C U R R E N T T O P I C

UDC: 615.381.03:612.419–003.93

Stem cells in the arrangement of bone marrow repopulation and regenerative medicine

Matične ćelije – primena u repopulaciji kostne srži i regenerativnoj medicini

Bela Balint*, Dragana Stamatović†, Milena Todorović‡, Miodrag Jevtić‖, Gordana Ostojić*, Mirjana Pavlović§, Zvezdana Lojpur¶, Miodrag Jocić*

Military Medical Academy, ***Institute of Transfusiology, †Clinic for Hematology, ‖Head Office, **Belgrade, Serbia; Clinical Center of Serbia, ‡Institute of Hematology, **Belgrade, Serbia; Florida Atlantic University,** §Center for Molecular Biology and Biotechnology, Department of Biology, **Florida, USA; ¶Clinical Center of Kragujevac, Kragujevac, Serbia**

Key words:
stem cells; bone marrow; hematopoiesis; hematologic diseases; myocardial infarction; therapeutics.

Ključne reči:
ćelije, matične; kostna srž; hematopoeza; hematološke bolesti; infarkt miokarda; lečenje.

Introduction

Hematopoiesis is a permanent and complex event in which a spectrum of different mature blood cells from a small population of toti/pluri/multipotent stem cells (SCs) are produced through a variety of proliferative and differentiative processes. Hematopoietic SCs are defined as the cells with extensive self–renewal and proliferative potential, together with their ability to differentiate into all blood–cell lineages. Many studies have demonstrated that a multifactorious network of interactive cytokines and other blood–derived mediators regulate the survival, maturation, and proliferation of SCs [1–5].

Hemobiology of stem cells

Compartment of SC can be divided into embryonic and "tissue specific" or adult – i.e. bone marrow and peripheral blood derived – cells. Generally, embryonic SCs are naturally capable of differentiation into all cell types. They are the most promising, but also the most controversial type of potentially transplantable SCs [4, 6].

When a sperm fertilizes an egg, it becomes what is known as zygote. Many scientists view the zygote as the ultimate SC because it can develop into any cell – not only of the embryo, but also of the surrounding tissues, such as placenta. The zygote has the highest degree of plasticity and it is referred to as a totipotent SC. Thirty hours after fertilization, the zygote begins to divide, and by the fifth or sixth day, the cells form a kind of a bubble or blastocyst. After the first week following fertilization, the cells begin to develop the coding sequence for specific functions, which makes isolating the SCs during the blastocyst state, imperative. When removed from the blastocyst, the cells can be cultured into embryonic SCs, but these cells are not embryos. They have the capability of developing into all three types of tissue cells: cells in the endoderm – which lines the digestive tract; cells in the ectoderm – the outermost layer of the tissue cells; and cells in the mesoderm – fills the space between the endoderm and the ectoderm with cells such as muscle tissue. These SCs are somewhat less plastic and more specialized than totipotent zygote SCs. Such SCs that can become any of the more than 200 types of cells in the body are called pluripotent SCs [4, 6–10].

Between the seventh and ninth day, the blastocyst attaches to the uterus, and begins to develop and grow. From this point up to about eight weeks it is generally referred to as an embryo; from eight weeks on, it is referred to as a fetus. From weeks eight to 12 fetal SCs are accumulated in the liver. Both embryonic and fetal SCs generate the developing tissues and organs. At this stage such SCs are designed as multipotent and they are more tissue–specific rather than generating all of the body's 200 different cell types. Such SCs are generally designated as multipotent. However, some researches suggest that at least some multipotent SCs may be more plastic than first thought and may, under the right circumstances, become pluripotent. Up to week 12, fetal SCs (as well as the embryonic SCs which preceded them) can be transplanted into an individual without being rejected. This is because they have little to none of immune-triggering pro-

Correspondence to: Bela Balint, Military Medical Academy, Institute of Transfusiology, Crnotravska 17, 11 040 Belgrade, Serbia. Tel.: +381 11 36 09 128. E-mail: belab@imi.bg.ac.yu

teins (Human Leukocite Antigens – HLA) on their surface. After the 12th week, fetal SCs acquire these proteins, and they remain present on SCs from this point on, including adult SCs [4, 6].

Thus, while some advocate therapeutic use of SCs derived from cord blood, adult bone marrow or the blood stream, these sources pose the problem of possible rejection reactions. Therefore, SCs derived from these sources may have therapeutic potential only when given to the individual from whom they were derived (autologous transplantation) or from an immunolgically matched donor (allogeneic transplantation) [4, 11–16].

