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# Role of Macrophages in Acute Lung Injury and Chronic Fibrosis Induced by Pulmonary Toxicants

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## ABSTRACT

A diverse group of toxicants has been identified that cause injury to the lung including gases (eg, ozone, chlorine), particulates/aerosols (eg, diesel exhaust, fly ash, other combustion products, mustards, nanomaterials, silica, asbestos), chemotherapeutics (eg, bleomycin), and radiation. The pathologic response to these toxicants depends on the dose and duration of exposure and their physical/chemical properties. A common response to pulmonary toxicant exposure is an accumulation of proinflammatory/cytotoxic M1 macrophages at sites of tissue injury, followed by the appearance of anti-inflammatory/wound repair M2 macrophages. It is thought that the outcome of the pathogenic responses to toxicants depends on the balance in the activity of these macrophage subpopulations. Overactivation of either M1 or M2 macrophages leads to injury and disease pathogenesis. Thus, the very same macrophage-derived mediators, released in controlled amounts to destroy injurious materials and pathogens (eg, reactive oxygen species, reactive nitrogen species, proteases, tumor necrosis factor α) and initiate wound repair (eg, transforming growth factor β, connective tissue growth factor, vascular endothelial growth factor), can exacerbate acute lung injury and/or induce chronic disease such as fibrosis, chronic obstructive pulmonary disease, and asthma, when released in excess. This review focuses on the role of macrophage subsets in acute lung injury and chronic fibrosis. Understanding how these pathologies develop following exposure to toxicants, and the contribution of resident and inflammatory macrophages to disease pathogenesis may lead to the development of novel approaches for treating lung diseases.

**Key words:** Macrophages; lung injury; fibrosis; inflammatory mediators; cytokines; oxidants.

The idea that macrophages accumulating at sites of injury play a role in the pathogenic response to xenobiotics was proposed more than 100 years ago by Eli Metchnikoff. Describing the inflammatory response as a "salutary reaction against some injurious influence," he hypothesized that "ferments" released by cells at inflammatory sites might contribute to tissue damage (Metchnikoff, 1968). Since that time, there have been numerous publications supporting this concept in all tissues of the body. In this review, we focus on the role of macrophages and inflammatory mediators they release in acute and chronic lung injury and disease pathogenesis induced by pulmonary toxicants. For brevity, we have not included discussion of the impact of exposure dose, duration or frequency, animal species, or mechanism of action. Although we recognize that these factors influence the inflammatory response, herein we present a general overview that may be more broadly applicable to multiple toxicants with distinct mechanisms of action.

## INFLAMMATORY MACROPHAGES

Inflammatory macrophages are mononuclear phagocytes that play an essential role in host defense and in innate immune

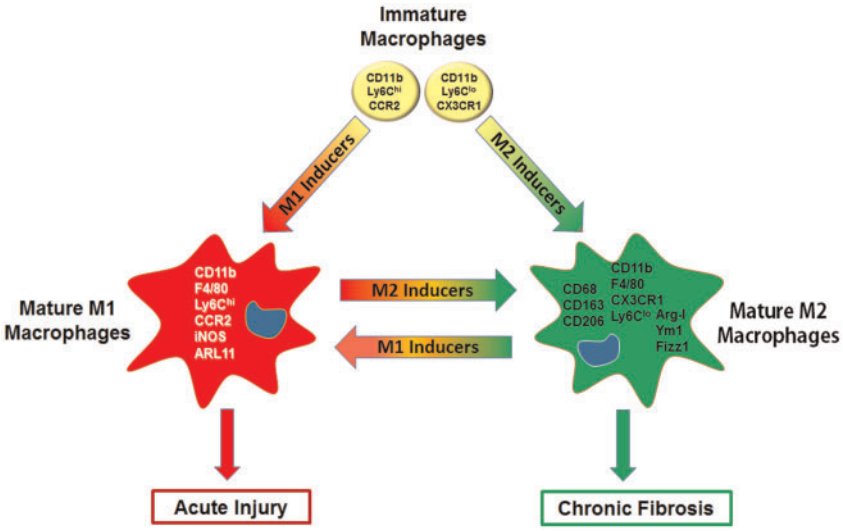


**Figure 1.** Proinflammatory/cytotoxic (M1) and anti-inflammatory/wound repair (M2) macrophages in acute injury and chronic fibrosis. Immature inflammatory mouse macrophages originating from blood and precursors express the integrin CD11b, high or low levels of the surface antigen Ly6C, and chemokine receptors, CCR2 or CX3CR1, respectively. In response to environmental cues (eg, cytokines, growth factors, TLR agonists, and lipids) and intracellular/nuclear regulatory pathways (eg, kinases, transcription factors, epigenetic regulators, metabolic factors) that are activated, these $CD11b^+$ cells mature into $F4/80^+Ly6C^{hi}CCR2^+$ proinflammatory/cytotoxic M1 macrophages, which express markers such as inducible nitric oxide synthase (iNOS) and ARL11, or $F4/80^+Ly6C^{lo}CX3CR1^+$ anti-inflammatory/wound repair M2 macrophages which express CD68, CD163, CD206, Arginase (Arg)-1, Ym1, and/or Fizz1. The extent to which macrophages develop into these subpopulations depends on the activities of M1 and M2 inducers/regulatory factors. As these vary over the course of the inflammatory response, macrophages exist along a continuum with subpopulations expressing varying levels of M1 and M2 markers and activities. The process of M1 and M2 macrophage activation is also highly dynamic; thus, as environmental cues, signaling molecules, transcription factors, and cellular metabolism change, macrophages readily modify their phenotype and function. The outcome of inflammatory responses to tissue injury depends on the relative activities of M1 and M2 macrophage subpopulations. In this context, overactivation of M1 macrophages and/or aberrant M2 anti-inflammatory/wound repair activity can exacerbate and perpetuate acute injury, while excessive activity of M2 macrophages can lead to chronic diseases such as fibrosis.

responses to noxious stimuli. In contrast to resident tissue macrophages (eg, alveolar macrophages), which originate mainly from embryonic precursors during development and self-renew during adulthood, inflammatory macrophages are largely derived from blood and bone marrow precursors. They express specific chemokine receptors such as CCR2 or CX3CR1 and accumulate in tissues in response to chemokines released from injured cells and tissues (Tsou *et al.*, 2007; Vannella and Wynn, 2017). Inflammatory macrophages also express the integrin CD11b, a marker of migratory cells. Once localized at sites of tissue injury, these newly recruited macrophages become activated by mediators they encounter in the tissue microenvironment developing into subpopulations exhibiting varying levels of pro-inflammatory/cytotoxic (M1) or anti-inflammatory/wound repair (M2) activity (Arora *et al.*, 2018; Hussell and Bell, 2014; Laskin *et al.*, 2011) (Figure 1). The process of macrophage activation into M1 and M2 subsets is tightly controlled and involves specific signaling pathways and transcription factors, and posttranslational regulatory networks (Lawrence and Natoli, 2011; Wang *et al.*, 2014a) (Table 1).

Proinflammatory/cytotoxic M1 macrophages, also referred to as classically activated macrophages, develop in response to interferon (IFN)γ alone, or in concert with toll-like receptor (TLR) 4 agonists (eg, lipopolysaccharide [LPS]; high-mobility group protein [HMGB1]) or other cytokines (eg, tumor necrosis factor [TNF]α and granulocyte macrophage-colony stimulating factor [GM-CSF]). Activated M1 macrophages release proinflammatory cytokines (eg, TNFα, interleukin [IL]-1β, IL-6, IL-12, IL-15, IL-23), and generate cytotoxic reactive oxygen species (ROS) and reactive nitrogen species (RNS), proteolytic enzymes, and bioactive lipids. The activity of M1 macrophages is balanced by M2 macrophages, which downregulate inflammation and initiate wound repair. These actions are mediated by anti-inflammatory cytokines (eg, IL-4, IL-10, IL-13), pro-resolving eicosanoids (eg, lipoxins, resolvins), and growth factors (eg, transforming growth factor [TGF]β, vascular endothelial growth factor [VEGF], epidermal growth factor [EGF], connective tissue growth factor [CTGF], fibroblast growth factor [FGF], platelet-derived growth factor [PDGF]). M2 macrophages have been subdivided into M2a macrophages, activated by IL-4 and IL-13, M2b macrophages, activated by immune complexes and LPS, M2c macrophages, activated by IL-10, TGFβ, or glucocorticoids, and M2d macrophages, activated by IL-6 and adenosines (Roszer, 2015). Although acute lung injury and persistent inflammation involves a prolonged or exaggerated response of M1 macrophages and defective M2 macrophage-mediated tissue repair, the development of chronic diseases such as fibrosis and cancer is thought to be a consequence of hyper-responsive subpopulations of M2 macrophages.

