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Review article

Structure activity relationships of engineered nanomaterials in inducing NLRP3 inflammasome activation and chronic lung fibrosis

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ABSTRACT

It has been demonstrated that certain engineered nanomaterials (ENMs) could induce chronic lung inflammation and fibrosis, however, the key structure activity relationships (SARs) that link the physicochemical properties and the fibrogenic effects have not been thoroughly reviewed. Recently, significant progress has been made in our understanding of the SAR, and it has been demonstrated that ENMs including rare earth oxides (REOs), graphene and graphene oxides (GO), fumed silica, as well as high aspect ratio materials (such as carbon nanotubes and cerium oxide nanowires) could trigger the NLRP3 inflammasome activation and IL-1β production in macrophages and subsequent series of profibrogenic cytokines, *i.e.* TGF-β1 and PDGF-AA *in vitro* and *in vivo*, resulting in synergistical cell-cell communication among macrophages, epithelial cells, and fibroblasts in a process named epithelial-mesenchymal transition (EMT) and collagen deposition in the lung as the adverse outcomes. Interestingly, different ENMs engage a range of distinct pathways leading to the NLRP3 inflammasome activation and IL-1β production in macrophages, which include frustrated phagocytosis, physical piercing, plasma membrane perturbation or damage to lysosomes due to high aspect ratio, particle structure, surface reactivity, transformation, *etc.* Furthermore, ENM physicochemical properties determine the biopersistence *in vivo*, which also play a major role in chronic lung fibrosis. Based on these progresses, we reviewed recent findings in the literature on the major SARs leading to chronic lung effects.

Contents

1. Introduction

Engineered nanomaterials (ENMs) with unique physicochemical properties have been used in many commercial products including foods, pharmaceuticals, cosmetics, electronic devices, sunscreens,

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paints, and other industrial applications (Nel et al., 2006; Chen and Mao, 2007; Jain et al., 2008; Sun et al., 2015a). However, with the rapid commercialization and increasing production and usage of ENMs, there is an increased exposure potential to human beings and environment, which has generated significant concerns that the ENMs could induce adverse health effects (Colvin, 2003; Oberdorster et al., 2005; Donaldson et al., 2004). Indeed, many types of ENMs have been shown to be not inherently benign that could induce adverse health effects similar to ambient ultrafine air pollution particles, asbestos, quartz, *etc.* It has been demonstrated that ENMs including metal and metal oxides (Zhang et al., 2011; Zhang et al., 2012a; Xia et al., 2011; George et al., 2011; Wang et al., 2014; Xia et al., 2013; Bonner et al., 2013; Zhang et al., 2014; Zhang et al, 2015), high aspect ratio materials including single-wall and multiwall carbon nanotubes (Jia et al., 2005; Wang et al., 2009; Wang et al., 2012; Wang et al., 2011; Sun et al., 2015b; Lin et al., 2014), rare earth metal oxides (Li et al., 2014a; Li et al., 2014b), fumed silica (Sun et al., 2015a; Zhang et al., 2012b; Sun et al., 2016), molybdenum disulfide (Wang et al., 2015a), graphene and graphene oxide (Wang et al., 2015b) could generate cytotoxicity and pro-inflammatory effects *in vitro* and *in vivo*. Detailed mechanistic studies further revealed that different ENMs could engage a range of distinct pathways leading to the NLRP3 inflammasome activation and IL-1β production (Wang et al., 2012), and subsequent series of profibrogenic cytokine production, resulting in synergistical cell-cell communication among macrophages, epithelial cells, and fibroblasts in a process named epithelial-mesenchymal transition (EMT), which leads to chronic collagen deposition in the lung (Wang et al., 2012).

In this review, we aim to discuss the chronic lung effects induced by ENMs and we summarize the major ENM physicochemical properties that lead to the NLRP3 inflammasome activation and lung fibrosis. Specifically, we focus on the detailed mechanisms or pathways that lead to the NLRP3 inflammasome activation, which will provide us strategies for using therapeutic modalities to alleviate the adverse effects. Importantly, it is also possible that through the use of established structure-activity relationships (SARs) and our understanding on the adverse outcome pathways (AOPs), we could use these knowledge for the safer design of ENMs, which will facilitate the sustainable development of nanotechnology (Nel et al., 2013; Sun et al., 2013a).

