The influence of hypoxia inducible factor-1 on immunomodulatory properties of human mesenchymal stromal cells

Mesenchymal stromal cells, also called mesenchymal stem cells (MSCs) are located in different tissues of mammalian organisms. This population can be successfully isolated from tissue fragments and multiplicated in the laboratory. MSCs can differentiate into several cell types and secrete factors which enhance regenerative processes. Moreover, they are able to affect the behavior of immune cells, what is called immunomodulation. The immune system is necessary to protect us against pathogens, but also has got some dark sides - causes grafts rejection and development of diseases associated with so called auto-aggression (autoimmunity). The most important cells responsible for those unfavorable activities of immune system are lymphocytes. It is know that MSCs cultured *in vitro* very strongly inhibit proliferation of activated lymphocytes. Furthermore, they increase a percentage of T regulatory cells, a subpopulation of lymphocytes that mediates immune tolerance and inhibit inflammatory response. Therefore, there are trials to use MSCs transplantations in the treatment of autoimmune diseases or to support tolerance induction after solid organ transplantations. For last several years, the significance of physical conditions on physiological and pathological processes in organism has gained a lot of interests. One of them is the partial oxygen pressure. The vast majority of *in vitro* experiments on cell cultures are conducted in the environment with atmospheric oxygen partial pressure (about 21%). Meanwhile, the physiological content of oxygen in the tissues is significantly lower - about 5-10%, and in pathological structures like solid tumor or ischemic tissues it is about 1%. It is known that hypoxic conditions affect the pattern of immune response. On the other hand, oxygen deprivation causes changes in MSCs activity. The major factor responsible for adaptation of cells to hypoxic conditions is hypoxia inducible factor 1 (HIF-1). Interestingly, the significance of HIF-1 activation for the interaction between MSCs and immune cells has not been extensively studied. The present project aims to determine the influence of HIF-1 activation on immunomodulatory properties of MSCs. Additionally, it is planned to conduct comparative experiments in the indicated field on MSCs isolated from different sources (adult vs perinatal).

The experiments will be performed with usage of human MSCs isolated from bone marrow (adult MSCs) and umbilical cord (perinatal MSCs). Because of eminently low HIF-1α stability, its activation will be held with 3 different methods: 1) cell culture in hypoxic conditions; 2) cell culture with addition of the chemical HIF-1α stabilizer; 3) introducing to MSCs a gene encoding the stabilized form of HIF-1α. After induction of HIF-1 activation, MSCs will be analyzed in comparison to control, untreated cells. The cells will undergo detailed evaluation to determine how the treatments influenced the expression of genes, proteins and what they secrete to the extracellular space. Next, MSCs will be co-cultured with immune cells (lymphocytes and macrophages) in controlled *in vitro* conditions. The aim of this part of the project will be to assess how HIF-1 activation affects those interactions. The complex analysis of obtained data with the usage of bioinformatic methods will allow to outline probable mechanisms responsible for the observed changes. Those working hypotheses will be verified in the next, fourth part of the project by blocking of candidate molecules in the culture with using of specific antibodies or chemical inhibitors and repeating of selected functional tests. The key experiments will be performed for both bone marrow- and umbilical cord-derived MSCs in parallel. This will allow for determination whether the MSCs' source (adult vs perinatal) affects the influence of HIF-1 activation on MSCs immunomodulatory properties

The immunomodulatory properties of MSCs raise a lot of hope. However, the results of previous clinical trials are not as promising as the *in vitro* data. The completion of the proposed project will lead to the better understanding of the immunomodulatory MSCs action both in physiological conditions and after the transplantation. If the experiments demonstrate that HIF-1 stabilization is associated with the improved immunomodulatory activity of MSCs, it will provide a basis for new approach aiming the enhancement of MSCs therapeutic potential in the treatment of immune-mediated disorders. Alternatively, if experiments demonstrate opposite effect (i.e. impairment of MSCs immunomodulation under control of HIF-1) this could help in elucidation of the differences observed in MSCs efficacy in various disorders and target tissues/organs.