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Defective Alveolar Macrophage Function in COPD and Novel Treatments to Restore the Defects

Mini Review

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Abstract

Alveolar macrophages (AM) are responsible for maintaining lung homeostasis through the clearance of bacteria, cell debris and apoptotic cells. In chronic obstructive pulmonary disease (COPD), AM functions are dysfunctional and dysregulated and this could drive disease pathogenesis. In COPD, bacterial clearance and apoptotic cell removal by AM is reduced which leads to the colonization of the lung with pathogenic bacteria. The mechanisms behind this defective AM function is not understood. Potential hypotheses surround alterations in surface receptor expression on AM, oxidative stress and exposure to environmental toxins. Identifying these molecular defects could pave the way for novel treatments for COPD. Here I will discuss COPD and the defective AM phenotype observed. Finally, I will discuss some recent therapeutic advances in COPD and the effects of these on macrophage function.

Keywords: *Alveolar Macrophage; COPD; Apoptotic Cell*

Chronic Obstructive Pulmonary Disease

COPD is a term used to describe the combination of three separate diseases-emphysema, small airways disease and chronic bronchitis. COPD is a heterogeneous disease with each patient presenting with a different aetiology. In all COPD patients however, there is a progressive decline in lung function over time and increasing airflow limitation that is only partially reversible [1]. In late stage COPD patients begin experiencing disease exacerbations [2]. These are usually caused by bacteria such as Non-typeable *Haemophilus influenzae, Moraxella catarrhalis* and *Streptococcus pneumoniae* [3]. However, respiratory viruses including human rhinovirus (HRV) and respiratory syncytial virus (RSV) are also isolated [3]. We now believe there is a link between respiratory virus infection and secondary bacterial infection in COPD because the latter can be detected post initial virus infection in a subset of patients [4]. Importantly, these microbes do not get cleared from COPD airways despite the presence of increased numbers of AM, especially in late stage disease [5,6]. This would suggest that AM functions in COPD are diminished and impaired which we now know to be true [7]. This has placed AM as central drivers of COPD disease pathogenesis but we still do not have a complete understanding of why they have defective functions in COPD.

Alveolar macrophages in COPD

In the past, the lung was described as a sterile organ and AM were critical to this sterility [8]. We now know that this is not the case and there is a distinct lung microbiome that can change with different diseases [9,10]. This challenges earlier concepts and suggests the

principal function of AM in the airways is a maintenance of homeostasis. During COPD AM functions such as phagocytosis are dysregulated altering the microbiome in these patients and potentially driving disease exacerbations [10]. However, the link between defective AM function in COPD and exacerbation has not been fully or clearly established (Arjomandi, personal communication) despite some suggestions [11]. In addition, in COPD AM homeostatic functions become diminished and they show a reduced potential to efferocytose apoptotic cells in the airways [7] leading to secondary necrosis and an exaggeration of the inflammatory response. This suggests that during COPD AM lack the ability to both phagocytose and resolve inflammation.

Defective alveolar macrophage phagocytosis in COPD

The initial suggestion that AM function in COPD could be diminished arose from experiments involving *Candida albicans*. Using this model, researchers demonstrated that AM in COPD patients phagocytosed and cleared less *C. albicans* compared to control patients [12,13]. Later studies very nicely demonstrated that AM from COPD patients were defective in their ability to internalize NTHi [14]. This defect appeared specific to AM and was not found for monocyte derived macrophages (MDMs) differentiated from the peripheral blood of these patients [14]. This was later challenged in a different study with a different cohort of patients showing that MDMs from COPD patients were defective in their ability to phagocytose both NTHi and *S. pneumoniae* [15]. This defect was not observed towards polystyrene beads [15] confirming earlier studies [7]. These results suggest that defective macrophage phagocytosis in COPD is not a global phagocytic shutdown but certain uptake pathways are diminished. These studies also offer the novel idea that the defective phagocytic phenotype is already present at the 'precursor' cell level in COPD patients and unmasks further upon differentiation into tissue macrophages. AM efferocytosis has also been shown to be defective in COPD. For COPD AM it has been demonstrated that the efferocytosis of apoptotic airway epithelial cells (AEC) was reduced [7]. The mechanism behind this was suggested to be related to either a failure of AM to recognize the AEC or a tolerance mechanism in the AM [7]. Further work identified that this observation was specific to COPD patients and not related to smoking status [16]. Despite these studies, the mechanism of this AM defect towards AEC and bacteria is still unknown.

