Reviews

Stem Cell Plasticity and Issues of Stem Cell Therapy

(stem cells / plasticity / transdiferentiation / cell fusion / stem cell therapy / myocardial infarction)

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Abstract. Today, there is much evidence suggesting that organ-specific stem cells need not rely completely on their own sources for maintenance and regeneration of an organism. In certain circumstances, mostly related to tissue damage, stem cell populations residing past the affected organ can contribute to its recovery – that means from different cell lines and also in tissues from another germ layer. The key factor in formation of self-renewing cellular clones is the presence of stem cells either from the tissue of origin or stem cells migrating from other areas and their successful settlement in an empty *niche* of the damaged tissue. Stem cell plasticity is the ability of adult tissue-specific stem cells to switch to new identities. The term plasticity also means stem cell phenotypic potential, which is broader than phenotypes of differentiated cells in their original tissues. Many laboratories have given evidence on stem cell plasticity; however, the presented results met with many objections from others. In the first part of our report we wish to refer to several issues associated with stem cell plasticity, transdifferentiation and fusion. Recent experimental results show that stem cells will play a key role in cell therapy. But there are still many questions to answer for scientists engaged in stem cell research. Is it possible to induce cells from one type of tissue to look and act as cells of another tissue? Do these changes occur naturally? Could plasticity be used in the treatment of fatal diseases? Cell therapy is one of the methods to treat damaged myocardial tissue. However, recent results with autologous bone marrow cells in the treatment of damaged myocardium show that this method has still many unanswered questions concerning cells, cytokines, microenvironment and other factors responsible for reparation. To date, there are many opinions either recommending or denying this method in different modifications. One question has not yet been definitely solved: What are the conditions for us to accept this method – its safety and efficacy? The future will show whether these our hopes and expectations will be fulfiled. Many experiments are needed before at least some of these questions may be answered and cell therapy become an important method for the benefit of our patients.

Presently, there is much evidence suggesting that organ-specific stem cells need not rely on their own sources in maintenance and regeneration of an organism. In some circumstances, mostly associated with tissue damage, stem cell populations normally residing past the damaged organ are able to contribute to the renewal of quite different cell lines also in tissues from a different germ layer (Raff, 2003; Quesenberry et al., 2004; Rutenberg et al., 2004). Plasticity can be described as a mutual ability to replace organ-specific stem cells. In a certain tissue, the organ-specific stem cell produces differentiated elements characteristic for the given tissue. Under certain conditions these stem cells can be made to produce elements that cannot be found in the original tissue (Quesenberry et al., 2005a). The term plasticity means phenotypical potential of tissue stem cells and is broader than phenotypes of differentiated cells in their initial tissue (Lakshmipathy and Verfaillie, 2005). For instance, it has been found that neural stem cells can give rise to haematopoietic (Björnson et al., 1999) or myogenous cells (Galli et al., 2000), bone marrow stromal stem cells can give rise to neural and glial cells (Kopen et al., 1999; Mezey et al., 2000), cardiomyocytes (Orlic et al., 2001a), pneumocytes (Krause et al., 2001), hepatocytes (Lagasse et al., 2000) and others (Quesenbery, 2005b, Lakshmipathy and Verfaillie, 2005). The ability of stem cell conversion was noted in cases when organ-specific stem cells produced cells of different type but of the same embryonic origin, i.e. organ-specific stem cells derived from the same germ layer (*intra-germ layer conversion).*

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Abbreviation: ES – embryonic stem.

Later, conversion was demonstrated of organ-specific stem cells into cells originating from another germ layer (*trans-germ layer conversion)* (Eisenberg et al., 2003). These facts make us think that plasticity represents a hierarchic system in which all elements of living organisms have their place. And the question is: What is the role of stem cells in the whole concept of plasticity? Are stem cells of use through the whole process or only at the beginning? That would mean that plasticity is a hierarchic, dynamic and, at the same time, balanced state of a living organism existing through the whole life span. This condition is probably associated with processes of regeneration, aging, tumour development and many others.

Anderson, Gage and Weissman presented a set of rules for the proof of plasticity (Anderson et al., 2001; Weissman et al., 2001). First, they asked for proper identification of all cells before starting experiments, because a single foreign cell in seemingly purified culture may lead to false results. The proof of expression of new proteins is not sufficient. So, to prove plasticity it has to be demonstrated that cells contribute to host tissue functions, for instance, to transmission of electric signals in the neural system or to removal of waste products from blood and liver. That means that a single, well-characterized donor cell must be able to produce a population of functional cells and not only a few scattered cells in the new tissue. Plasticity should be a natural phenomenon, which means that cells must function in the host tissue without their changing during the culture (Anderson et al., 2001; Weissman et al., 2001). These conditions have led to a heated discussion (Goodell, 2003) and several scientists in this field agree that cells must be better characterized, their functionality confirmed and predictive factors associated with plasticity described (Filip et al., 2005). Presently, in spite of marked progress, no agreement has been reached in that of how many functional jumps these cells must make before plasticity could be considered (Filip et al., 2004a).

