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# Stem cells: an alternative to organ transplantation in chronic, degenerative and infectious diseases?

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**SUMMARY**

Even in the absence of damage or illness mature animals need billions of new cells every single day of their lives in order to survive and renew circulating blood cells and intestinal and skin lining. This task is accomplished by undifferentiated cells residing in most adult organs. These cells are designated adult stem cells (ASC) since they represent the adult counterpart, present in almost every organ, of the embryonal stem cells (ES) from which the entire human body develops.

Scientists first hypothesized the existence of stem cells over a century ago, and haematopoietic stem cells (HSC) have been exploited for the therapy of human diseases for two decades. Other types of stem cells also circulating in the bloodstream have been described. We briefly describe the potential uses of each of these types of cells, including autologous circulating stem cells, for disease therapy and in particular for the possible reversal of liver failure due to chronic hepatitis and/or cirrhosis.

*KEY WORDS:* Stem Cells, Monocytes, Macrophages, Liver Cells, Autologous Transplantation

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## INTRODUCTION

Stem cells are of paramount importance to living organisms. Two characteristics distinguish them from other cell types: first, they are the never ending source of parental cells needed for continuous replication of specialized cells, i.e. cells terminally differentiated into many cell populations (Stem cells, 2001). A good example of this is the gut epithelial cell; the rapid rate of cell turnover throughout the intestinal tract demands that undifferentiated stem cells constantly produce new epithelial cells (Chandross and Mezey, 2001). Second, stem cells have to give rise to different cell types in response to local signals (Stem cells, 2001; Slack, 2000). Thus, under certain physiologic or experimental conditions, stem cells are induced to decide their fate, becoming cells with specialized functions (e.g. myocytes in muscle, or insulin-producing cells of the pancreas) (Stem cells, 2001). Stem cells are therefore required to have the potential to develop into many different cell types in the body, and the potential to replace injured tissues (Stem cells, 2001; Chandross and Mezey, 2001; Slack, 2000; Zhao *et al.*, 2003). When a stem cell divides, a new cell has the potential either to remain a stem cell or to become another type of cell with a more specialized function, such as a muscle cell, a red blood cell or a neuron (Stem cells, 2001; Uzan, 2004).

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Adult mammals, including humans, consist of more than two hundred kinds of cells. All of these cells are generated from a single fertilized egg - *the zygote*. This is defined as totipotent because it has the potential to generate all the cells and tissues that make up an embryo. The term pluripotent describes stem cells that can give rise to cells of the mesoderm**,** endoderm and ectoderm (Stem cells, 2001; Zhao *et al.*, 2003). The term bipotent indicates a stem cell capable of differentiating along two lineages. Undamaged tissues are typically created by unipotent cells and give rise to just one cell type under normal conditions; these unipotent cells are capable of differentiating along one lineage only. If, however, the tissue becomes damaged and the replacement of multiple cell types is required**,** pluripotent stem cells (PSC) may become activated to repair the damage (Stem cells, 2001; Uzan, 2004). The adult stem cell is an undifferentiated (unspecialized) cell that is found in differentiated (specialized) tissues. It can renew itself and become specialized to yield all of the specialized cell types of the tissue from which it originated.

Adult stem cells (ASC) are capable of self-renewal throughout the lifetime of the organism. Sources of adult stem cells have been found in the bone marrow, peripheral blood, umbilical cord, cornea and retina of the eye, dental pulp of the tooth, liver, skin, gastrointestinal tract and pancreas (Stem cells, 2001; Zhao *et al.*, 2003). Investigators have studied stem cells from simple organisms, such as *Caenorrhabditis elegans* and drosophila, and from higher forms of life, such as birds, mice, and humans; both embryonic stem cells and adult stem cells have been intensively sought (Stem cells, 2001; Buttery *et al.,* 2001).

Researchers discovered ways to obtain or derive stem cells from mouse ( *Mus musculus*) embryos many years ago, and a methodology for isolating and culturing human stem cells from embryos was first published in 1998. Human embryonic stem cells were termed hES (Stem cells, 2001; Brook and Gardner, 1997). In the past, human embryos for research studies were obtained from fertility clinics. Attempts to overcome a couple's infertility through *in vitro* procedures generated a surplus of fertilized eggs, and as most of these remained unused they were available for research purposes (Stem cells, 2001; Slack, 2000; Bongso *et al.,* 1994a).). More recently, however, ethical restraints have been placed on this approach.