Although originally considered two phenotypically and functionally distinct subpopulations at opposite ends of the activation spectrum, it is now recognized that macrophages exist on a continuum with subpopulations in between expressing varying levels of M1 and M2 markers and activities. Moreover, the extent of macrophage activation toward an M1 or M2 phenotype is regulated not only by environmental cues including cytokines, growth factors, TLR agonists, and lipids, but also by various signaling pathways (ie, kinases), downstream transcription factors (eg, nuclear factor-kappa B [NF-κB], activator protein [AP]-1, interferon regulatory factor [IRF]s, signal transducer and activator of transcription [STAT]s, peroxisome proliferator-activated receptor [PPAR]γ), and epigenetic regulators (eg, microRNAs, chromatin modifiers) that are activated within the cells, as well as by posttranslational regulatory networks, and changes in

**Table 1.** Regulators of Macrophage Activation and Polarization

| Regulator | M1 | M2 | Supplementary References |
|---|---|---|---|
| **Extracellular** | | | |
| Cytokines | IL-1$\beta$, IL-6, IL-12, IL-23, IFN$\gamma$, TNF$\alpha$, MIP-1$\alpha$ | IL-4, IL-13, IFN$\alpha$, IL-1 RA | Martinez and Gordon (2014), Amici *et al.* (2017), Murray (2017), Parisi *et al.* (2018) |
| Growth factors | GM-CSF | M-CSF, TGF$\beta$ | Martinez and Gordon (2014), Wynn and Vannella (2016) |
| Eicosanoids/bioactive lipids | $LTB_4$; 12-HETE, 5-HETE | Lipoxins, resolvins, thromboxane, $PGI_2$ | Masoodi *et al.* (2015), Robb *et al.* (2016), Amici *et al.* (2017) |
| TLR-4 agonists | DAMPs, PAMPs, LPS | | Martinez and Gordon (2014) |
| NOD agonists | Peptidoglycans | | Zhou *et al.* (2015) |
| **Intracellular** | | | |
| Oxidative stress | ROS, RNS | | Martinez and Gordon (2014), Amici *et al.* (2017), Murray (2017), Parisi *et al.* (2018) |
| Metabolism | Anaerobic glycolysis, glucose uptake, fatty acid synthesis | Oxidative glucose metabolism, fatty acid oxidation, and uptake | Jha *et al.* (2015), Murray (2017); Van den Bossche *et al.* (2017); Van den Bossche and Saraber (2018) |
| Signaling pathways | AKT2, NOTCH1/2 | AKT1, RTKs | Amici *et al.* (2017) |
| **Nuclear** | | | |
| Transcription factors | NF$\kappa$B, AP-1, STAT1, IRF1, IRF5, IRF8, HIF-1$\alpha$ | IRF4, KLF-4, c-myc, PPAR$\gamma$, RXRs, LXRs, STAT3, STAT6, IRF3, IRF4, HIF2$\alpha$ | Escribese *et al.* (2012), Pello *et al.* (2012), Martinez and Gordon(2014); Roszer (2015), Chistiakov *et al.* (2018), Parisi *et al.* (2018), Saradna *et al.* (2018) |
| **Epigenetic** | | | |
| MicroRNA | miRNA-155, miRNA-125b, miRNA-27b, miRNA-127, miRNA-223, miRNA-106a | miRNA-146a/b miRNA-21, miRNA-511-3p, miRNA-124, miRNA-125a/b, miRNA-24, miRNA-34a, let-7c | Ponomarev *et al.* (2011), Amici *et al.* (2017), Parisi *et al.* (2018), Saradna *et al.* (2018), Shapouri-Moghaddam *et al.* (2018) |
| Histone modifications | HDAC3 | | |
| DNA Methylation | DNMT3b, DNMT1 | H3K27 demethylase, DNMT3a, DNMT3al | |

Abbreviations: AKT, protein kinase B; AP, activator protein; DAMP, damage-associated molecular pattern; DNMT, DNA methyltransferase; GM-CSF, granulocyte-macrophage colony stimulating factor; HDAC, histone deacetylase; HETE, hydroxyeicosatetraenoic acid; HIF, hypoxia-inducible factor; IFN, interferon; IL, interleukin; IRF, interferon regulatory factor; KLF, Kruppel-like factors; LPS, lipopolysaccharide; LT, leukotriene; LXR, liver X receptor; M-CSF, macrophage colony stimulating factor; MIP, macrophage inflammatory protein; miRNA, micro-RNA; NF-$\kappa$B, nuclear factor-kappa B; NOTCH, neurogenic locus notch homolog protein; PAMP, pathogen-associated molecular pattern; PG, prostaglandin; PPAR, peroxisome proliferator-activated receptor; RNS, reactive nitrogen species; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; RXR, retinoid X receptor; STAT, signal transducer and activator of transcription; TGF, transforming growth factor; TNF, tumor necrosis factor.

intracellular metabolism (Murray, 2017) (Table 1). It should be noted, however, that M1 and M2 macrophage activation is a highly dynamic process; thus, as environmental cues, signaling molecules, transcription factors, epigenetic regulators, and cellular metabolism change in response to pathophysiologic conditions, macrophages readily modify their phenotype and function. Accordingly, cells that initially promote proinflammatory and cytotoxic reactions, can undergo phenotypic switching, subsequently participating in the resolution of inflammation and injury (Porcheray *et al.*, 2005; Parisi *et al.*, 2018). In this context, the transition of macrophages from a proinflammatory M1 to an anti-inflammatory/wound repair M2 phenotype appears to be critical for the progression of normal wound healing and tissue regeneration. This is exemplified by findings that noxious pulmonary stimuli such as hypoxia impair M1 to M2 macrophage phenotypic switching, a response associated with prolonged tissue injury (Faulknor *et al.*, 2017). Also of importance, is the observation that while there are some unique characteristics of the activation profile of M1 and M2 macrophages, there are also many overlapping activities. These findings underscore the dynamic nature of the macrophage activation process and the notion that within any inflamed tissue, mixed phenotype macrophages co-exist with M1 and M2 macrophages, their specific function depending on the balance of activating and inhibiting activities and the tissue microenvironment (Martinez and Gordon, 2014).

## INFLAMMATORY MACROPHAGES IN ACUTE LUNG INJURY AND REPAIR

Acute injury to the lung is associated with disruption of endothelial and epithelial barriers (Muller-Redetzky *et al.*, 2014; Tam *et al.*, 2011). It is characterized by an accumulation of protein-rich edema fluid, sloughing of the bronchial epithelium, the appearance of necrotic or apoptotic type I cells, denuded basement membrane, enlarged edematous interstitium, injured endothelial cells, and an accumulation of cellular debris in the tissue. Cellular characteristics include loss of alveolar-capillary membrane integrity, transepithelial migration of neutrophils and macrophages, and increases in proinflammatory/cytotoxic proteins. The outcome of acute lung injury depends on the nature of the toxicant, the dose and duration of exposure and the specific tissue location. Although after some exposures, lung structure and function return to normal, in other instances, there is persistence and/or progression of injury, leading to pulmonary vascular destruction, fibrosing alveolitis, multiple organ failure and death.

