Heterogeneity of Lung Mononuclear Phagocytes in Chronic Obstructive Pulmonary Disease

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Key Words

Chemokines • Macrophages • Pattern recognition receptors

Abstract

Chronic obstructive pulmonary disease (COPD) is a disease defined by an aberrant inflammatory response to inhaled cigarette smoke and other noxious particles. The factors triggered in the lungs that drive inflammation and lung tissue destruction are not fully understood, but mononuclear phagocytes play a central role by releasing mediators that promote both inflammation and tissue-destructive emphysema. Although conflicting studies on alveolar macrophages exist regarding chronic cigarette smoke exposure and its effects on macrophage polarization patterns, we have recently identified a cell type in mice defined by CX3CR1 expression. The population of this cell type expands in the lungs and elaborates M1 signature cytokines in response to cigarette smoke exposure *in vivo*. In addition, the absence of functional CX3CR1 provides protection from tissue-destructive emphysema in a murine model of chronic cigarette smoke exposure. The heterogeneity and plasticity of discrete macrophage subsets, in terms of immunophenotype and function, may explain the seemingly disparate findings showing a suppressed inflammatory profile on the one hand and a heightened inflammatory response on the other. This

review examines the evidence that discrete mononuclear phagocyte subsets develop in response to cigarette smoke exposure, and that the spatial cues provided by the lung tissue microenvironment in which the mononuclear phagocytes reside may influence the distribution and function of these subsets.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide and cigarette smoke exposure is cited as the most important risk factor in the development of COPD [1]. COPD is characterized by an aberrant inflammatory response to inhaled cigarette smoke and other noxious particles [2, 3]. While it is defined physiologically by airflow limitation that is not fully reversible, it includes a spectrum of disease ranging from inflammation of the larger airways (termed chronic bronchitis), remodeling of the small airways, and parenchymal tissue destruction with airspace enlargement (defined as emphysema). In addition, COPD contributes to systemic manifestations affecting skeletal muscles, bone and the cardiovascular system [4, 5]. Although the phenotypic expression of COPD is quite het-

erogeneous, the small airway walls and emphysematous lung tissue have consistently shown a persistent inflammatory response with mononuclear phagocytes being a key player [6–8].

Recent studies, however, have also shown that alveolar macrophages from smokers show a ‘noninflammatory’ or suppression of M1 phenotype [9, 10]. This dichotomy of findings may be explained by the plasticity and heterogeneity of lung mononuclear phagocytes and the development of macrophage subsets in different compartments of the lung. Depending on spatial cues that are dictated by the lung tissue microenvironment and are influenced by additional endogenous or exogenous triggers, there is evidence to suggest that mononuclear phagocytes in the lungs may undergo distinct activation programs acquiring polarized phenotypes and function to facilitate protection or repair on the one hand, but at times also result in damage to tissues [9]. While macrophages in the lungs serve a critical line of host defense against inhaled environmental particles and pathogens, our understanding of how cigarette smoke exposure alters the distribution and function of discrete subsets of mononuclear phagocytes remains incomplete. Many pathways have been studied and ever-increasing numbers of mediators and processes are now currently being investigated (detailed comprehensively elsewhere [5]). In this review, we focus upon the available evidence for the role of mononuclear phagocytes, specifically macrophages, underlying the pathogenesis of COPD and emphysema. In particular, with regard to cigarette smoke exposure, we highlight the experimental evidence which supports the concept of the local tissue microenvironment within the lungs influencing the distribution and function of these discrete subsets.

