

## EXPANSION AND IMMUNOSUPPRESSIVE ABILITY OF MESENCHYMAL PROGENITOR CELLS FROM HUMAN BONE MARROW CULTURED IN A NOVEL SERUM AND ANIMAL-FREE CULTURE MEDIUM

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**Introduction** Human Mesenchymal progenitor cells (MPCs) are an important cellular source for cell therapy. MPCs are typically cultured in medium containing fetal bovine serum which is problematic when the cells are to be utilized in clinical applications. We have developed a humanized, serum and animal-component free medium (MesenCult<sup>®</sup>-ACF) for the expansion of human MPCs and studied the proliferation, differentiation and immunosuppressive potential of the MPCs generated in this medium

**Methods** Clonogenic growth of MPCs cultured in serum-containing medium (MesenCult<sup>®</sup>) or in MesenCult<sup>®</sup>-ACF was analyzed by low density plating of Bone Marrow mononuclear cells for outgrowth of mesenchymal colonies (colony forming unit-fibroblast, CFU-F). Proliferation of MPCs cultured in serum or in MesenCult<sup>®</sup>-ACF was examined by serial passage *in vitro*, with cell surface phenotype assessed at each passage by flow cytometry. Differentiation potential was examined by plating cells in osteogenic, chondrogenic or adipogenic differentiation media. For testing of immunosuppressive effects, MPCs generated in MesenCult<sup>®</sup>-ACF or in medium with serum were treated with mitomycin C prior to co-culture with T-cells which were purified from peripheral blood using EasySep<sup>®</sup> immunomagnetic separation and fluorescently labeled using carboxy-fluorescein-diacetate (CFSE). CFSE labeled T-cells ( $2 \times 10^5$  cells/well) were cultured with  $1 \times 10^5$  or  $1.25 \times 10^4$  MPCs in serum-free X-VIVO-15 medium supplemented with IL-2. T-cells were activated by the addition of antibodies to CD3 $\epsilon$ , CD28 and CD2 crosslinked in tetrameric antibody complexes. On days 3 and 7 cells were harvested, stained with anti-CD45 and propidium iodide and the T-cell division history analyzed by flow cytometry.

**Results** MPCs cultured in MesenCult<sup>®</sup>-ACF or serum-containing medium revealed similar CFU-F frequencies ( $119 \pm 33$  and  $109 \pm 16$  and per  $10^6$  cells plated;  $\bar{x} \pm SD$ , n=3), but colonies generated in MesenCult<sup>®</sup>-ACF were twice as large as those in serum-containing medium (average diameter  $5.7 \pm 0.3$  mm vs  $2.8 \pm 0.1$  mm;  $\bar{x} \pm SD$ , p<0.05 t-test; n=3). MPCs cultured for 9 passages in MesenCult<sup>®</sup>-ACF showed an average fold expansion of  $8.5 \pm 1.4$ ;  $\bar{x} \pm SD$  (n=3), at each subculture, which was ~3-fold higher than the average expansion in serum-containing media ( $2.7 \pm 0.8$  fold;  $\bar{x} \pm SD$ ; n=3). The phenotype of MPCs was similar in both media, with most cells expressing CD105, CD90 and CD73 and lacking expression of CD45 and CD33. Osteogenic, adipogenic and chondrogenic differentiation was detected in cultured MPCs at early and late passages. MesenCult<sup>®</sup>-ACF cultured MPCs suppressed T-cell proliferation more robustly than MPCs cultured in serum. On day 3 of co-culture, MesenCult<sup>®</sup>-ACF MPCs completely abrogated T-cell proliferation, whereas serum-MPCs suppressed T-cell proliferation by only 50%. By day 7 the % proliferating T cells and the number of T-cell divisions were substantially lower in co-cultures with MesenCult<sup>®</sup>-ACF MPCs compared to co-cultures with serum- MPCs (20% vs. 80% proliferating T-cells; <3 vs. >3 T-cell divisions, respectively).

**Conclusion** MPCs expand faster in animal-component free MesenCult<sup>®</sup>-ACF medium than in serum-containing media, retain their adipogenic, osteogenic and chondrogenic differentiation ability and show robust immunosuppressive activity *in vitro*. The ability to expand human mesenchymal cells in an animal-component free medium will enable further use of MPC-derived cell populations for development of clinical cell therapy and tissue engineering applications.