Five days post fertilization the future embryo is called a blastocyst, and it contains a bulk of 20-50 cells representing the "stem cells" for the future individual. From this bulge all tissues originate, i.e. it allows for the differentiation of multiple specialized cell types that will generate the heart, lung, skin and all other tissues (Stem cells, 2001; Buttery *et al.,* 2001; Brook and Gardner, 1997; Bongso *et al.,* 1994a; Bongso *et al.,* 1994b; Bongso *et al.,* 1995; Bongso, 1996; Itskovitz-Eldor *et al.,* 2000; Thomson and Odorico, 2000; Pedersen, 1999; Brustle *et al.,* 1999; Thomson *et al.,* 1998; Reubinoff *et al.,* 2000; Odorico *et al.,* 2001; Doetschman *et al.,* 1985; Marshak *et al.,* 2001; Call *et al.,* 2000). In addition, in certain adult tissues, such as in bone marrow, liver, muscle and brain, discrete existing populations of adult pluripotent stem cells generate replacements for individual cells lost through normal aging processes, injury, or diseases (Stem cells, 2001; Chandross and Mezey, 2001; Slack, 2000; Zhao *et al.*, 2003; Uzan, 2004; Draper *et al.,* 2004). It has been proposed that stem cells will become the basis for treating chronic and degenerative diseases. Among these, special attention has been devoted to central nervous system diseases such as Parkinson's disease or amyotrophic lateral sclerosis. Other chronic diseases that have been considered are diabetes, chronic hepatitis and cirrhosis, heart disease and autoimmune diseases, HIV infection, osteoporosis, etc. (Stem cells, 2001; Uzan, 2004). Valuable studies of stem cells in several laboratories have led to gratifying results, including the ability to grow them *in vitro*, and to stimulate them to differentiate into specialized cell types that possess the normal functions of adult "normal" cells (Stem cells, 2001; Draper *et al.,* 2004; Evans and Kaufman, 1981). The possibility of using stem cells for therapy of human diseases generates optimistic forecasts, and high expectations have been placed on hES (Stem cells, 2001; Evans and Kaufman, 1981). However, the hES human therapy approach remains controversial for ethical as well as technical reasons (Slack, 2000). As already noted, the use of PSC from aborted human foetuses, umbilical cords or embryonic tissues derived from *in vitro* fertilized eggs raises both ethical and legal questions.

Furthermore, the use of PSC could pose a risk of transmitting infections, and they may be ineffective because of immune rejection (Draper *et al.,* 2004). A way to circumvent these problems is to use autologous graft of adult stem cells, preferably from an accessible tissue, such as haematopoietic PSCs from adult bone marrow or peripheral blood (Draper *et al.,* 2004). The continuous generation of mature blood cells from primitive multi-potent progenitor cells requires a highly complex series of cellular events that are still poorly understood. Haematopoietic PSCs are therefore an important resource for research, and potentially for drug discovery and transplantation (Stem cells, 2001; Chandross and Mezey, 2001; Slack, 2000; Zhao *et al.,* 2003; Uzan, 2004; Draper *et al.,* 2004). The longevity of stem cells and their great capacity for tissue regeneration raises hopes for the treatment of various pathological conditions (Draper *et al.,* 2004). Since PSCs are detected in peripheral autologous blood, no ethical controversy surrounds the use of these cells (Draper *et al.,* 2004).

**Stem cells: general features**

Any organism, in order to survive, must replace dying cells with new ones. The new cells are generated by cell division; however specialized cells do not themselves divide, but are replenished from stem cells.

A stem cell has the ability to self replicate indefinitely during the organism's life, and the right local conditions induce stem cells to specialize. Stem cells have the potential to develop into mature cells that have characteristic forms and specialized functions, such as liver, heart, skin or nerve cells (Stem cells, 2001; Chandross and Mezey, 2001; Slack, 2000). Stem cells have the capacity to undergo an asymmetric division such that of the two daughter cells one retains the properties of a stem cell while the other differentiates into a more specialized cell type.