Macrophages as Mediators of Tissue Destruction

Macrophages are the resident phagocytes representing a major line of defense to noxious particles and pathogens in the respiratory bronchioles and airspaces. Cigarette smokers show an accumulation of pigmented macrophages within the respiratory bronchioles that is rarely observed in nonsmokers [11]. This respiratory bronchiolitis was postulated by Niewoehner et al. [11] in 1974 as a precursor of centriacinar emphysema (defined by the presence of irreversible airspace enlargement without obvious fibrosis that is due to lung tissue destruction affecting the region of the respiratory bronchiole) and is responsible for subtle functional abnormalities found in

young smokers prior to the development of clinically overt disease. Finkelstein et al. [12] later showed that increased numbers of macrophages and T lymphocytes within the alveolar walls correlate with mild-to-moderate emphysema, as opposed to the numbers of neutrophils which show a negative correlation with emphysema. The positive correlation between macrophages and T lymphocytes led the authors to propose an interaction between these two cell types that promotes lung tissue destruction [12]. Although the interaction between innate and adaptive immune cells collaborating to promote aspects of disease is supported by recent findings [6, 8, 13], the understanding of distinct macrophage activation programs elicited by cigarette smoke and how they contribute to disease in some individuals is less clear.

Proteinase:anti-proteinase imbalance, where cigarette smoke exposure promotes repeated proteolytic injury directed against the extracellular matrix in the face of inadequate antiproteinase defenses, has been a dominant paradigm explaining the pathogenesis of cigarette-smoke-induced emphysema. The evolution of this paradigm has been intimately linked to the recognition of the macrophage as a major effector cell. Neutrophils, releasing neutrophil elastase (an enzyme that breaks down elastin), a major protein component of the lung extracellular matrix [14], were originally implicated as the principle cell in mediating cigarette-smoke-induced emphysema. This was primarily based upon the fact that individuals with a deficiency of α 1-anti-trypsin (the major inhibitor of neutrophil elastase) are predisposed to the development of early-onset emphysema and that intra-tracheal instillation of elastase resulted in emphysema in experimental animals [15, 16]. However, the concept of neutrophil elastase as the sole arbiter of tissue-destructive disease in cigarette-smoke-induced emphysema is less clear and was challenged when mice expressing the human collagenase (matrix metalloproteinase-1; MMP-1) transgene developed pulmonary emphysema [17]. Moreover, mice deficient in macrophage elastase (i.e. macrophage metalloproteinase-12), the predominant MMP in murine macrophages, were completely protected from developing cigarette-smoke-induced emphysema [18]. Further supporting the involvement of macrophages in the proteinase:antiproteinase imbalance, another study showed that alveolar macrophages from COPD subjects release significantly less of the tissue inhibitor of metalloproteinase (TIMP)-1 (an important antielastolytic molecule) than smokers without COPD or nonsmokers [19].

Murine lungs are anatomically distinct from human lungs, as they show less airway branching and lack respiratory bronchioles [20]. Despite these differences, murine models of emphysema provide a powerful tool to examine underlying mechanisms and possibly test early therapeutic strategies, and are particularly helpful in studying novel targets or pathways that may be relevant in human disease. Indeed, studies examining human alveolar macrophages and bronchoalveolar lavage fluid (BALF) have confirmed that a proteinase:antiproteinase imbalance exists in cigarette-smoke-induced emphysema or COPD [19, 21, 22]. In particular, macrophages from the BALF of patients with emphysema showed increased mRNA transcripts of MMP-1 (collagenase) and MMP-9 (gelatinase B) associated with increased collagenase and elastolytic capacity when compared with macrophages from normal subjects [21]. Although mice overexpressing human MMP-9 in macrophages developed emphysema [23], a subsequent study showed that MMP-9 deletion did not confer protection in a murine model of cigarette-smoke-induced emphysema [24]. Furthermore, no correlation exists between macrophage MMP-9 production, the major source of MMP-9 in severe COPD, and local emphysema severity in human lung tissues [24]. It is important to note that these investigators concede that, under the right conditions, MMP-9 overexpression may contribute to the development of emphysema in humans; an MMP-9-overexpressing polymorphism was associated with upper lobe-predominant emphysema in a Japanese cohort [24, 25].

The MMP-12 gene is induced in the alveolar macrophages of cigarette smokers [26] and a functional polymorphism that reduces MMP-12 expression is associated with a reduced risk of COPD in adult smokers [27]. However, studies have not confirmed increased MMP-12 expression in human emphysema [21, 28]. While the precise molecular targets responsible for the proteinase:antiproteinase imbalance have been the source of continued debate, both human and mouse studies have confirmed the importance of macrophages as mediators of tissue destruction.

