCA cycle\textsuperscript{168} and is therefore a key substrate for ROS production via NOX.\textsuperscript{169} Activation of macrophages results in the accumulation of endogenous metabolites capable of adopting immunomodulatory roles such as succinate\textsuperscript{170} and itaconate\textsuperscript{171-173}. Recently, our laboratory identified itaconate as an endogenous anti-fibrotic in the human and murine lung. In patients with IPF, there were reduced levels of airway itaconate, and decreased expression of ACOD1 (which controls the synthesis of itaconate) in AMs compared to healthy controls. Acod1 deficiency in mice leads to more severe disease pathology and exogenous itaconate limits fibroblast activity\textsuperscript{174}. These data indicate that AM metabolites may play a key role in the pathogenesis of lung fibrosis and may be exploited for the development of anti-fibrotic therapies.

ROS production is a key feature of AMs in IPF\textsuperscript{175} and can occur during OXPHOS, by the membrane bound NOX or by reaction of hydrogen peroxide with intracellular iron\textsuperscript{176}. NOX, and subsequent superoxide production, is activated by binding GTP-bound Rac\textsuperscript{177} which is secreted from AMs in IPF\textsuperscript{170} and can also activate the mTOR signalling hub\textsuperscript{179}. Superoxide produced by NOX can further react with NO to form peroxynitrite (ONOO⁻), another type of ROS. At the expense of NADPH, NO is produced in the mitochondria by iNOS\textsuperscript{182} which is upregulated in pro-inflammatory macrophages\textsuperscript{185} and in IPF-AMs leading to increased levels of the cytotoxic ONOO⁻ in IPF AMs\textsuperscript{187}. In the bleomycin mouse model of pulmonary fibrosis, increased levels of superoxide, NO and ONOO⁻ were measured in AMs\textsuperscript{182}. MiROS is furthermore linked to expression of PPAR-γ coactivator 1-alpha (PGC-1α), which induces metabolic reprogramming to FAO and is regulated by the mitochondrial calcium uniporter (MCU), which is increased in IPF AMs\textsuperscript{187}. MCU has furthermore been shown to regulate expression of the fatty acid transporter CPT-1, which is increased in AMs from IPF patients and bleomycin exposed mice\textsuperscript{183}. While human IPF-AMs have increased levels of MCU, mitochondrial calcium and expression of PGC-1α, bleomycin exposed mice utilised increased FAO\textsuperscript{168}, which is reduced in mice expressing dominant-negative MCU\textsuperscript{185}. Furthermore, these mice were protected from bleomycin induced pulmonary fibrosis. These findings highlight calcium transport and FAO as pathways to target in IPF AMs; however, a better understanding of the linking mechanism will be necessary.

IPF AMs have also been shown to be iron laden\textsuperscript{184}, which further induces oxidative stress and ROS production\textsuperscript{185}. Using RNA-sequencing, Lee et al. showed furthermore, that macrophage activation is increased in iron laden AMs in IPF, suggesting that iron accumulation plays a role in macrophage activation\textsuperscript{185}. The proportion of AMs expressing transferrin receptor (CD71), importing transferrin bound iron into the cell, are decreased in IPF AMs, leading to an extracellular accumulation of transferrin. Furthermore, numbers of CD71-negative macrophages are an independent predictor of survival in IPF\textsuperscript{186}. Iron metabolism is therefore likely a key pathway in IPF-AMs and targeting it would be a viable option to decrease ROS, oxidative stress and macrophage activation.

Recently, therapies targeting metabolic processes in IPF are of considerable interest. While antioxidant therapy in IPF was promising in vivo, the double-blind placebo controlled PANTHER trial, administering either N-acetylcysteine or placebo to IPF patients for 60 weeks did not show a change in lung function parameters\textsuperscript{187}. Another arm of this study investigated the combined potential of corticosteroid prednisone, immunosuppressant azathioprine and N-acetylcysteine but was stopped prematurely due to increased mortality and adverse effects without evidence of benefit\textsuperscript{188}. Another randomized, double-blind clinical trial assessed the safety and tolerability of N-acetylcysteine in...
patients already receiving pirfenidone anti-fibrotic therapy. While this trial showed that N-acetylcysteine in combination with pirfenidone was safe, no change in FVC, 6-minute walk test or occurrence of adverse effects was detected\(^{189}\). Another promising therapeutic avenue was the use of metformin, a potent metabolic remodelling drug often prescribed for type II diabetes. While on a global level metformin lowers the amount of blood sugar in diabetic patients, on a cellular level metformin activates AMP-activated protein kinase (AMPK) leading to inhibition of TGF-\(\beta\) induced NOX activity\(^{190}\). Sato et al. have shown that metformin inhibited TGF-\(\beta\) induced NOX activity via AMPK leading to inhibition of myo-fibroblast differentiation in vitro and reversed bleomycin induced collagen deposition in vivo\(^{191}\). Consistent with this, Rangarajan et al. showed that metformin treatment reversed bleomycin induced pulmonary fibrosis via AMPK activation, while in IPF patients AMPK phosphorylation was decreased\(^{192}\). A posthoc analysis study of the effect of metformin in IPF patients however showed no change in clinically outcomes\(^{193}\), once again showing the difficulty of translating in vitro and in vivo findings into the clinic. Another study investigating the NOX-NRF2 imbalance as a therapeutic target showed that in vivo knockdown of NOX4 and NOX1/4 inhibited the capacity of fibrosis resolution in aged mice\(^{194}\). Furthermore, treatment with nitrated fatty acids, reversed pulmonary fibrosis in a mouse model by promoting collagen uptake by AMs and dedifferentiating myofibroblasts\(^{195}\).