Adult SCs are at a more advanced stage of development. These SCs are not capable of differentiating into the endoderm, ectoderm or mesoderm, because they are already at a developed stage as one of the three types of tissues and cannot be rejuvenated back to an early developmental stage. They can be found in the blood, cornea, bone marrow, dental pulp of the tooth, brain, skeletal muscle, skin, liver, pancreas and gastrointestinal tract. These cells are capable of making identical copies of themselves, and usually divide to make progenitor or precursor cells capable to develop into specific cell lines [1–4].

Adult SCs are cells that can be derived from the different parts of the body and, depending on where they are from, have different properties. They exist in several different tissues including bone marrow, blood and the brain. Some studies have suggested that adult SCs are very versatile and can develop into many different cell types. Adult SCs have already been used for more than 20 years as bone–marrow transplants to reconstitute the immune systems of patients with cancer and to treat blood cancers such as leukemia. Using the body's own SCs means the immune system's rejection reflex will not be aroused. However, other studies have concluded that adult SCs are only able to develop into a limited number of cell types related to the tissue that the SCs originally came from. Although a great deal of information on adult SCs has already accumulated, scientists still do not understand completely their specific properties. Research continues with the hope of one day being able to use these cells to restore or replace damaged tissues or organs. More recently, their use in treatment of cor ischemic diseases and liver disorders has also been explored. The potential for hematopoietic SCs to produce cell types other than blood cells has become the subject of intense scientific controversy, since it is still not clear whether they could be used on a clinical scale to restore tissues and organs other than blood and the immune system [3, 17–21].

Clinical application of stem cells

Thanks to the above mentioned properties (self-renewal, differentiation and proliferation), SCs are capable of providing complete and long-term reconstitution of hematopoiesis in hematological disorders, as well as altered/distorted immunity, that was the basis for the clinical use of SC transplant in allogeneic or autologous settings [22–29]. In a few words, SC transplants involve the administration of high-dose chemotherapy (myeloablative or immunoablative treatment) with subsequent (re)infusion of SCs.

Historically, bone marrow was the primary source of SCs for transplant – however peripheral blood and umbilical (cord) blood are also used as SC sources [14–16, 27–30]. Therefore, SCs could be collected by multiple aspirations from spongious bones (e.g. posterior iliac crest) or by apheresis from blood after mobilization by chemotherapy plus cytokines (rHuG–CSF) in autologous setting or by rHuG–CSF alone in allogeneic setting [4, 11–13]. The collected cells should be administered immediately after the collection through catheter placed in subclavian or some peripheral vein. If necessary, cell harvest can be subjected to different purification methods or stored in liquid or frozen state (cryopreservation) [12]. Peripheral blood transplant can be characterized by the absence of the risk of bone infections, general or epidural anesthesia, as well as by faster reconstruction of hematopoiesis. Nowadays peripheral blood SC-harvests are ever more applied in both, allogeneic and autologous transplant settings.

The intensifying of pre-transplant myeloablative therapy and the increase of the clinical use of SCs, that is $CD34^+$ cells, as well as the introduction of the novel cell–mediated curative approaches (e.g., adoptive cell-therapy) resulted in the increased needs for both specific blood-derived cells, and practical operating procedures inducing minimized cell damages during their collection or processing and storage in liquid or frozen state [5, 11, 30–32]. Therefore, a successful performance of SC transplants requires both efficient collection and (cryo) preservation procedures for obtaining an acceptable cell yield and post–thaw recovery.

As mentioned, immature adult SCs have high potential of differentiation not just into all blood cells, but into several somatic cell types (trans-differentiation or lineage-plasticity), such as osteocytes, chondrocytes, hepatocytes, myocytes, cardiomyocytes and even endothelial cells. Thanks to the above mentioned ability, toti/pluripotent SCs (having ”unlimited“ biological capacity), as well as mesenchymal and endothelial precursors are clinically applicable for the cell-therapy in the field of regenerative medicine – that is for the treatment of patients with myocardial, brain, vascular, liver, pancreas and some other tissue damages. In this way, preclinical studies showed that ”implantation“ of immature SCs into damaged/ischemic area induces their homing and subsequent trans-differentiation into the cell lineages of host organ, including collateral vessel formation. To be precise, angiogeneic growth-factors (or genes encoding for these proteins) promote the development of collateral arteries, the process known as therapeutic angiogenesis or neovascularization [17–21].

