Role of oxidized phospholipids in promoting pro-fibrotic alternative activated macrophages and driving pulmonary fibrosis

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Pulmonary fibrosis is often a progressive disease characterized by accumulation of scared tissue in the lung interstitium that results in loss of alveolar function and respiratory distress. There are many known causes of pulmonary fibrosis including radiotherapy, cigarette smoking, inhalation of environmental particles, autoimmunity and sacrcoidosis, however, idiopathic pulmonary fibrosis is associated with unknown etiologies. Although injured or dysfunctional alveolar epithelium are central in driving aberrant repair responses leading to fibrosis, the pathogenesis of fibrosis is complex and involves abnormal inflammatory-immune responses through cross talk of epithelial cells, macrophages, fibroblasts and T cells. Alveolar macrophages (AMs) display remarkable plasticity and can change their functional phenotype depending on the environmental cues they receive. Based on their function, macrophages are classified into three main categories: classical activated (M1) macrophages, anti-inflammatory/immunoregulatory macrophages (M2) and wound healing macrophages (also referred as M2). Wound healing macrophages regulate tissue repair by secreting TGF-β, promoting proliferation of epithelial cells and stimulating extracellular matrix synthesis, however, prolong activation of M2 macrophages is linked with aberrant tissue repair or scarring leading to fibrosis.
The microenvironment surrounding the macrophages could induce shift in their functional phenotype, nevertheless, the signaling molecules and molecular mechanisms that govern this process remain poorly understood. Wealth of data implicates persistent oxidative stress induced by chronic exposure to stressors is involved in injuring alveolar epithelial cells, leading to cell membrane lipid peroxidation (Oxidized PhosphoLipids, OxPLs) and cell death. However, our knowledge of oxidative stress dependent signals/factors that mediates the cross-talk between injured lung epithelium and AMs and subsequent reprogramming of macrophages into wound healing phenotype are poorly understood.
AIM - to determine whether the uptake of OxPLs by macrophage affects its effector function by activation towards M2-like phenotype and regulates pro-fibrotic process.
METHODS - Mouse AMs (B6 cell line SV40 transformed) and murine Bone Morrow-Derived Macrophages (BMDMs differentiated in L-929 medium) were cultured in serum free medium or complete medium for 24 hr prior to incubation with OxPLs (POVPC=1-Palmitoyl-2-(5'-oxo-Valeroyl)-sn-Glycero-3-phosphocholine, 10 ug/mL, Avanti polar lipids) or unoxidized PLs (PAPC=1-Palmitoyl-2-Arachidonoyl-sn-Glycero-3-Phosphocoline, 10 ug/mL, Avanti polar lipids) with 0.1% FBS medium, with or without co-treatment with IL-4 (10 ng/mL, Thermo Scientific). M2-like macrophage markers was assessed by quantitative PCR (arginase 1, ARG1) or Flow cytometry (FACS) analysis (CD206, also known as Mannose Receptor, and CD209).
RESULTS - Mouse AMs treated with OxPLs in absence of IL-4 showed a slight increased expression of ARG1 compare to PBS and PAPC treatment, whereas, AMs treated with OxPLs in presence of IL-4 markedly increased (>2-fold) the expression of ARG1 when compared to PBS or PAPC exposed IL-4-stimulated macrophages. Murine BMDMs treated with OxPLs in presence of IL-4 for 24h showed an increased expression of ARG1, 5 times more than PBS or PAPC exposed IL-4-stimulated macrophages. FACS analysis of CD206 and CD209 showed that incubation of BMDMs with OxPLs significantly increased M2 macrophages markers as compared to PBS or PAPC exposed macrophages following IL-4 stimulation.
CONCLUSION - In our pilot study using both *in-vitro* and *ex-vivo* model, we found that OxPLs could be important for driving M2-like macrophage activation, which could be necessary for tissue repair. Data collected from both models suggest that OxPLs could have a synergistic effect with IL-4, important cytokine involved in macrophages M2-type activation and intrinsically linked to wound repair andfibrosis.