Initial evidence suggesting a role of macrophages in acute lung injury was based on findings that numbers of these cells increased in the tissue following exposure of animals to diverse pulmonary toxicants (eg, ozone, particulate matter, bleomycin, endotoxin, nanomaterials, mustard vesicants, silica, asbestos, and radiation) (Bekki *et al.*, 2016; Hiraiwa and van Eeden, 2013; Laskin *et al.*, 2011; Weinberger *et al.*, 2011). In addition, macrophages accumulating in the lung early after injury induced by pulmonary toxicants were found to be activated toward a proinflammatory M1 phenotype, as evidenced by morphologic changes including increased size and vacuolization, and expression of inducible nitric oxide synthase (iNOS) and TNFα, prototypical marks of proinflammatory/cytotoxic macrophages (Aggarwal *et al.*, 2014; Laskin *et al.*, 2011; Malaviya *et al.*, 2017; Sugiura and Ichinose, 2011). Macrophages responding to toxicant-induced injury also generated increased quantities of cytotoxic and proinflammatory mediators, which induce and/or amplify the pathogenic response, including ROS, RNS, IL-1, IL-6, IL-18, TNFα, chemokines, eicosanoids, proteases, and bioactive lipids.

Direct evidence for a role of proinflammatory/cytotoxic M1 macrophages in acute lung injury comes from findings that tissue damage is directly correlated with macrophage functional status. Thus, in a number of experimental models using a variety of approaches (eg, pharmacologic, genetic, or macrophage-deficient mice), pulmonary damage is ameliorated or prevented by suppressing or depleting macrophages (Table 2). For example, when M1 macrophage cytotoxic/proinflammatory activity is blocked with anti-inflammatory steroids, lung damage induced by ozone, silica, residual oil fly ash, bleomycin, sulfur mustard, endotoxin, oleic acid, or hydrogen sulfide is reduced (Al-Harbi *et al.*, 2016; Chen *et al.*, 2006; Geng *et al.*, 2018; Huang *et al.*, 2014; Laskin *et al.*, 2011; Samet *et al.*, 2000; Wigenstam *et al.*, 2009). Similarly, the accumulation of macrophages in the lung and subsequent toxicity of ozone, cigarette smoke, carbon nanotubes, particulate matter, radiation, and silica are mitigated by pretreatment of animals with gadolinium chloride, which blocks M1 macrophage activation or clodronate liposomes, administered intravenously, which depletes newly recruited inflammatory macrophages (Frank *et al.*, 2015; Laskin *et al.*, 2011; Marchini *et al.*, 2016; Nemmar *et al.*, 2005; Perez-Rial *et al.*, 2013; Poole *et al.*, 2012). Bleomycin-induced acute lung injury and expression of iNOS are also abrogated in CCR4 knockout mice, which cannot generate M1 macrophages (Trujillo *et al.*, 2008). Similar protective effects against dust have been described in IL-18R knockout mice, and against ozone in TNFR1 and galectin-3 knockout mice, which also display defective M1 macrophage development (Bauer *et al.*, 2013; Laskin *et al.*, 2011; Sunil *et al.*, 2015). Production of inflammatory mediators and lung injury induced by endotoxin are also reduced in mice lacking CD40, a cell surface receptor required for M1 macrophage activation and iNOS expression (Hashimoto *et al.*, 2004). Mice deficient in TLR4, a key receptor/signaling pathway regulating M1 inflammatory macrophage activation, are also protected from toxicity induced by ozone, endotoxin, and particulate matter (Connor *et al.*, 2012; Hollingsworth *et al.*, 2004). Likewise, loss of downstream TLR4 signaling molecules including MyD88 or NF-κB, protects animals from lung injury induced by ozone, bleomycin, silica, and particulate matter (He *et al.*, 2016; Laskin *et al.*, 2011; Re *et al.*, 2014).

Protection against damage induced by ozone has also been described in animals treated with cyclophosphamide, which depletes bone marrow-derived monocytic precursors (Bhalla *et al.*, 1992), consistent with the origin of proinflammatory macrophages. Analogous protection against LPS-induced lung toxicity, has been observed in CD11b-diptheria toxin (DT) receptor mice, depleted of blood monocyte precursors by administration of DT (Dhaliwal *et al.*, 2012; Lu *et al.*, 2018). CCR2 knockout mice, which are defective in M1 macrophage migratory activity to sites of injury, are also protected from oxidative stress and tissue injury induced by ozone, radiation, bleomycin, and endotoxin (Francis *et al.*, 2017; Gharaee-Kermani *et al.*, 2003; Laskin *et al.*, 2011; Wiesemann *et al.*, 2018; Yang *et al.*, 2010).

Additional support for proinflammatory M1 macrophage involvement in the pathogenesis of acute lung injury comes from findings that activation of these cells exacerbates tissue damage induced by pulmonary toxicants. Hence, pretreatment of rodents with macrophage activators such as LPS or *Bacillus Calmette-Guerin* augments acute lung injury induced by endotoxin, radiation, bleomycin, and ozone (Laskin *et al.*, 2011). Similar increases in toxicity in response to radiation, ozone, and bleomycin have been observed in mice lacking the pulmonary collectin, surfactant protein D, which under homeostatic conditions, functions to suppress lung macrophage proinflammatory activity (Casey *et al.*, 2005; Groves *et al.*, 2012; 2013; Malaviya *et al.*, 2015a).

The resolution of acute injury and the return to normal lung structure and function is an active coordinated process. Evidence suggests that it is largely mediated by M2 macrophages, which stimulate counter-regulatory mechanisms that suppress the release of proinflammatory mediators and activate tissue repair processes. Anti-inflammatory/wound repair M2 macrophages have been reported to increase in the lung following exposure of animals to ozone, mustards, bleomycin, particulate matter, radiation, endotoxin, cigarette smoke, silica and asbestos (Arora *et al.*, 2018; Duru *et al.*, 2016; Francis *et al.*, 2017; Kambara *et al.*, 2015; Laskin *et al.*, 2011; Malaviya *et al.*, 2015b; Murthy *et al.*, 2015; Venosa *et al.*, 2016; Xiang *et al.*, 2016). However, their appearance is delayed relative to M1 macrophages, consistent with a role for these cells in tissue repair. These macrophages express prototypical M2 markers including CD68, CD163, CD206, Ym-1, Arg-1, and Fizz-1.

Increases in M2 macrophages in the lung following exposure to ozone, endotoxin, mustard, diesel exhaust, asbestos, silica, or hyperoxia are correlated with upregulation of IL-4, IL-10, and IL-13 (Hussell and Bell, 2014; Laskin *et al.*, 2011; Venosa *et al.*, 2016). These cytokines dampen macrophage production of proinflammatory/cytotoxic mediators and stimulate the generation of extracellular matrix proteins and growth factors important in wound healing (Wynn and Vannella, 2016). Notably, administration of IL-13, which is key for M2 macrophage development, protects mice from lethal endotoxemia, whereas anti-IL-13 antibodies significantly reduce survival of these mice (Matsukawa *et al.*, 2000). Additionally, cytotoxicity and mortality are increased in response to hyperoxia in IL-13 null mice (Bhandari *et al.*, 2007). Increases in susceptibility to ozone have also been described in mice deficient in IL-10, a potent anti-inflammatory cytokine known to activate M2 macrophages (Backus *et al.*, 2010). The importance of M2 macrophages in suppressing inflammation and initiating wound repair is also evidenced by findings that depletion of these cells by administration of DT to CD206-DT receptor transgenic mice is associated with increased expression of proinflammatory cytokines including TNFα, IL-1β, IL-6, and MCP-1/CCL2 following endotoxin administration, a response correlated with exacerbation of lung inflammation and injury (Kambara *et al.*, 2015).

The origin of M2 macrophages has not been clearly established. The fact that their appearance in injured tissues is, like

M1 macrophages, dependent in large part on CCR2/CCL2, suggests that they are derived from bone marrow and monocyte precursors. However, whether they originate from M1 macrophages via phenotypic switching or from a separate monocytic precursor pool is unclear. In this regard, recent studies have demonstrated an accumulation of bone marrow-derived macrophages in the lung following ozone inhalation which co-express the M2 macrophage chemokine receptor, CX3CR1, and CD206 (Francis *et al.*, 2017). It has also been suggested that at least some populations of M2 repair macrophages are derived from proliferating resident lung macrophages (Alber *et al.*, 2012; Tighe *et al.*, 2011). In this regard, while both monocyte and tissue-derived M2 macrophages express Arg1, Ym-1, and Fizz-1, only monocyte-derived M2 macrophages express CX3CR1, CD206, Raldh2, and PD-L2 (Gundra *et al.*, 2014).

