Description for the general public (in English)

Human amniotic cells (hAC) possess features of stem cells and multiple unique immunomodulatory properties. This makes them a very attractive and safe material for clinical applications in regenerative medicine and transplantology, alternative or supporting classical drug immunosuppression.

The aim of this study is a comparative assessment of the skin allo- (mouse-to-mouse) and xeno- (rat-to-mouse) grafts in terms of immunosuppression dependent on Cyclosporine A or activated hAC. We are going to verify a hypothesis that immunomodulatory properties of amniotic cells enhanced by *ex vivo* activation are comparable to, or more pronounced than that of Cyclosporine A in terms of sufficiency for inhibition of graft rejections. We want assess also the effectiveness of a way of hAC administration (*via* blood *vs.* transdermal injections) with comparison to Cyclosporine A transdermal injections.

The experiment will be performed on BALB/c mice, being the recipients for the skin allografts taken from C57BL/6 mouse donors or xenografts taken from Wistar rat donors. Next, the recipient mice will be observed during 15 days of immunosuppression evoked by Cyclosporine A or hAC administration. After 15 days breeding or at the time when the graft massive rejection is observed, mice will be euthanized and the skin transplant and a probe of blood will be taken for further analyzes. Viability and histological structure of the graft, especially a presence of stem cells in hair follicles and inflammation markers in the recipient mouse will be assessed.

In the experiment, recipient mice will be divided into three groups receiving injections of: 1. placebo (control group); 2. Cyclosporine A; 3. suspension of cells isolated from human amniotic membrane and activated *ex vivo*, into a tail vein or subcutaneously - around the graft. Groups 1-3 will be subdivided into allo- (mouse-to-mouse) and xeno- (rat-to-mouse) skin transplantations.

Before the cells will be injected to the recipient mouse, amniotic cells will be activated *ex vivo,* by adding cytokines IL1β and INFγ to the culture medium, to increase hAC immunomodulatory properties. The activation procedure will involve 96 hours cell cultures in specific medium dedicated to stem cells. At four time points the analysis of chosen immunomodulatory markers, especially markers of immunosupressory properties, will be performed. Based on these results, a time point of a highest immunomodulatory hAC activity will be indicated. Before hAC are injected into mice the cells will be cultured again for a defined period of time.

A new approach to the immunosuppression in planned animal model experiments is the usage of *ex vivo* activated human amniotic cells for maintenance the vital functions of the skin allo- and xenografts. This strategy is consistent with current course of experimental researches consisting of *ex vivo* stem cell phenotypic modifications induced under specified culture conditions, before their introduction to the recipient. Recently, the studies with the use of activated hAC for immunosuppression have not been published. Previous experiments in which non-activated hAC were injected to animals did not give a definite answer how big is an immunosuppressive potential of hAC *in vivo*. Application of isolated hAC as a source of immunosuppressive factors could be a very interesting alternative or a way to support classical drug immunosuppression which may cause many side effects. Unfortunately, conducting an experiment with the use of hAC is not possible in humans without preliminary studies on the animal model. On the other side, the results of experiments performed *in vitro* are a source of important but rather supplementary data. The only method for experimental reconstruction of a tissue niche for the human skin graft is to place it into the mammal skin.

In this project the effectiveness of immunomodulatory activity of hAC in the animal skin model of allo- and xenotransplantations will be assessed. In the future, such models could be adopted to the studies concerning human skin xenotransplantantions.