Until now, none of the studies wanting to demonstrate plasticity satisfied the rigorous criteria given by Weissman et al. (2001). Let us remind experiments of Krause et al., who used the cells that did not change during culture and showed that a single hematopoietic stem cell may give rise to many types of cells (Krause et al., 2001). However, there is still the problem how to prove cell fusion. For instance, Lagasse et al. remain sceptical, they presume that cells from the first transplant recipient „were not very well characterized“ (Lagasse et al., 2001). Other authors, such as Verfaillie et al., express their opinion that studies „actually do not show an important contribution to any organ“ (Verfaillie, 2002; Verfaillie et al., 2003). Presently, there were only small groups of cells present without any function. In spite of various opinions and efforts to find the truth, until now this experiment has not been verified. Weissman et al. reported that when he and his team tried to repeat the above-mentioned experiment with carefully selected blood stem cells, they found only expected bone and blood derivatives, six liver cells and one cerebellar cell – this Purkinje cell had the DNA content twice higher, which means that it could be a local cell fused with one of the labelled cells (Weissman et al., 2001). However, we can say that these different results achieved by the groups of Krause and Weissman led to an important question – how to design a basic experiment where plasticity would be either confirmed or excluded. The link between the transdifferentiation process and the influence of environment where stem cells reside is difficult to find. To understand this relationship, predictive factors, besides others, have an important role involving morphology, function and microenvironment (Filip et al., 2004b; Filip et al., 2005). Stem cells, probably, spend the whole time of their existence in G_o phase, keep certain active relation to different forms of microenvironment and can influence it back (Krause et al., 2001; Weissman et al., 2001; Krause, 2002). Furthermore, it seems that haematopoietic stem cells (HSCs) may travel into some tissues and organs and can influence their regeneration, such as liver, lungs, GIT, vessels and heart (Harraz et al., 2001; Krause et al., 2001; Anversa et al., 2003). Mesenchymal stem cells (MSCs) have the capacity to add cells to blood, lung, liver and intestines (Jiang et al., 2002). Stem cell populations in brain and fat tissue also show previously unforseen potentiality (Clarke et al., 2000; Zuk et al., 2001). For all that, these studies did not convince many scientists and they claim that there is still sufficient reason for scepticism (Anderson et al., 2001; Hawley and Sobieski, 2002; Lemischka, 2002; Orkin and Zon, 2002; Goodell, 2003).

Plasticity, Cell Fusion and Transdifferentiation

One of the main arguments used to challenge the reports on stem cell plasticity is that previous results aimed to prove transdifferentiation were in fact the proof of cell fusion (Hawley and Sobieski, 2002; Wurmser and Gage, 2002; Medvinsky and Smith, 2003). This issue has come forward with recent observations that tissue-derived stem cells may undergo fusion with other cell types (Terada et al., 2002; Wang et al., 2003). Studies on cell fusion are often cited to challenge the existence of stem cell transdifferentiation (Wurmser and Gage, 2002). Based on newer studies on fusion, it has been concluded that cell fusion in previous reports may be explained as stem cell plasticity. Besides, the proof of cell fusion is being described as an invalidation of the concept of stem cell transdifferentiation (Wurmser and Gage, 2002; Medvinsky and Smith, 2003). It is not clear, however, why cell fusion and transdifferentiation should be controversial phenome-

na. After all, the development of skeletal muscle involves both – cell differentiation and fusion.

Although all scientific hypotheses, to be fully accepted, require rigorous scientific proof, doubts exist whether the rules proposed by Weissman, Anderson, and Gage are always suitable for determinations of stem cell plasticity (Raff, 2003; Filip et al., 2004a). The first rule suffers from overconfidence that studies on living animals are able to solve problems with stem cell potential. While definite positive results *in vivo* are always the best variant, interpretation of negative results is often problematic. If a certain stem cell population shows uncapable of regenerating target tissue, does it mean that these cells do not have the right tissue potential? Actually, negative results may indicate disability of donor stem cells to settle down or integrate with target tissue. Moreover, it is difficult to distinguish in an experiment whether it is the real phenotypic potential of donor cells or supportive microenvironment of the host tissue that is just being studied. The culture of cells is a useful addition to studies on living animals because experimental conditions are much better controlled than in the *in vivo* environment. At first sight it may appear as a sensible declaration that „donor cells should produce robust and permanent regeneration of target tissue to determine exactly the stem cell plasticity". However, let us examine the meaning of this criterion in a broader context. For instance, for many years it had been thought that adult myocardium was postmitotic, but now it is known that there is continuous though slow regeneration (Anversa and Nadal-Ginard, 2002; Anversa et al., 2003). This normal rate of myocyte replacement is far from robust, although probably sufficiently high to maintain normal homeostasis in myocardium during the whole life span. In the meantime, good evidence has been described, but not confirmed, suggesting that extracardiac cells generate spare myocytes in the adult heart (Jackson and Goodell, 1999; Orlic et al., 2001b; Laflamme et al., 2002; Quiani et al., 2002). To give an example of required functionality, let us presume that progeny of transplanted haematopoietic stem cells has fully integrated into contractile myocardium and demonstrated multiple muscle proteins in the myocardial sample. Would determination of functionality be necessary for us to show that transdifferentiation occured? Functional analysis would be needed for evaluation of clinical usefulness of transplantation but not for verification of transdifferentiation (Filip et al., 2004a).

