

Institutionen för Medicin  
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# The role of red blood cells in inflammation and remodeling

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## Abstract

Besides being carriers of oxygen and carbon dioxide, red blood cells (RBCs) also have a scavenger function, binding inflammatory mediators to surface receptors. Animal and experimental models have suggested a role for RBCs in inflammatory and fibrotic responses and patients with idiopathic pulmonary hemosiderosis, a disease characterized by lung hemorrhage, frequently develop fibrosis. Fibroblasts, the resident cell in the connective tissue, play an active role in tissue remodeling by proliferating and migrating. These cells have the capacity to produce and secrete matrix components, matrix metalloproteinases (MMPs), tissue inhibitors of matrix metalloproteinases and a variety of cytokines and growth factors. Alteration in these fibroblast functions may lead to impaired repair, resulting in loss in lung function. Given the ability of RBCs to interact with extracellular matrix in lung disorders, the present thesis is based on the hypothesis that RBCs might play a role in repair and progression of fibrosis.

We used a model where human lung fibroblasts were cultured in floating three-dimensional collagen gels, a method that reflects the contractile process typical of tissue remodeling. RBCs, hemolyzed RBCs and RBC-conditioned medium (RBC-CM) stimulated fibroblast-mediated collagen gel contraction in a time and concentration dependent manner. RBCs and RBC-CM also stimulated fibroblast fibronectin secretion. In addition, transforming growth factor- $\beta_1$  was increased in culture supernatants when fibroblasts were co-cultured with RBCs, whereas the concentration of prostaglandin- $E_2$  did not change.

Accumulation of neutrophils is a regular feature in many inflammatory and fibrotic lung disorders. Neutrophil elastase, a serine protease released by neutrophils, further augmented RBC-induced fibroblast-mediated collagen gel contraction. Both contraction induced by neutrophil elastase and RBCs was attenuated by a pan-MMP-inhibitor, indicating a role for fibroblast-derived MMP in the contractile process. This was supported by increased activity of MMP-1, -2 and -3 in gel culture supernatants.

Both RBCs and RBC-CM were able to stimulate fibroblast release of interleukin-8 (IL-8), a chemoattractant for neutrophils. The pro-inflammatory cytokines interleukin- $1\beta$  (IL- $1\beta$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) also stimulated fibroblast IL-8 secretion and when the cytokines were added together with RBCs, a synergistic stimulatory effect on fibroblast IL-8 secretion was observed. Transwell, an *in vitro* model for cell migration, showed that supernatants from fibroblasts cultured with IL- $1\beta$ /TNF- $\alpha$  stimulated neutrophil migration. When RBCs were added, neutrophil migration was further enhanced.

Cell proliferation and apoptosis is crucial in order to maintain fibroblast homeostasis. RBCs inhibited fibroblast proliferation measured by three different *in vitro* methods. In addition, RBCs increased fibroblast susceptibility to staurosporine-induced apoptosis. RBC-CM did not affect fibroblast proliferation and apoptosis, indicating that cell-cell contact is necessary to mediate these effects.

We conclude that RBCs can act on several fibroblast functions important in tissue remodeling. Since micro hemorrhage occurs in many inflammatory and fibrotic lung disorders, the present thesis support a role for RBCs in regulating repair mechanisms after injury.

Keywords: Red blood cells, fibroblast, inflammation, remodeling, fibrosis

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