While these treatment approaches targeted metabolic changes during pulmonary fibrosis, none was specific to AMs. Targeting macrophage specific metabolic reprogramming, which sustains ROS and TGF-\(\beta\) production and contributes to dysregulated wound healing in IPF would therefore be a promising approach.

**Respiratory tract infections**

During respiratory tract infections, activation of pattern recognition receptors expressed by AM can elicit a variety of pro-inflammatory host responses\(^{21}\). For example, severe Coronavirus disease-19 (COVID-19) associated pneumonia patients may exhibit features of systemic hyper-inflammation also known as macrophage activation syndrome or “cytokine storm” which is associated with sustained elevation of macrophage/monocyte-derived pro-inflammatory cytokines (e.g., IL-6, IL-8, TNF-\(\alpha\), IL-1\(\beta\)) leading to acute respiratory distress syndrome (ARDS)\(^{196,198}\). Using single cell approaches a recent study demonstrated that highly inflammatory, monocyte recruited AMs, rather than quiescent pulmonary resident AMs, predominate in the BAL in COVID-19 patients with severe pathology, implicating these cells in COVID-19-associated ARDS\(^{199}\). Rather than direct infection of AMs, AM:AEC cross-talk has been identified as a major mechanism for control of many respiratory viral infections\(^{200}\) and AEC have been shown to be a key source of pro-inflammatory cytokines, modulating AM phenotype\(^{196,201}\). For example, Rhinovirus (RV), the causative agent of the common cold, primarily infects the upper airways, however prior infection with RV attenuates subsequent AM antibacterial responses\(^{202}\). Although AMs are susceptible to influenza A viral infection (IAV), replication within AMs has been shown to be minimal with the exception of several highly virulent strains\(^{203-205}\). Here, we will focus on *Mycobacterium tuberculosis* (*Mtb*) infection, as AMs are the primary infected cell type and metabolic changes in response to *Mtb* infection are well studied.

**Tuberculosis.** Tuberculosis (TB) is a contagious, chronic disease and one-third of the world’s population is infected with *Mtb*, the causative agent of TB, resulting in -2 million deaths per year (2009 World Health Organization Report)\(^{206}\). During infection, *Mtb* colonises AMs intracellularly and disables innate intracellular defence mechanisms such as the phagolysosome and inflammation and acquires macrophage intracellular nutrients\(^{207}\). AM host defence mechanisms against *Mtb* include production of ROS and reactive nitrogen species (RNS) for bacterial killing and fusing mycobacteria-containing phagosomes with lysosomes as well as autophagy and apoptosis\(^{208}\). However, virulent or multi-drug resistant strains can evade these host responses e.g. by preventing phagolysosome fusion and surviving ROS/RNS\(^{209}\).

During *Mtb* infection, AMs shift their metabolic programme from OXPHOS to aerobic glycolysis, which is regulated by HIF-\(\alpha\) and interferon-gamma (IFN-\(\gamma\)). This metabolic shift and subsequent enhanced glycolytic flux in infected AMs is crucial to control infection. Mice lacking HIF-\(\alpha\) in the myeloid lineage are more susceptible to infection and show decreased cytokine and antimicrobial effector production\(^{210}\). To support this metabolic reprogramming, key glycolysis genes are upregulated in the early stages of granuloma formation in mice, supporting the shift towards aerobic glycolysis\(^{211}\). Blocking this shift on the other hand results in decreased levels of IL-1\(\beta\), increased IL-10 and subsequent increased bacterial survival\(^{212}\). Gleeson et al. suggest furthermore that infection-induced glycolysis limits *Mtb* survival through the induction of IL-1\(\beta\) during infection with drug susceptible *Mtb*, as absence or inhibition of the IL-1 receptor (IL-1R) negated the effect of aerobic glycolysis\(^{212}\). In contrast, during infection with multi-drug resistant *Mtb*, AM metabolic reprogramming and induction of glycolysis is regulated through IFN-\(\beta\) as overexpression of *Mtb* cell wall lipids blocks the IL-1 receptor type 1 pathway\(^{213}\). These findings suggest that infection-induced glycolysis is necessary for control of bacterial intracellular replication though it remains to be investigated whether glycolytic reprogramming could be further induced to support AM defence.

*M. tuberculosis* has developed several mechanisms to evade host defence. Using mice deficient of cystathionine-gamma-lyase (CSE), which catalyses the synthesis of hydrogen sulphide (H\(_2\)S), H\(_2\)S has been identified as a regulator for central carbon metabolism during *Mtb* infection in AM. CSE\(^{-/-}\) mice had increased flux through the glycolysis and pentose phosphate pathway in AM, while *Mtb* infected WT mice produced increased levels of H\(_2\)S, reducing HIF-\(\alpha\) levels, glycolysis and host defence\(^{214}\). Howard et al. show that multi-drug resistant *Mtb* can drive augmented cell wall lipids synthesis, thereby bypassing the IL-1 receptor pathway and resulting in induction of IFN-\(\beta\) signalling, reprogramming host metabolism\(^{215}\). Furthermore, increased expression of MIF-21 in BMDMs and human MDMs upon exposure with *Mtb*, resulted in decreased glycolytic response and facilitated bacterial survival by targeting phosphofructokinase isomor M (PFK-M) and limiting IL-1\(\beta\) production. IFN-\(\gamma\) however inhibits MIF-21, forcing an isozyme switch in the PFK-M complex and rescuing glycolysis and host defence\(^{215}\).

