Review

Adult Stem Cells and Their Importance in Cell Therapy

(adult stem cells / embryonic stem cells / cell therapy / ethical problems)

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Abstract. For their unique properties stem cells promise to be of universal use in clinical medicine, especially in regeneration of many organs and tissues in the human body. This attractive subject receives an ever growing attention of specialists from different branches of science and, no doubt, will present one of the most studied trends in medicine in the new millennium. In this communication, the authors discuss two main sources of human stem cells potentially suitable for cell-based therapy. The first are the cells obtained from embryonic tissues – embryonic stem cells, the second are the cells derived from adult tissues – adult stem cells. Presently, harvesting and therapeutic use of embryonic stem cells are associated with many problems both methodical and ethical. Utilization of adult stem cells in cell-based therapy is a certain solution in the current state of replacement therapy. Still, we have to be aware that this is not a compromise but one of the most prospective ways to treat a variety of serious diseases. To date, it is not yet clear which way would be more suitable and it is up to us which way we choose for the benefit of millions of patients. Considering the current state of knowledge, it is impossible yet to predict which stem cells – embryonic or adult – or therapeutic approaches would yield the best results. Much research is to be done and verified in practice and, at the same time, ethical problems must be resolved.

Stem cells are an object of intensive and ever increasing interest for their biologic properties and potential significance in medicine. To date, no general definition of stem cells has been agreed on (Hall and Watt, 1989; Potten and Loeffler, 1990). The minimalistic definition says that stem cells have a capacity for both self-renewal and development of differentiated progeny. Although it is inadequate in many aspects, it immediately elucidates several important problems: How can a stem cell pass over its "stem" properties at least to one of two daughter cells at each division? And what determines whether divisions of stem cells will be self-regenerating or differentiating?

Different properties are often ascribed to stem cells besides renewing and differentiation potentials, such as the ability to undergo asymmetric division, extensive self-renewing capacity, the possibility to exist in mitotic silent form and clonally regenerate all different types of cells making up the tissue where they exist (Hall and Watt, 1989; Potten and Loeffler, 1990). In the following text, we show that stem cells are not universal but their properties differ in various tissues or organs. This helps to distinguish fundamental issues in stem cell biology from issues which are highly relevant but specific only for certain systems. This also illustrates the difficulty to reach a generally applicable definition of stem cells.

At present, we can define stem cells as a unique population of cells generated at the beginning of ontogenesis and persisting throughout the life of an organism, which are the basis of individual tissues and organs maintaining their structure and function in a multicellular system. Stem cells are undifferentiated cells (without specialization) capable of renewing themselves indefinitely (Morrison et al., 1999).

A new era in stem cell biology began in 1998 with the derivation of cells from human blastocysts and foetal tissue with the unique ability to differentiate into cells of all germ layers, the pluripotent cells. Two research teams developed methods for culturing embryonic stem (ES) cells (Thomson et al., 1995) and embryonic germ (EG) cells (Shamblot et al., 1998). In the work done by Thomson, pluripotent stem cells were isolated directly from the inner cell mass of human embryos at the blastocyst stage. Embryos received were from an *in vitro* fer-

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Abbreviations: CNS – central nervous system, EG cell – embryonic germ cell, ES cell – embryonic stem cell, HSC – haemopoietic stem cell, NSC – neural stem cell.

tilization clinic – they were in excess of the clinical need for infertility treatment. The embryos were made for purposes of reproduction, not research. Informed consent was obtained from the donor couples. Thomson isolated the inner cell mass, cultured the cells and produced a pluripotent stem cell line. In contrast, Gerhart isolated pluripotent stem cells from foetal tissue obtained from terminated pregnancies. Informed consent was obtained from the donors after they had independently made the decision to terminate their pregnancy. Gerhart took cells from the region of the foetus that was destined to develop into the testes or ovaries. The developed cells, although they were derived from different sources, appeared to be very similar.