The demand that stem cell plasticity must be demonstrated in „natural conditions" seems especially unsuitable for understanding the biological importance of transdifferentiation and plasticity (Raff, 2003). The greatest cellular regeneration occurs during the process of wound healing and, therefore, transdifferentiation studies during organ reparation may be the right field for research. This criterion is also controversial to many studies in cell biology because traditional models of stem cell differentiation are based on studies investigating the capacity of haematopoietic cells to reconstitute the blood system after lethal irradiation in recipient animals (Graf, 2002; Orkin and Zhon, 2002; Wang et al., 2003). The call for greater scientific severity in studying data on stem cell plasticity seems reasonable, but these demands considerably complicate further debate on this theme and so complicate experimental proof of plasticity. The demand for every experiment to give the most exact evidence has its meaning only when advocates of stem cell plasticity as a group will defend the alternative to the traditional view on stem cells. But this is not the problem brought up by the knowledge on stem cell plasticity. Instead, the plasticity studies show that traditional biologic models of stem cells are not sufficient to explain the development of diversification of cell phenotypes in vertebrate organisms (Anversa et al., 2003). There is an imense number of reports on plasticity that must be confronted because not each of them deals with transdifferentiation. It was the knowledge that at least some plasticity data were valid which led to experiments integrating this knowledge into the traditional theory on stem cells.

The term transdifferentiation is often used for the potential of stem cell plasticity. For instance, it may be considered that stem cells, normally generating blood cells, undergo transdifferentiation if they produce cardiomyocytes. However, this is an older definition of transdifferentiation used for conversion of one differentiated cell type to another. The most frequent examples of transdifferentiation are: regeneration of extremities in amphibians and conversion of pigment epithelium into lens and retinal neural cells (Tsonis and Del Rio-Tsonis, 2004). In these cases, differentiated tissue dedifferentiates into cells with a clear stem cell phenotype prior to their metamorphosis into other differentiated cell phenotypes. Also, there are other cases of transdifferentiation, such as conversion of pancreatic cells into hepatocytes and vascular epithelium into smooth muscle (Frid et al., 2000; Shen et al., 2003). Besides this, data indicate that bone marrow macrophages may transdifferentiate into the phenotype of cardiomyocytes when cultured in the presence of myocardial tissue (Orlic et al., 2001b; Eisenberg and Eisenberg, 2003).

In adults, generation of newly differentiated cells is significantly increased as a response to wound healing. Generally, the view has been accepted that stem cells sense tissue damage and migrate from the distance to the site of injury (Theise et al., 2000; Harraz et al., 2001; Mahmood et al., 2001). Nevertheless, it has been observed that activation of immune response increases regeneration of cells and tissues when tranplanted to the host (Krause et al., 2001; Laflamme et al., 2002; Quaini et al., 2002). For instance, in the study on male heart tissue after transplantation of female heart, the greatest numbers of Y-positive cardiomyocytes were found at

the sites of acute rejection (Laflamme et al., 2002). These results are surprising because the first wave of cells recruited to the wound are *immune response cells* as the first step to stop further and more serious damage at the site of trauma. If these immune cells may later be the source of new cells for wound repair, then their differentiation may compensate for the need to mobilize secondary cellular population, such as stem cells, to the damaged tissue. This hypothesis is in accordance with the results and indicates that macrophages entering the myocardial tissue may contribute to the generation of new myocytes (Eisenberg and Eisenberg, 2003). Further evidence that cells may transdifferentiate are studies on the properties of monocytes which are the cells usually thought of as immediate macrophage progenitors. Several studies have shown that monocytes may transdifferentiate into endothelial cells (Fernandez Pujol et al., 2000; Harraz et al., 2001). For instance, monocytes contribute as endothelial cells to new vessel developing in the injured extremities during regeneration (Harraz et al., 2001). A newer study suggested that the phenotypical potential of monocytes may also spread to other cell lines (Zhao et al., 2003). The endothelial potential of monocytes should be considered in context with one of the oldest controversies in stem cell biology about the link between blood and endothelial cell lines. Interest was focused on the existence of a multipotent stem cell – *haemangioblast*, which gives rise to both haematopoietic and endothelial cell lines (Robb and Elefanty, 1998). Although this debate still goes on, it seems clear now that stem cells with haemangioblast properties exist in the embryo as well as in the adult (Choi, 1998). But the finding that a cell, such as monocyte which is presumed to be fully committed to myeloid blood lines, is also capable of generating endothelial cells, has significant consequences for stem cell biology. The importance of these observations is in their divergence from standard hierarchic models of stem cells which show diversification of lines at the level of multipotent stem cells. If it is possible to redirect unipotent progenitor cells to multiple cell fates, then what is the difference between multipotent and unipotent stem cells?

The ability of both differentiated cells and cells characterized as highly committed progenitors, i.e. monocytes, to transdifferentiate into other cell types suggests that current diversification models need not sufficiently represent the increase of cellular phenotypes. Although there are few examples where existence of transdifferentiation of differentiated cell phenotypes was clearly demonstrated, the evidence in these cases is definitive (Tsonis and Del Rio-Tsonis, 2004) and establishes a precedent that regenerated tissue does not always develop from stem cells along the pathways of hierarchic differentiation. Despite the definitive evidence of transdifferentiation, its existence is usually considered a special case with little relevance for discussions on stem cell biology and tissue regeneration. However, a new evidence (Frid et al., 2000; Shen et al., 2003) suggests that transdifferentiation may have a broader meaning for understanding mammal biology. Although differentiation may be higher than dedifferentiation and transdifferentiation among the phenotypes, ensuring the direction towards cell lines, the importance of the transdifferentiating model of cell diversification is that all cellular phenotypes in an organism are part of continuity (Eisenberg and Eisenberg, 2004; Kucia et al., 2005).

