Tracking towards GMP conditions: Replacing FBS with Human Umbilical Cord Blood Serum for the culture of human MSC

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Background: A major stumbling block in the utilization of MSC in the clinic is the use of animal sera or serum components in the culture media. In this study we describe the use of human umbilical cord blood serum (CBS) as a replacement for fetal bovine serum (FBS) for culturing MSC from different sources.

Methods: MSC from human and swine bone marrow and human umbilical cord blood were cultured in the presence of DMEM/F12 containing either 10%FBS or 10%CBS. Human MSC were characterized by the presence of CD105 and 73 markers and the absence of CD45 marker. Swine MSC were characterized by the absence of CD45 marker and their ability to differentiate into chondrocytes.

Results: Human MSC cultured in presence of FBS or CBS showed typical fibroblast like morphology, which is characteristic of MSC. 99% of the cells cultured in FBS had a CD73+/CD105+/CD45- phenotype compared to 96% of cells cultured in CBS. Cells cultured in CBS had a significantly higher cell count as compared to cells cultured in FBS. Swine Bone Marrow MSC cultured in the presence of FBS & CBS were morphologically & phenotypically similar.

Generation of antigen presenting cells (APC) from hES cells

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We described for the first time the generation of functional antigen presenting cells (APCs) from hES cells through an intermediate embryoid body formation step. hES cells were cultured to form EBs and plated in tissue culture plates along with recombinant cytokines. Non-adherent and adherent fractions were analyzed over a 6 week period using functional and phenotypic characterization. Both myeloid and erythroid clonogenic elements could be generated. No lymphoid lineage cells were identified. Phentoptypically, cells expressed antigens associated with APCs. Morphological

examinations revealed presence of dendritic cells and macrophages. The generated cells elicited a proliferation of T cells in a mixed lymphocyte reaction (MLR), indicating that they were functional. hES cell-derived APCs could in theory be used to induce antigen-specific immune tolerance for tissue generated from the same hES cells.

Self-renewal and Wnt signaling in hES cells

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In mES cells LIF is known to be involved in self-renewing proliferation. The factors(s) responsible for the same in hES cells are not clear. We wanted to determine if Wnt signaling is necessary and sufficient in promoting self renewal of hES cells. Performing blocking experiments with Wnt antagonists we found that Wnt is not necessary in maintaining hES self-renewal. On addition of recombinant Wnt3a we determined that in absence of feeder-derived factors, Wnt 3a promoted cell proliferation but not self-renewal. hES cells gradually differentiated as determined by functional and molecular characterization. Using a functional reporter assay we found that β-catenin mediated transcriptional activation was minimal in undifferentiated hES cells and greatly up-regulated with Wnt and several other methods. We propose a new model of Wnt in undifferentiated hES cells and highlight the limitations of assay systems and the need for more robust means of interpreting experimental observations in the hES system.