**What are stem cells?**

Stem cell research has developed from the pivotal concept of the single totipotent cell attributed to *the zygote*. The fertilized egg divides and differentiates until it produces a mature organism. Adult mammals, including humans, consist of very many kinds of cells, including neurons, myocytes, epithelial cells, blood cells (erythrocytes, monocytes**,** lymphocytes, etc.), bone cells (osteocytes) and cartilage cells (chondrocytes). Other cells, which are essential for embryonic development but are not incorporated into the embryo's body form the extraembryonic tissues of the placenta, yolk sac and umbilical cord. As stated before, all of these cells are generated from a single totipotent cell, called *the zygote*. The three embryonic germ layers, mesoderm**,** endoderm**,** and ectoderm (Chandross and Mezey, 2001), are the source of all the cells of the body. Thus the many different kinds of specialized cells that make up the body are all derived from these pluripotent cells, as can be observed in the natural course of embryonic development and under certain laboratory conditions. *Unipotent* stem cell is a term usually applied to a cell in a fully developed adult organism, and these cells are only capable of differentiating along one lineage. Undamaged tissues are typically unipotent and give rise to just one cell type under normal conditions. If, however, tissue is damaged and the replacement of multiple cell types is required**,** pluripotent stem cells may become activated to repair the damage (Slack, 2000). The adult PSC is an undifferentiated (unspecialized) cell that is present in a fully differentiated (specialized) tissue, can renew itself and become specialized, and can yield all of the specialized cell types of the tissue from which it originated. Sources of ASC have been found in the bone marrow, peripheral blood, cornea and retina of the eye, the dental pulp of the tooth, liver, skin, gastrointestinal tract and pancreas (Zhao *et al.,* 2003).

**Properties shared by every stem cell**

Stem cells differ from other cell types in the body. Regardless of their source, they have three general properties: they are capable of dividing and renewing themselves for long periods, they are unspecialized and they can give rise to specialized cell types (Stem cells, 2001).

Unlike embryonic stem cells (ES), however, adult stem cells cannot proliferate for long periods in the laboratory setting without differentiating. Several studies have been undertaken to investigate the phenomenon of stem cell mediated tissue regeneration, and the factors that normally regulate this stem cell proliferation and self-renewal (Stem cells, 2001; Uzan, 2004; Draper *et al.*, 2004; Buttery *et al.,* 2001).

**Properties of embryonic stem cells**

Embryonic cells have been isolated from the blastocyst, which is the stage of embryonic development at 4-5 days post-fertilization, prior to implantation in the uterine wall (Uzan, 2004; Draper *et al.*, 2004; Buttery *et al.,* 2001). At this stage of embryonic development the number of cells ranges from 200 to over 300, but only a quarter of them are the remaining ESs.

Studies of ES derived from mouse blastocysts became possible 20 years ago with the discovery of techniques that would allow these cells to be grown in the laboratory. Embryonic-like stem cells, called embryonic germ (EG) cells, can also be derived from primordial germ (PG) cells (the cells of the developing mouse or human fetus) (Brook and Gardner, 1997; Bongso *et al.,* 1994a; Bongso *et al.,* 1994b; Bongso *et al.,* 1995; Bongso, 1996).

ES cells closely resemble the cells of the preimplantation embryo, but are not in fact the same (Buttery *et al.,* 2001; Itskovitz-Eldor *et al.,* 2000; Odorico, *et al.,* 2001; Pedersen, 1999). These cells proliferate extensively in the embryo, show and maintain a stable, full (diploid), normal karyotype, are capable of differentiating into all the cell types that occur in the adult, and can be isolated and grown *ex vivo* (outside the organism), where they continue to replicate and show the potential to differentiate (Brustle *et al.,* 1999).

Pluripotent ES cells can give rise to differentiated cell types that derive from all three primary germ layers of the embryo (endoderm, mesoderm, and ectoderm). *In vitro,* single ES cell can give rise to a colony of genetically identical cells, or clones, which have the same properties as the original cell (Thomson *et al.,* 1998; Reubinoff *et al.,* 2000; Odorico *et al.,* 2001). The cell cycle of the ES also seems to incorporate a step that prevents differentiation (Doetschman *et al.,* 1985); Marshak *et al.,* 2001). From studies of these signaling pathways, it is clear that many factors must be balanced in a particular way for ES cells to remain in a self-renewing state. If the balance shifts, ES cells begin to differentiate. The transcription factor Oct-4 is a protein expressed by mouse and human ES cells *in vitro* and also by mouse inner cell mass cells *in vivo*. Indeed, ES cells express the transcription factor Oct-4, which then activates or inhibits a host of target genes and maintains ES cells in a proliferative, non-differentiating state (Stem cells, 2001; Marshak *et al.,* 2001).