2. Critical role of the NLRP3 inflammasome activation in determining the chronic lung fibrosis

Inflammasome is an intracellular multi-protein complex assembled upon various stimuli, which control the activation of caspase-1 and modulate the secretion of cytokines including interleukin-1β (IL-1β) and interleukin-18 (IL-18) in innate immune system (Gross et al., 2011; Schroder and Tschopp, 2010). The NLRP3 inflammasome is the most studied one in the inflammasome family and it is also the most versatile one that could be induced by various stimuli that are distinct in nature (Sun et al., 2013b), including RNA, ATP, uric acid, asbestos, α-quartz, and ENMs (Eisenbarth et al., 2008; Dostert et al., 2008; Sun et al., 2013c; Sun and Shen, 2015). It has been demonstrated that NLRP3 inflammasome activation is associated with many acute and chronic inflammatory diseases (Strowig et al., 2012), including Alzheimer's disease (Halle et al., 2008), gout (Martinon et al., 2006), type II diabetes (Masters et al., 2010), atherosclerosis (Duewell et al., 2010), and lung fibrosis (Wang et al., 2012). Recent studies have suggested that many ENMs could activate the NLRP3 inflammasome, which plays an important role in the generation of chronic granulomatous inflammation and fibrosis in the lung. Mechanistic studies revealed that NLRP3 inflammasome activation induced by ENMs involves frustrated phagocytosis, plasma membrane perturbation and potassium efflux, oxidative stress, lysosomal damage and subsequent cathepsin B release that provides signals for the assembly of the NLRP3 inflammasome (Wang et al., 2012; Ji et al., 2012; Lin et al., 2014; Sun et al., 2013b). IL-1β has been shown to play a major role in triggering the chronic lung effects (Liu, 2008) (Table 1).

Table 1
ENM physicochemical properties and NLRP3 inflammasome activation pathways in the lung.

ENMs	Physicochemical properties	NLRP3 inflammasome activation pathways
CNTs	Dispersion, charge, surface functionalization	Lysosomal damage, cathepsin B release (Nel et al., 2013; Ji et al., 2012; Lin et al., 2014; Arts et al., 2007; Nash et al., 1966; Marks, 1957)
GO	Size, surface functional groups	TLR4 activation, lysosomal damage, cathepsin B release, ROS generation (Ma et al., 2015; Trommer and Bergmann, 2015; Qu et al., 2013)
Fumed silica	Surface silanol, three-membered ring	Plasma membrane perturbation, potassium efflux, ROS generation (Zhang et al., 2012b; Sun et al., 2015a)
REOs	Dissolution, phosphate binding, transformation	Lysosomal damage, cathepsin B release (Li et al., 2014a; Li et al., 2014b)

Chronic lung fibrosis or fibrotic reactions in the airways or the lung interstitium constitute a common pathologic outcome following exposure to toxic substances such as inhaled particles, fibers, and metals (Dostert et al., 2008; Bonner, 2010). There is cumulative understanding of the importance of cooperation among epithelial cells, macrophages, and fibroblasts in the pathogenesis of lung fibrosis (Wang et al., 2012; Wang et al., 2011; Bonner, 2010). Epithelial injury can lead to EMT, which represents a gradual cellular transformation process in which the epithelial cells acquire reversible mesenchymal features that could culminate in their differentiation to myofibroblasts or fibroblasts (Wang et al., 2012; Wang et al., 2011; Bonner, 2010). The release of chemokines by injured epithelial cells attracts macrophages contributing to EMT through the production of IL-1β, TGF-β1, PDGF-AA, and proteases (Wang et al., 2012; Wang et al., 2011; Bonner, 2010). Triggering of IL-1β production in macrophages by xenobiotics or ENMs involves the activation of various signaling pathways leading to the assembly of the NLRP3 inflammasome, which is responsible for converting pro-IL-1β to IL-1β. IL-1β acts in synergy with epithelial produced TGF-β1 and PDGF-AA to promote EMT, which culminates in the formation of fibroblast-like cells that deposit collagen in the lung (Fig. 1) (Wang et al., 2012; Wang et al., 2011; Bonner, 2010; Nel et al., 2013). In addition, biopersistence of ENMs also plays an important role in determining their chronic profibrogenic potential. Studies have demonstrated that surface properties, *e.g.*, charge, hydrophobicity, affect the retention of ENMs in the lung. Prolonged retention of ENMs in the lungs can have a significant toxicological effect due to increased production of reactive oxygen species (ROS) and, in turn, increased profibrogenic potential (Valle et al., 2014).

3. Surface reactivity of carbon nanotubes is responsible for lysosome damage and NLRP3 inflammasome activation

One type of engineered nanomaterial that has been widely studied and demonstrated that could induce chronic lung inflammation and lung fibrosis is carbonaceous nanomaterials. Engineered carbonaceous nanomaterials (ECNs) include single-wall carbon nanotubes (SWCNTs), multiwall carbon nanotubes (MWCNTs), graphene, and graphene oxides (GO). ECNs are drawing greater attention because of their widely potential applications in electronics, optics, and drug delivery due to their unique physicochemical properties including high conductivity, tensile strength, surface area, flexibility as well as hydrophilicity and dispersibility in aqueous solutions when surfaces are properly functionalized (Nel et al., 2013; Jariwala et al., 2013). However, the extensive use of ECNs also raises safety concerns, and literature shows that ECNs are capable of inducing both acute and chronic lung injury. For instance, SWCNTs and MWCNTs generate acute lung injury as well as subchronic granulomatous inflammation and fibrosis in the rodent lung, whether

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