## INFLAMMATORY MACROPHAGES AND PULMONARY FIBROSIS

Chronic pulmonary fibrosis refers to a range of disorders characterized by irreversible destruction and remodeling of lung architecture that occurs as a consequence of excess deposition of collagen and other extracellular matrix components. This leads to scarring of the airways and difficulty in breathing. Evidence suggests that dysregulated wound repair is a key factor contributing to pulmonary fibrosis, and that this is due, at least in part, to an imbalance in the actions of M1 and M2 macrophages after prolonged inflammation or pneumonitis (Alber *et al.*, 2012; Byrne *et al.*, 2016; Herold *et al.*, 2011) (Figure 1). This imbalance is associated with reduced production of antifibrotic cytokines (eg, CXCL10) and MMPs by M1 macrophages, which promote the resolution of scarring and matrix degradation, and excessive release of profibrotic mediators (eg, TGFβ, CTGF) by M2 macrophages, which induce fibroproliferative tissue remodeling.

The importance of overactive macrophages in the development of pulmonary fibrosis has been recognized since the mid-1980s, and macrophage phenotype has more recently emerged as an important contributor to the process (Laskin *et al.*, 2011). This is largely based on findings that macrophages are persistently increased in the lung in close proximity to collagen producing fibroblasts, during the aberrant wound healing phase of fibrogenesis in animals treated with bleomycin, asbestos, silica, mustards, carbon nanotubes, or radiation (Duke and Bonner, 2018; Huang *et al.*, 2017; Laskin *et al.*, 2011; Malaviya *et al.*, 2015b; Murthy *et al.*, 2015), and that these macrophages express markers of an anti-inflammatory/profibrotic M2 phenotype including Arg-1, Fizz-1, Ym-1, CD68, CD163, and/or mannose receptor (CD206). In a number of these experimental models, macrophages are also enlarged and foamy in appearance, a characteristic feature of a profibrotic phenotype (Romero *et al.*, 2015; Venosa *et al.*, 2016). Increases in M2 macrophages in areas of fibrotic tissue have similarly been noted in lungs of patients with idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease and cystic fibrosis; moreover, number of these cells in the tissue correlate directly with a worsening prognosis.

M2 macrophages accumulating in the lung during fibrogenesis in both humans and rodents have been identified as the major source of a number of key profibrotic mediators (eg, TGFβ, CTGF, and CCL18), which stimulate fibroblast proliferation and collagen synthesis (Table 3) (Bickelhaupt *et al.*, 2017; Prasse *et al.*, 2006; Pulichino *et al.*, 2008; Shvedova *et al.*, 2005). M2 macrophages are also thought to promote fibrosis through the release of TNFα, IL-1, IL-10, IL-13, IL-33, PDGF, FGF, and fibronectin, which are increased in patients and animals with fibrotic lung disease (Wynn and Vannella, 2016). Arginase, which favors polyamine and proline biosynthesis and promotes cell growth and collagen formation, is also upregulated in M2 macrophages localized in fibrotic lesions (Murthy *et al.*, 2015).

The strongest evidence supporting a role of hyperactive M2 macrophages in the development of pulmonary fibrosis comes from findings that the response to fibrogenic toxicants (eg, bleomycin, radiation, silica, carbon nanotubes) is exacerbated in animals overexpressing IL-10 or IL-13, or by exogenous administration of IL-33, which promote M2 macrophage activation (Table 3) (Fulkerson *et al.*, 2006; Laskin *et al.*, 2011; Li *et al.*, 2014; Lumsden *et al.*, 2015; Luzina *et al.*, 2013; Wang *et al.*, 2014b). Conversely, collagen production and fibrosis are reduced in animals lacking M-CSF or those treated with chlodronate liposomes or anti-CSF1R antibody, which deplete M2 macrophages (Baran *et al.*, 2007; Gibbons *et al.*, 2011; Meziani *et al.*, 2018; Murray *et al.*, 2011), or serum amyloid P, which inhibits the development of M2 macrophages in the lung (Laskin *et al.*, 2011; Murray *et al.*, 2011; Pilling *et al.*, 2007), and in mice deficient in the M2 macrophage-inducing cytokines IL-4, IL-10, IL-13, IL-18, IL-33, or their receptors (Hoshino *et al.*, 2009; Huaux *et al.*, 1998, 2003; Laskin *et al.*, 2011; Li *et al.*, 2014; Liu *et al.*, 2004; Lumsden *et al.*, 2015; Wang *et al.*, 2014b; Zhao *et al.*, 2018), chemokine receptor CCR4 (Trujillo *et al.*, 2008), Fizz-1, which induces M2 macrophage recruitment and myofibroblast differentiation (Liu *et al.*, 2014), or C/EBP homologous protein, which is required for M2 macrophage activation and TGFβ production (Buck and Chojkier, 2011; Yao *et al.*, 2016). A role for IL-1R1 and MyD88 signaling has also been described in bleomycin-induced lung fibrosis (Gasse *et al.*, 2007).

There is also some experimental evidence that proinflammatory monocyte-derived M1 macrophages drive pulmonary fibrosis (Byrne *et al.*, 2016; Gibbons *et al.*, 2011). Thus, prednisone is effective in reducing TGFβ production and collagen deposition in bleomycin-induced fibrosis, but only if it is administered beginning one day after bleomycin administration, a time associated with a prominent proinflammatory/cytotoxic M1 macrophage response (Chaudhary *et al.*, 2006). Additionally, selective depletion of circulating proinflammatory monocytes, defined in mice by expression of high levels of the surface marker Ly6C ($Ly6C^{hi}$), leads to decreases in M2 macrophages in the lung, and abrogation of bleomycin-induced pulmonary fibrosis; moreover, adoptive transfer of these cells during fibrogenesis exacerbates fibrosis (Gibbons *et al.*, 2011; Ji *et al.*, 2016). Studies with mice lacking CCR2 or CCL2, or wild-type mice treated with MCP-1 (CCL2) neutralizing antibodies, which have blunted M1-monocyte-derived macrophage emigration to sites of injury, have confirmed the importance of these cells in the development of fibrosis. Thus, in these experimental models, significantly fewer M2 macrophages are observed in the lung following administration of bleomycin or radiation, a response correlated with reduced TGFβ, CTGF, and fibrosis (Baran *et al.*, 2007; Gharaee-Kermani *et al.*, 2003; Gibbons *et al.*, 2011; Groves *et al.*, 2018). In contrast, when proinflammatory monocytes are adoptively transferred into mice during the acute reversible inflammatory response to bleomycin, they have a resolution promoting role (Gibbons *et al.*, 2011). These data demonstrate that there is considerable heterogeneity in lung macrophage functioning during different phases of the fibrogenic process (Misharin *et al.*, 2013).

A clear causal link between inflammatory monocyte-derived lung macrophages and the pathobiology of fibrosis has recently