ES cells can continue to proliferate or can be induced to differentiate. ES cells spend most of their time in the S phase of the cell cycle, during which they synthesize DNA. Unlike differentiated somatic cells, ES cells do not require any external stimulus to initiate DNA replication, and do not show chromosome X inactivation. By contrast, one of the two X chromosomes becomes permanently inactivated in every somatic cell of a female mammal (Stem cells, 2001; Evans and Kaufman, 1981).

**Embryonic stem cells are pluripotent cells**

ES cells have been experimentally injected into adult mice to determinine their pluripotency. The usual implantation site is under the skin or within the kidney capsule. In the host animal, the injected ES cells sometimes develop into benign tumors called teratomas. These tumors contain cell types derived from all three primary germ layers of the embryo -endoderm, mesoderm, and ectoderm. Teratomas typically contain gut-like structures such as layers of epithelial cells and smooth muscle, skeletal or cardiac muscle (which may contract spontaneously), neural tissue, cartilage or bone, and sometimes hair. Thus, ES cells that have been maintained for a long period *in vitro* can behave as pluripotent cells *in vivo*.

They can participate in normal embryogenesis by differentiating into any cell type in the body, and they can also differentiate into a wide range of cell types in an adult animal. However, normal mouse ES cells do not generate trophoblast tissues *in vivo* (Smith, 2001).

An *in vitro* technique for demonstrating the pluripotency of ES cells is to allow mouse cells to differentiate spontaneously, or to direct their differentiation along specific pathways. The former is usually accomplished by removing feeder layers and adding leukemia inhibitory factor (LIF) to the growth medium (Stem cells, 2001; Smith, 2001; Wiles and Keller, 1991). A few days after changing the culture conditions ES cells aggregate and tend to form embryoid bodies (EBs). In many ways EBs in the culture dish resemble the teratomas that can be observed in the animal. EBs consist of a disorganized array of differentiated or partially differentiated cell

types that are derived from the three primary germ layers of the embryo (Smith, 2001).
The techniques for culturing mouse ES cells from the inner cell mass of the preimplantation blastocyst were first reported more than 20 years ago. Versions of these standard procedures are used today in several laboratories around the world (Evans and Kaufman, 1981; Martin, 1981).

**Embryonic stem cell can differentiate into a single cell type *in vitro***

Research on embryonic stem cells has demonstrated that they can generate specialized cells, such as liver cells, neurons, myocardiocytes, endothelial cells of blood vessels and insulin secreting cells similar to those found in the pancreas. The possibility to use these stem cells for therapy of human diseases has generated high expectations of human ES. However, hES human therapy remains controversial for ethical reasons (Slack, 2000). In addition tailoring any production/differentiation process of ES cells *ad personam* would be impratical and prohibitively expensive, and it could also be extremely dangerous.
The most common way to obtain differentiation was to modify the growth conditions of the ES cells, for example by adding growth factors to the culture medium or by changing the chemical composition of the surface on which the ES cells were growing. It was shown that ES cells differentiate after specific stimulation. The plastic culture dishes used to grow both mouse and human ES cells can be treated with a variety of substances that either allow the cells to adhere to the surface of the dish or alternatively avoid adherence and allow them to float in the culture medium. In general, an adherent substrate prevents the cells from interacting and differentiating; but a nonadherent substrate allows the ES cells to aggregate and interact with each other. Cell-cell interactions are critical to normal embryonic development. Therefore, when these "natural" *in vivo* interactions occur the culture dish is set to allow differentiation. This is a fundamental strategy for inducing mouse or human ES cell differentiation *in vitro*. The addition of specific growth factors to the culture medium triggers the activation (or inactivation) of specific genes in ES cells that initiate a series of molecular events and induce the cells to differentiate along a particular pathway (Call *et al.,* 2000; Wiles and Keller, 1991).
Another way to direct differentiation of ES cells is to introduce a foreign gene into the cells by transfection or other methods (Wiles *et al.,* 2000). The result of these strategies is to add an active gene to the ES cell genome and trigger the cells to differentiate along a particular pathway. This approach appears to be a precise way of regulating ES cell differentiation, but it will only work if it is possible to identify which gene must be active at which particular stage of differentiation. The gene must then be activated at the right time - i.e. during the correct stage of differentiation - and must be inserted into the genome at the proper location (Wiles *et al.,* 2000).
Another approach to generate mouse ES cells uses cloning technology. The therapeutic cloning consists in transferring the nucleus of somatic stem cells isolated from the patient into an enucleated oocyte, and allowing the blastocyst development from which ES cells can be derived. In theory, the nucleus of a differentiated mouse somatic cell might be reprogrammed by injecting it into an oocyte. The resultant pluripotent cell would be immunologically compatible because it would be genetically identical to the donor cell (Odorico *et al.,* 2001).
Embryonic stem cells have been shown to differentiate into a variety of cell types. For example, mouse ES cells can be directed *in vitro* to yield vascular structures, neurons that release dopamine and serotonin (Lee *et al.,* 2000), or endocrine pancreatic islet cells (Lumelsky *et al.,* 2001). Also, the onset of mouse ES cell differentiation can be triggered by withdrawing the cytokine leukocyte inhibitory factor (LIF) which promotes the division of undifferentiated mouse ES cells. In addition, when directed to differentiate, ES cells aggregate and a change in their three-dimensional environment occurs that presumably allows some of the cell-cell interactions to occur *in vitro* that would occur *in vivo* during normal embryonic development (Yamashita *et al.,* 2000). Collectively, these three observations provide some of the best examples of directed differentiation of ES cells (Lee *et al.,* 2000; Lumelsky *et al.,* 2001; Yamashita *et al.,* 2000).
Under specific culture conditions, ES cells can be committed to a variety of differentiation pathways, giving rise to large amounts of cells cor-

