ImmunoTools *special* Award 2017

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Influence of Mesenchymal Stem Cells on Macrophage Migration and Differentiation in a 3D Cell Culture Model of Sepsis

Sepsis is a major cause of death in intensive care and affects 1.8 million people each year worldwide with an overall mortality rate of more than 50%. At the onset of sepis, endotoxins (such as lipopolysaccharide (LPS)) in the case of gram-negative bacteria start to activate monocytes/macrophages, neutrophils, and endothelial cells. Activated monocytes start to produce pro-inflammatory cytokines, bind to the endothelium and penetrate as macrophages into the surrounding tissue. During the progression of sepsis, macrophages phagocytose bacteria and generate series of pro-inflammatory cytokines, which initiate the innate immune response. Macrophages can be pro-inflammatory (M1-polarized) or anti-inflammatory (M2-polarized), depending on the type of stimulation or interaction with other cell types. In patients with severe sepsis, the higher circulating concentrations of M1-type cytokines, the higher mortality could be observed. However, M2-polarized macrophages protect bodies from inflammatory or infectious disease to a certain extent. It has been reported that mixed M1/M2 phenotype macrophages resulted in higher survival rate in baboon experimental peritonitis. Therefore, regulating macrophage polarization emerges as a potential therapeutic approach for effective treatment of inflammatory diseases such as sepsis.

Mesenchymal stem/stromal cells (MSC) are known to have angiogenic and immunomodulatory potential. Recently, several studies demonstrated that functional interactions occur between MSC and macrophages. It was shown that macrophages shifted from the M1 to M2 phenotype in activated mouse bone marrow-derived macrophages in vitro. Thus, the use of MSC opens new possibilities for the treatment of sepsis.

The precise interaction between MSC and macrophage activity during sepsis is insufficient explained, as appropriate models for investigating sepsis-specific immune responses of macrophages to blood vessels is missing. We recently established an innovative artificial 3D blood vessel for sepsis-specific investigations that simulates natural human blood vascular conditions, especially in terms of morphology and flow

conditions, which is necessary for the reconstruction of natural blood vessel *ex vivo*. In this project, the artificial blood vessel will allow to investigate initial steps in the human septic response controlled by macrophages that is otherwise not possible.

In this project close to nature 3D blood vessel shall be colonized with macrophages in order to investigate the interaction of endothelial cells and mesenchymal stem cells and to clarify the influence in the immune response of sepsis.

Co-cultures of (i) endothelial cells (EC)/macrophages, (ii) EC/MSC/macrophages, and (iii) MSC/macrophages will be stimulated with an LPS stimulation medium. Differentiation into M1 or M2 macrophages is examined after 0h, 4h, 16h and 24h. Differentiation into M1 macrophages is performed by the detection of specific cell surface proteins CD68, CD86, CD282, CD 284, iNOS, and MHCII, while the M2 expression in macrophages is analysed by the detection of CD163, CD200R, and CD206 by flow cytometric analysis. Various differentiations during migration are examined using immunohistochemical methods. The results of the expression differences will be verified by qRT-PCR. Selected antibodies or recombinant cytokines from ImmunoTools will be used for monocyte characterization isolated from whole blood (CD3, CD4, CD19, CD14, CD16,..), stimulation of monocytes for differentiation into macrophages (IL-4, IL-10, TGF-beta), and for characterization of endothelial cells (CD54, CD62,..), MSC (CD105) macrophages (CD86).

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