Innate Immune Activation of Macrophages by Repeated Cigarette Smoke Exposure

Experimental evidence has shown that components of cigarette smoke, either directly or through the induction of endogenous danger signals, can be sensed by pattern recognition receptors such as Toll-like receptors (TLR)

and trigger an innate activation program in macrophages. Cigarette smoke condensate activates bone marrow-derived macrophages in mice to produce tumor necrosis factor (TNF)- α in vitro in a dose-dependent manner that is independent of LPS but requiring TLR4, interleukin (IL)-1R1, and MyD88 signaling [29]. Moreover, heat shock protein (hsp)-70 has been identified in the BALF of mice following acute cigarette smoke exposure, indicating that endogenous danger signals are released, trigger the TLR4/IL-1R1 signaling pathway and produce lung inflammation [29]. It has been recognized that persistent inflammation occurs in the lungs of COPD patients even with tobacco cessation [7], although the mechanism by which this occurs is not entirely clear. Mineo et al. [30] showed that nonsmoking emphysematous patients manifest higher systemic TNF- α and IL-6 concentrations when compared to healthy nonsmoking volunteers. Following the removal of inflammatory emphysematous tissue by lung volume reduction surgery, there was a reduction in both cytokine concentrations in these patients. This finding supports the concept of persistent inflammation long after smoking cessation and that TNF- α and IL-6 may be cytokine signatures of this activation state.

Churg et al. [31] have shown that TNF- α contributes to the majority of cigarette-smoke-driven emphysema in a mouse model, and TNF- α is a central cytokine of acute smoke-induced inflammation resulting in connective tissue breakdown [32]. TNF- α in COPD has been studied extensively, and there is substantial evidence that this cytokine is increased in the sputum and serum of COPD patients [5]. However, a randomized, double-blind, placebo-controlled trial in moderate-to-severe COPD patients showed no benefit from infliximab, an anti-TNF- α antibody, in health-related quality-of-life assessments measured by the Chronic Respiratory Questionnaire total score [33]. This suggests that targeting one single mediator is unlikely to provide clinical improvements in a complex, heterogeneous disease such as COPD, and that focusing on the underlying biology of cell types which amplify and contribute to an altered lung microenvironment may prove to be more useful in providing the foundation of knowledge for rational therapeutic design.

Suppression of M1 Polarization Pattern in Alveolar Macrophages with Chronic Cigarette Smoking

Recent studies in humans have highlighted the fact that not all macrophages show a program consistent with innate activation. Comparing alveolar macrophages ob-

tained by bronchoalveolar lavage of healthy nonsmokers, healthy smokers and COPD smokers, Shaykhiev et al. [9] showed that cigarette smoke exposure is associated with the progressive downregulation of genes encoding for type 1 chemokines CXCL9, CXCL10, CXCL11 and CCL5 and progressive activation of genes such as MMP-2 and MMP-7. Some studies have designated MMPs as characteristic of M2 macrophage polarization [9, 34], particularly in the setting of tumor-associated macrophages [35]; currently, however, it is not clear whether MMPs in the setting of COPD are distinctive M2 signatures because they show both proinflammatory and tissue-destructive potential. Investigators nevertheless concluded that alveolar macrophages likely contribute to COPD pathogenesis in a noninflammatory manner due to their smoking-induced reprogramming towards an M1-deactivated, partially M2-polarized state [9].

Alveolar macrophages from smokers in another study presented a distinctive activation program that could be differentiated from nonsmokers, with increases observed in phospholipase A2 group 7, MMP-12, CCR5 and osteopontin genes [26]. Still others have shown that the BALF from smokers expressed higher numbers of cells than nonsmokers, but with lower IL-6 concentrations [36] and a depressed capacity for LPS-induced TNF- α and IL-6 release. Chen et al. [10] expanded upon these findings and showed that cigarette smoking inhibits proinflammatory but not anti-inflammatory cytokine production in the alveolar macrophages of smokers stimulated with TLR2 and TLR4 agonists. These findings suggest that macrophages within the alveolar spaces show a response that does not conform to a classically-activated M1 phenotype with cigarette smoking, but whether COPD (independent of active cigarette smoking) is defined strictly by M2 polarization is not so clear-cut.

