

**THE IMMUNOSUPPRESSIVE POTENTIAL OF MESENCHYMAL STEM CELLS; EFFECTS OF TIME IN CULTURE AND GROWTH FACTOR SUPPLEMENTATION.**

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Allogeneic hematopoietic stem cell transplantation (SCT) is the only curative therapy for many patients with hematologic malignancies. In addition to delivering effective anti-cancer treatment, the therapeutic potential of allogeneic SCT relies on graft-versus-tumor (GVT) effects, which eradicate residual malignant cells via immunologic mechanisms. Unfortunately, GVT effects are closely associated with the development of graft-versus-host disease (GVHD).

Preliminary experimental data show that a family of bone marrow-derived, multi-potent cells that are of non-hematopoietic origin can be readily identified, isolated and expanded and possess powerful immunomodulatory and tissue regenerative properties. Mesenchymal stem cells (MSC) fall within this category of cells. These findings are significant because they identify potential targets for novel cellular therapeutic strategies that are non-cross reactive with standard immunosuppressive approaches and therefore have the potential to reduce GVHD severity while maintaining immune reconstitution and GVT effects have been shown to inhibit immune alloreactivity *in vitro* through secretion of cytokines.

The purpose of these experiments was to characterize the immunosuppressive activity of human MSCs grown for different periods of time and under different conditions.

MSCs from 5 different volunteer donors were expanded for up to 90 days (13 passages) with or without fibroblast growth factor-2 (FGF-2) supplementation. At every passage during this culture period, aliquots of MSCs were cryopreserved. First, fourth, seventh, tenth and thirteenth passage cryopreserved hMSCs were thawed and allowed to recover in growth medium then trypsinized, counted and seeded in 6-well plates where they were stimulated with interleukin-1 $\beta$  (IL-1 $\beta$ ) to generate 24-hour conditioned media. These conditioned media were tested in interferon-gamma (Ifn- $\gamma$ ) EliSpots. The activity of each conditioned medium was determined by the decrease in the number of Ifn- $\gamma$ -positive spots compared to control wells.

Control MSCs reached, on average, 26.3 $\pm$ 4.7 population doublings by passage 13 while FGF-treated cells reached 44.2 $\pm$ 3.9; in fact, FGF-treated hMSCs reached 27.3 $\pm$ 2.4 population doublings (the maximum expansion obtained with control cells) by passage 6 in approximately 35 days. All the conditioned media exhibited immunosuppressive properties as indicated by lower number of spots in the assay; however, different preparations exhibited different levels of inhibition ranging from 30 to 80%. In three of the cell preparations tested, the immunosuppressive activity was well maintained over the passages tested while in the other two the activity sharply decreased after four passages, even in these preparations, FGF-treated cells exhibited equivalent or superior immunosuppressive activity compared to population doubling-matched controls. Overall, when all five cell preparations are considered, there is a trend towards decreased immunosuppressive activity as a function of time in culture.

It is noteworthy, particularly in those preps with sustained activity over time, that expansion in FGF-supplemented medium would result in a 3,500-fold increase in the number of cells obtained by passage 7 and 240,000-fold increase by passage 13 compared to expansion in control conditions, without loss of immunosuppressive activity. However, because the immunosuppressive activity, as measured *in vitro*, is not universally preserved, caution must be exercised when significant expansion of the MSCs is desired or required; in these cases, the activity of the final cell population should be verified prior to its therapeutic application.