Stem cell therapy

Experimental biology and medicine have used stem cells in cell therapy for more than 20 years. An *in vitro* method has been developed to culture embryonic stem (EC) cells acquired at abortions or from „surplus“ embrya left after *in vitro* fertilizations, and immediately evoked ideas how to direct the development and differentiation of these cells and utilize them in regeneration of damaged tissues (Filip et al., 2003; Lisker, 2003; Filip et al., 2004a). Still, the cell therapy faces a difficult task how to detect, harvest and culture stem cells for treatment of several diseases (Lemischka, 1999; Lemischka, 2002). Will it be possible to use adult stem cells in therapy of a broader spectrum of diseases?

Diseases due to destruction and dysfunction of a certain limited number of cell types, such as *diabetes mellitus* (with selective damage to β-cells in Langerhans islets) or Parkinson's disease (destruction of dopaminergic neurons in *substantia nigra*) can be treated by transplantation of differentiated derivatives of ES cells. Animal studies show that transplantation of pluripotent stem cells or foetal cells can successfully treat a number of chronic diseases, such as diabetes, Parkinson's disease, traumatic spinal cord injuries, Purkinje's cellular degeneration, liver failure, heart failure, Duchenne's muscular dystrophy, *osteogenesis imperfecta*, and others (Horwitz et al., 2001; Soria et al., 2001; Snyder et al., 2004; Kajstura et al., 2005). Although marked progress has been achieved in human transplant therapy, there are still several main set-backs limiting broad application of cells in the routine therapy, such as the need of massive doses of immunosuppressive drugs to prevent rejection of transplanted tissue and also lack of organs from dead donors. Despite all these set-backs, strategy based on human ES cells may allow production of unlimited amounts of cells, eventually tissues, and their sufficient supply from an abundant, renewable and quickly available source. Moreover, ES cells according to their adaptability for stable genetic modification could be treated so as to avoid or inhibit the host immune response.

The first step to develop successful therapy based on human ES cells is to demonstrate their capability to differentiate into a certain, for us interesting, cell type, and to purify this line from a mixed population. In the sec-

ond step, it would be necessary to critically examine that differentiated cell derivatives function in a normal physiologic way, for example, that secretion of insulin in the cells of Langerhans islets is normal and responds to the glucose level. The third step and most important milestone on the route to clinical tests will be the proof of efficiency of model diseases on guinea-pigs and big animals. The fourth step is to exclude formation of tumours developing from derivatives after differentiation of ES cells and transplanted to human recipients. Considering that progress in this direction goes forward in big strides, other problems will certainly show which may limit the therapeutic use of cells. The effort of scientists to treat diseases at present untreatable, the pressure of patients and their families, as well as political pressure may complicate the development of new therapies. Important is to keep a „clear mind“, get rid of emotions and respect scientific and ethical rules.

Prospective trends in the cell therapy are: therapeutic cloning, ES cells, therapy of the foetus, adult stem cells, use of humoral agents for control of stem cell behaviour and, eventually, genetic stem cell modification. At the beginning it appeared that adult stem cells may represent a certain „ ethical compromise“ to embryonic cells. Today, however, we understand that individual approaches are closely linked together and this, consequently, delays the answers to bioethical issues. Scientists have already shown that a number of cell types, such as neurons and muscle cells (Kehat and Gepstein, 2003), pancreatic cells (Soria et al., 2001; Vogel, 2001) and others can be obtained by culture of ES cells. Today, stem cells may be used in quite unexpected cases, such as renal diseases (Mollura et al., 2003; Schachinger and Zeiher, 2005) and immunologic repair in AIDS patients (Scadden, 2003).

Stem cell therapy and myocardial infarction

Stem cell biology and cell therapy, apparently, are coming to age and raise new hopes as well as problems some of which we would like to illustrate on myocardial infarction. Myocardial infarction is a disease leading to the loss of tissue and impairment of heart performance. Residual cardiomyocytes are not able to reconstitute the necrotic tissue and heart function gets worse with time. According to some theories, distant stem cells activate damage to the target organ, migrate to the injured site and undergo there alternative differentiation (Ferrari et al., 1998; Eglitis and Mezey, 1999; Jacksonet al., 1999). The size of infarction is the main determinant of morbidity and mortality because massive infarctions affecting 40% or more of the left ventricle are associated with unmanageable cardiogenic shock or fast development of congestive heart failure. In the past, regeneration of heart function depended in full on the activity of the residual, unaffected part of the ventricle. Nevertheless, hypertrophied infarcted heart succumbs to progressive impairment, dilating myopathy, terminal failure, and death (Lee et al., 2004a; Ozbaran et al., 2004).

