

Commentary

Macrophages—the immune effector guardians of the lung: impact of corticosteroids on their functional responses

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Lung macrophages (LMs) are key immune effector cells that protect the lung from inhaled particulate matter, noxious gases and pathogens. In Chronic Obstructive Pulmonary Disease (COPD), there is an abundance of macrophages in airspaces and lung tissues suggesting that they play an important role in the pathogenesis of the disease. Furthermore, macrophage phenotype and functional properties are altered in COPD toward a more pro-inflammatory state, characterized by reduced pathogen recognition and processing ability and dysfunctional tissue repair qualities. Inhaled corticosteroids (ICSs), used in the management of COPD, has been shown to reduce acute exacerbations of COPD but is also associated with increased occurrence of pneumonia. Corticosteroids treatment altered LM phenotypic characteristics and their functional properties, and this commentary discusses current knowledge and also the gaps in our understanding of the impact of ICS on LMs phenotype and function. A better understanding of how ICSs impact the immune-inflammatory responses in the lung, in particular ICSs' effects on LMs, could allow more selective personalized tailoring of the use of ICSs in COPD to improve disease progression, morbidity and mortality.

Chronic Obstructive Pulmonary Disease (COPD) is caused by the chronic inhalation of toxic particles and gases, including cigarette smoke (CS) [1]. These exposures elicit a persistent innate and eventually an adaptive immune response in the airspaces and lung tissues characterized initially by stimulation and overproduction of mucus in the central airways (chronic bronchitis), chronic tissue inflammation resulting in fibrosis and obstruction of small airways and eventual destruction of the lung parenchyma causing emphysema [2]. These chronic destructive immune responses have features of impaired tissue repair and tissue remodeling that ultimately lead to a progressive disease phenotype [3]. Furthermore, the chronic inflammatory process in lung tissues, increase vulnerability for viral and bacterial infections causing acute exacerbations which are associated with a poor long-term outcome [4].

Airspace macrophages are the first immune cells encountering inhaled toxins and are key innate immune effector cells that identify, engulf and process inhaled particles and noxious gases, including CS and ambient environmental particulate matter (PM) [5]. The number of macrophages in bronchial alveolar lavage fluid (BAL) and lung tissues are increased in COPD [6] suggesting a potential key role for these cells in the chronic inflammatory process and pathogenesis of COPD. The inflammatory microenvironment in the lung triggered by inhalation exposures alters the phenotype and function of lung macrophages (LMs). The 'classical' activated M1 macrophages have enhanced phagocytic capabilities and antigen-presenting properties, producing cytokines such as interleukin (IL)-1 β (IL-1 β), IL-6 and IL-12 and tumor necrosis factor- α (TNF- α), all important for clearance of intracellular and bacterial pathogens [7]. M2 macrophages or 'alternative activated macrophages', are characterized by the expression of distinguishable surface markers such as mannose receptor (CD206), and differentiation further into M2a

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macrophages driven predominantly by IL-4 and IL-13, M2b by immune complexes and lipopolysaccharides (LPSs), M2c by glucocorticoids and transforming growth factor β (TGF- β), and M2d by IL-6 and adenosines [8]. Therefore, the microenvironment in the lung has the ability to shape and reshape LMs differentiation depending on their microenvironment, highlighting the strong plasticity of these cells [9] in order to promote a delicate balance between excessive inflammation and tissue repair in response to innocuous stimuli and to maintain homeostasis.

Exposure of LMs to noxious stimuli such as PM and pathogens, skew macrophages toward a more M1 phenotype that produces pro-inflammatory cytokines, chemokines and oxygen radicals [7]. However with a more chronic persistent exposure (such as in COPD induced by cigarette smoking) the chronic inflammatory microenvironment compromises these macrophages' phagocytosis and efferocytosis abilities [10,11]. Furthermore, this chronic exposure also results in an abundance of macrophages in COPD tissues [5,6] contributing to the inflammatory burden in the lung [2] and to the downstream detrimental effects of reducing macrophage phagocytosis and efferocytosis abilities [11]. Several recent studies however have shown a larger spectrum of phenotypes of macrophages in airspaces and lung tissues in COPD [12]. Specifically, there are population of macrophages that express markers for both M1 and M2 macrophages (dual positive) and a significant population that does not express any classical M1 or M2 markers (M0 macrophages). Furthermore, Dewhurst and co-workers also showed the importance of LM location, cell size and function such as cytokine production and phagocytosis [13]. The function and the role of these different macrophage phenotypes in the pathogenesis of COPD is still unclear.