Derivation of all human pluripotent cell lineages was the ground for the initial conception saying that there are two main types of embryonic stem cells potentially suitable for cell therapy – ES and EG cells. Currently, a major goal of ES cell research is to control the differentiation of human ES and EG cell lines into specific kinds of cells – an objective that must be met if the cells are to be used as the basis for therapeutic transplantation. Perhaps the most far-reaching potential application of human pluripotent stem cells is the generation of cells of a tissue that could be used for so-called cell therapies. Pluripotent stem cells, stimulated to develop into specialized cells, offer the possibility of a renewable source of replacement cells and tissues to treat a myriad of diseases, conditions, and disabilities including Parkinson's disease, diabetes, spinal cord injury, stroke, burns and others. Harvesting and therapeutic use of ES cells are associated with a number of problems both methodical and ethical. The utilization of adult stem cells for cell-based therapy is a certain solution of the current state. However, we have to bear in mind that it is not a compromise but one of the possible ways to treat many severe diseases. Presently, it is not quite clear which way would be more suitable. It is up to us which way we choose.

In the following text, we will characterize adult stem cells in more detail and, above all, focus our attention on haemopoietic stem cells (HSCs) and their plasticity. In the end we wish to point out some ethical problems associated with the utilization of therapeutic cloning in ES cells. Considering these problems, our team took a clear stand on preferring the use of adult stem cells and elaborated a method for adult stem cell harvesting from the bone marrow and adipous tissue for cell-based therapy. Our first experience, as of other authors, shows that adult stem cells are irreplaceable in this kind of therapy.

Adult stem cells

As noted earlier, multipotent stem cells can be found in some types of adult tissue. In fact, stem cells are needed to replenish the supply of cells in our body that normally wear out. An example that was mentioned previously is the blood stem cell.

Adult stem cells, like all stem cells, share at least two characteristics. First, they can make identical copies of themselves for long periods of time; this ability to proliferate is referred to as long-term self-renewal. Second, they can give rise to mature cell types that have characteristic morphologies and specialized functions. Unlike ES cells, which are defined by their origin in the inner cell mass of the blastocyst, adult stem cells share no such definitive means of characterization. In fact, no one knows the origin of adult stem cells in any mature tissue. Alternatively, adult stem cells may differentiate into a tissue that during normal embryonic development would arise from a different germ layer. For example, bone marrow-derived cells may differentiate into neural tissue, which is derived from embryonic ectoderm (Brazelton et al., 2000; Mezey et al., 2000). And reciprocally, neural stem cell (NSC) lines cultured from adult brain tissue may differentiate into haemopoietic cells (Björnson et al., 1999) or even give rise to many different cell types in a chimeric embryo (Clarke et al., 2000). In both cases cited above, the cells would be deemed to show plasticity, but in the case of bone marrow stem cells generating brain cells, the finding is less predictable.

Research on human adult stem cells suggests that these multipotent cells have potential for use in both research and in the development of cell therapies. Multipotent stem cells have not been found for all types of adult tissues, but discoveries in this area of research are increasing. For example, until recently, it has been thought that stem cells were not present in the adult nervous system. The experience in humans is more limited. In humans, NSCs have been isolated from foetal tissue, and a kind of cell that may be an NSC had been isolated from the adult brain tissue that was surgically removed for treatment of epilepsy. Until recently, there was little evidence in mammals that multipotent cells, such as blood stem cells, could change course and produce skin cells, liver cells or any cell other than a blood stem cell or a specific type of blood cell; however, research in animals is leading scientists to question this view.

The use of adult stem cells for such cell therapies would certainly reduce or even avoid the practice of using stem cells that were derived from human embryos or humal foetal tissue, sources that trouble many people on ethical grounds.

Haemopoietic stem cells and their plasticity

To date, the most worked out evolutionary system is haemopoiesis. At present, it is already known that in the bone marrow one HSC is in every 10-15 thousands of cells (Weissman, 2000). Terminally differentiated haemopoietic cells make up at least 8 lines, each having a different function, morphology and cell kinetics. For

example, HSCs are constantly being generated in the bone marrow, where they differentiate into mature types of blood cells. Indeed, the primary role of HSCs is to replace blood cells (Domen and Weissman, 1999).

Various populations of HSCs are being studied, exploring cell surface marker expression, such as Sca-1, c-kit, CD34 and lin (Jackson et al., 2002).