The idea to regenerate the damaged heart tissue by addition of cells is not new, but to find cells able to fulfil this task is difficult. The solution may come from a selected group of bone marrow cells. Myocardial infarction results in death of cardiac cells and leads to the formation of a scar at the damaged area, further impairing heart performance. Every year gradual progression from heart attack to heart failure is a cruel reminder for millions of people that current pharmacotherapy is not able to replace the loss of living contractile cells. Lately, in case of heart failure, doctors have taken to heart transplantation. Replacement of dead cardiac cells would be an attractive alternative, but its realization is still prevented by many biologiccal, technical and ethical problems (Lee and Makkar, 2004).

Damaged hearts in animal models were gradually transplanted with cell populations from donors who were considered suitable for regeneration. Suitable for transplantation are elements of skeletal musculature, immortalized cells from heart atrium, smooth muscle myocytes, bone marrow cells and cardiomyocytes. All these cells can be obtained from the embryo, foetus, and adult (Abbott and Giordano, 2003; Lee et al., 2004; Lee and Makkar, 2004). The success of transplantation depends on survival, maturation and electromechanic connection of donor cells with existing heart cells of the recipient and on their effect on heart function. These are high requirements for any cell population, so it is not surprising that experiments, until now, brought different results depending on selected cell types.

The idea to use bone marrow stem cells for heart regeneration is particularly attractive. They are pluripotent, i.e. able to differentiate into several distinct cell types. As for the heart, these pluripotent cells might be capable of forming heart muscle as well as vessels for alimentation support of the damaged area and for repopulation with muscle cells. Harvesting of cells from bone marrow in adults is easy and routine and does not present any ethical problems connected with the use of embryonic and foetal tissues. The therapy with cells from the patient’s own bone marrow eliminates the fear of tissue rejection (a great problem with cells from another donor). Furthermore, it is known that transfer of marrow cells into the scar in the damaged heart improves the heart function if the cells are cultured one week before and then treated to induce expression of muscle proteins (Tomita et al., 1999; Ozbaran et al., 2004).

Orlic et al. separated Lin^{-} $c\text{-}kit^{+}$ cells of bone marrow from transgenic mice expressing enhanced green fluorescent protein (EGFP) (Orlic et al., 2001a; Orlic et al., 2001b). Failure to reconstitute infarction was ascribed to difficulties with transplantation of cells into

the tissue with high contractile frequency. Also immunologic reaction of female mice to male bone marrow transplant might be the cause for insufficient regeneration in some female recipients. Local transplant of Lin^- $c\text{-}kit^+$ bone marrow cells showed high capacity for differentiation into cardiac tissue. They led to the formation of new cardiomyocytes, endothelial cells and smooth muscle cells and formed *de novo* myocardium with coronary arteries, arterioles and capillaries. Partial regeneration of infarcted myocardium means that transplanted cells responded to signals of injured myocardium and induced migration, proliferation and differentiation in the necrotic area of the ventricular wall. Differentiating cardiomyocytes may express nuclear and cytoplasmic proteins typical of heart tissue (Orlic et al., 2001b) but also atypical, such as nestin (Mokrý et al., 2004).

The repair of damaged myocardium may be evoked by application of autologous bone marrow cells (Strauer and Kornowski, 2003; Eisenberg and Eisenberg, 2004), autologous muscle cells (Fuchs et al., 2001) and application of some cytokines such as SCF and G-CSF (Strauer and Kornowski, 2003; Deten et al., 2005) and others (Lee and Makkar, 2004).

Critically evaluating to date published clinical studies on cell therapy of myocardial infarction we have to realize that it will not be easy to find a cell population or cytokine cascade which would enable us to better utilize the possibilities that cell therapy offers. The results of these studies are different in both, clinical and biological aspects – numbers of patients are small, application of cells (myocardial injection or intracoronary infusion) is also different and different is the transplant itself (either bone marrow cells, muscle-obtained myoblasts, or separated progenitor cells). Application of bone marrow cells had only minimum complications, such as supraventricular tachycardia and one death, but not due to arrhythmia (Lee et al., 2004). More serious complications were described in patients given muscle-obtained myoblasts – here complications were also arrhythmias and ventricular tachycardias, and one death at cell application (Lee et al., 2004). Some other reports preferring the use of marrow cells transdifferentiated into cardiomyocytes with the help of some growth factors, such as G-CSF (Takano et al., 2003) are very interesting and show the possibilities to combine cytokines, for instance. Each of the presented methods has its advantages and disadvantages. With application of bone marrow cells, both, repair of myocardium and its revascularization is presumed. Contrary to this, revascularization is much smaller with muscle cell application. We must not forget to mention late complications of which we do not know much (Abbott and Giordano, 2003; Eisenberg and Eisenberg, 2004).

In conclusion we can say that cell therapy represents a new therapeutic method for myocardial tissue damage. Recent results with autologous bone marrow cells show that this method has still many unanswered issues concerning cells, cytokines, microenvironment, and other factors responsible for reparation. Presently, there are many opinions either recommending or not recommending this method in its different modifications. However, the most important problem has not been solved yet – what are the conditions to accept this method – safety and fulfilment of the hopes we put in it. Many experiments will be needed before it becomes an important part of the therapy of myocardial infarction.

References

Abbott, J. D., Giordano, F. J. (2003) Stem cells and cardiovascular disease. *J. Nucl. Cardiol*. **10,** 403-412.