Corticosteroids are one of the cornerstones of the chronic management of COPD (inhaled corticosteroids or ICSs) and management of acute exacerbations [14,15]. The glucocorticoid receptor function on macrophages harvested from COPD lung tissues are unchanged (compared with controls) [16] and the corticosteroid-induced reduction in pro-inflammatory mediator and oxygen radical production by macrophages in COPD are thought to be one of the main beneficial anti-inflammatory effects of ICS in COPD [17]. In this issue, Higham and co-workers extended their previous work using monocyte-derived macrophages (MDM) for studies on macrophages harvested from fresh lung tissues [18]. They explore other potential beneficial mechanisms how glucocorticoids could impact macrophage functional properties to support the large body of clinical evidence supporting the use of ICS in COPD. Their studies support the concept that corticosteroids modulate LM to a more M2 phenotype, specifically an M2c phenotype with increased expression of CD163, CD206 and MERTK. CD163 is the high affinity scavenger receptor for the hemoglobin-haptoglobin complex and functions as innate immune sensor for Gram-positive and Gram-negative bacteria [19]. The authors postulate that enhanced scavenging of hemoglobin-haptoglobin complexes by CD163 macrophages reduces iron bioavailability for bacterial growth, thereby reducing bacterial colonization in airspaces of COPD. CD206, a mannose receptor is expressed on the surface of macrophages and immature dendritic cells, where it acts as a pattern recognition receptor interacting with a variety of proteins and glycolipids on the surfaces of pathogens, including viruses, fungi and bacteria, thereby playing a key role in immune recognition of pathogens [20]. Both these molecules (CD163 and CD206) will promote pathogen recognition and removal but the authors were unable to show enhanced phagocytosis of a variety of opsonized bacteria by corticosteroid-treated macrophages. This is a critically important issue that requires further study and investigation. Numerous studies have shown that LM phagocytosis is compromised in COPD [10,11] and the mechanism(s) resulting in this reduced phagocytosis are still unclear. Recent studies have shown that this reduction in phagocytosis of macrophages is global and not restricted to specific macrophage phenotype [21]. The airways of subjects with COPD are known to be prone to be colonized with a variety of bacteria, and colonization is associated with more frequent acute exacerbation of COPD, events known to cause progression of disease and increase mortality [22,23]. Using of ICSs in subjects with COPD reduces COPD exacerbation which are mostly triggered by either viral or bacterial infections [24]. Therefore this beneficial effect of ICS in COPD is not via promoting phagocytosis of microorganisms [18] but via other mechanisms such as reducing airway inflammatory responses. Chronic ICS use in COPD is also associated with more frequent pneumonias [25]. This suggests that the immune-modulating effects of ICS on macrophages (such as its impact on phagocytosis of microorganisms) may differ significantly in airways and alveolar spaces. Harvesting macrophages from these specific geographical regions for phenotyping and functional studies, could resolve this critical important issue. At last, Higham and co-workers showed that MERTK was up-regulated by exposure LM to corticosteroids. Tyrosine-protein kinase MER or MERTK are involved in cell survival, migration, differentiation and phagocytosis of apoptotic cells (efferocytosis). The lack of functional MERTK protein in macrophages compromise their ability to appropriately engulf apoptotic cells. This inefficient clearance of dead cells can lead to promoting inflammation and even development of autoimmunity [26], a hypothesis suggested as a reason for the progressive nature of COPD [27]. Due to the chronic inflammatory response present in COPD lung tissue, the burden of apoptotic cells (both structural cells such as epithelial cells, fibroblast etc. and immune cells such as neutrophils and eosinophils) in lung tissues are high [28]. Furthermore, LM ability to clear these apoptotic cells are reduced in COPD (compared with controls)

