

Facilitation of engraftment of human mesenchymal stem cells in immunocompetent mice by down regulation of MHC class I antigens.

Shoshan Knaän-Shanzer¹, Anabel S. de la Garza-Rodea¹, Marieke C. Verweij², Hester Boersma¹, Ietje van der Velde¹, Dirk W. van Bekkum¹, Rob C. Hoeben¹, Antoine A.F. de Vries¹, Emmanuel J.H.J. Wiertz²

¹Virus and Stem Cells Biology Laboratory, Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, the Netherlands

²Department of Medical Microbiology, Leiden University Medical Center, Leiden, the Netherlands

The observation that MSC do not elicit immune responses *in vitro*, gave rise to the proposition, that these cells could be used for transplantation across histocompatibility barriers. Repair of tissue damage that requires *in situ* differentiation of MSC into specific cell types and their long-term persistence can, at present, only be attained with autologous/syngeneic MSC or with allogeneic MSC in immunocompromised recipients. As MSC do not normally express surface MHC class II, we attempted to overcome rejection of MSC by down regulation of surface MHC class I proteins through the use of viral immune evasion strategies.

Bone marrow derived human MSC were transduced with bicistronic retroviral vectors coding for one of 4 different *immuno-evasin* genes and for the enhanced green fluorescent protein (eGFP)

FACS analysis for HLA-ABC antigens revealed that the human cytomegalovirus (HCMV) US11 protein strongly inhibits MHC class I expression on the plasma membrane of hMSC. The HCMV US2 protein, which acts through a similar mechanism, was less effective. In contrast, inhibitors of the transporter associated with antigen processing encoded by the Epstein-Bar virus *BNLF2a* gene and the bovine herpesvirus *UL49.5* gene, both potent blockers of MHC class I complex assembly in a variety of human and murine cells had no such effect. US11-mediated inhibition of MHC I persisted for over three months in cultured hMSCs. Importantly, the US11 protein did not compromise their growth rate, proliferation capacity or differentiation potential.

In vivo studies were performed with MSC expressing LacZ only, or LacZ and US11/. Cells were injected subcutaneously into the pinna and the ears were analysed at intervals by beta Glo assay for the number of surviving hMSC.

In immunocompetent mice rejection of US11 expressing cells was accelerated, but depletion of NK cells of the recipients resulted in a similar survival of the US11 transduced cells as seen in immune deficient NOD/SCID mice. In contrast, NK cell depletion did not prevent immune rejection of MHC I positive MSC

These encouraging findings demonstrate the potential of viral *immunoavoidance* proteins for developing off-the-shelf cellular therapeutics for degenerative disorders . It also shows that MHC I down regulation needs to be supplemented with measures that interfere with recognition by NK cells.