Anderson, D. J., Gage, F. H., Weissman, I. L. (2001) Can stem cells cross lineage boundaries? *Nat. Med*. **7,** 393-395.

Anversa, P., Kajstura, J., Nadal-Ginard, B., Leri, A. (2003) Primitive cells and tissue regeneration. *Circ. Res.* **92,** 692-699.

Anversa, P., Nadal-Ginard, B. (2002) Myocyte renewal and ventricular remodeling. *Nature* **415**, 240-243.

Björnson, C. R., Rietze, R. L., Reynolds, B. A., Magli, M. C., Vescovi, A. L. (1999) Turning brain into blood: a hematopoietic fate adopted by neural stem cells in vivo. *Science* **283,** 534-537.

Choi, K. (1998) Hemangioblast development and regulation. *Biochem. Cell Biol*. **76,** 947-956.

Clarke, D. L., Johansson, C. B., Wilbertz, J., Veress, B., Nilsson, E., Karlstrom, H., Lendahl, U., Frisen, J. (2000) Generalized potential of adult stem cells. *Science* **288,** 1660-1663.

Deten, A., Volz, H. C., Clamos, S., Leiblein, S., Briest, W., Marx, G., Zimmer, H. G. (2005) Hematopoietic stem cells do not repair the infarcted mouse heart. *Cardiovasc. Res.* **65,** 52-63.

Eglitis, M. A., Mezey, E. (1999) Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc. Natl. Acad. Sci. USA* **94,** 4080-4085.

Eisenberg, L. M., Eisenberg, C. A. (2003) Stem cell plasticity, cell fusion, and transdifferentiation. *Birth Defect Res. Part. C. Embryo Today* **69,** 209-218.

Eisenberg, L. M., Eisenberg, C. L. (2004) Adult stem cells and their cardiac potential. *Anat. Rec.* **276A,** 103-112.

Fernandez Pujol, B., Lucibello, F. C., Gehling, U. M., Lindemann, K., Weidner, N., Zuzarte, M. L., Adamkiewicz, J., Elsasser, H. P., Muller, R., Havemann, K. (2000) Endothelial-like cells derived from human CD14 positive monocytes. *Differentiation* **65,** 287-300.

Ferrari, G., Cusella-De Angelis, G., Coletta, M., Paolucci E, Stornaiuolo A, Cossu G, Mavilio F. (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* **279,** 1528-1530.

Filip, S., Mokrý, J., Hruška, I. (2003) Adult stem cells and their importance in cell therapy. *Folia Biol. (Praha)* **49,** 9-14.

Filip, S., English, D., Mokrý, J. (2004a) Issues in stem cell plasticity. *J. Cell. Mol. Med*. **8,** 572-577.

Filip, S., Mokrý, J., Karbanová, J., Vávrová, J., English, D. (2004b) Local environmental factors determine hematopoietic differentiation of neural stem cells. *Stem Cells Dev.* **13,** 113-120.

Filip, S., Mokrý, J., Karbanová, J., Vávrová, J., Vokurková, D., Bláha, M., English, D. (2005) The transplantation of neural stem cells and predictive factors in hematopoietic recovery in irradiated mice. *Transfus. Apher. Sci.* **32,** 157-166.

Frid, M. G., Kale, V. A., Stenmark, K. R. (2000) Mature vascular endothelium can give rise to smooth muscle cells via endothelial-mesenchymal transdifferentiation: in vitro analysis. *Circ. Res*. **90,** 1189-1196.

Fuchs, S., Baffour, R., Zhou, Y. F. (2001) Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. *J. Am. Coll. Cardiol.* **37,** 1726-1732.

Galli, R., Borello, U., Gritti, A. (2000) Skeletal myogenic potential of human and mouse neural stem cells. *Nat. Neurosci.* **10,** 986-991.

Goodell, M. A. (2003) Stem-cell „plasticity“: befuddled by the muddle. *Curr. Opin. Hematol*. **10,** 208-213.

Graf, T. (2002) Differentiation plasticity of hematopoietic cells. *Blood* **99,** 3089-3099.

Harraz, R. G., Jiao, C., Hanlon, H. D., Hartley, R. S., Schatteman, G. C. (2001) CD34(-) blood-derived human endothelial cell progenitors. *Stem Cells* **19,** 304-312.

Hawley, R. G., Sobieski, D. A. (2002) Somatic stem cell plasticity: to be or not to be. *Stem Cells* **20,** 195-197.

Horwitz, E. M., Prockop, D. J., Gordon, P. L., Koo, W. W., Fitzpatrick, L. A., Neel, M. D., McCarville, M. E., Orchard, P. J., Pyeritz, R. E., Brenner, M. K. (2001) Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta. *Blood* **97,** 1227-1231.

Jackson, K. A., Mi, T., Goodell, M. A. (1999) Hematopoietic potential of stem cells isolated from murine skeletal muscle. *Proc. Natl. Acad. Sci. USA* **96,** 14482-14486.

Jiang, Y., Jahagirdar, B. N., Reinhardt, R. L., Schwartz, R. E., Keene, C. D., Ortiz-Gonzalez, X. R., Reyes, M., Lenvik, T., Lund, T., Blackstad, M., Du, J., Aldrich, S., Lisberg, A., Low, W. C., Largaespada, D. A., Verfaillie, C. M. (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* **418,** 41-49.

