Myogenesis in Hemopoietic Tissue Mesenchymal Stem Cell Culture

S. N. Gornostaeva*, A. A. Rzhaninova*, and D. V. Gol’dstein**,***

Translated from Kletochnye Tekhnologii v Biologii i Medicine, No. 2, pp. 63-69, April, 2006
Original article submitted March 6, 2006

The myogenic differentiation capacity of prenatal mesenchymal stem cells from the main sites of hemopoiesis (bone marrow, thymus, liver, and spleen) was studied. Myogenesis was observed in all studied cell cultures except splenic mesenchymal stem cells. Differentiating cells from the thymus, bone marrow, and liver were positively stained for skeletal muscle markers (myogenin and MyoD). Autonomous contracting structures positively stained for cardiomyopinin I and slow muscle myosin, were detected in the same cultures. Our experiments revealed differences in differentiation of mesenchymal stem cells from hemopoietic organs depending on the source of cells.

Key Words: mesenchymal stem cells; myogenesis; differentiation

Mesenchymal stem cells (MSC) were for the first time derived from adult donor bone marrow, due to their capacity to adhere to plastic [7]. Recent studies showed that multipotent stromal cells differentiating into many cell lines can be isolated from various tissues [3,4,6]. These cells are precursors of stromal elements maintaining hemopoiesis in vivo and ex vivo; they produce matrix components, cytokines, and growth factors involved in migration, proliferation, and differentiation of hemopoietic cells.

Proliferation and differentiation of hemopoietic stem/progenitor cells during human ontogeny is realized in histologically different microenvironment. In adult human bone marrow MSC are closely related to hemopoietic cells. The liver, spleen, thymus, and bone marrow are well-known sites of active hemopoiesis during certain periods of prenatal development.

The data on in vitro myogenic differentiation of MSC are scanty and contradictory. Differentiation of MSC into cardiomyoblasts induced by cytotoxic agents (5-azacitidine), co-culturing, and addition of cardiomyogenic differentiation media was demonstrated [8,9,13]. No spontaneous differentiation was described. In vitro predifferentiation of undifferentiated stem cells into cardiomyogenic cells was attained by stem cell co-culturing with cardiomyocytes and END-2 cells or by using myogenic agents, for example, 5-azacitidine and other compounds [11]. However, the safety of this predifferentiation is not proven, and we do not know whether it will have a negative impact on stem cell capacity to migration, division, or alternative differentiation into other cell types. The capacity of MSC to differentiate into muscle cells was not persuasively demonstrated.

The study of prenatal MSC behavior in culture and specific features of their differentiation will help to understand the ontogenesis of these cells and presumably open new potentialities for cell and gene therapy.

MATERIALS AND METHODS

Isolation of mesenchymal stem cells from the bone marrow, thymus, liver, and spleen. Bone marrow MSC cultures were derived from adult donors (n=6),
prenatal bone marrow (n=9), and thymus (n=8) as described previously [1,2].

The spleens (n=9) and livers (n=9) were separated from adjacent connective tissue and washed in Hanks’ solution (PanEco) with gentamicin (50 µg/ml). The organs were mechanically crushed with scissors and disaggregated in 0.2% dispase solution (Invitrogen Corp.) for 20 min at 37°C. The resultant suspension was centrifuged for 5 min at 800 rpm (4°C), and the supernatant was discarded. Cold DMSI medium with 1% fetal calf serum was added to the precipitate. Cell suspension was filtered through a stainless steel sieve and centrifuged. Cell precipitate was suspended in DMSI (Invitrogen Corp.) with 10% FCS, selected for optimal growth of low-density cultures (HyClone, Fetal clone I, Lot No. AND18477), 2 mM L-glutamine, and 10 µg/ml gentamicin. Cell suspension was inoculated in 90-mm plastic Petri dishes (Nunclon) in a concentration of 10^4 mononuclear cells/ml and incubated at 37°C in a CO₂ incubator at 5% CO₂. After 24 h nonadherent cells were removed, adherent cells were washed twice in Hanks’ solution and incubated until confluence in the growth medium containing additio-

![Fig. 1. Formation of myotubule-like structures in mesenchymal stem cell (MSC) culture. a) prenatal bone marrow MSC; b) thymus MSC; c) adult donor bone marrow MSC; d) liver MSC; e) splenic MSC (no myotubules formed in this culture). Relief phase contrast, ×100.](image)