Karolinska Institutet

Department of Laboratory Medicine

Engineering More Efficient Multipotent Mesenchymal Stromal (Stem) Cells for Systemic Delivery as Cellular Therapy

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Big Lecture Hall at Novum Building (4th floor, Hälsovägen 7, Karolinska University Hospital Huddinge).

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ABSTRACT

Do mesenchymal progenitor cells naturally circulate *in vivo*? Are they fundamentally compatible with blood? What mechanism allows them to be in contact with blood? How do we make therapeutic cells with blood-compatible properties? Can we optimise their survival and therapeutic function upon systemic delivery? How should we best isolate and condition therapeutic multipotent mesenchymal stromal (stem) cells (MSCs) before infusion, to achieve an optimum and sustainable clinical response in patients? This thesis covers many aspects related to these questions. It describes how MSCs interact with the instant blood-mediated inflammatory reaction (IBMIR) upon infusion. The IBMIR was first documented and characterised after infusion of islet cells. More then a decade ago, clinicians observed a cascade of innate immune responses occurring after islet cell infusion into the portal vein of diabetic patients in an attempt to reverse insulin dependence. This response was characterised by the instant activation of the complement and coagulation systems, which was accompanied by platelet adhesion to the graft, effector cell infiltration, and rapid graft destruction. The reaction resulted in a massive cell loss; 80-90% of the infused cells were destroyed within hours of infusion. We wondered if similar events occur after systemic intravenous infusion of MSCs? Expression profiling showed that MSCs express typical hemostatic regulators, similar to those produced by endothelial cells, but display higher amounts of pro-thrombotic tissue / stromal factors on their surface, which trigger the IBMIR after blood exposure. This process was dependent on the cell dose, the choice of MSC donor, and particularly the cell passage number. Freshly harvested, short-term expanded MSCs triggered only weak blood responses *in vitro*, while cryostorage and freeze-thawing, extended culture, and co-culture with activated lymphocytes increased their pro-thrombotic properties. Particularly thawed cells, as used in many clinical applications, displayed impaired immunomodulatory and blood regulatory properties. Thawed cells showed reduced responsiveness to pro-inflammatory and impaired production of anti-inflammatory mediators, an increased triggering of the IBMIR, and a particularly strong activation of the complement cascade, which resulted in twice as efficient lysis after serum exposure. Triggering of IBMIR was augmented when the cells were washed and resuspended in human AB plasma before blood exposure, as done during clinical cell graft preparation. After infusion to patients, we found increased formation of blood activation markers, but no formation of hyperfibrinolysis marker D-dimer or acute phase reactants with the currently applied dose of 1-3 x 10^6 cells per kilogram, demonstrating product safety. Triggering of IBMIR could be reduced by culturing MSCs with human platelet lysate, or antagonised by cell surface heparin-modification and use of soluble anticoagulants. We conclude, that currently applied doses of low-passage clinical grade MSCs are safe and elicit only minor systemic effects, but higher cell doses, and particularly higher passage cells, should be handled with care. This deleterious reaction can compromise the survival, engraftment, and function of these therapeutic cells.

Key words: MSC, multipotent mesenchymal stromal / stem cell, immunomodulation, tissue repair, tissue engineering, cell therapy, cryopreservation, systemic cell delivery, innate immune response, instant blood-mediated inflammatory reaction (IBMIR), complement, coagulation, ABO blood group, xenoantigen

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