Kajstura, J., Rota, M., Whang, B., Cascapera, S., Hosoda, T., Bearzi, C., Nurzynska, D., Kasahara, H., Zias, E., Bonafe, M., Nadal-Ginard, B., Torella, D., Nascimbene, A., Quaini, F., Urbanek, K., Leri, A., Anversa, P. (2005) Bone marrow cells differentiate in cardiac cell lineages after infarction independently of cell function. *Circ. Res.* **96,** 127-137.

Kehat, I., Gepstein, L. (2003) Human embryonic stem cells for myocardial regeneration. *Heart Failure Rev.* **8,** 229-236.

Kopen, G. C., Prockop, D. J., Phinney, G. (1999) Marrow stromal cells migrate throughout forebrain and cerebellum and they differentiate into astrocytes after injection into neonatal mouse brains. *Proc. Natl. Acad. Sci. USA* **96,** 10711-10716.

Krause, D. S., Theise, N. D., Collector, M. I., Henegariu, O., Hwang, S., Gardner, R., Neutzel, S., Sharkis, S. J. (2001) Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* **105,** 369-377.

Krause, D. S. (2002) Plasticity of marrow-derived stem cells. *Gene Ther.* **9,** 754-758.

Kucia, M., Ratajczak, J., Ratajczak, M. Z. (2005) Are bone marrow stem cells plastic or heterogenous – that is the question. *Exp. Hematol.* **33,** 613-623.

Laflamme, M. A., Myerson, D., Saffitz, J. E., Murry, C. E. (2002) Evidence for cardiomyocyte repopulation by extracardiac progenitors in transplanted human hearts. *Circ. Res*. **90,** 634-640.

Lagasse, E., Connors, H., Al-Dhalimy, M., Reitsma, M., Dohse, M., Osborne, L., Wang, X., Finegold, M., Weissman, I. L., Grompe, M. (2000) Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. *Nat. Med*. **6,** 1229-1234.

Lagasse, E., Shizuru, J. A., Uchida, N., Tsukamoto, A., Weissman, I. L. (2001) Toward regenerative medicine. *Immunity* **14,** 425-436.

Lakshmipathy, U., Verfaillie, C. (2005) Stem cell plasticity. *Blood Rev.* **19,** 29-38.

Lee, M. S., Makkar, R. R. (2004) Stem-cell transplantation in myocardial infarction: a status report. *Ann. Intern. Med.* **140,** 729-737.

Lee, M. S., Lill, M., Makkar, R. R. (2004a) Stem cell transplantation in myocardial infarction. *Rev. Cardiovasc. Med.* **5,** 82-98.

Lemischka, I. (1999) The power of stem cells reconsidered? *Proc. Natl. Acad. Sci, USA* **96,** 14193-14195.

Lemischka, I. (2002) A few thoughts about the plasticity of stem cells. *Exp. Hematol*. **30,** 848-852.

Lisker, R. (2003) Ethical and legal issues in therapeutic cloning and the study of stem cells. *Arch. Med. Res*. **34,** 607-611.

Mahmood, A., Lu, D., Wang, L., Li, Y., Lu, M., Chopp, M. (2001) Treatment of traumatic brain injury in female rats with intravenous administration of bone marrow stromal cells. *Neurosurgery* **49,** 1196-1203.

Medvinsky, A., Smith, A. (2003) Fusion brings down barriers. *Nature* **422,** 823-825.

Mezey, E., Chandross, K. J., Harta, G., Maki, R. A., McKercher, S. R. (2000) Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. *Science* **290,** 1779-1782.

Mokrý, J., Čížková, D., Filip, S., Ehrmann, J., Österreicher, J., Kolář, Z., English, D. (2004) Nestin expression by newly formed human blood vessels. *Stem Cells Dev.* **13,** 658-664.

Mollura, D. J., Hare, J. M., Rabb, H. (2003) Stem cell therapy for renal diseases. *Am. J. Kidney Dis.* **42**, 891-905.

Orkin, S. H., Zon, L. I. (2002) Hematopoiesis and stem cells: plasticity versus developmental heterogenity. *Nat. Immunol.* **3,** 323-328.

Orlic, D., Kajstura, J., Jakoniuk, I., Anderson, S. M., Li. B., Pickel, J., McKay, R., Nadal-Ginard, B., Bodine, D. M., Leri, A., Anversa, P. (2001a) Bone marrow cells regenerate infarcted myocardium. *Nature* **410,** 701-705.

Orlic, D., Kajstura, J., Chimenti, S., Limana, F., Jakoniuk, I., Quaini, F., Nadal-Ginard, B., Bodine, D. M., Leri, A., Anversa, P. (2001b) Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc. Natl. Acad. Sci. USA* **98,** 10344-10349.

Ozbaran, M., Omay, S. B., Nalbantgil, S., Kultursay, H., Kumanlioglu, K., Nart, D., Pektok, E. (2004) Autologous peripheral stem cell transplantation in patients with congestive heart failure due to ischemic heart disease. *Eur. J. Cardiothorac. Surg.* **25,** 342-351.

Quaini, F., Urbanek, K., Beltrami, A. P., Finato, N., Beltrami, C. A., Nadal-Ginard, B., Graf, T. (2002) Differentiation plasticity of hematopoietic cells. *Blood* **99,** 3089-3099.