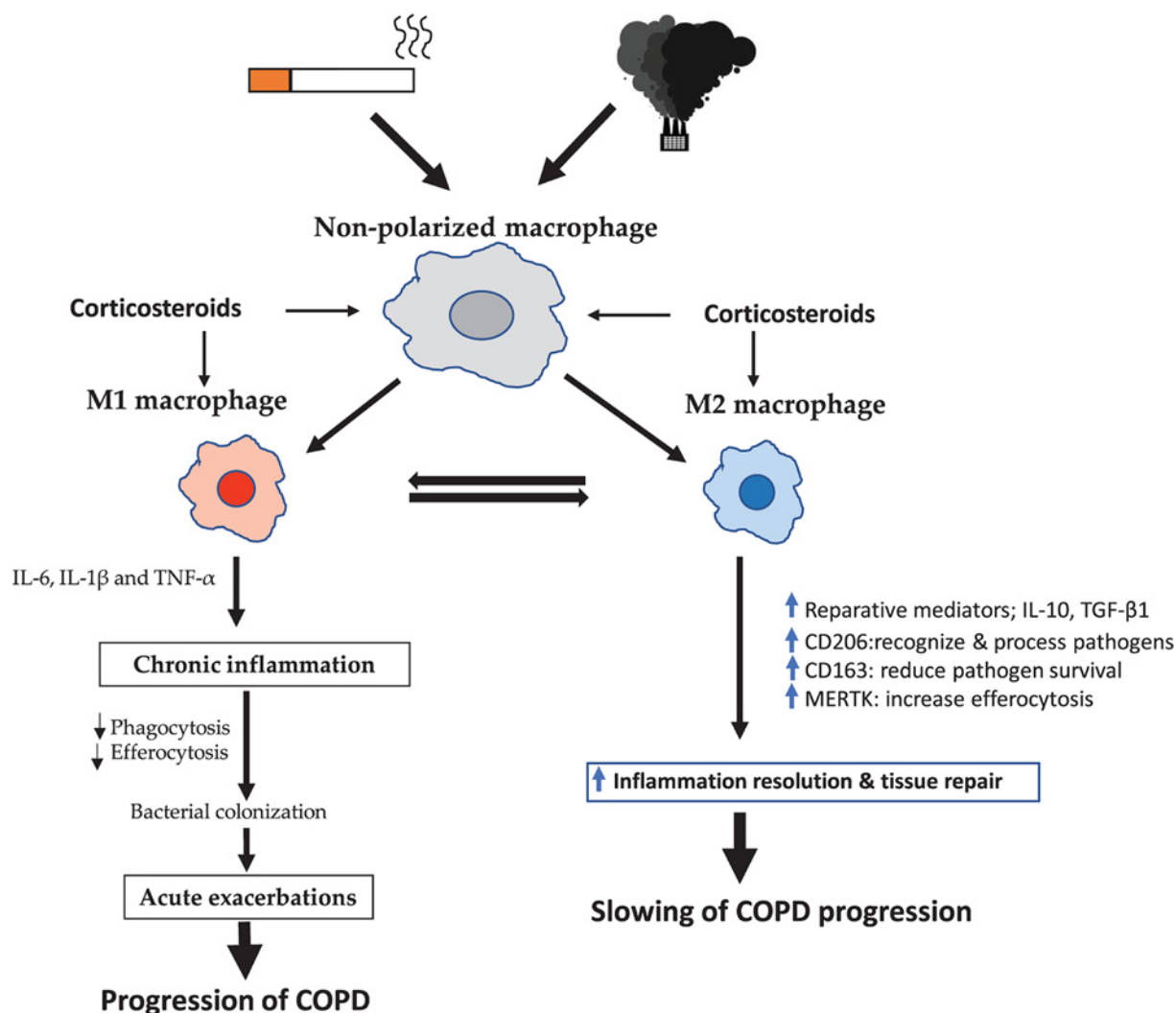


Figure 1. The impact of noxious gases and particulate matter (CS and ambient air pollutants) on LM phenotype and disease progression in COPD: effect of corticosteroids