Several signalling metabolites have been identified to be important for host defence in AMs during *Mtb* infection. Decreased levels of fumarase result in accumulation of bacterialum fumarate, which can modify metabolites and proteins through succinification\(^{216}\). Furthermore, the antimicrobial metabolite itaconate is increased during *Mtb* infection, although it can be disabled by \(\beta\)-hydroxyacyl CoA lyase in *Mtb* and used as a nutrient. Wang et al. show that deletion of this enzyme resulted in attenuated *Mtb* infection in mice\(^{217}\). Similarly, *Mtb* exploit intracellular iron as a nutrient. This is highlighted by worse TB outcome with increased dietary ingestion of iron and inhibition of *Mtb* growth when iron is unavailable\(^{218,219}\). Abreu et al. show that heparin reduced hepatic expression in macrophages infected with *Mtb* while heparin-treated macrophages had increased expression of ferroportin and subsequent iron export, limiting iron availability for intracellular bacilli\(^{206}\). *Mtb* furthermore induce ferroptosis, associated with reduced levels of GSH, superoxide and increased free iron. The ferroptosis inhibitor ferostatin-1 (Fer-1) as well as iron chelation decreased necrotic cell death of *Mtb*-infected macrophages in vitro, while in vivo treatment with Fer-1 reduced bacterial load\(^{220}\). *Mtb* can cope in low iron environments however...
| Disease | Metabolic change/Functional change | Ref. |
|---------|----------------------------------|------|
| Asthma  | ↑ HO-1, ↑ ROS production          | 83, 86 |
|         | ↑ 15-LOX 5-HETE and leukotrienes | 239  |
|         | ↑ Leukotriene B4/E4 Bronchial constriction, AHR | 85–87 |
|         | ↑ Prostaglandin E2 AM phagocytosis | 78   |
|         | ↑ Fatty acid oxidation (CPT) ↑ FAO metabolism | 84, 89, 90 |
| COPD    | ↑ ROS, ↑ mtROS, ↑ Superoxide Oxidative stress | 108–110 |
|         | ↓ mtROS after challenge Impaired bacterial clearance | 108 |
|         | ↓ Glutamyl cysteine ligase Loss of GSH synthesis | 111 |
|         | ↓ iNOS NO production | 117 |
|         | ↓ Mt membrane potential Impaired phagocytosis | 114 |
|         | ↑ HIF1α ↑ Glycolysis | 119 |
|         | ↓ Compensatory glycolysis Dysfunctional metabolism and macrophage phenotype | 7 |
|         | ↓ Proton leak | 7 |
|         | ↑ A2BR ↑ Adenosine metabolism | 118 |
| CF      | ↑ Arginase Impaired phagocytosis & efferocytosis | 132 |
|         | ↑ ROS release, ↑ GSH Oxidative stress | 136, 137 |
|         | ↓ Superoxide, ↓ NOX phos. ↓ Oxidative burst | 141 |
|         | ↓ Lipoxin A4 ↓ Anti-inflammatory potential | 147 |
|         | ↓ Acod11 Persistent P. Aeruginosa infection | 143 |
|         | ↓ CFTR-PTEN complex ROS production, succinate release | 144 |
|         | ↓ Iron ↑ ROS production | 138 |
|         | ↑ IRE-a pathway Increased glycolysis & Mt. function | 149 |
|         | ↑ HO-1 Loss of oxidative response | 240 |
|         | ↑ Secreted Rac1 NOX, superoxide, mTOR activation | 178, 179 |
|         | ↑ INOS NO and OONO⁻ production | 181, 182 |
|         | ↑ HIF1α Loss of oxidative response | 184, 185 |
|         | ↑ % of CD71⁺ AM Accumulation of transferrin | 186 |
|         | ↑ AKT Activation of HIF1α | 241 |
|         | ↑ GLUT-1, ↑ Glucose uptake NADPH production, superoxide | 166–168 |
|         | ↑ Glycolysis, glycolysis genes M2-like AM profile | 166 |
|         | ↑ FAO | 166 |
|         | ↑ MCU, ↑ Calcium, ↑ PGC-1α FAO reprogramming, mtROS | 166 |
|         | ↑ ROS/RNS production Bacterial killing | 208 |
|         | ↑ HIF1α Aerobic glycolysis, IFN-γ host defence | 210 |
|         | ↑ Warburg shift, ↑ glycolysis genes ↑ IL-1β and bacterial killing | 211, 215 |
|         | ↑ Host MiR-21 ↓ Glycolytic response, ↑ bacterial survival | 215 |
|         | ↑ Host MiR-33 ↓ autophagy, FAO, ↓ host defence | 224 |
|         | ↓ Hydrogen sulphide ↑ Glycolysis & PPP | 214 |
|         | ↑ fumarate Bactericidal | 216 |
|         | ↑ itaconate Antimicrobial, modulates host response | 217 |
|         | ↑ Heparin ↓ Hepcidin Decreased iron availability to bacilli | 206 |
|         | ↑ Iron, superoxide, lipid perox. Ferroptosis, bacterial spread | 220 |
|         | ↑ FAO, ↑ Lipid accumulation ↑ Host response, ↑ Bacterial burden | 223, 242 |
|         | PPAR-α activation FAO, autophagy & host defence | 222 |
|         | ↑IDO, ↑ tryptophan ↓ host defence | 225 |
|         | ↑ Glutaminolysis ↑ cytokine profile | 243 |
|         | ↑ Arg1 NO production | 244 |
|         | ↑ NAD⁺, creatine, GSH Host defence | 245 |

*Upward arrow represents increased expression, downward arrow represents decreased expression.*
by downregulating their non-essential protein content via specific sRNA.221