Although identification of surface antigens in both mature and foetal HSCs is improving all the time, a reliable isolation of a true HSC population is difficult (Hole, 1999). Only recently, a common myeloid progenitor cell was characterized, the existence of which was not confirmed previously in developmental lines of blood elements (Akashi et al., 2000). This progenitor has a clonogenic character and gives rise to both committed monocyte-granulocyte progenitor or committed progenitor of megakaryocyte-erythrocyte lineages. At present, multipotent stem cells obtained from bone marrow were identified as lin^{-} $c\text{-}kit^{+}$ capable of regenerating the damaged myocardium. However, one question still remains – whether a so defined stem cell is really a true stem cell. But certainly, it is multipotent (Orlic et al., 2001).

However, recently rediscovered observations of Altman from the 1960's (Altman, 1969) show that neurogenesis is in progress in certain regions of the adult brain and this is accompanied by a high wave of activity at identification of progenitor cells responsible for both embryonic and postnatal neural development (Gage et al., 1995; Weiss et al., 1996). Stem cells in the neural crest and in embryonic central nervous system (CNS) (Morrison et al., 1997) were identified by *in vitro* assays where differentiation and regenerative capacities of individual progenitor cells were demonstrated by subclonal experiments. Clonal populations of NSCs repopulated the haemopoietic system after transplantation. It has been found that after transplantation into irradiated hosts, genetically labelled NSCs produced different types of blood cells including myeloid and lymphoid, as well as immature haemopoietic cells. Thus it seems that NSCs have a wider differentiation potential than was previously thought (Björnson et al., 1999). The differentiation potential of NSCs is surprisingly broad. After transplantation into chick and mouse embryos, NSCs participated in the creation of tissues derived from all three primary germ layers. Thus generated cell populations resided in epidermis, heart muscle, liver, gastrointestinal tract, mesonephros, notochord, and CNS (Clarke et al., 2000). The concept of stem cell plasticity received strong support from a recent observation that extensively passaged clonally derived NSCs could contribute to haemopoiesis. Results of some authors show that haemopoietic competence is a consistent property of intravenously infused NSCs. However, the consistent changes that occurred during extended passaging are compatible with genetic or epigenetic alteration and suggest that rare fusion events may account for the neural to blood fate switch originally reported (Morshead et al., 2002).

Interesting results were also achieved with transplantation of skeletal muscle stem cells that contributed to haemopoietic system regeneration (Jackson and Goodell, 1999). Current results indicate that muscle-derived HSCs are likely derived from the haemopoietic system and are a result not of transdifferentiation of myogenic stem cells but instead of the presence of substantial numbers of HSCs in the muscle (McKinney-Freeman et al., 2002).

After HSC transplantation, plastic stem cells may even give rise to a hepatocyte population, which may allow survival of mice with severe liver damage (Lagasse, 2000). In turn, cells obtained from the bone marrow may regenerate the damaged myocardium (Orlic et al., 2001). Multipotent cells have also been obtained from lipoaspirate. These cells showed a multilineage potential and differentiated into adipogenic, osteogenic, chondrogenic, and myogenic lines (Zuk et al., 2001). Experiments indicated that cells derived from the bone marrow or other tissues may be effective in formation of mesoderm-derived tissues. Human bone marrow is derived from embryonic mesoderm and is made up of the HSC population supported by mesenchymal stroma (Friedenstein et al., 1974). Bone marrow stroma in animals and in humans is heterogenous and consists of several cell populations including mesenchymal stem cell population. These cells are able to differentiate into adipocytes and other cell types (Paul et al., 1991).

At present, we already know that under certain conditions stem cells are capable of producing a whole spectrum of cell types, regardless whether these tissues are derived from the same germ layer or not. This ability is called the stem cell plasticity. As yet there is no formally accepted name for this phenomenon in the scientific literature. It is variously referred to as "plasticity" (Krause et al., 2001), "unorthodox differentiation" (Bianco and Cossou, 1999), "transdifferentiation" (Lagasse et al., 2000) or "reprogramming".

