

## Abstract

Cardiac diseases are still the leading cause of death worldwide. There are only palliative therapies to treat the symptoms but not to prevent or to cure pathological cardiac conditions. Left ventricular hypertrophy (LVH) that is characterized by chronic myocardial inflammatory reactions, is an adaptive response to chronic work overload. LVH is accompanied by various molecular and cellular modifications leading to impaired left ventricle function. Pathologically induced and untreated LVH increases the risk to suffer cardiovascular diseases, e.g. heart failure or stroke, and even sudden death. In general, inflammation involves cell invasion including the recruitment of macrophages among others. Macrophages are divided in inflammatory M1 and anti-inflammatory M2 cells including the distinct anti-inflammatory subsets M2a, M2b and M2c with their distinguished transcriptional profiles, functions, duration and prevalence in initiation and resolution of inflammation. During the initiation, inflammatory M1 macrophages are the dominant cells while the switch to anti-inflammatory M2 macrophages is essential for the resolution. However, the role of anti-inflammatory macrophage subtypes in cardiac diseases is still elusive.

Currently, mesenchymal stem cells (MSCs) are in focus of cellular and regenerative medicine due to their unique abilities of self-renewal, multipotency and their immunomodulatory potential. Clinical studies have verified the safety of MSC-based therapies in human but with regard to their efficiency only limited results were gained by MSC transplantation. Since it is not known how MSCs exert their immunomodulatory potential and thereby improve cardiac function, it is pivotal to understand the underlying mechanisms involved in this beneficial effect to achieve a breakthrough in MSC-based therapies.

Therefore, a new cardiac hypertrophy *in vitro* model was developed in this study using induced-pluripotent stem cell-derived cardiomyocytes (iPS-CM) as valid counterpart to cardiomyocytes *in vivo*. Hypertrophy progression was examined in respect of modulating the inflammatory response using M1- and M2-polarized macrophages as well as MSC-mediated macrophage phenotypes that might ameliorate hypertrophy progression. Here, IFN- $\gamma$  plus IL-1 $\beta$ -activated MSCs revealed an improved immunosuppressive capacity in respect of mediating macrophage polarization demonstrating that the immunomodulatory potential of MSCs is not innate but requires previous activation. Macrophages are able to change their physiology in response to environmental stimuli (M1/M2 Shift) and accordingly also through the presence of immunosuppressive MSCs. The transition to an anti-inflammatory macrophage phenotype was found to depend on soluble nitric oxide (NO) and prostaglandin E2 (PGE2) released by pre-activated MSCs. Besides, functional IL-6 signaling was identified as the key factor for polarization towards cells with anti-inflammatory characteristics. Since an IL-6 knockdown cannot be

conducted in MSCs without affecting their secretome, macrophages deficient for IL-6 receptor alpha (IL-6R $\alpha$ ) were used. The importance of IL-6 signaling was further confirmed by stimulation of wildtype macrophages with recombinant IL-6 under anti-inflammatory culture conditions. Macrophages were found to up-regulate M2b-specific genes, whereas the marker genes for M2a and M2c were not affected. Hence, macrophages co-cultured with previously activated MSCs differentiated into cells with a general anti-inflammatory phenotype when exposed to an inflammatory environment (IFN- $\gamma$  +LPS), whereas a strong regulatory M2b macrophage phenotype was provoked by MSC-secreted IL-6 under anti-inflammatory culture conditions (IL-4 stimulation). In contrast, non-pre-activated MSCs had only minor effects on macrophage polarization.

These newly gained insights regarding the improved capacity of pre-activated MSCs to mediate macrophage phenotypes were applied on a cardiac hypertrophy model. iPS-CM were treated with phenylephrine (PE) to induce hypertrophy. These cells were found to have an enlarged cell size as well as an increased AKT kinase activity and nuclear hormone receptor 1 (Nor1)-expression. The gap junction protein connexin 43 (Cx43) was found to be increased in the first 24 h of PE-treatment but was down-regulated in a progressed state of hypertrophy (48 h PE-treatment). The presence of anti-inflammatory macrophages and MSC-mediated macrophage phenotypes resulted in a decrease in cell size of hypertrophic iPS-CM that correlated with the cell size of control iPS-CM indicating a reverse in hypertrophy progression. Moreover, the AKT kinase activity as well as Nor1-expression were found to be down-regulated to baseline levels under same culture conditions. Considering the beneficial impact of macrophages with anti-inflammatory characteristics (e.g. due to co-culture with pre-activated MSCs), it can be assumed that the observed modifications in Cx43-expression also contribute to hypertrophy regression by providing functional intercellular communications. Notably, these effects were mediated by soluble factors while direct cell interactions appeared to attenuate the MSC-mediated impact.

Finally, in this study it was demonstrated that macrophages with MSC-mediated anti-inflammatory characteristics contribute to hypertrophy regression. It has to be emphasized that pre-activation of MSCs might represent a better strategy for their future application in clinical treatment by an improved immunomodulatory potential. Besides, MSC-mediated macrophage polarization should be considered as an interesting approach in treating cardiac hypertrophy by monitoring the inflammatory response.