Several changes in fatty acid metabolism of Mtb infected AMs were identified recently. Compared to interstitial macrophages during Mtb infection, which are reliant on glycolysis, AMs utilise FA, which is induced by PPAR-α and have a lower burden of Mtb infection.222 To escape host defence, Mtb has developed a mechanism inhibiting pathways related to autophagy, lysosomal function and FAO in support of replication by inducing microRNA-33 (MiR-33) in the host cell. Silencing of MiR-33 however induced AM lipid catabolism and autophagy and rescued host defence.223 Furthermore, amino acid metabolism is altered during Mtb infection. In mice and macaque lungs, indoleamine 2,3-dioxygenase (IDO), which is involved in tryptophan catabolism, was increased during Mtb infection, while inhibition of IDO in a macaque model of TB decreased bacterial burden and pathology, as tryptophan metabolites suppress host immunity.224

While Mtb relies on host lipids as energy source, existing therapies such as targeting PPAR transcription factors or cyclosporine have been successful mainly in animal models226–229, whereas retrospective human studies, which investigated the effect of statins in diabetich TB patients did not show any results.230 As Mtb can also utilise iron as a substrate, another approach is to prevent iron accumulation. Treatment of Mtb infected human MDMs and primary AM with iron chelator Desferrioxamine (DFX) ex vivo induced the expression of glycolytic enzymes and enhanced glycolysis, as well as IL-1β, thereby supporting host defence231 and offers a novel therapeutic approach, which will need to be investigated in clinical trials. Together, these findings highlight the distinct phenotype of AMs during Mtb infection, which counteracts intracellular infection through aerobic glycolysis, but is also heavily exploited by Mtb bacteria feeding on host lipids and iron.

Targeting metabolism during chronic lung disease
Many potential targets have been identified recently that could rewire macrophage metabolic and phenotypic changes driving chronic lung disease. Since all cells depend on oxidative phosphorylation or cytoplasmic glycolysis to synthesize ATP, there is the potential for unwanted side effects by targeting specific metabolic processes. However, it is becoming increasingly apparent that it is possible to safely target metabolic pathways in patients. For example, dimethyl fumarate, a known regulator of inflammatory processes, has developed as a therapeutic pathway in relapsing multiple sclerosis.232 Indeed, metabolic processes are highly plastic with significant redundancy, modulation of these processes may have the added benefit of selectively targeting cells with high metabolic demands.233 Targeted delivery to AMs may add another layer of selectivity, improving efficacy, sustained drug release and evading capture by mucus.234 Systems for inhaled AM targeted drug delivery include the use of micro- and nanocarriers, including liposomes, which are phagocytosed by AMs. Rifampicin-loaded microspheres as a therapeutic approach for Mtb have been described235, and have been further refined to allow a one-step assembly for rifampicin containing microspheres.236 Recently, aerosolised delivery of siRNA, which post-translationally downregulates gene expression, has been developed to target AMs specifically237 whilst mannose coated microspheres have been developed which exploit the phagocytic activity of AMs.238 Many of these delivery vehicles have been developed to transport antibiotics targeting intracellular AM bacterial infections, which are helpful for treating TB, however other drugs could be incorporated into aerosolised micro- or nano delivery systems. Specifically, treatment with iron chelators, antioxidants and nitrated fatty acids has shown to rewire AM phenotype and improve diverse chronic lung disease; these may be ideal candidates to develop novel, aerosolised vehicle-assisted drug delivery to AMs during chronic lung disease.

CONCLUSION
In the last decade enormous strides have been made regarding our understanding of how adaptations in metabolic pathways underlie macrophage phenotype and function. AMs are remarkably plastic cells, orchestrating not only pathogen defence and efferocytosis, but also pulmonary tolerance and resolution. It has become increasingly clear that AMs tailor their metabolic profile to fit their local niche generating ROS for pathogen defence, utilising aerobic glycolysis to rapidly generate cytokines, employing the TCA cycle to fuel inflammatory responses and generating metabolites with secondary signalling functions such as citrate, itaconate, succinate and fumarate. Work elucidating the complexities of AM metabolic alterations in the context of CLDs has highlighted many potential therapeutic targets (summarized in Table 1). Indeed, a lack of understanding of shared cellular mechanisms, which underlie CLDs has been a major obstacle in respiratory biology; identification of common AM-metabolic pathways/metabolites which directly influence core features of CLDs would be a significant advance on the route to devising new AM-directed strategies to treat pulmonary diseases which affect millions worldwide.

ACKNOWLEDGEMENTS
We thank Clare M. Lloyd for critically reading this review and providing valuable feedback. P.P.O. is supported by a Studienstiftung des Deutschen Volkes fellowship, A.J.B. is supported by a Joan Bending, Evelyn Bending, Mervyn Stephens + Olive Stephens Memorial fellowship (AUK-SNF-2017-381).

AUTHOR CONTRIBUTIONS
P.P.O. and A.J.B. conceived of and wrote the manuscript.

