CHAPTER V

DISCUSSION

A major focus in experimental hematology is the delineatin of conditions that would allow HSC to be manipulated in vitro in such a way that could expand in number yet maintain all of the characteristics that define and HSC. Recent reports have shown that extrinsic signals, such as soluble factors and adhesion/matrix proteins provided by the stem cell microenvironment or stem cell "niche," play a crucial role in the asymmetric division of stem cells and maintenance of the stem cell pool[95-101]. Thus far, the majority of studies that have demonstrated expansion of long-term engrafting HSC have accomplished this by employing culture systems that combine cytokine stimulation with support of feeder cell layers.

In the last two decades, a number of xenogeneic stromal cell lines, especially nurine stromal cell lines, have been used as tools to study the mechanisms regulating early stages of hematopoiesis, some of which were reported to posses the ability to maintain or expand stem cell activity in vitro[73, 102-105]. However, xenogeneic stromal cell lines may have been infected by microorganisms and cause transmission of infectious diseases, when expanded cells are inoculated to patients. FCS, if supplemented in culutre, could be another source of infectious microorganisms. The adventages of being able to using MSC layers of human origin that could be harvested either from the patient of from allogeneic sources and the serum-free culutre medium Stemlinell would have significant clinical application, since it would reduce the potential for contamination of infectious agents.

FL were used since they are know to positive influence self-renewal, proliferation, and preservation from apoptosis of the more primitive hematopoietic cells, especially in synergy with several other cytokine[106, 107]. In 1997, Piacibello et al. demonstrate the culture system compose of two hematopoietic growth factor (FL and TPO), in the absence of stromal cells, which can be sustaining for more than 6 months both proliferation and renewal of CB primitive stem cells, with out any sign of their exhaustion[41]. In this studies they used a population of adult CB cells enriched for the surface marker CD133 which is expressed only in the $CD34^{bright}$ subset of human

hematopoietic progenitor. This population are enrich with SCID-repopulating cells and were shown to engraft successfully in fetal sheep transplantation model of primary and secondary recipient suggesting that these cell fraction has long-term repopulating potential. Thus, these data suggest that CD133 could be a specific marker for hematopoietic stem and progenitor cell population.

In our study we investigated the ability of human MSC layers obtained from normal randomly selected marrow donors, in the presence of IL-1α or TPO and FL to support the ex vivo expansion/maintenance of human CB-derived HSC. We demonstrated that human MSCs from healthy donors could efficiently support expansion of progenitor cells in synergy with IL-1α or FL and TPO under serum-free culture condition.

The mechanisms of the supportive effects of MSCs on the expansion of primitive progenitors are not known at this time. One possibility is that membrane-bound cytokines or those sequestrated in the extracellular matrix of MSCs were responsible. Cytokines secreated by MSCs only upon contact with progenitor cells may have been essential. Another possibility is that cell-cell contact of stromal cells anad progenitors was essential.

In our studies we were able to successfully expand CB CD34+ cells and achieved a 93.77-fold and 35.37-fold in condition 3 and 1 respectively. Furthermore, we were also able to expand the more primitive CD133+Cd34+ cells and CD34+CD38- cells and achieved a 33.28-fold (condition3) and 12.52-fold (condition1) and 46.23 (condition3) and 10.01 (condition1), respectively in crease in this population in CB cultures.

We were also able to maintain/expand the clonogenic potential of HSC populations in the system described. The total fold increase in CFU-GM colonies per 104 cells at day16 was 3,155-, 3,198-, and 3,717-fold in condition1, 3 and 4, respectively. CFU-GEMM colonies per 104 cells at day 12 was 4,704-, 5,018-, and 5,417-fold in condition1, 3, and 4,respectively. We show that the clonogenic potential in condition described is not significantly differentiation.

Although the both culture conditions utilized here resulted in the generation of similar numbers of assayable progenitor cells, the most extensive

expansion of total nucleated cells occurred almost exclusively in the MSC with TPO and FL culture system.

The cells produced under the conditions mentioned in these studies were shifted towards the myeloid lineage as can be seen with giemsa staining which indicated that the morphology of the most expanded cell tend to be cell with high granularity. Although the differentiative potential of CB cells coculture with human MSC was primarily shifted towards the myeloid lineage with no $CD5^+$ and $CD19^+$ cell populations, CB cells showed an increase in percentage of $CD7^+$ cells with time in culture; by day 20 the percentage of $CD7^+$ cells was 40%-48%. Thus, we conclude that MSC treated with IL-1α or TPO and FL culture system is also able to expand cells with an early lymphocytic phenotype. CD7 is the earliest antigen marker expressed in the T lineage, being found on T cell precursors in fetal liver and thorax prior to thymic colonization, and in thymus and BM[108-110].

In vivo studies in mouse model of human hematopoiesis are being performed to address the question of whether the expansion system described herein will allow normal engraftment and differentiation of expanded cell population.