As phagocytosis involves the recognition and engagement of particles with various cell surface receptors it has been suggested that reduced receptor expression could drive an impaired AM response in COPD. To begin to understand why efferocytosis of apoptotic cells was decreased in COPD, researchers began looking at critical receptors. A study in 2006 identified that CD206 or the mannose receptor was decreased in expression on COPD AM compared to controls [17] with no effect on CD11b [18]. This could explain partially why efferocytosis was diminished because CD206 is crucial for efferocytosis. Various studies have highlighted that CD14, which is more highly expressed on peripheral monocytes is increased in expression in AM in COPD patients [16,19]. Furthermore, a population of small AM have been identified in COPD patients expressing CD14 and human leukocyte antigen-DR (HLA-DR) at higher levels compared to control patients [16,19]. This could suggest that the defective AM phagocytic response in COPD patients does not arise in the tissue AM but is a property of these smaller AM that have a lower phagocytic capacity. However, the expression of CD14 is still debatable and other reports suggest that CD14 expression in COPD AM is not affected and similar to control patients [18,20]. A range of other surface receptors have also been explored but the results are more conflicting. In the Lofdahl study they reported no differences in surface expression of CD58, CD80, CD7, HLA-DR and CD11b but reduced expression of CD86 and CD11a [18]. In 2007 the team of Sandra Hodge suggested that CD71 was reduced on COPD AM as was CD31, CD91 and CD44 [16]. This conflicted with an earlier study suggesting that CD44 expression was not altered on COPD AM [20]. This same study also suggested that CD36, CD61, CD86 and CD40 were not affected on COPD AM but CD80 and HLA-DR was [20]. Toll like receptors (TLR) have also been explored and reduced expression in COPD has been reported for some TLR such as TLR2 [21] but not for others including TLR4 [22]. Similar studies with COPD MDMs have not been as clear. Although COPD MDMs demonstrate defective bacterial phagocytosis this was not related to differences in cell surface receptor expression [15]. Taken together, it would seem that while reduced cell surface receptor expression might explain some of the phagocytic defects on COPD macrophages they do not explain the entire story. In addition, a lot more detailed analysis is required to overcome the conflicting reports in different patient cohorts. It is necessary to keep in mind however, that as phagocytosis is a complex process and can involve multiple

receptors [23] a change in one or two would probably not result in large differences in phagocytosis.

In summary, all of these reports prove and strengthen the idea that there is a defective phagocytic and efferocytic function of AM in COPD towards specific targets but rule out a defect in the response towards inert particles. This suggests that a) specific uptake pathways are affected in COPD AM and b) different recognition mechanisms have a role in regulating this defect. Further research to dissect the mechanism behind this defective response and the driver of the defect in AM and other macrophage populations in COPD is needed.

Pharmacological restoration of phagocytosis and other macrophage functions in COPD

As defective AM phagocytosis could drive COPD pathophysiology, agents that restore their functions could prove beneficial for future patient treatment. Current treatments for COPD only partially improve airflow limitation [24]. A lot of the current treatments including anticholinergics can modulate macrophage functions in vitro [25,26] with select responses [27,28]. Therefore, over recent years there has been a lot of interest in potential new therapeutics for COPD that target macrophages and especially macrophage phagocytosis and inflammatory responses in COPD.