ADDITIONAL INFORMATION
Competing interests: The authors declare no competing interests.
Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

REFERENCES
1. Lloyd, C. M. & Marsland, B. J. Lung homeostasis: influence of age, microbes, and the immune system. Immunity 46, 549–561 (2017).
2. Byrne, A. J., Mathie, S. A., Gregory, L. G. & Lloyd, C. M. Pulmonary macrophages: key players in the innate defence of the airways. 1189–1196. https://doi.org/10.1136/thoraxjnl-2015-207020 (2015).
3. Ginhoux, F. & Jung, S. Monocytes and macrophages: developmental pathways and tissue homeostasis. Nat. Rev. Immunol. 14, 392–404 (2014).
4. Galván-Peña, S. & O’Neill, L. A. J. Metabolic reprogramming in macrophage polarization. Front. Immunol. 5, 1–6 (2014).
5. Bossche, J. Van, den, O’Neill, L. A. & Menon, D. Macrophage immunometabolism: where are we (going)? Trends Immunol. 38, 395–406 (2017).
6. Tzouvelekis, A. Metabolic disorders in chronic lung diseases. Front. Med. 4, 1–9 (2018).
7. O’Beirne, S. L. et al. Alveolar macrophage immunometabolism and lung function impairment in smoking and chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. 125899, 1–14 (2019).
8. Russell, T. & Bell, T. J. Alveolar macrophages: plasticity in a tissue-specific context. Nat. Rev. Immunol. 14, 81–93 (2014).
9. Lambrecht, B. N. Alveolar macrophage in the driver’s seat. Immunity 24, 366–368 (2006).
10. Koivisto, L., Bj, J., Häkkinen, L. & Larjava, H. Integrin avβ6: structure, function and role in health and disease. Int. J. Biochem. Cell Biol. 99, 186–196 (2018).
11. Byrne, A. J., Maher, T. M. & Lloyd, C. M. Pulmonary macrophages: a new therapeutic pathway in fibrosing lung disease? Trends Mol. Med. 22, 303–316 (2016).
12. Misharin, A. V., Morales-Nebreda, L., Mutlu, G. M., Budinger, G. R. S. & Perlman, H. Flow cytometric analysis of macrophages and dendritic cell subsets in the mouse lung. Am. J. Respir. Cell Mol. Biol. 49, 503–510 (2013).
13. Zaynagetdinov, R. et al. Identification of myeloid cell subsets in murine lungs using flow cytometry. Am. J. Respir. Cell Mol. Biol. 49, 180–189 (2013).
Macrophage metabolic reprogramming during chronic lung disease
PP Ogger and AJ Byrne

14. Guth, A. M. et al. Lung environment determines unique phenotype of alveolar macrophages. Am. J. Physiol. Lung Cell. Mol. Physiol. 296, 936–946 (2009).
15. Snegurov, R. J. et al. A critical function for CD200 in lung immune homeostasis and the severity of influenza infection. Nat. Immunol. 9, 1074–1083 (2008).
16. Schulz, C. et al. A lineage of myeloid cells independent of Myb and hemato-poietic stem cells. Science. 336, 86–90 (2012).
17. Guilliams, M. et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. J. Exp. Med. 210, 1977–1992 (2013).
18. Hashimoto, D. et al. Tissue-resident macrophages self-maintain locally throughout adult life with minimal contribution from circulating monocytes. Immunity 39, 792–804 (2013).
19. van de Laar, L. et al. Yolk sac macrophages, fetal liver, and adult monocytes can colonize an empty niche and develop into functional tissue-resident macrophages. Immunity 44, 755–768 (2016).
20. Svedberg, F. R. et al. The lung environment controls alveolar macrophage metabolism and responsiveness in type 2 inflammation. Nat. Immunol. 20, 571–580 (2019).
21. Gihouëx, F. & Guilliams, M. Tissue-resident macrophage ontogeny and homeostasis. Immunity 44, 439–449 (2016).
22. Tan, S. Y. S. & Krasnow, M. A. Developmental origin of lung macrophage diversity. Development 143, 1318–1327 (2016).
23. Patel, A. A. et al. The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. J. Exp. Med 214, 1913–1923 (2017).
24. Shi, C. & Pamer, E. G. Monocyte recruitment during infection and inflammation. Nat. Rev. Immunol. 11, 762–774 (2011).
25. Gibbons, S. L. et al. Transcriptome analysis highlights the conserved difference between embryonic and postnatal-derived alveolar macrophages. Blood 126, 1357–1366 (2015).
26. Bittmann, L. et al. Cellular chimerism of the lung after transplantation: an interphase cytogenetic study. Am. J. Clin. Pathol. 115, 525–533 (2000).
27. Eguíluz-Gracia, I. et al. Long-term persistence of human donor alveolar macrophages in lung transplant recipients. Thorax 71, 1006–1011 (2016).
28. Nayak, D. K. et al. Long-term persistence of donor alveolar macrophages in human lung transplant recipients that influences donor specific immune responses. Am. J. Transpl. 16, 2300–2311 (2017).
29. Hunninghake, G. W. et al. The human alveolar macrophage. Methods Cell Biol. 21, 95–112 (1980).
30. Thomas, E. D., Ramberg, R. E., Sale, G. E., Sparks, R. S. & Golde, D. W. Direct evidence for a bone marrow origin of the alveolar macrophage in man. Science 192, 1016–1018 (1976).
31. Byrne, A. J. et al. Dynamics of human monocytes and airway macrophages during healthy aging and after transplant. J. Exp. Med 217, 1–11 (2020).
32. Murciano-Gorriti, S. et al. Identification of a novel monocyte subset in peripheral blood that differentiates into alveolar macrophages in steady state. Cell 165, 1565–1580 (2016).
33. Warnatz, J. et al. Cytokine network of human monocytes and alveolar macrophages. Cell. Immunol. 114, 242–251 (1988).
34. Sredni, S. et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. J. Exp. Med. 210, 1977–1992 (2013).
35. Tannahill, G. M. et al. Succinate is an in...
75. Jenkins, S. J. et al. Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. Science 332, 1284–1288 (2011).

76. Byrne, A. J. et al. A critical role for IRF5 in regulating allergic airway inflammation. Mucosal Immunol. 10, 716–726 (2017).

77. Draijer, C. et al. Human asthma is characterized by more IRF5+ M1 and CD206+ M2 macrophages and less IL-10+ M2-like macrophages around airways compared with healthy airways. J. Allergy Clin. Immunol. 140, 280–283.e3 (2017).