**Table 2.** Approaches for Assessing the Role of Macrophages in Acute Lung Injury and Fibrosis

| **Proinflammatory/cytotoxic M1 macrophages in acute injury** | | | |
|---|---|---|---|
| **Approach** | **M1 Macrophage Suppression** | **Outcome: Decreased Injury** | **Supplementary References** |
| **Pharmacologic** | Glucocorticoids | Ozone, silica, PM, bleomycin, mustard, endotoxin, oleic acid, hydrogen sulfide | Haddad *et al.* (1995), DiMatteo and Reasor (1997), Samet *et al.* (2000), Chen *et al.* (2003b), Chen *et al.* (2006), Wigenstam *et al.* (2009), Huang *et al.* (2014), Wang *et al.* (2014a), Al-Harbi *et al.* (2016); Geng *et al.* (2018) |
| | Gadolinium chloride | Ozone, hyperoxia, PM, endotoxin | Jankov *et al.* (2003), Arimoto *et al.* (2005), Laskin *et al.* (2011), Duke-Novakovski *et al.* (2013) |
| | Chlodronate liposomes | Cigarette smoke, carbon nanotubes, endotoxin, PM, silica | Elder *et al.* (2004), Nemmar *et al.* (2005), Laskin *et al.* (2011), Dhaliwal *et al.* (2012), Poole *et al.* (2012), Perez-Rial *et al.* (2013), Frank *et al.* (2015), Marchini *et al.* (2016) |
| | Cyclophosphamide | Ozone, silica | Bhalla *et al.* (1992), Bassett *et al.* (2001), Nemmar *et al.* (2005) |
| | Anti-CCR2 antibody | Endotoxin | Dhaliwal *et al.* (2012) |
| | $CCl_2$-specific inhibitor | Radiation | Wiesemann *et al.* (2018) |
| **Genetic** | CCR4$^{-/-}$ mice | Bleomycin | Trujillo *et al.* (2008) |
| | IL-18R$^{-/-}$ mice | PM | Bauer *et al.* (2013) |
| | TNF$\alpha^{-/-}$ or TNFR$^{-/-}$ mice | Ozone | Cho *et al.* (2007), Fakhrzadeh *et al.* (2008b) |
| | Galectin-3$^{-/-}$ mice | Ozone | Sunil *et al.* (2015) |
| | CD40$^{-/-}$ mice | Endotoxin | Hashimoto *et al.* (2004) |
| | TLR2$^{-/-}$ and TLR4$^{-/-}$ mice | Ozone, endotoxin, PM | Kleeberger *et al.* (2000, 2001), Gilmour *et al.* (2004), Hollingsworth *et al.* (2004), Cho *et al.* (2005), Williams *et al.* (2007), Garantziotis *et al.* (2010), Bauer *et al.* (2011), Li *et al.* (2011), Connor *et al.* (2012); Connor *et al.* (2013) |
| | MyD88$^{-/-}$ mice | Ozone, PM, bleomycin, silica | Gasse *et al.* (2007), Williams *et al.* (2007), Li *et al.* (2011), Bauer *et al.* (2013), Re *et al.* (2014), He *et al.* (2016a) |
| | NF-$\kappa$Bp50$^{-/-}$ mice | Ozone | Fakhrzadeh *et al.* (2004b) |
| | CD11b-DT transgenic mice | Endotoxin | Dhaliwal *et al.* (2012), Lu *et al.* (2018) |
| | CCR2$^{-/-}$ mice | Ozone, radiation, bleomycin, endotoxin | Moore *et al.* (2001), Gharaee-Kermani *et al.* (2003), Maus *et al.* (2003), Okuma *et al.* (2004), Yang *et al.* (2010), Francis *et al.* (2017), Wiesemann *et al.* (2018) |
| | IL1R$^{-/-}$ mice | Bleomycin | Gasse *et al.* (2007) |
| | IL13$^{-/-}$ mice | Hyperoxia | Bhandari *et al.* (2007) |
| **Macrophage-deficient mice** | MAFIA | Ricin | Lindauer *et al.* (2009) |
| **Approach** | **M1 Macrophage Activation** | **Outcome: Increased Injury** | **Supplementary References** |
| **Pharmacologic** | Endotoxin | Endotoxin, radiation, ozone | Wollert *et al.* (1994), Johnston *et al.* (2004), Haque *et al.* (2009) |
| | *Bacillus Calmette-Guerin* | Endotoxin, bleomycin | Chyczewska *et al.* (1993), Tasaka *et al.* (1995) |
| | Anti-IL-13 antibody | Endotoxin | Matsukawa *et al.* (2000) |
| **Genetic** | SP-D$^{-/-}$ mice | Ozone, radiation, bleomycin | Casey *et al.* (2005), Kierstein *et al.* (2006), Groves *et al.* (2012), Groves *et al.* (2013), Malaviya *et al.* (2015a) |
| | IL-10$^{-/-}$ mice[a] | Ozone | Backus *et al.* (2010) |
| | CD206-DT transgenic mice | Endotoxin | Kambara *et al.* (2015) |
| **Anti-inflammatory/profibrotic M2 macrophages in fibrosis** | | | |
| **Approach** | **M2 Macrophage Suppression** | **Outcome: Decreased Fibrosis** | **Supplementary References** |
| **Pharmacologic** | Chlodronate liposomes | Bleomycin | Gibbons *et al.* (2011) |
| | Anti-CSF-1R antibody | Radiation | Meziani *et al.* (2018) |
| | Serum amyloid P | Bleomycin | Pilling *et al.* (2007), Laskin *et al.* (2011) |

Table 2. (continued)

| Anti-inflammatory/profibrotic M2 macrophages in fibrosis | | | |
|---|---|---|---|
| **Approach** | **M2 Macrophage Suppression** | **Outcome: Decreased Fibrosis** | **Supplementary References** |
| | Glucocorticoid | Bleomycin | Chaudhary *et al.* (2006) |
| | Liposome-encapsulated spironolactone | Bleomycin | Ji *et al.* (2016) |
| | IL-13-recombinant immunotoxin | Silica | Ferreira *et al.* (2013) |
| | Anti-IL-33 antibody | Bleomycin | Li *et al.* (2014) |
| **Genetic** | IL-4$^{-/-}$ or IL-4R$^{-/-}$ mice | Silica | Huaux *et al.* (2003), Laskin *et al.* (2011) |
| | IL-10$^{-/-}$ mice | Silica | Huaux *et al.* (1998), Barbarin *et al.* (2004) |
| | IL-13R$\alpha$2 overexpression | Bleomycin | Lumsden *et al.* (2015) |
| | IL-33$^{-/-}$ or IL-33R (ST2)$^{-/-}$ mice | Carbon nanotubes, bleomycin | Li *et al.* (2014), Wang *et al.* (2014b) |
| | Fizz-1$^{-/-}$ mice | Bleomycin | Liu *et al.* (2014) |
| | C/EBP$^{-/-}$ mice | Bleomycin | Buck and Chojkier (2011), Yao *et al.* (2016) |
| | CCR2 or CCL2$^{-/-}$ mice[b] | Bleomycin, radiation | Moore *et al.* (2001), Gharaee-Kermani *et al.* (2003), Okuma *et al.* (2004), Baran *et al.* (2007), Groves *et al.* (2018) |
| | CX3CR1$^{-/-}$ mice | Bleomycin | Tighe *et al.* (2011), Ishida *et al.* (2017) |
| | CCR4$^{-/-}$ mice | Bleomycin | Trujillo *et al.* (2008) |
| | IL-18$^{-/-}$ or IL18R$\alpha^{-/-}$ mice | Bleomycin | Hoshino *et al.* (2009) |
| | MyD88$^{-/-}$ mice | Bleomycin | Gasse *et al.* (2007) |
| | IL1R$^{-/-}$ mice | Bleomycin | Gasse *et al.* (2007) |
| | M-CSF$^{-/-}$ mice | Bleomycin | Baran *et al.* (2007) |
| **Macrophage-deficient mice** | MAFIA | Bleomycin | Redente *et al.* (2014) |
| **Approach** | **M2 Macrophage Activation** | **Outcome: Increased Fibrosis** | **Supplementary References** |
| **Pharmacologic** | IL-33 | Bleomycin | Luzina *et al.* (2013), Li *et al.* (2014) |
| **Genetic** | IL-10 over-expressing mice | Silica | Barbarin *et al.* (2005) |
| | MyD88$^{-/-}$ mice | Silica | Re *et al.* (2014) |
| | SP-D$^{-/-}$ mice | Bleomycin | Casey *et al.* (2005), Kierstein *et al.* (2006), Groves *et al.* (2012, 2013), Malaviya *et al.* (2015a) |

PM, particulate matter (ash, dust, diesel exhaust).
[a]M1 macrophage activation secondary to loss of M2 macrophages.
[b]Indirect suppression.
Abbreviations: CCL, chemokine ligand; CCR, chemokine receptor; C/EBP, CCAAT/enhancer-binding protein; CSF-1R, colony-stimulating factor-1 receptor; DT, diphtheria toxin; Fizz, found in inflammatory zone; IL, interleukin; LPS, lipopolysaccharide; MAFIA, macrophage Fas-induced apoptosis; M-CSF, macrophage-colony stimulating factor; SP-D, surfactant protein-D; TLR, Toll-like receptor; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor.