Inhaled toxins and particles are processed by airspace macrophages which shift them to a more M1 phenotype with the production of pro-inflammatory mediators including, IL1- β , IL-6, TNF- α which promote the recruitment of polymorphonuclear leukocytes such as neutrophils and eosinophils as well as monocytes into the airspaces to clear these toxins and particulate matter from the airspaces. However, with chronic exposure, resident M1 macrophages and new MDM phagocytic and efferocytosis functions are compromised leading to chronic low-grade inflammation and eventual bacterial colonization of airspaces. This chronic airway inflammation and bacterial colonization are associated with frequent infectious exacerbation that lead to COPD disease progression. M2 macrophages are involved in inflammation resolution, tissue remodeling and repair, but this phenotype and their function are suppressed by the chronic inflammatory insult of inhaled toxins and particulate matter. Corticosteroids exposure (either inhaled or systemic) reduce pro-inflammatory cytokine production by macrophages and shift macrophage phenotype more toward M2 macrophage phenotype and function which increase tissue reparative mediators (IL-10 and TGF- β 1), increase CD206 expression (to increase pathogen recognition and processing), CD163 (to reduce pathogen survival by scavenging iron ions) and up-regulating MERTK (to enhance cell efferocytosis of apoptotic cells and cell debris). This macrophage phenotype shift will promote resolution of inflammation and tissue repair, reduce bacterial colonization thereby slowing disease progression in COPD.

[29] and Higham and co-workers have shown that the increase in MERTK expression in macrophages treated with corticosteroids was associated with enhanced uptake of apoptotic neutrophils. This suggests that at least one of the mechanisms how ICSs reduce airspace and lung inflammation in COPD via promoting the clearance function of LMs. They speculate that corticosteroids push the LM response more toward M2 macrophage functional response

promoting efferocytosis and tissue repair. This, however, still needs to be established *in vivo* in subjects using chronic ICSs for COPD management. This study of Higham and co-workers [18] however have enhanced our understanding of the potential benefits of corticosteroids in the treatment of COPD and also highlight gaps in our knowledge that need further study. Key questions are:

- 1) How does the effect of corticosteroids differ between macrophages in the airways, alveolar spaces and lung tissues (interstitial macrophages)? Higham and co-workers used macrophages harvested from all the three compartments [17], macrophages from which region of the lung are the most sensitive to the beneficial effects of corticosteroids?
- 2) Do macrophages phenotype impact the beneficial effects of corticosteroids?
- 3) How do corticosteroids impact other key macrophage functions such as chemotaxis, proliferation and survival?
- 4) ICSs are associated with increased pneumonias in COPD. Do corticosteroids have a detrimental effect on macrophages from alveolar spaces, such as reduce their phagocytic function?
- 5) Do other anti-inflammatories used in COPD management such as macrolides and phosphodiesterase inhibitors impact macrophage phenotype and function?

LMs are key initiators of lung innate immunity and possess high phagocytic activity to clear particulate antigens and pathogens to regulate the response to infection and inflammation. The resident and recruited macrophages remove apoptotic cells and cell debris to drive tissue remodeling and repair following the downstream inflammatory-induced epithelial and tissue damage. These functional responses of LM are highly regulated by their micro-environment to establish tissue homeostasis. If this delicate balance between pro-inflammatory response to clear pathogens and particulate matter and resolution of tissue inflammation is perturbed, these LM could contribute to pathological tissue remodeling characteristic of diseases such as COPD (Figure 1). Furthermore, this chronic inflammatory milieu in lung tissues also increase the vulnerability to acute and chronic airways colonization and infections leading to exacerbations of COPD that further lead to disease progression. Modulating the function(s) of these immune effector cells in COPD [30] have the potential to slow the progression in tissue damage, reduce the vulnerability to infectious exacerbations improving disease morbidity and mortality.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

COPD, chronic obstructive pulmonary disease; CS, cigarette smoke; ICS, inhaled corticosteroid; IL, interleukin; LM, lung macrophage; PM, particulate matter.

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