**NOVEL UNDERSTANDING OF THE PATHOGENESIS OF JUVENILE IDIOPATHIC ARTHRITIS: FOCUS ON MESENCHYMAL STEM CELLS IMPAIRMENT, SENESCENCE AND IMMUNOREGULATORY FUNCTION**

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**Background**:

Juvenile idiopathic arthritis (JIA) is a well-known chronic rheumatic disease of childhood characterised by progressive joint destruction and severe systemic complications.

Immune cells are known to trigger the pathophysiological cascade in JIA, but there is little information regarding the contribution made by Mesenchymal stem cells (MSCs). These cells are able to modulate the immune response and decrease the level of pro-inflammatory cytokines. With addition of regenerative property it makes MSCs potential candidates for clinical application as immunosuppressants in treatment of autoimmune diseases.

**Objectives:**

To investigate MSCs proliferation, viability and immunomodulatory function in JIA and healthy children.

**Methods:**

MSCs were separated from peripheral blood (PB) and synovial fluid (SF) of JIA patients and healthy controls. Cell proliferation rate was counted by Population doublings per day (PDD) during 9 days, in the last of which alamarBlue™ assays were performed to assess cell viability. Due to measure senescence MSCs were stained with SA-*β*-galactosidase. Immunofluorescence was used to examine the expression of p16, p21, p53. Oxidative stress was measured with DCFH-DA. Cell cycle analysis was evaluated with Propidium Iodide and analysed by Accuri® C6 Flow Cytometer.

Commercially-available bone marrow mesenchymal stem cells (BM-MSCs) were treated with graded concentrations of pro-inflammatory cytokines (0.1-100 ng/ml) with following examination of cell viability. Mixed lymphocyte reactions (MLR) were performed to measure MSC immunomodulatory ability *in vitro.*

**Results:**

The growth kinetics of JIA-MSCs were different from healthy controls. JIA-MSCs divided slowly and appeared disorganised with large cytoplasm and loads of outgrowth. They demonstrated a decrease in cell proliferation (negative PDD) and metabolic activity. Difference in growth kinetics and metabolic activity were found inside the JIA PB group with some evidence of response following biological treatment. Thus, PB-MSCs from patients treated with TNFα and anti-IL6 medications had notably higher cell proliferation and metabolic activity against JIA patients received other therapy. Considering this difference, it was hypothesised that cytokines obtained in a high amount in PB and SF of JIA patients may influence MSCs viability. To prove this BM-MSCs were treated with cytokines and demonstrated a dose-dependent decrease in metabolic activity significantly after TNFα and IL1, no significantly after treatment with IL6. Both BM-MSCs treated with cytokines and JIA-MSCs displayed high level of reactive oxygen species.

Cell cycle analysis revealed that JIA-MSCs were arrested in G0/G1 phase with low number of mitotic cells. In addition, the number of senescence-associated SA-β-gal-positive cells was notably higher in JIA-MSCs. Furthermore, JIA-MSCs expressed high level of immunofluorescence for p16, p21 and p53 which played an important role in regulating the senescence progress of MSCs.

Results of MLR showed the ability of BM-MSCs to decrease the percentage of activated T-helpers, T-suppressors, B-cells and natural killers proliferation, while JIA-MSCs lost this property.

**Conclusion:**

Taken together current research has demonstrated that under the influence of proinflammatory cytokines JIA-MSCs suffered from oxidative stress and disruption of metabolic activity acquire senescent morphology, shorten of telomere length, arrest in G0 phase of cell cycle and finally loss of immune regulation. We are continuing our research to determine the mechanisms that are responsible for the impaired phenotype with the aim of identifying new therapeutic strategies for the treatment of JIA.