We have previously analyzed our results of peripheral blood vs. bone marrow transplants based on the hematopoietic reconstitution efficiency. SC transplants were used for the treatment of patients with severe aplastic anemia (SAA), acute lymphoblastic leukemia (ALL), acute non-lymphoblastic leukemia (ANLL), chronic myeloid leukemia (CML), multiple myeloma (MM), Hodgkin's and non-Hodgkin's lymphoma, breast and ovarian cancer, extragonadal non–seminal germ cell tumor, and severe multiple sclerosis. The

mononuclear cells (MNC) yield was 10.1×10^8/kgbm in allogeneic and 7.6×10^8/kgbm in autologous setting on the average. The mean $CD34^+$ yields for allogeneic and autologous transplantats were 16.7×10^6/kgbm and 11.8×10^6/kgbm, respectively.

specific kinetics of the programmed cooling for the efficacy of cryopreservation. We confirmed that the best survival rate of very primitive hematopoietic SCs (MRA cells) was achieved when 10% DMSO was combined with controlled–rate freezing by compensation of released heat of fusion [32].

Table 1

Mononuclear cell (MNC) and bone marrow nucleated cell (TNC) harvests for peripheral blood stem cell transplantation (PBSCT) and cardiac repair (CR)

Type of transplantation		Blood volume Processed [in L]	MNC$^\Psi$ or TNC* harvest [in mL]	MNC count	$CD34^+$ cell count	$CD133^+$ cell count
PBSCT	allogeneic	13.1±2.5	257.0±40.8	$10.8\pm6.1 \times 10^8$/kg	$16.7\pm9.8 \times 10^6$/kg	/
	autologous	16.0±4.2	284.6±55.3	$7.6\pm4.6 \times 10^8$/kg	$11.8\pm6.5 \times 10^6$/kg	$17.9\pm10.6 \times 10^6$
	total	15.5±4.1 (10.2–37.8)	279.6±53.7$^\Psi$	$8.2\pm5.9 \times 10^8$/kg	$12.7\pm7.6 \times 10^6$/kg	/
CR		/	236.6±58*	$8.4\pm6.1 \times 10^8$/kg	$10.4\pm7.5 \times 10^6$/kg	$7.16\pm4.6 \times 10^6$

Hematopoietic reconstitution were evidently superior when peripheral blood vs. bone marrow transplants were compared, as it is presented in Figure 1 [13, 19].

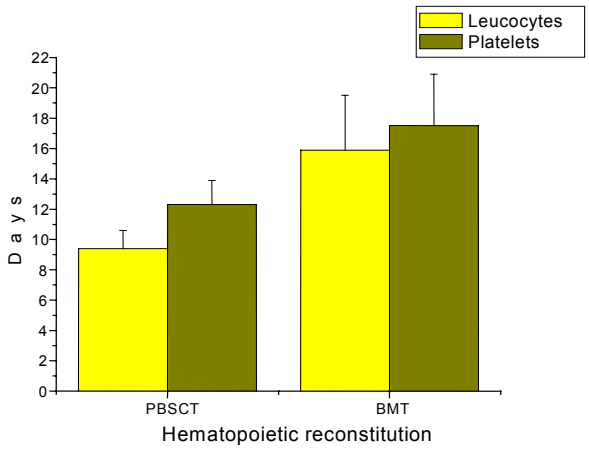

Fig. 1 – Hematopoietic reconstitution after peripheral blood stem cell transplantation (PBSCT) and bone marrow transplantation (BMT). Data are expressed as mean values ± standard deviations (SD).

For a successful autologous SC transplant, that is SC cryopreservation not only the use of an optimized freezing strategy (with cooling rate specific for each cryobiosystem), but the selection of the most appropriate cryoprotective agent is required. For SC, lymphocyte and platelet freezing, dimethyl sulfoxide (DMSO) is commonly used, although in different concentrations. Nowadays a variety of experimental and clinical protocols is used in blood–derived cell freezing practice, confirming that the most effective cryopreservation system is not yet determined [31].

Microprocessor-controlled (controlled-rate) freezing is more efficient than uncontrolled-rate (without programmed cooling) procedure due to better cell recovery. Our earlier results obtained for cryopreserved bone marrow cells and peripheral blood mononuclear cells were in agreement with these findings [5, 13]. These cryoinvestigations pointed out the importance of the

The use of the selection reduces T-cell and/or tumor cell counts with ≥ 3 Log^{10} or more. At the same time, $CD34^+$ cell count should be at least 70% of the total cell number present before purging [11, 13]. Recently, a novel antigen, CD133 (formerly named as AC133), present in a very primitive SC population has been described. The results obtained suggest that $CD133^+$ cell selection for cell-therapy perhaps could be a better option than CD34-selection. Our results obtained by the use of positive SC selection with Isolex 300i are in accordance with data from the literature. Namely, the $CD34^+$ recovery and purity was 62.4±4.2% and 82.4±6.3%, respectively [3, 19]. Although these methods are originally designed to purify the SCs, their appliance also removes unwanted red blood cells and residual plasma, too.