been established by Misharin *et al.* (2017). Using novel lineage tracking systems, these investigators showed that lung fibrosis is ameliorated when inflammatory monocytes are driven to necroptosis during their differentiation into macrophages. A similar attenuation of pulmonary fibrosis was observed using an inducible transgenic system, which specifically depletes proinflammatory M1 macrophages (McCubbrey *et al.*, 2018). These data provide support for the notion that profibrotic M2 macrophages are largely derived from proinflammatory M1 macrophages. This is in accord with reports that proinflammatory monocytes are also precursors of circulating fibrocytes, which have been shown to be important in pulmonary fibrosis (Gibbons *et al.*, 2011). It should be noted, however, that at present, direct evidence for a precursor relationship between proinflammatory M1-like (Ly6C$^{hi}$CCR2$^{+}$) monocytes and M2 macrophages in the lung during fibrosis is limited. In this context, it cannot be ruled out that M2 macrophages are derived from anti-inflammatory monocytes, which express low levels of Ly6C (Ly6C$^{lo}$) and chemokine receptor CX3CR1, and migrate into the lung from the blood (Gibbons *et al.*, 2011). The fact that reduced number of M2 macrophages are observed in the lungs of bleomycin-treated mice lacking CX3CR1 and that fibrosis is blunted, are consistent with this idea (Ishida *et al.*, 2017). It is also possible that, at least some M2 macrophages originate from proliferating resident alveolar macrophages. This idea is in accord with reports that M2 macrophage accumulation in the lung is reduced in bleomycin-treated CSF-1 knockout mice, which have defective resident macrophage proliferation relative to wild-type mice, a response characterized by reduced CTGF expression and collagen deposition (Baran *et al.*, 2007).

Of note are findings that the M1 macrophage marker, iNOS, as well as the M1-associated chemokine, CXCL10/IP10, are increased in M2-suppressed animals, consistent with the idea that M2 macrophages are important in downregulating M1 macrophage activity, and that it is a balance in the activity between these cell populations that dictates the outcome of the response to fibrogenic stimuli. This is supported by findings that the development of fibrosis in a granulomatous lung disease model is associated with upregulation of arginase-positive M2 macrophages, and that administration of the proinflammatory

**Table 3.** Macrophage Mediators Implicated in Lung Injury and Fibrosis

| Mediator | Toxicant | Supplementary References |
|---|---|---|
| **Proinflammatory/cytotoxic M1 macrophage mediators** | | |
| ROS (eg, superoxide anion, hydrogen peroxide, hydroxide radicals) | Ozone | Fakhrzadeh *et al.* (2004a), Connor *et al.* (2012), Sunil *et al.* (2012), Kumarathasan *et al.* (2015), Zhu *et al.* (2016), Francis *et al.* (2017) |
| | Radiation | Rabbani *et al.* (2005), Lee *et al.* (2015) |
| | Endotoxin | Dang *et al.* (2018) |
| | Bleomycin | Yamazaki *et al.* (1998), Gu *et al.* (2015), Zhou *et al.* (2018) |
| | PM | Morio *et al.* (2001), Laskin *et al.* (2003), Li *et al.* (2003), Naota *et al.* (2010), Shvedova *et al.* (2013), Bekki *et al.* (2016), He *et al.* (2017) |
| | Asbestos | Simeonova and Luster (1995), Fattman *et al.* (2006), Blake *et al.* (2007), Nagatomo *et al.* (2007), He *et al.* (2011) |
| | Silica (Talc) | Rimal *et al.* (2005), Hu *et al.* (2006), Kaewamatawong *et al.* (2006), Nagatomo *et al.* (2006), Langley *et al.* (2011), Shim *et al.* (2015) |
| | Nanoparticles | Shvedova *et al.* (2005), Chou *et al.* (2008), Coccini *et al.* (2012) |
| | Chemical warfare agents | Malaviya *et al.* (2010), McGovern *et al.* (2011), Malaviya *et al.* (2015b), Lam *et al.* (2016), Elfsmark *et al.* (2018) |
| RNS (eg, NO, peroxynitrite) | Ozone | Pendino *et al.* (1995), Kleeberger *et al.* (2001), Laskin *et al.* (2001), Fakhrzadeh *et al.* (2002), Fakhrzadeh *et al.* (2004b), Malaviya *et al.* (2010), Sunil *et al.* (2012) |
| | Radiation | Nozaki *et al.* (1997), Malaviya *et al.* (2015a) |
| | Endotoxin | Pendino *et al.* (1993b), Wizemann *et al.* (1994), Kristof *et al.* (1998), Moncao-Ribeiro *et al.* (2011), Tu *et al.* (2017), Dang *et al.* (2018) |
| | Bleomycin | Yamazaki *et al.* (1998), Gurujeyalakshmi *et al.* (2000), Chen *et al.* (2003a), Genovese *et al.* (2005) |
| | PM | Morio *et al.* (2001), Becher *et al.* (2007), Kumarathasan *et al.* (2015) |
| | Asbestos | Tanaka *et al.* (1998), Dorger *et al.* (2002), Zeidler and Castranova (2004) |
| | Silica | Srivastava *et al.* (2002), Zeidler *et al.* (2004), Rimal *et al.* (2005), Cruz *et al.* (2016) |
| | Nanoparticles | Coccini *et al.* (2012), Lee *et al.* (2012) |
| | Chemical warfare agents | Martin *et al.* (2003), Malaviya *et al.* (2010), Venosa *et al.* (2016), Weinberger *et al.* (2016) |
| Proinflammatory cytokines and chemokines (eg, TNF$\alpha$, IL-1, IL-2, IL-6, IL-12, IL-18, CCL2, CCL3, CCL4, CCL5, CCL17, CXCL1, CXCL2, CXCL5, CXCL10) | Ozone | Johnston *et al.* (2000), Kenyon *et al.* (2002), Yu *et al.* (2002), Fakhrzadeh *et al.* (2004b), Cho *et al.* (2007), Hollingsworth *et al.* (2007), Fakhrzadeh *et al.* (2008a), Backus *et al.* (2010), Tighe *et al.* (2011), Gabehart *et al.* (2014), Francis *et al.* (2017) |
| | Radiation | Zhang *et al.* (2008), Lee *et al.* (2015), Sohn *et al.* (2015), Li *et al.* (2017) |
| | Endotoxin | Jordan *et al.* (2001), Moncao-Ribeiro *et al.* (2011), Takashima *et al.* (2014), Tu *et al.* (2017), Dang *et al.* (2018) |
| | Bleomycin | Failla *et al.* (2006), Baran *et al.* (2007), Gasse *et al.* (2007), Trujillo *et al.* (2008), Hoshino *et al.* (2009), Yu *et al.* (2015), Wei *et al.* (2016), Zhou *et al.* (2018) |
| | PM | Morio *et al.* (2001), Becher *et al.* (2007), Gowdy *et al.* (2008), Churg *et al.* (2009), Naota *et al.* (2010), Hiraiwa and van Eeden (2013), Shvedova *et al.* (2013), Snow *et al.* (2014), Bekki *et al.* (2016), He *et al.* (2016b), He *et al.* (2017) |
| | Asbestos | Simeonova and Luster (1995), Fisher *et al.* (2000) |
| | Silica | Srivastava *et al.* (2002), Zeidler *et al.* (2004), Barbarin *et al.* (2005), Rimal *et al.* (2005), Hu *et al.* (2006), Langley *et al.* (2011), Ferreira *et al.* (2013), Kawasaki (2015), Cruz *et al.* (2016) |
| | Nanoparticles | Shvedova *et al.* (2005), Chou *et al.* (2008), Hsieh *et al.* (2012), Frank *et al.* (2015), Sager *et al.* (2016) |
| | Chemical warfare agents | Sunil *et al.* (2011), Malaviya *et al.* (2015b), Venosa *et al.* (2016), Elfsmark *et al.* (2018) |
| Proteases (eg, MMPs, TIMPs, cathepsin) | Ozone | Kenyon *et al.* (2002), Sunil *et al.* (2012) |
| | Radiation | Flechsig *et al.* (2010), Lee *et al.* (2015), Li *et al.* (2017) |
| | Endotoxin | Franco *et al.* (2002), Moncao-Ribeiro *et al.* (2011), Takashima *et al.* (2014) |
| | Bleomycin | Yu *et al.* (2015), Liu *et al.* (2017) |
| | PM | Adamson *et al.* (2003), Zhang *et al.* (2010), Shimada *et al.* (2015), Bekki *et al.* (2016) |
| | Asbestos | Tan *et al.* (2006) |
| | Silica | Langley *et al.* (2011), Cruz *et al.* (2016) |
| | Nanoparticles | Hsieh *et al.* (2012) |
| | Chemical warfare agents | Guignabert *et al.* (2005), Anderson *et al.* (2009), Malaviya *et al.* (2010), Sunil *et al.* (2014), Venosa *et al.* (2016) |
| | Hydrogen sulfide | Wang *et al.* (2014a) |
| | Oleic acid | Yeh *et al.* (2009) |