Resveratrol, a naturally occurring polyphenol inhibits inflammatory responses almost as well as glucocorticoids [29-31]. This mechanism appears to occur via resveratrol's ability to directly target phosphodiesterase type 1 (PDE1), type 3 (PDE3) and type 4 (PDE4) isoforms and extracellular regulated kinase (ERK). It also shows a unique inhibitory effect on the phosphorylation of c JUN N terminal kinase (JNK) [32-34]. For COPD patients, it is particularly interesting because it can suppress inflammation induced by NTHi through upregulating short myeloid differentiation primary response 88 (MyD88s) and inhibiting extracellular regulated kinase 1/2 (ERK1/2) phosphorylation [35]. These authors demonstrated that treating mice with resveratrol post NTHi infection reduced inflammation [35]. This supports the idea that this polyphenol could be used to reduce NTHi induced inflammation at COPD exacerbation and even before patients progress to exacerbation [35].

Resolvin D1 is an aspirin derived specialized pro-resolving mediator (SPM). It has been found to dampen NTHi induced lung inflammation and promote bacterial clearance through an increase in M2 macrophages [36]. In mice treated with resolvin D1 the majority of systemic NTHi infection was absent [36]. This represents the first pulmonary model where an SPM was pro-resolving with effects on inflammation, lung physiology and bacterial clearance. This opens up a new avenue in COPD research and could become a novel treatment to reduce inflammation and improve lung function.

Curcumin, a natural plant ligand has been extensively reported to have anti-inflammatory effects [37-39]. Recently curcumin was found to inhibit NTHi induced inhibitor of nuclear factor kappa B kinase subunit beta (IKK β) phosphorylation and upregulate mitogen activated protein kinase phosphatase 1 (MKP-1) expression leading to decreased C-X-C motif chemokine ligand 5 (CXCL5) secretion [40]. In vivo it blocked neutrophil infiltration into the middle ear during otitis media [40]. The ability of curcumin to block CXCL5 secretion could suppress inflammation and curcumin is readily emerging as a therapeutic to treat NTHi induced inflammatory disease [40].

In earlier research procysteine was shown to restore the phagocytic ability of AM and tissue associated macrophages [41]. This has now been extended to thymoquinone which has antioxidant and anti-inflammatory effects [42,43]. Thymoquinone improved macrophage efferocytic and phagocytic ability [44]. The mechanism of action of thymoquinone is under investigation but preliminary data suggests it could modulate the sphingosine 1 phosphate (S1P) signaling system [44]. This is potentially interesting because the S1P system is critical for various macrophage functions [45]. As thymoquinone is already in trials for various diseases this drug will be worth considering as a novel treatment for inflammation and phagocytic defects in COPD in the future.

Inhibition of pathways actively contributing to COPD offer novel routes to treating and reversing the disease. There are multiple trials showing the benefits of inhaled p38 inhibitors [46,47]. In murine models, administration of an aerosolized phosphoinositide 3 kinase gamma/delta (PI3K γ / δ) inhibitor suppressed lung inflammation [48]. Rho associated protein kinase (ROCK) inhibition has anti-inflammatory effects on airways [49]. A current study demonstrated that inhibition of the p38 mitogen activated protein kinase (MAPK)

pathway by two structurally different chemotypes did not alter AM bacterial phagocytosis, killing or efferocytosis [50]. This is in agreement with other studies [51]. Inhibition of the PI3K pathway by three subunit inhibitors did not alter phagocytosis or intracellular killing of *S. pneumoniae* or *Haemophilus influenzae* by AM suggesting differential regulation [50]. Inhibition of ROCK did not alter phagocytosis or intracellular killing of bacteria by AM but led to an increase in the efferocytosis of apoptotic neutrophils [50]. This supports the notion that ROCK inhibition reverses the defect in efferocytosis seen in the COPD lung and highlights even further that there are very different pathways regulating bacterial phagocytosis and efferocytosis in COPD [50]. In summary, these studies support the notion of using kinase inhibitors as novel treatments for COPD because they do not further impact on the suppressed immune responses of COPD macrophages and these approaches will not have a negative effect on bacterial clearance and the removal of apoptotic bodies.