In the treatment of our 24 patients with acute or chronic cardiac infarction and myocardial failure, as well as coronary artery by-pass, cell-therapy using autologous bone marrow derived SCs was performed. Harvested cells were initially filtered, afterwards processed and resuspended in serum–free culture medium up to optimized hematocrit value as previously described [31]. In myocardial cell therapy setting, the number of MNC, $CD45^+/CD34^+$ and $CD34^+/CD133^+$ are quantified [3]. The total count of applied MNC and their viability was $8.4\pm6.1 \times 10^8$ and 98.2%. The mean number of $CD45^+/CD34^+$ and $CD34^+/CD133^+$ cell was also high, 10.4×10^6 and 7.2×10^6, respectively (Table 1). Cell suspensions were administered as an intermittent infusion into the infarcted artery across coronary catheter after previously completed primary percutaneus coronary intervention (invasive cardiological setting) or directly into myocardium (during coronary by-pass grafting). The results obtained have demonstrated that trans-differented SCs regenerated cardiac tissue in post-infarcted hearts by inducing neovascularization and myocyte-generation. Consequently, in our preclinical study, cell-therapy resulted in a considerably improved myocardial perfusion and systolic function in all patients. They tolerated the use of intensive cell-mediated treatment well, without any adverse effects.

Conclusion

The concept of SC lineage-plasticity has generated enormous interest in recent years. Although the mechanisms by which cells can be trans-differentiated or "reprogrammed" as a result of the action of external (extrinsic) or endogenous (intrinsic) factors are still poorly understood, the above presented clinical results and facts suggest that appropriate populations of SCs could be used as a way of recovery of reduced/lost functions of damaged tissues. Thus, the most important application of human SCs in a new millennium is the generation of cells and tissues that could be used safely in cell-therapy or regenerative medicine. Although regenerative medicine presents one really complex process and the number of potential questions is higher than the number of possible answers, considering an ever increasing use of different cell-mediated curative approaches, it should also find its rightful place in the medical practice in our country.