78. Huynh, M. L. N. et al. Defective apoptotic cell phagocytosis attenuates prostaglandin E2 and 15-hydroxyeicosatetraenoic acid in severe asthma alveolar macrophages. Am. J. Respir. Crit. Care Med. 172, 972–977 (2005).

79. Lappalainen, U., Whitsett, J. A., Wert, S. E., Tichelaar, J. W. & Bry, K. Interleukin-1 oxygen species metabolism of airspace cells and airway inflammation follow antigen challenge in human asthma. J. Allergy Clin. Immunol. 86, 306–313 (1990).

80. Lee, I. & Yang, C. Role of NADPH oxidase / ROS in pro-inflammatory mediators-induced airway and pulmonary diseases. Biochem. Pharmacol. 84, 581–590 (2012).

81. Park, H. S., Kim, S. R. & Lee, Y. C. Impact of oxidative stress on lung diseases. Respir. Physiol. 14, 27–38 (2009).

82. Harju, T., Soini, Y., Pääkkö, P. & Kinnula, V. L. Up-regulation of heme oxygenase-1 causes pulmonary inflammation, emphysema, and airway remodeling in the adult murine lung. Am. J. Respir. Cell Mol. Biol. 32, 311–318 (2005).

83. Calhoun, W. J., Bush, R. K., Salisbury, S. M. & Stevens, C. A. Enhanced reactive oxygen species metabolism of airspace cells and airway inflammation follow antigen challenge in human asthma. J. Allergy Clin. Immunol. 86, 306–313 (1990).

84. T. Byrne, A. J. et al. A critical role for IRF5 in regulating allergic airway in the adult murine lung. Am. J. Respir. Crit. Care Med. 172, 972–977 (2005).

85. Chavis, C., Godard, P., Michel, F. B., Paulet, A. Cde & Damon, M. Sulphate transport in human alveolar macrophages from patients with nocturnal asthma but not control subjects: Relations to inflammatory mechanisms in patients with chronic obstructive pulmonary disease. Eur. Respir. J. 20, 209–215 (1998).

86. Mayatepek, E. et al. Synthesis and metabolism of leukotrienes in human alveolar macrophages. Respir. Med. 100, 1025–1032 (2006).

87. Huynh, M. L. N. et al. Defective apoptotic cell phagocytosis attenuates proinflammatory cytokine release from human alveolar macrophages. J. Immunol. 180, 854–865 (2008).

88. Vercelli, D. Arginase: Marker, effector, or candidate gene for asthma? Trends Immunol. 20, 27–32 (2019).

89. Lappalainen, U., Whitsett, J. A., Wert, S. E., Tichelaar, J. W. & Bry, K. Interleukin-1 oxygen species metabolism of airspace cells and airway inflammation follow antigen challenge in human asthma. J. Allergy Clin. Immunol. 86, 306–313 (1990).

90. Chang, C.-I., Zoghi, B., Liao, J. C. & Kuo, L. The involvement of tyrosine kinases, cyclic AMP/protein kinase A, and p38 mitogen-activated protein kinase in IL-13–induced expression of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by alveolar macrophages from patients with chronic obstructive pulmonary disease. Am. J. Respir. Cell Mol. Biol. 26, 602–609 (2002).

91. Donnelly, L. E. & Barnes, P. Defective phagocytosis in airways disease. Chest 141, 1055–1062 (2012).

92. Grablec, A. M. & Russell, T. The role of airway macrophages in apoptotic cell clearance following acute and chronic lung inflammation. Semin. Immunopathol. 38, 409–423 (2016).

93. Beasley, M. A. et al. Impaired mitochondrial microbicidal responses in chronic obstructive pulmonary disease macromolecules. Am. J. Respir. Crit. Care Med. 196, 845–855 (2017).

94. Rahman, I. & Macnee, W. Role of oxidants/antioxidants in smoking-induced lung diseases. Free Radic. Biol. Med. 21, 669–681 (1996).

95. Xia, T., Kovochich, M. & Nel, A. E. Impairment of mitochondrial function by particle matter (PM) and their toxic components: Implications for PM-induced cardiovascular and lung disease. Front. Biosci. 12, 1238–1246 (2007).

96. Harju, T., Kaarteenaho-Wik, R., Soini, Y., Sormunen, R. & Kinnula, V. L. Diminished immunoreactivity of y-galactosylcycteine synthase in the airways of smokers’ lung. Am. J. Respir. Crit. Care Med. 166, 754–759 (2002).

97. Cloonan, S. M. et al. The iron-y of iron overload and iron deficiency in chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. 196, 1103–1112 (2017).

98. Philipopt, O. et al. Increased iron sequestration in alveolar macrophages in chronic obstructive pulmonary disease. PLoS One 9, e92240 (2014).

99. Belchamber, K. B. R. et al. Defective bacterial phagocytosis is associated with dysfunctional mitochondria in COPD macromolecules. Eur. Respir. J. 54, 1802244 (2019).

100. Eapen, M. S., Sharma, P. & Sohal, S. S. Mitochondrial dysfunction in macrophages: A key to defective bacterial phagocytosis in COPD. Eur. Respir. J. 54, 1901641 (2019).

101. O’Beirne, S. L. et al. Alveolar macrophage immunometabolism and lung function impairment in smoking and chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. 201, 735–738 (2020).

102. Ichinose, M., Sugiuira, H., Yamagata, S., Koarai, A. & Shiraio, K. Increase in reactive nitrogen species production in chronic obstructive pulmonary disease airways. Am. J. Respir. Crit. Care Med. 162, 701–706 (2000).

