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21772 | Decoding Immunomodulatory Interactions: Exploring the crosstalk between bone marrow-derived mesenchymal stem cells (BM-MSCs) out the bag and peripheral blood mononuclear cells (PBMCs) for potential in-vivo immunosuppression

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Background & Aim: Allogeneic hematopoietic stem cell transplant is vital for haematological diseases, yet chronic complications arise as donor immune cells start attacking patient tissues. A lab barrier to studying this alloreactivity is the absence of the hematopoietic stem cell niche. Here, we employed BM-MSCs from discarded collection bags [1] to solve this hurdle. The aim was to modulate PMBC proliferation *in vitro*, downregulating alloreactivity and proposing immunomodulation mechanisms. **Methods:** Responder PBMCs were labelled with CFSE dye, and stimulator cells irradiated. Co-cultured in a 2:1 ratio to mimic alloreactivity in MLR assays (n=3) for 6 days, with or without BM-MCs. MSC phenotype examined pre- and post-co-culture [2]. Flow cytometry examined PBMC proliferation through CFSE histograms and assessed surface marker expression of CD45+, CD3+, CD4+, CD8+, CD19+, CD56+ and CD14+. Resazurin assays tracked metabolic activity. CFSE immunofluorescence recorded PBMC proliferation, while phase-contrast images showed PBMC-MSC physical interactions. Data is shown as mean ± standard deviation. Statistical significance assessed with Two-Way ANOVA test ($p<0.05$). **Results:** BM-MSCs expanded successfully, maintaining ideal phenotype and function. CD44+ expression increased significantly in MSCs post-culture. CFSE expression aligned only with PBMC format, whilst phase-contrast images revealed closer alignment in co-culture. Metabolic activity showed enhanced metabolism in PBMCs/MSCs co-culture, with a 15% reduction in proliferating responder cells. March's pending immunophenotyping results will enhance understanding of the observed immunosuppression. **Conclusions:** Expanding BM-MSCs from discarded bags unravelled immunosuppression in alloreactive conditions. BM-MSCs, lacking CD44, had increased expression due to *in-vitro* cultivation [3]. Reduced alloreactive PBMC proliferation by 15% highlights MSCs' immunosuppressive potential. Upcoming immunophenotyping insights will detail the immunosuppression seen.

Keywords: Hematological Disease, Peripheral Blood Mononuclear Cells, Bone Marrow Derived-Mesenchymal Stem Cells, Immunomodulation, Flow Cytometry.

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