Cell-based therapy – stem cell transplantation

The idea of adult stem cell harvesting is based on the assumption that these multipotent cells are present in an adult organism and represent a residuum of embryogenesis. Tissue specific stem cells may have a common embryonic origin and a capacity to activate various genetic programmes and responses to different stimuli (Seale and Rudnicki, 2000). Every adult brings with him in his development both – a pool of stem cells committed to certain tissues and able to regenerate and a pool of embryonic cells that can reside in different tissues and, with proper stimuli, can proliferate and differentiate into various cell lines not identical with their original tissue. Considering many possibilities of using these cells for

cell-based therapy, we must bear in mind several important cell definitions. Stem cell – a stem cell is a cell from the embryo, foetus, child, adult or elderly people that has, under certain conditions, the ability to reproduce itself for long periods or, probably, throughout the life of the organism. It can also give rise to specialized cells that make up the tissues and organs of the body. Much basic understanding about ES cells has come from animal research. In the laboratory, this type of stem cell can proliferate indefinitely, a property that is not shared by adult stem cells. Pluripotent stem cell – a single pluripotent stem cell has the ability to give rise to the types of cells that develop from the three germ layers (mesoderm, endoderm, ectoderm) from which all cells of the body arise. The only known sources of human pluripotent stem cells are those isolated and cultured from early human embryos and from foetal tissue that was destined to be part of the gonads. ES cell – an embryonic stem cell is derived from a group of cells called the inner cell mass which is part of the early (4–5 day-old) embryo called the blastocyst. Once removed from the blastocyst, the cells of the inner cell mass can be cultured into ES cells. These ES cells are not themselves embryos. In fact, evidence is emerging that these cells do not behave in the laboratory as they would in the developing embryo, that is the conditions in which these cells develop in culture and are likely to differ from those in the developing embryo. EG cell – an embryonic germ cell is derived from foetal tissue. Specifically, they are isolated from the primordial germ cells of the gonadal ridge of the 5–10 week-old embryo. Later in development, the gonadal ridge develops into the testes or ovaries and the primordial germ cells and EG cells are pluripotent, but they are not identical in their properties and characteristics. Adult stem cell – an adult stem cell is an undifferentiated (unspecialized) cell that occurs in a differentiated (specialized) tissue, renews itself and its progeny becomes specialized to yield all of the specialized cell types of the tissue from which they originated. Adult stem cells are capable of making identical copies of themselves for the lifetime of the organism. This property is referred to as self-renewal. Adult stem cells usually divide to generate progenitor or precursor cells, which then differentiate or develop into mature cell types that have characteristic shapes and specialized functions. Sources of adult stem cells include the bone marrow, blood, cornea and retina of the eye, brain, skeletal muscle, dental pulp, liver, skin, Lieberkuhn crypts of the gastrointestinal tract and pancreas. The most abundant information about adult human stem cells comes from studies of HSCs isolated from the bone marrow and blood. These adult stem cells have been extensively studied and applied therapeutically to various diseases. There are insufficient numbers of cells available for transplantation and stem cells do not replicate indefinitely in culture.

Traditional procedures of harvesting HSCs – such as bone marrow collection or stem cell cytapheresis with positive selection – present one of the techniques for adult stem cell therapy. Presently, it is possible to obtain $lin^-/c\text{-}kit^+$ cells (Orlic et al., 2001), $CD34^-/low$, $c\text{-}kit^+$, $Sca\text{-}1^+$ (Jackson et al., 2001) and other populations of pluripotent cells with demonstrated plasticity; e.g., when transplanted, a regeneration of ischemic myocardium was achieved. This procedure can yield sufficient amounts of stem cells needed for haemopoiesis repair, e.g., after chemotherapy. Still, the question remains if this method can yield sufficient numbers of cells capable to generate, for instance, the muscle tissue for transplantation. The routine procedure of HSC mobilization in combination with chemotherapy and growth factors shows to be less suitable. *Ex vivo* expansion may be one of the possible solutions. Currently, there are few tissue systems that may provide sufficient amounts of adult stem cells with a remarkable plasticity: bone marrow HSCs, muscle stem cells, and adipose tissue stem cells.