Chitin, a polymer of $\beta(1-4)$ -linked N-acetyl-D-glucosamine and a component of exoskeletons of fungi, helminths, crustaceans and insects [37, 38], mediates alternative macrophage activation in mice [37]. Although acidic mammalian chitinase (AM-Case) is one of two chitinases encoded in the human genome that degrade chitin [38] and is implicated in asthma [39], chitotriosidase (the other chitinase and also referred to as chitinase 1) is the primary active form in human lungs and macrophages increase its expression in cigarette smokers [38]. Chitotriosidase transcripts are elevated in lung tissue explants from patients with severe-to-very severe COPD even in the absence of active smoking, and plasma levels correlate with the degree of physiologic airway obstruction in one study [40]. In another study, BALF chitotrio-

sidase levels are increased in smokers with COPD compared to smokers without COPD or never-smokers [41]. This same study showed that chitotriosidase levels correlated with increased levels of IL-1 β , CXCL8, TNF- α and TNF-RII in the BALF of smokers with COPD and that TNF- α directly increased chitotriosidase levels in the alveolar macrophages of smokers with COPD but not in those of smokers without COPD or of never-smokers [41]. Furthermore, the addition of chitotriosidase further enhanced proinflammatory CXCL8 and CCL2 chemokine production and MMP-9 from the alveolar macrophages of smokers with COPD [41]. YKL-40, a chitin-binding protein without chitinolytic activity, is also increased in the BALF and appears to enhance the release of CXCL8, CCL2, CCL3 and MMP-9 in the alveolar macrophages of smokers with COPD compared to smokers without COPD and never-smokers [42]. So far, chitotriosidase and YKL-40 appear to exert a proinflammatory and tissue-destructive role in COPD, amplifying the effects of TNF- α rather than serving as a distinct signature of M2 polarization in humans.

How do we reconcile the difference between the prevailing concept that COPD develops as a result of a persistent inflammatory response and some of the findings in human alveolar macrophages? One possibility is that many of the studies examining underlying mechanisms utilized animal models (particularly murine models), and these may differ from responses in humans. Woodruff et al. [26] have shown the transcriptome profiles of macrophages obtained from transgenic mice that had spontaneously developed emphysema did not predict the gene expression alterations observed in the human alveolar macrophages from cigarette smokers. One limitation of their study was that cigarette smoke exposure in mice was not directly compared with cigarette smoking of humans, and that the spontaneous development of emphysema in transgenic mice may not adequately reflect cigarette-smoke-induced activation programs.

Although human and murine macrophages do not show identical polarization patterns [43–45], another possibility that we present is that different macrophage subsets develop in response to environmental triggers that are influenced by the existing phenotype of the mononuclear phagocyte (e.g. the chemokine receptor repertoire, the level of MHC II expression), the maturation status (i.e. transitioning blood monocyte vs. fully committed alveolar macrophage) and the spatial cues provided by the local tissue microenvironment in which the mononuclear phagocytes reside (i.e. airway wall, tissue bound to the interstitium and airspaces). Thus, while

alveolar macrophages in the face of cigarette smoking can show a suppression of the M1 phenotype, it is possible that a distinct subset of mononuclear phagocytes within the lungs can develop and sustain the inflammatory response. COPD likely reflects an altered lung microenvironment where habitual cigarette smoking induces inflammation, but also, over time, impairs the ability of the alveolar macrophage to respond to subsequent microbial triggers [46]. This impairment in host lung defense may provide the opportunity for microbial pathogens to colonize and in some cases infect the lower respiratory tract, creating the nidus for the recruitment of additional innate and adaptive immune cells, and driving the progression of small airway remodeling known to occur in individuals with COPD [7, 10, 46].