103. Zhou, Y., Murthy, J. N., Zeng, D., Belardinelli, L. & Blackburn, M. R. Alterations in adenosine metabolism and signaling in patients with chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. PLoS One 5, e92240 (2010).

104. Russell, K. E. et al. The MIF antagonist ISO-1 attenuates corticosteroid-insensitive inflammation and airways hyperresponsiveness in an o-zone-induced model of COPD. PLoS One 11, e0150990 (2016).

105. Harvey, C. J. et al. Targeting NFκB signaling improves bacterial clearance by alveolar macrophages in patients with COPD and in a mouse model. Sci. Transl. Med. 3, 78ra32 (2011).

106. Cloonan, S. M. et al. Mitochondrial iron chelation ameliorates cigarette smoke-induced bronchitis and emphysema in mice. Nat. Med. 22, 163–174 (2016).

107. Hodige, S. et al. Cigarette smoke-induced changes to alveolar macrophage phenotype and function are improved by treatment with procysteine. Am. J. Respir. Cell Mol. Biol. 44, 673–681 (2011).

108. Wine, J. J. et al. Progress in understanding mucus abnormalities in cystic fibrosis. J. Cyst. Fibros. 14, 535–539 (2018).

109. Dhoooge, B., Noel, S., Huaux, F. & Leal, T. Lung inflammation in cystic fibrosis: pathogenesis and novel therapies. Clin. Biochem. 47, 539–546 (2014).

110. Roesch, E. A., Nichols, D. P. & Chmiel, J. F. Inflammation in cystic fibrosis: an update. Pediatr. Pulmonol. 53, 530–550 (2018).

111. Nichols, D. P. & Chmiel, J. F. Inflammation and its genesis in cystic fibrosis. Pediatr. Pulmonol. 50, 539–556 (2015).

112. Brennan, S. et al. Alveolar macrophages and CC chemokines are increased in children with cystic fibrosis. Eur. Respir. J. 34, 655–661 (2009).

113. Dakin, C. J. et al. Inflammation, infection, and pulmonary function in infants and young children with cystic fibrosis. Am. J. Respir. Crit. Care Med. 165, 904–910 (2002).