These considerations were based on experimental results indicating the existence of bone marrow-derived myogenic progenitors that may migrate into a degenerating muscle, participate in the regeneration process and enable the development of fully differentiated muscle fibres, as well as muscle regeneration with bone marrow progenitor cells (Ferrari et al., 1998). Another experiment showed that cells derived from skeletal muscle are capable of haemopoietic differentiation, because they contributed to all major blood lines at least for three months after the transplantation (Jackson et al., 1999). The fact that a close relationship exists between these two systems was confirmed by the results showing that stem cells derived from the bone marrow may be able to undergo a myogenic differentiation (Bittner et al., 1999). The capacity of HSCs to participate in myogenesis and, in turn, the ability of myogenic stem cells and NSCs to reside in the haemopoietic system indicates that they may be common developmental progenitors for these muscle-specific stem cells (Seale et al., 2000). The ability of embryonic vasculature to give rise to muscle satellite cells certainly supports this idea (De Angelis et al., 1999). Adult stem cells might be obtained from fatty tissue as well. And human lipoaspirate may yield a cellular fraction able to make up many mesodermal cell lines. This cellular fraction consists of cells similar to fibroblasts. Cells from lipoaspirate also showed a multilineage potential and differentiated into adipogenic, osteogenic, chondrogenic lines (Zuk et al., 2001).

Stem cells in therapy and ethical problems

A clonal organism is a kind of a new biologic entity and therapeutic cloning brings many ethical problems, for example: What is the moral statute of an organism

developed by cloning? Is it permissible to generate such a human entity and to destroy it later? Is it right to obtain human eggs for therapeutic cloning? What are the problems associated with persons whose cells are cloned? There are many ethical questions with very difficult solutions that are in contrast with the needs to develop these new technologies for treatment of many severe diseases. The results of several recent studies enhance the hope for a new form of therapy called regenerative medicine. It is expected that ES cells will be harvested from an embryo several days old, in the stage when the embryo is barely visible. These cells give rise to all tissues in the adult body. However, this depends on the condition that we are able to recognize the right natural signals and make ES cells produce any cellular type. Current studies with pancreatic cells carried out by Nadya Lumensky, Ron McKay and others of the National Institute of Health and other laboratories lay foundations for a new therapy of diabetes type I with transplantation of ES cells. This method means a new chance for millions of diabetics all over the world (Lumensky et al., 2001). Presently, many discussions are going on, namely about financing of similar projects.

Despite the hopes given to these methods using human ES cells, serious ethical problems and legal complications are likely to arise. Surely, there would be protests from moralists opposing interruptions as was presented in the article of N. Wade in The New York Times, of April 27, 2001. Protests may also come from donors of embryos, even if they wished to help with the therapy of severe and currently untreatable diseases. The question would be how to safeguard these good intensions from possible misuse? Even approved continuation of this research in private institutions may be problematic, as it would lack the benefit of public supervision.

Persisting controversy in ethical issues may bring legal difficulties especially in multinational communities, such as EU. European Committee (EC), according to declaration, refuses cloning of human embryos for medical and reproductive purposes (with the exception of sterility). On November 26, 2001, the Czech Press Agency reported that "European Committee is against cloning of human embryos but has no right to order anything". On the other hand, health protection and progress of means and methods in medicine are fully in competence of individual states. Then, how could EC act as the "ethical guardian" of EU?

Newly developing projects of harvesting stem cells for regenerative medicine, however, may not represent the only way of embryonic cell utilization. There are other promising methods as well, which are not opposed. Stem cells in various stages of development can be found in mature tissues of adult subjects. There they remain as a residuum of their own embryonic development and from here they can be isolated. At present, their yields for therapeutic purposes are not sufficient, and effective stimulation methods are still to be found. Another problem under discussion concerns the fact that mature stem cells are less adaptable and, therefore, difficult to turn into tissues for treatment of diabetes (Soria et al., 2001) or Parkinson's disease (Dunnett and Björklund, 1999). It is thought that both, research and practical use of stem cells may compete with time in more than one respect. Would it be possible to find out a medically more difficult, "non-embryonic" way in the near future? Would utilization of ES cells be more advanced in the countries where research, even under strict conditions, is permitted (UK, Sweden)? Where, ultimately, modern medicine and therapy would go, after all? In the USA, therapies from adult and embryonic stem cell research are studied. To date, adult stem cell research, which is federally funded, has resulted in the development of a variety of therapeutic treatments of human diseases. Although ES cell research has not yet produced similar results, many scientists believe that ES cell research holds a promise over time because of the capacity of ES cells to develop into any tissue in the human body.

In the meantime, our team, with regard to all these problems, prefers to collect and transplant adult stem cells.

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