Tissue-Bound Interstitial Mononuclear Phagocytes

It has previously been shown that the lung tissue microenvironment determines the function and distribution of discrete subsets of mononuclear phagocytes in rodents [47]. In addition, alveolar macrophages transition from blood monocytes, but they require an obligate intermediate stage found within the lung interstitium [48]. This transitional intermediate mononuclear phagocyte population has been previously recognized [47, 49], but it appears to be heterogeneous and plastic to environmental challenges [49, 50]. Whether functional subsets of mononuclear phagocytes develop, amplify and are spatially confined within discrete compartments of the lungs following cigarette smoke exposure is not entirely clear, but recent findings appear to suggest this possibility.

CX3CR1 is highly expressed by resident murine mononuclear phagocytes in both resting and inflamed tissues [51], harbors the immediate potential to differentiate into macrophages and dendritic cells [51–53], and exhibits the ‘patrolling behavior’ required for rapid tissue invasion at the site of an infection [51]. The relevance of the CX3CL1–CX3CR1 pathway to human disease is supported by a comprehensive gene expression profiling study examining the lung tissues of smokers with COPD compared to those of smokers without COPD [54]. CX3CL1 was the only chemokine found among the 327 differentially regulated genes identified from a group of >26,000 transcripts examined by serial analysis of gene expression (SAGE) and represents a biologically plausible candidate implicated not only in inflammation [51, 55, 56], but also in cell survival [57, 58] and the amplification of T helper 1 (Th1) responses [59]. Expanding upon the human data, we have

previously shown that increases in chemokine CX3CL1 gene expression are associated with the recruitment of CX3CR1+ mononuclear phagocytes in murine lungs during cigarette-smoke-induced emphysema [60].

CX3CR1+ cells normally comprise approximately 10–15% of the mononuclear phagocyte population in the murine lung interstitium [50]. We previously defined tissue-bound interstitial mononuclear phagocytes as those mononuclear phagocytes isolated from collagenase-digested lung tissue after the removal of alveolar macrophages by lung lavage and blood monocytes by saline perfusion of the pulmonary circulation. The ‘tight tethering’ [51] or tissue-bound state exhibited by CX3CR1+ mononuclear phagocytes allow for their separation into the ‘interstitial’ rather than the alveolar compartment. It is currently not known whether they are stationed (i.e. tissue-bound) along the continuum of the interstitium representing populations that are adherent on the luminal surface of the endothelium and within the interstitial space. Unlike these tissue-bound mononuclear phagocytes, very few, if any, conventional alveolar macrophages express CX3CR1 under homeostatic conditions and following cigarette smoke exposure in the mouse. Even though a cleaved ligand is detectable in the airspaces, CX3CR1+ cells account for <1% of the BALF cells examined. However, it is conceivable that these CX3CR1+ cells are present in the airspaces but are tightly bound to the surface of the alveolar epithelium due to as of yet unidentified adhesive interaction and, thus, are not easily lavageable. Regardless of the underlying mechanism for the CX3CR1 tissue-bound state, these findings suggest that mononuclear phagocyte subsets are present in the lungs and can be distinguished not only by function but perhaps also by their physical compartmentalization. Others have previously identified lung mononuclear phagocytes confined to the interstitium with either a suppressive phenotype [61, 62] or producing immunoregulatory cytokines such as IL-12 [63], IL-1 and IL-6 [64], depending upon the response to distinct environmental challenges. These subsets arise, expand and attest to the plasticity of mononuclear phagocytes in vivo. It is yet unclear whether interstitial mononuclear phagocytes represent distinct subset(s) in human lungs. We have previously shown that macrophages express CX3CR1 in human lung tissue [60], but it remains to be seen whether human lung macrophages show similar function and compartmentalization in the healthy and diseased states.