**Table 3.** (continued)

| Mediator | Toxicant | Supplementary References |
|---|---|---|
| Bioactive lipids (eg, lipid peroxides, PAF, prostaglandins and leukotrienes) | Ozone | Giri *et al.* (1975), Tan and Bethel (1992), Pendino *et al.* (1993a), Kaneko *et al.* (1995), Stevens *et al.* (1995), Devlin *et al.* (1996), Kinney *et al.* (1996), Nakano *et al.* (2000), Fakhrzadeh *et al.* (2002), Connor *et al.* (2012), Sunil *et al.* (2012), Francis *et al.* (2017) |
| | Radiation | Lee *et al.* (2015) |
| | Endotoxin | Ermert *et al.* (2000), Inoue *et al.* (2004a) |
| | Bleomycin | Chen *et al.* (2003a), Chen *et al.* (2006), Failla *et al.* (2006) |
| | PM | Kuhn *et al.* (1993), Samet *et al.* (2000), Shvedova *et al.* (2013), Yanamala *et al.* (2013), Bekki *et al.* (2016), Salama *et al.* (2016) |
| | Silica | Mohr *et al.* (1992) |
| | Nanoparticles | Shvedova *et al.* (2005), Coccini *et al.* (2012), Lee *et al.* (2012) |
| | Chemical warfare agents | Wigenstam *et al.* (2009), Malaviya *et al.* (2010), McGovern *et al.* (2011), Sunil *et al.* (2014), Venosa *et al.* (2016) |
| **Anti-inflammatory/profibrotic M2 macrophage mediators** | | |
| Profibrotic cytokines and Chemokines (eg, IL-4, IL-10, IL-13, IL-17, IL-33 CXCL-9, fractalkine, CCL-18) | Ozone | Backus *et al.* (2010), Zhu *et al.* (2016) |
| | Radiation | Inoue *et al.* (2004b), Chung *et al.* (2016), Groves *et al.* (2016), Zhang *et al.* (2018) |
| | Hyperoxia | Bhandari *et al.* (2007) |
| | Endotoxin | Matsukawa *et al.* (2000), Hocke *et al.* (2006), D'Alessio *et al.* (2016), Tu *et al.* (2017) |
| | Bleomycin | Pochetuhen *et al.* (2007), Li *et al.* (2014), Wei *et al.* (2016) |
| | PM | Gowdy *et al.* (2008) |
| | Silica | Barbarin *et al.* (2004), Barbarin *et al.* (2005), Ferreira *et al.* (2013) |
| | Nanoparticles | Shvedova *et al.* (2005) |
| | Chemical warfare agents | Venosa *et al.* (2016) |
| Growth factors (eg, CTGF, TGF$\beta$, TGF$\alpha$, PDGF$\alpha$, M-CSF) | Radiation | Rube *et al.* (2000), Chung *et al.* (2014), Lee *et al.* (2015), Sohn *et al.* (2015), Bickelhaupt *et al.* (2017), Li *et al.* (2017), Zhang *et al.* (2018) |
| | Endotoxin | Tu *et al.* (2017) |
| | Bleomycin | Baran *et al.* (2007), Yu *et al.* (2015), Wei *et al.* (2016), Yao *et al.* (2016), Liu *et al.* (2017), Xie *et al.* (2017) |
| | PM | Yanamala *et al.* (2013) |
| | Asbestos | Nishimura *et al.* (2013), Murthy *et al.* (2015) |
| | Silica | Barbarin *et al.* (2004), Barbarin *et al.* (2005), Ferreira *et al.* (2013), Cruz *et al.* (2016) |
| | Nanoparticles | Shvedova *et al.* (2005) |
| | Chemical warfare agents | Sunil *et al.* (2011), Venosa *et al.* (2016) |

Abbreviations: CCL, chemokine ligand; CTGF, connective tissue growth factor; IL, interleukin; M-CSF, macrophage-colony stimulating factor; MMPs, matrix metalloproteinases; NO, nitric oxide; PAF, platelet-activating factor; PDGF, platelet-derived growth factor; PM, particulate matter; RNS, reactive nitrogen species; ROS, reactive oxygen species; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinases; TNF, tumor necrosis factor.

cytokine, IL-12, results in increases in M1 macrophages, overexpression of iNOS, and reduced fibrosis (Hesse *et al.*, 2000).

Evidence suggests that proinflammatory cytokines produced by M1 macrophages including IL-1 and TNF$\alpha$ play a role in the development of pulmonary fibrosis. Thus, following administration of silica, bleomycin, or nitrogen mustard, IL-1 and TNF$\alpha$ are rapidly upregulated in the lung (Byrne *et al.*, 2015). Moreover, administration of IL-1$\beta$ or transient overexpression of IL-1$\beta$ in the lung recapitulates many of the features of bleomycin-induced fibrosis in mice, while blockade or loss of IL-1R1 reduces fibrosis (Byrne *et al.*, 2016; Gasse *et al.*, 2007). Silica-induced fibrosis in rodents is also blunted by neutralization of IL-1$\beta$ (Guo *et al.*, 2013). Similarly, although infusion of recombinant TNF$\alpha$ produces alterations characteristic of pulmonary fibrosis including fibroblast proliferation, collagen deposition, and cell necrosis, blockade or loss of TNF$\alpha$ activity early in the pathogenic process mitigates fibrosis induced by bleomycin, silica, asbestos, and mustard vesicants, a response associated with decreased expression of TGF$\beta$ (Laskin *et al.*, 2011; Liu and Brody, 2001; Malaviya *et al.*, 2015b; Ortiz *et al.*, 1998). Conversely, pulmonary delivery of TNF$\alpha$ to mice with established fibrosis reduces their fibrotic burden and improves lung function and architecture, a response linked to decreases in profibrotic M2 macrophages (Redente *et al.*, 2014). These data demonstrate that the contribution of M1 macrophages to fibrosis is distinct during different phases of the fibrogenic process.

## MACROPHAGE-DERIVED MEDIATORS IMPLICATED IN ACUTE LUNG INJURY AND CHRONIC FIBROSIS

Mediators released by inflammatory macrophages are generally not target specific. Thus, their actions depend on the amount and location of release and their persistence in the tissue. In this regard, they may exacerbate acute tissue injury and/or promote chronic fibrosis. Among the more well-established proinflammatory/cytotoxic mediators implicated in tissue injury are ROS (eg, superoxide anion, hydrogen peroxide, hydroxyl radicals) and RNS (eg, nitric oxide, peroxynitrite), proteases (eg, MMPs, TIMPs), lipid mediators (eg, lipid peroxides, $PGE_2$, PAF), and cytokines (eg, TNF$\alpha$, IL-1, IL-6, IL-12, IL-18)/chemokines (eg, CCL2, CCL3, CCL4, CCL5, CXCL1). Macrophage mediators

implicated in chronic fibrosis include cytokines (eg, IL-4, IL-13, IL-33) and growth factors (eg, TGFβ, CTGF, VEGF). As illustrated in Table 3, similar macrophage-derived mediators contribute to the pathogenic effects of diverse toxicants with distinct mechanisms of action. Complicating the understanding of the role of macrophage mediators in the response to toxicants is the fact that they likely act in concert to promote tissue damage and fibrosis. It should also be noted that macrophages are not the only source of proinflammatory/cytotoxic and profibrotic mediators in the lung. Thus, following toxicant exposure, airway and alveolar epithelial cells and fibroblasts also have the capacity to generate cytotoxic/proinflammatory and profibrotic mediators, a response due in part to macrophage-derived products (Hiraiwa *et al.*, 2013). The contribution of mediators released by these cells to pulmonary toxicity, relative to macrophages, remains to be established.