Quesenberry, P. J., Abedi, M., Aliotta, J., Colvin, G., Demers, D., Dooner, M., Greer, D., Hebert, H., Menon, M. K., Pimentel, J., Paggioli, D. (2004) Stem cell plasticity: an overview. *Blood Cells Mol. Dis.* **32,** 1-4.

Quesenberry, P. J., Dooner, G., Colvin, G., Adebi, M. (2005a) Stem cell biology and the plasticity polemic. *Exp. Hematol.* **33,** 389-394.

Quesenberry, P. J., Colvin, G. A., Adebi, M., Dooner, G., Dooner, M., Aliotta, J., Keaney, P., Luo, L., Demers, D., Peterson, A., Foster, B., Greer, D. (2005b) The stem cell continuum. *Ann. N. Y. Acad. Sci.* **1044,** 228-235.

Raff, M. (2003) Adult stem cell plasticity: fact or artifact? *Annu. Rev. Cell. Biol.* **19,** 1-22.

Robb, L., Elefanty, A. G. (1998) The hemangioblast – an elusive cell captured in culture. *Bioessays* **20,** 611-614.

Rutenberg, M. S., Hamazaki, T. Singh, A. M., Terada, N. (2004) Stem cell plasticity, beyond alchemy. *Int. J. Hematol.* **79,** 15-21.

Scadden, D. T. (2003) Stem cells and immune reconstitution in AIDS. *Blood Rev.* **17,** 227-231.

Schachinger, V., Zeiher, A. M. (2005) Stem cells and cardiovascular and renal disease: today and tomorrow. *Am. Soc. Nephrol.* **16,** 2-6.

Shen, C. N., Horb, M. E., Slack, J. M., Tosh, D. (2003) Transdifferentiation of pancreas to liver. *Mech. Dev.* **120,** 107-116.

Snyder, E. Y., Daley, G. Q., Goodell, M. (2004) Taking stock and planning for the next decade: realistic prospects for stem cell therapies for the nervous system. *J. Neurosci. Res.* **76,** 157-168.

Soria, B., Skoudy, A., Martin, F. (2001) From stem cells to beta cells, new strategies in cell therapy of diabetes mellitus. *Diabetologia* **44,** 407-415.

Strauer, B. E., Kornowski, R. (2003) Stem cell therapy in perspective. *Circulation* **107,** 929-934.

Takano, H., Ohtsuka, M., Akazawa, H., Toko, H., Harada, M., Hasegawa, H., Nagai, T., Komuro, I. (2003) Pleiotropic effects of cytokines on acute myocardial infarction: G-CSF as a novel therapy for acute myocardial infarction. *Curr. Pharm. Des.* **9,** 1121-1127.

Terada, N., Hamazaki, T., Oka, M., Hoki, M., Mastalerz, D. M., Nakano, Y., Meyer, E. M., Morel, L., Petersen, B. E., Scott, E. W. (2002) Bone marrow cells adopt the phenotype of other cells by spontaneous fusion. *Nature* **416,** 542-545.

Theise, N. D., Nimmakayalu, M., Gardner, R., Illei, P. B., Morgan, G., Teperman, L., Henegariu, O., Krause, D. S. (2000) Liver from bone marrow in humans. *Hepatology* **32,** 11-16.

Tomita, S., Li, R. K., Weisel, R. D., Mickle, D. A., Kim, E. J., Sakai, T., Jia, Z. Q. (1999) Autologous transplantation of bone marrow cells improves damaged heart function. *Circulation* **100,** 247-256.

Tsonis, P. A., Del Rio-Tsonis, K. (2004) Lens and retina regeneration: transdifferentiation, stem cells and clinical application. *Exp. Eye Res.* **78,** 161-172.

Verfaillie, C. M. (2002) Adult stem cells: assessing the case for pluripotency. *Trends Cell. Biol*. **12,** 502-508.

Verfaillie, C. M., Schwartz, R., Reyes, M., Jianf, Y. (2003) Unexpected potential of adult stem cells. *Ann. N. Y. Acad. Sci.* **996,** 231-234.

Vogel, G. (2001) Stem cells are coaxed to produce insulin. *Science* **292,** 615-617.

Wang, X., Willenbring, H., Akkari, Y., Torimaru, Y., Foster, M., Al-Dhalimy, M., Lagasse, E., Finegold, M., Olson, S., Grompe, M. (2003) Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature* **422,** 897-901.

Weissman, I. L., Anderson, D. J., Gage, F. (2001) Stem and progenitor cells: origins, phenotypes, lineage, commitment, and transdifferentiations. *Annu. Rev. Cell. Dev. Biol*. **17,** 387-403.

Wurmser, A. E., Gage, F. H. (2002) Cell fusion causes confusion. *Nature* **416,** 485-487.

Zhao, Y., Glesne, D., Huberman, E. (2003) A human peripheral blood monocyte-derived subset acts as pluripotent stem cells. *Proc. Natl. Acad. Sci USA* **100,** 2426-2431.

Zuk, P. A., Zhu, M., Mizuno, H., Huang, J., Futrell, J. W., Katz, A. J., Benhaim, P., Lorenz, H. P., Hedrick, M. H. (2001) Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Eng.* **2,** 211-228.