Using mice in which *egfp* is expressed at the locus of the *cx3cr1* gene, we showed that CX3CR1+ mononuclear phagocytes are tissue-bound to the interstitium and are

Fig. 1. Hypothesized role of CX3CR1+ mononuclear phagocytes in COPD. Individuals prone to COPD show a heightened response in the lungs to repetitive cigarette smoking characterized by activation of the transmembrane ligand CX3CL1 (in red). Transmembrane CX3CL1 may transduce a cell survival signal [69, 70], or promote adhesive interactions and retention of CX3CR1+ mononuclear phagocytes. The principal cell type producing transmembrane CX3CL1 has still to be identified in human lungs, but the bronchial epithelium [71], airway smooth muscle cells [72, 73] and endothelial cells [71, 74] have been previously implicated. MMPs such as A disintegrin and metalloproteinase (ADAM)17 or ADAM10 cleave transmembrane CX3CL1 [75–77] and the soluble chemokine form of CX3CL1 may promote inflammatory cytokine release, tissue damage and amplify the recruitment of other cell types such as neutrophils or T lymphocytes.

comprised of transitioning monocytes, macrophages and likely dendritic cells heterogeneous in their expression of CD11c and MHC II expression [50]. Our data suggest that, in response to cigarette smoke exposure *in vivo*, these ‘tissue resident’ mononuclear phagocytes initiate an innate immune response by producing the key cytokines TNF- α and IL-6, amplify divergent populations of CD11b+ cells regardless of CX3CR1 expression, and directly contribute to emphysema. We speculate that cigarette smoke exposure itself triggers CX3CL1 activation in the lungs and amplifies an M1 phenotype in a subset of mononuclear phagocytes, but the actual constituent in tobacco smoke that is causing this response is still to be determined. A hypothesized role for CX3CR1+ mononuclear phagocytes is depicted in figure 1.

Based upon the nomenclature approved by the International Union of Immunological Societies and the World Health Organization, human blood monocytes, the precursors of lung mononuclear phagocytes, are divided into 3 subsets defined by CD14 (the coreceptor for LPS recognition) and CD16 (Fc γ R III) surface expression [65]: (1) classic CD14++CD16–, (2) intermediate CD14++CD16+ and (3) nonclassic CD14+CD16++. The intermediate and nonclassic subsets, both of which express CD16, are often described together. CD16+ monocytes have previously been shown to rise in numbers during acute and chronic inflammatory conditions and are the major producers of TNF- α [66] and implicated in HIV-1 infection and atherosclerosis [67]. Ancuta et al. [68] showed that CX3CL1 preferentially mediates the arrest and migration of human CD16+ monocytes, providing the first evidence that human CD16+ subsets express CX3CR1. Previously, we published that CX3CR1+ mononuclear phagocytes are present in human lung tissue [60], but whether they show a similar M1 activation response or spatial restraint in COPD lungs is currently unknown and should be the basis for future study.

Conclusion

The mononuclear phagocyte, principally the macrophage, has been recognized as a contributor of proteolytic injury and inflammatory responses in the lungs with repeated cigarette smoke exposure. We have identified a mouse cell type defined by CX3CR1 expression and previously implicated in cell survival that is tissue-bound to the interstitium. The population of this cell type expands and elaborates M1 signature cytokines in response to cigarette smoke exposure *in vivo*. We speculate that discrete macrophage subsets such as those defined by CX3CR1 expression may explain, in part, the seemingly disparate findings of studies showing a heightened inflammatory response on the one hand and a suppressed inflammatory profile on the other. Indeed, many questions arise from this hypothesis and serve as the basis for future investigation: are CX3CR1+ mononuclear phagocytes overrepresented in the lungs of COPD subjects and do they show similar spatial restraint to that in mouse lungs? Do CX3CR1+ mononuclear phagocytes from human COPD lungs show an M1 polarization pattern and account for the persistent inflammation long after smoking cessation? What is the principal cell type responsible for CX3CL1 ligand activation in COPD lungs? CX3CL1 is the sole physiologic ligand for the receptor CX3CR1

and CX3CR1 is the sole receptor for CX3CL1. A better understanding of the macrophage subsets that contribute to a maladaptive inflammatory response in COPD is central in developing specific therapeutics targeting the persistent inflammation that continues long after tobacco cessation and is perhaps augmented by host-pathogen interactions.

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