Macrolide antibiotics are moving forwards as novel treatments for COPD. Azithromycin and telithromycin have been shown to have anti-viral effects and/or effects during respiratory exacerbations [52-56]. Azithromycin was found to improve efferocytosis of AEC by normal and COPD AM [17]. A similar effect was observed with apoptotic neutrophils [17,57,58]. This was particularly exciting and clinically important for COPD which presents increased apoptosis of bronchial epithelial cells and AEC [59] and where the inflammation leads to an ineffective repair of the injured epithelium and a loss in structural integrity [17]. The use of azithromycin is promising because smokers demonstrate increased pulmonary internalization of antibiotics that concentrate in macrophages [60]. Follow up studies by the Hodge team found that azithromycin increased the surface expression of the mannose receptor on AM by 50% [61]. This result was very strongly backed up by patient studies. The team administered low dose azithromycin to a cohort of COPD patients for 12 weeks and took samples at the end to monitor inflammatory markers [61]. They confirmed that the AM from those patients given azithromycin exhibited better phagocytosis and increased expression of the mannose receptor [61]. This is critical as the mannose receptor is important in apoptotic cell phagocytosis.

Recently, novel macrolides with virus induced interferon (IFN) augmenting potential were identified [62]. The authors identified Mac5 as having stronger effects compared to azithromycin [62]. Further analysis identified several analogues of Mac5 with antiviral, anti-inflammatory and broad spectrum anti-bacterial properties [62]. This work paves the way to identify novel therapeutics for COPD by using macrolide structures as the chemical starting points [62]. However, this work is not without small caveats. Initial small studies surrounding the ability of macrolide antibiotics to reduce exacerbation frequency produced conflicting data [63-67]. A report from 2011 using a larger patient cohort showed that subjects at an increased risk of exacerbation who received azithromycin daily for 1 year showed a decreased exacerbation frequency [68]. This was accompanied by a decrease in the incidence of colonisation with respiratory pathogens and improved quality of life but there were side effects including increased colonisation with macrolide resistant organisms [68].

Alpha 1 antitrypsin has become interesting as a therapeutic after the discovery that it exhibits an anti-inflammatory function in bacterial infected macrophages [69]. Recently during experimental cigarette smoke exposure, application of alpha 1 antitrypsin was shown to reduce HRV viral load [70]. This could have wide reaching effects as alpha 1 antitrypsin augmentation therapy is currently used as an approach to restore alpha 1 antitrypsin levels in COPD with alpha 1 antitrypsin deficiency as it can reduce disease severity and exacerbation frequency [71-73].

It has been shown that calcium can restore phagocytosis in macrophages [74]. Calcium treatment improved NTHi phagocytosis [74] by increasing expression of CD16 and MARCO at the cell surface on MDMs from COPD patients and was mainly due to a greater per cell expression. In addition, calcium showed effects on macrophage activation and cytokine production [74]. The above data is also backed up by a previous study that showed if COPD AM were treated with sulforaphane then the levels of MARCO on the cell surface increased [75]. This treatment improved bacterial phagocytosis, clearance and antibacterial defenses [75]. This improved phagocytosis was found to be common to NTHi and *Pseudomonas aeruginosa* and this was found to be through a balance between the increased transcription and expression of MARCO induced by sulforaphane and also an increase in antioxidants [75].

In summary, all of these novel molecules will prove important for COPD treatment and research in the future. They all have the potential to improve patient outcome in COPD but their clinical utility still remains to be verified and fully established. But, they demonstrate that by improving our understanding of the disease it is possible to develop new therapeutics that will benefit patients in the future.

Concluding Remarks

COPD is on the increase in the developed world. It is clear that AM in COPD patients are defective in phagocytosis and clearance of respiratory pathogens and airway debris. The role of receptors in mediating this defect has proven conflicting and aside from this there is no downstream mechanistic data about what regulates defective AM phagocytosis in COPD and this area of research represents a big gap in the field. Given this, we must now begin to dissect the effect that pathogens have on macrophage phagocytic capabilities in COPD in order to better dissect the molecular details behind their defective functions in this disease. Lastly, it is important to consider how the interaction of macrophages with other cell types in the COPD lung environment could influence their functions.

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Competing Interests

JJ has no competing interests.

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