114. Meyer, M. et al. Azithromycin reduces exaggerated cytokine production by M1 alveolar macrophages in cystic fibrosis. Am. J. Respir. Cell Mol. Biol. 41, 590–602 (2009).
Riquelme, S. A., Wong Fok Lung, T. & Prince, A. Pulmonary pathogens adapt to
Ferrari, E. et al. Cysteamine re-establishes the clearance of Pseudomonas aer-
Al-Turkmani, M. R., Freedman, S. D. & Laposata, M. Fatty acid alterations and n-3
Ringholz, F. C. et al. Reduced 15-lipoxygenase 2 and lipoxin A4/leukotriene B4
Assani, K. et al. Human cystic
Nair, S. et al. Irg1 expression in myeloid cells prevents immunopathology during
Riquelme, S. A. et al. Pseudomonas aeruginosa utilizes host-derived itaconate to
Andersson, C., Zaman, M. M., Jones, A. B. & Freedman, S. D. Alterations in
Bruscia, E. M. et al. Macrophages directly contribute to the exaggerated
Lloyd-Still, J. D. et al. Bioavailability and safety of a high dose of docosahex-
Vizia, B. D. E. et al. Effect of an 8-month treatment with u-3 fatty acids (eico-
sapentaenoic and docosahexaenoic) in patients with cystic fibrosis. J. Parenter.
130. Bruscia, E. M. et al. Macrophages directly contribute to the exaggerated
131. Tarique, A. A. et al. CFTR-dependent defect in alternatively-activated macro-
132. Murphy, B. S. et al. Characterization of macrophage activation states in patients
133. Riquelme, S. A. et al. Pseudomonas aeruginosa by macrophages bearing the cystic
134. Assani, K. et al. Human cystic
135. Gaggar, A. et al. Series
136. Ghio, A. J. et al. Iron accumulates in the lavage and explanted lungs of cystic
137. Roum, J. H., Buhl, R., McElvaney, N. G., Borok, Z. & Crystal, R. G. Systemic de
138. Ghio, A. J. et al. Iron accumulates in the lavage and explanted lungs of cystic
139. Koli, K., Myllärniemi, M., Keski-Oja, J. & Kinnula, V. L. Transforming growth factor-
140. Gauthier, T. W. et al. Impaired defenses of neonatal mouse alveolar macrophage
141. Assani, K. et al. Human cystic
142. Nair, S. et al. Irg1 expression in myeloid cells prevents immunopathology during
143. Riquelme, S. A. et al. Pseudomonas aeruginosa utilizes host-derived itaconate to
144. Andersson, C., Zaman, M. M., Jones, A. B. & Freedman, S. D. Alterations in
145. Bruscia, E. M. et al. Macrophages directly contribute to the exaggerated
146. Andersson, C., Zaman, M. M., Jones, A. B. & Freedman, S. D. Alterations in
147. Ringholz, F. C. et al. Reduced 15-lipoxygenase 2 and lipoxin A4/leukotriene B4
148. Gauthier, T. W. et al. Impaired defenses of neonatal mouse alveolar macrophage
149. Assani, K. et al. Human cystic
150. Bruscia, E. M. et al. Macrophages directly contribute to the exaggerated
151. Tazi, M. F. et al. Elevated Mrcl1/Mir17-92 cluster expression negatively regulates
152. Ferrari, E. et al. Cysteamine re-establishes the clearance of Pseudomonas aer-
153. Coste, T. C. et al. An overview of monitoring and supplementation of omega 3
154. Al-Turkmani, M. R., Freedman, S. D. & Laposata, M. Fatty acid alterations and n-3
155. Bivienllet, S. Van et al. Oral DHA supplementation in Δ508 homozygous cystic
156. Lloyd-Still, J. D. et al. Bioavailability and safety of a high dose of docosahex-
157. Vizia, B. D. E. et al. Effect of an 8-month treatment with u-3 fatty acids (eico-
sapentaenoic and docosahexaenoic) in patients with cystic fibrosis. J. Parenter.
158. Beharry, S. et al. Long-term docosahexaenoic acid therapy in a congenic murine
159. Raghu, G. Idiopathic pulmonary fibrosis: Guidelines for diagnosis and clinical
160. Selman, M. & Parado, A. Withholding the pathogenic and aging-related mechanisms of the
161. Zhang, L. et al. Macrophages: Friend or foe in idiopathic pulmonary fibrosis?
162. Mahalanobish, S., Saha, S., Dutta, S. & Sil, P. C. Matrix metalloproteinase: An
163. Willems, S. et al. Multiplex protein profiling of bronchoalveolar lavage in idio-
164. Dancer, R. C. A., Wood, A. M. & Thickett, D. R. Metalloproteinases in idiopathic
165. Tsubura, E. et al. Accumulation of damaged mitochondria in alveolar macro-
166. Lewis, C. A. et al. Tracing compartmentalized NADPH metabolism in the cytosol
167. Babior, B. M. NADPH oxidase. Curr. Opin. Immunol. 16, 42–47 (2004).
168. Mills, E. & O'Neill, L. A. Sucinate: a metabolic signal in inflammation. Trends Cell
169. Cordes, T. et al. Inflammatory gene 1 and itaconate inhibit succinate dehydrogenase to moderate intracellular succinate levels. J. Biol. Chem. 291, 14274–14284 (2016).
170. Mills, E. L et al. Iotacone is an anti-inflammatory metabolite that activates NrF2 via activation of KEGP1. Nature 556, 113–117 (2018).
171. Lampropoulou, V. et al. Iotacone links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. Cell Metab. 24, 158–166 (2016).
172. Ogger, P. P. et al. Iotacone controls the severity of pulmonary fibrosis. Sci. Immunol. 5, eabc1884 (2020).
173. Gonzalez-Gonzalez, F. J., Chandel, N. S., Jain, M. & Budinger, G. R. S. Reactive oxygen species as signaling molecules in the development of lung fibrosis. Transl. Res. 190, 61–68 (2017).
174. Soares, M. P. & Hamza, I. Macrophages and iron metabolism. Immunity 44, 492–504 (2016).
175. Bedard, K. & Krause, K. H. The NOX family of ROS-generating NADPH oxidases: Physiology and pathophysiology. Physiol. Rev. 87, 245–313 (2007).
176. Murphy, S. et al. Modulation of reactive oxygen species by Rac1 or catase prevents asbestos-induced pulmonary fibrosis. Am. J. Physiol. Lung Cell. Mol. Physiol. 297, 846–855 (2009).
177. Saci, A., Cantley, L. C. & Carpenter, C. L. Rac1 regulates the activity of mTORC1 and mTORC2 and controls cellular size. Mol. Cell 42, 50–61 (2011).
178. Fung, E. et al. Delta-like 4 induces Notch signaling in macrophages: implications for inflammation. Circulation 115, 2948–2956 (2007).
179. Saleh, D., Barnes, P. J. & Giaid, A. Increased production of the potent oxidant peroxynitrite in the lungs of patients with idiopathic pulmonary fibrosis. Am. J. Respir. Crit. Care Med. 155, 1763–1769 (1997).
180. Yamazaki, C. et al. Production of superoxide and nitric oxide by alveolar macro-
181. Lee, J. et al. Bronchoalveolar lavage (BAL) cells in idiopathic pulmonary fibrosis express a complex pro-inflammatory, pro-repair, angiogenic activation pattern, likely associated with macrophage iron accumulation. PLoS One 13, 1–18 (2015).
182. Alden, S. J. et al. The transferrin receptor CD71 delineates functionally distinct airway macrophage subsets during idiopathic pulmonary fibrosis. Am J. Respir. Crit. Care Med. 200, 209–219 (2019).
Macrophage metabolic reprogramming during chronic lung disease

PP Ogger and AJ Byrne
240. Ye, Q. et al. Decreased expression of haem oxygenase-1 by alveolar macrophages in idiopathic pulmonary fibrosis. *Eur. Respir. J.* **31**, 1030–1036 (2008).

241. Larson-Casey, J. L., Deshane, J. S., Ryan, A. J., Thannickal, V. J. & Carter, A. B. Macrophage Akt1 Kinase-mediated mitophagy modulates apoptosis resistance and pulmonary fibrosis. *Immunity* **44**, 582–596 (2016).

242. Stüve, P. et al. De novo fatty acid synthesis during mycobacterial infection is a prerequisite for the function of highly proliferative T cells, but not for dendritic cells or macrophages. *Front. Immunol.* **9**, 1–22 (2018).

243. Koeken, V. A. C. M. et al. Role of glutamine metabolism in host defense against mycobacterium tuberculosis infection. *J. Infect. Dis.* **219**, 1662–1670 (2019).

244. Qualls, J. E. & Murray, P. J. Immunometabolism within the tuberculosis granuloma: amino acids, hypoxia, and cellular respiration. *Semin. Immunopathol.* **38**, 139–152 (2016).

245. Vrieling, F. et al. Analyzing the impact of mycobacterium tuberculosis infection on primary human macrophages by combined exploratory and targeted metabolomics. *Sci. Rep.* **10**, 1–13 (2020).