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Multi-Walled Carbon Nanotubes Stimulate Arachidonate 5-Lipoxygenase-dependent M1 polarization of Macrophages to Promote Proinflammatory Response *in vitro*

Chol Seung Lim,[1] Michael Kashon,[2] Dale Porter,[2] and Qiang Ma[2]

[1]NIOSH, CDC; and [2]NIOSH

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Polarization of the macrophages regulates the acute inflammation and its resolution in a time-dependent manner. Multi-walled carbon nanotubes (MWCNTs) induce the polarization of M1 and M2 macrophages in mouse lungs. The molecular and cellular pathways leading to the macrophage polarization induced by MWCNTs are incompletely understood. Here we examined the molecular mechanism by which MWCNTs induce M1 polarization using *in vitro* assays and the murine macrophage cell line, J774A.1. Carbon black (CB, Printex90) was used as an amorphous, carbon-based particle control. Treatment of macrophages with MWCNTs (Mitsui-7), but not CB, increased the expression of arachidonate 5-lipoxygenase (Alox5) mRNA and protein in a concentration- and time-dependent manner. MWCNTs, but not CB, induced the expression of CD68, a M1 cell surface marker, and the expression and activity of inducible nitric oxide synthase, a M1 intracellular marker, indicating M1 polarization. Induction of CD206, a M2 marker, was not observed, indicating polarization to M2 macrophages was not induced. Consistent with polarization to M1 macrophages, MWCNTs induced the production of the proinflammatory cytokines tumor necrosis factor-α and interleukin-1β, and the proinflammatory lipid-mediators leukotriene B4 (LTB4) and prostaglandin E2 (PGE2). Moreover, the cell-free media from MWCNT-polarized macrophages induced the migration of neutrophilic cells (differentiated from HL-60). MWCNT-induced chemotaxis was blocked by inhibition of leukotriene A4 hydrolase or the LTB4 receptor, but not cyclooxygenase 2, revealing LTB4 as a major mediator of neutrophilic chemotaxis from MWCNT-polarized macrophages. Knockdown of Alox5 using specific small hairpin-RNA suppressed MWCNT-induced M1 polarization, LTB4 secretion, and migration of neutrophils, revealing a critical role of Alox5. Taken together, these findings demonstrate that MWCNTs induce the polarization of M1 macrophages *in vitro* and that induction of Alox5 is an important mechanism by which MWCNTs promote proinflammatory responses by boosting M1 polarization and production of proinflammatory lipid mediators.

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