## RESIDENT ALVEOLAR MACROPHAGES IN ACUTE LUNG INJURY AND FIBROSIS

Phenotypically and functionally distinct subpopulations of macrophages are localized throughout the lung of healthy individuals and experimental animals; these resident tissue macrophages have been identified as alveolar macrophages, interstitial macrophages, pleural macrophages, intravascular macrophages, and airway macrophages (reviewed in Laskin *et al.*, 2015). The largest and most well characterized are alveolar macrophages, which play a key role in recycling of surfactant. Resident alveolar macrophages are also central to pulmonary immune defense, serving as sentinels, strategically located to respond to invading pathogens and inhaled toxicants. Like other tissue-resident macrophages, they possess an M2-like phenotype, which is key to their role in maintaining homeostasis and protecting against injury and infection (Alber *et al.*, 2012; Morales-Nebreda *et al.*, 2015). In healthy lung, alveolar macrophages are sustained in a restrained state by their interaction with the alveolar epithelium and molecules such as surfactant protein D, CD200, IL-10, TGFβ and a transmembrane glycoprotein, MUC-1, via macrophage receptors like TLR4, CD200R, and SIRPα (Herold *et al.*, 2011; Morales-Nebreda *et al.*, 2015; Snelgrove *et al.*, 2011). Disturbances in the alveolar macrophage-epithelium contact are considered critical events in early inflammatory signaling (Alber *et al.*, 2012). Evidence suggests that resident alveolar macrophages play a role in triggering acute inflammatory responses to noxious stimuli. They have the capacity to recognize danger signals (eg, DAMPs) released from injured and/or necrotic epithelial cells and initiate the inflammatory phase of wound healing. It has been suggested that following injury, epithelial cells release calcium which upregulates macrophage NADPH oxidase leading to hydrogen peroxide production; this triggers the release of HMGB1 and other DAMPs (eg, ATP, hyaluronan, heat shock proteins, heparan sulfate) from necrotic cells (Minutti *et al.*, 2017). DAMPs are recognized by macrophage TLR4 and purigenic receptors; binding to these receptors initiates a signaling process resulting in the release of inflammatory mediators and chemokines and the recruitment of inflammatory cells to sites of injury.

Resident alveolar macrophage activation following pulmonary exposure to noxious stimuli can also occur via inflammasomes (eg, NLRP3). These are cytosolic multiprotein complexes that oligomerize and activate caspase-1 resulting in the generation and release of IL-1 and IL-18, which promote inflammation. Full activation of the NLRP3 inflammasome requires both priming and activating stimuli. Although priming is initiated by DAMPs binding to pattern recognition receptors (PRR) such as TLR4, which causes upregulation of pro-IL-1 and pro-IL-18 and other components of the inflammasome (adaptor protein ASC, procaspase-1), activation involves the assembly of these components into the inflammasome structure followed by production of proinflammatory interleukins. In sterile inflammation, NLRP3 activation occurs following phagocytosis of crystals (eg, cholesterol, urate), particles (eg, silica, titanium), or nanomaterials (eg, carbon nanotubes) (Hosseinian *et al.*, 2015; Nakayama, 2018). Studies suggest that the NLRP3 inflammasome contributes to acute lung injury induced by ozone, titanium nanoparticles, asbestos, silica, and particulate matter (Che *et al.*, 2016; Kim *et al.*, 2017; Michaudel *et al.*, 2016; Sayan and Mossman, 2016).

There are also data showing that the inflammasome and inflammasome-linked cytokines released from resident alveolar macrophages are involved in the development of chronic lung diseases including asthma, COPD, and fibrosis (Grailer *et al.*, 2014). In this regard, acute pulmonary fibrotic changes are associated with increased levels of IL-1β and IL-18 in alveolar macrophages from patients with idiopathic pulmonary fibrosis, as well as asbestos-induced fibrosis (Sayan and Mossman, 2016). Fibrogenic toxicants including cigarette smoke, asbestos, silica, carbon nanotubes, engineered nanomaterials, and bleomycin have all been shown to directly activate the NLRP3 inflammasome in alveolar-resident macrophages leading to IL-1β secretion (Sayan and Mossman, 2016). IL-1β stimulates the release of TGFβ, which triggers activation, proliferation, and differentiation of epithelial cells and fibroblasts into collagen-producing myofibroblasts (dos Santos *et al.*, 2012). Recent studies have demonstrated that depletion of resident macrophages just prior to the onset of bleomycin-induced fibrosis did not alter the severity of the pathology (Misharin *et al.*, 2017). These data suggest that the contribution of resident macrophages to fibrogenesis is more prominent in early phases of the disease process.

Consistent with a role of resident alveolar macrophages in early responses to toxicants and pathogens are recent findings demonstrating that these cells are prone to undergo regulated cell death (ie, pyroptosis, necroptosis, METosis) following toxic exposures or infections (Doster *et al.*, 2018; Ginhoux *et al.*, 2017). These tightly regulated cell death pathways are highly inflammatory and immunogenic. They can be induced by a variety of factors including TLR4 ligands (eg, LPS, HMGB1), RAGE activation, inflammasomes and cytokines like IL-1β and TNFα (Li *et al.*, 2016; Xu *et al.*, 2014), and involve caspase-1 receptor-interacting serine/threonine-protein kinases (RIPK1, RIPK3), mixed lineage kinase domain-like protein, and gasdermin D (Doster *et al.*, 2018; Ginhoux *et al.*, 2017). Resident alveolar macrophage death is thought to be key in triggering the recruitment of circulating monocytes and neutrophils to sites of injury and infection and initiating inflammatory responses (Fan and Fan, 2018). There is increasing recognition that macrophage death and inflammation reciprocally affect one another, forming an auto-amplification loop which exaggerates inflammation (Linkermann *et al.*, 2014).

## CONCLUSIONS AND FUTURE DIRECTIONS

Because the respiratory tract is in direct contact with the external environment, it is particularly vulnerable to the adverse effects of inhaled pathogens and toxicants. Macrophages represent an essential host immune defense mechanism against these harmful xenobiotics. However, effective host defense,

wound healing, and restoration of homeostasis requires that the activity of macrophages be carefully controlled. In the absence of operative control mechanisms, macrophages become overactivated, resulting in exacerbation of acute injury and/or the development of chronic lung disease. This is complicated by the fact that macrophages do not consist of one homogeneous cell population; rather subpopulations with unique phenotypic and functional properties. Moreover, as macrophages are highly plastic cells, they have the capacity to rapidly change their phenotype. The use of approaches that allow identification and characterization of individual macrophages within a mixed population (eg, single cell RNAseq or western blotting), as well a conditional knockdown of macrophages (eg, Cre-lox) at different times during the pathogenic process will be particularly valuable for assessing the role of these cells in the development of lung disease. Also important will be a focus on the interactions between lung macrophages and epithelial cells in the response to toxicants. In this regard, recent studies demonstrating that epithelial cell-derived microvesicles generated following hyperoxia or toxicant-induced lung injury are key in proinflammatory activation of macrophages, represent an exciting new avenue of research (Lee *et al.*, 2016, 2017). Another area of interest for future investigations is the role of extracellular traps (fibers composed of DNA and proteins) released from inflammatory macrophages (METs) in response to ROS, proteases, and TNF$\alpha$ in the pathogenic response to pulmonary toxicants (Boe *et al.*, 2015; Doster *et al.*, 2018). It has been suggested that a failure to remove METs may prolong inflammatory responses contributing to chronic tissue injury and disease (Boe *et al.*, 2015; King *et al.*, 2017). Understanding pathways regulating macrophage activation and the mediators they release may lead to more efficacious approaches for treating lung diseases and disorders caused by inhaled toxicants.

## SUPPLEMENTARY DATA

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