R E F E R E N C E S

1. *Petakov M, Balint B. Bugarski D.* Hematopoietic stem cells biology. In: *Balint B,* editor. Transfusiology. Belgrade: Zavod za udžbenike i nastavna sredstva; 2004. p. 497–523. (Serbian)
2. *Pavlović M, Balint B.* The use of stem cells to repair cardiac tissue. Anest Reanim Transfuziol 2006; 34: 129–50.
3. *Balint B, Todorović M, Pavlović M, Elez M, Urošević I, Obradović S,* et al. Stem cells – application for marrow repopulation and in regenerative medicine. Anest Reanim Transfuziol 2006; 34: 151–6.
4. *Balint B, Pavlović M.* Stem cells – biology, harvesting, extracorporeal purification and some aspects of their clinical application. Bilt Transf 2006; 52(2–3): 2–10.
5. *Balint B.* Coexistent cryopreservation strategies: microprocessor–restricted vs. uncontrolled–rate freezing of the "blood–derived" progenitors/cells. Blood Banking Transf Med 2004; 2(2): 62–71
6. *Vats A, Bielby RC, Tolley NS, Nerem R, Polak JM.* Stem cells. Lancet 2005; 366(9485): 592–602.
7. *Todorović V, Nikolić I, Glibetić M, Balint B.* Humane stem cells – previous knowledge. Anest Reanim Transfuziol 2006; 34: 109–127.
8. *Lysdahl H, Gabrielsen A, Minger SL, Patel MJ, Fink T, Petersen K,* et al. Derivation and characterization of four new human embryonic stem cell lines: the Danish experience. Reprod Biomed Online 2006; 12(1): 119–26.
9. *Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A.* Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. Nat Biotechnol 2000; 18(4): 399–404.
10. *Hwang WS, Ryu YJ, Park JH, Park ES, Lee EG, Koo JM,* et al. Evidence of a pluripotent human embryonic stem cell line derived from a cloned blastocyst. Science 2004; 303(5664): 1669–74.
11. *Balint B.* Stem and progenitor cell harvesting, extracorporeal "graft engineering" and clinical use – initial expansion vs. current dillemas. Clin Appl Immunol 2005; 3(5): 445–54.
12. *Balint B, Stamatović D, Andrić Z.* Hematopoietic stem cells transplantation. In: *Balint B,* editor. Transfusiology. Belgrade: Zavod za udžbenike i nastavna sredstva; 2004. p. 525–47. (Serbian)
13. *Balint B. Bugarski D, Petakov M, Stamatović D.* Quality control in haematopoietic SC collection and cryopreservation. In: *Walterova L, Kretschmer V, Bogdanović G, Rossi U,* editors. The contribution of clinical medicine to blood safety. Proceedings of the European School of Transfusion Medicine Residential Course; 2003 Oct 8–12; Belgrad, Serbia. Milano: ESTM; 2003. p. 91–8.
14. *Škorić D, Balint B, Petakov M, Sindić M, Rodić P.* Collection strategies and cryopreservation of umbilical cord blood. Transfus Med 2007; 17(2): 107–13.
15. *Warwick R, Armitage S.* Cord blood banking. Best Pract Res Clin Obstet Gynaecol 2004; 18(6): 995–1011.
16. *Rogers I, Casper RF.* Umbilical cord blood stem cells. Best Pract Res Clin Obstet Gynaecol 2004; 18(6): 893–908.
17. *Martin-Rendon E, Watt SM.* Exploitation of stem cell plasticity. Transfus Med 2003; 13(6): 325–49.
18. *Dai W, Kloner RA.* Myocardial regeneration by embryonic stem cell transplantation: present and future trends. Expert Rev Cardiovasc Ther 2006; 4(3): 375–83.
19. *Balint B.* Stem cells – unselected or selected, unfrozen or cryopreserved: marrow repopulation capacity and plasticity potential in experimental and clinical settings. Maked Med Pregl 2004; 58 (Suppl 63): 22–4.
20. *Obradović S, Balint B, Rusović S, Ristić–Anđelkov A, Romanović R, Baškot B,* et al. The first experience with autologous bone marrow derived progenitor cell transfer for myocardial regeneration after acute infarction. Anest Reanim Transf 2004; 32(1–2): 39–50.
21. *Liu F, Pan X, Chen G, Jiang D, Cong X, Fei R,* et al. Hematopoietic stem cells mobilized by granulocyte colony-stimulating factor partly contribute to liver graft regeneration after partial orthotopic liver transplantation. Liver Transpl 2006; 12(7): 1129–37.
22. *Sevilla J, Fernandez-Plaza S, Diaz MA, Madero L; Paediatric Disease Working Party of the EBMT.* Hematopoietic transplantation for bone marrow failure syndromes and thalassemia. Bone Marrow Transplant 2005; 35 Suppl 1: S17–21.
23. *Notarangelo LD, Forino C, Mazzolari E.* Stem cell transplantation in primary immunodeficiencies. Curr Opin Allergy Clin Immunol 2006; 6(6): 443–8.
24. *Uzunel M, Remberger M, Sairafi D, Hassan Z, Mattsson J, Omazic B,* et al. Unrelated versus related allogeneic stem cell transplantation after reduced intensity conditioning. Transplantation 2006; 82(7): 913–9.
25. *Nademanee A, Forman SJ.* Role of hematopoietic stem cell transplantation for advanced-stage diffuse large cell B-cell lymphoma-B. Semin Hematol 2006; 43(4): 240–50.
26. *Dey BR, Spitzer TR.* Current status of haploidentical stem cell transplantation. Br J Haematol 2006; 135(4): 423–37.
27. *Specchia G, Pastore D, Mestice A, Liso A, Carluccio P, Leo M,* et al. Early and long-term engraftment after autologous peripheral stem cell transplantation in acute myeloid leukemia patients. Acta Haematol 2006; 116(4): 229–37.
28. *Lindvall O, Kokaia Z.* Stem cells for the treatment of neurological disorders. Nature 2006; 441(7097): 1094–6.
29. *Su L, Xu J, Ji BX, Wan SG, Lu CY, Dong HQ,* et al. Autologous peripheral blood stem cell transplantation for severe multiple sclerosis. Int J Hematol 2006; 84(3): 276–81.
30. *Parkins MD, Bahlis N, Brown C, Savoie L, Chaudhry A, Russell JA,* et al. Overnight storage of autologous stem cell apheresis products before cryopreservation does not adversely impact early or long-term engraftment following transplantation. Bone Marrow Transplant 2006; 38(9): 609–14.
31. *Balint B.* Cryopreservation of blood cells. In: *Balint B,* editor. Transfusiology. Belgrade: Zavod za udžbenike i nastavna sredstva; 2004. p. 479–95. (Serbian)
32. *Balint B, Ivanović Z, Petakov M, Taseski J, Jovčić G, Stojanović N,* et al. The cryopreservation protocol optimal for progenitor recovery is not optimal for preservation of marrow repopulating ability. Bone Marrow Transplant 1999; 23(6): 613–9.

The paper was received on April 24, 2007.