responding to different tissues (neurons, cardiomyocytes, skeletal muscle, etc.). However, producing these tissues from already established ES cell lines would lead to immune rejection when transplanted to patients (Dani *et al.,* 1997; Bain, *et al.,* 1995; Fairchild *et al.,* 2000; Fraichard *et al.,* 1995; Itskovitz-Eldor *et al.,* 2000; Kramer *et al.,* 2000).

Alternatively, stem cells offer substantial opportunities for providing well-defined differentiated cells for drug discovery, toxicology and regenerative medicine, though the development of efficient techniques for their large-scale culture under defined conditions, and for controlling and directing their differentiation, presents substantial challenges. As regards ES, markers for defining the undifferentiated cells are well established, based upon previous studies of embryonal carcinoma (EC) cells, their malignant counterparts derived from teratocarcinomas. These markers are valuable tools for monitoring human ES cultures and their state of differentiation (Draper *et al.,* 2004).

It has been proposed to use the expertise accumulated by animal cloning by nucleus transfer, and to apply the technique to human ES cells. Compatible tissues will then be produced from these stem cells. While it is then theoretically possible to reimplant the cloned blastocyst into a surrogate mother and obtain a baby genetically identical to the donor ("reproductive cloning") this raises worrying ethical and legal questions (Uzan, 2004).

Current culture techniques are suboptimal and involve the use of poorly defined culture media and the use of feeder cells. Over time, the cells may also acquire karyotypic changes, reflecting genetic selection and adaptation to *in vitro* culture conditions. Originally, human ES cells were derived and maintained in medium containing fetal calf serum. More recently human ES lines have been cultured in medium free of fetal calf serum (Draper *et al.*, 2004). These cells nevertheless retain the features of undifferentiated human ES cells, including a capacity for differentiation. Although these cells also carried karyotypic changes, further research has focused upon understanding the mechanisms that control self-renewal, apoptosis, and commitment to differentiation. This will facilitate the definition of culture conditions that minimize genetic change and optimize results (Andrews *et al.,* 1984; Andrews, 1988; Andrews, 1998).

## What do we need to know about human embryonic stem cells?

The first requirement for a research center is to identify, through optimum knowledge of ES cell culture systems, which mechanisms allow human ES cells *in vitro* to proliferate without differentiating (Odorico *et al.,* 2001). Once the mechanisms that regulate human ES proliferation are known, it will in all likelihood become possible to apply this knowledge to the longstanding challenge of improving the *in vitro* self-renewal capabilities of adult stem cells. This appears to be a major obstacle to the success of therapeutic use of human ES.

Whether the cultural conditions of human ES cells play any significant role in maintaining the cells, directing the differentiation and determining their suitability for transplant is as yet unclear. During the growth of mouse blastocysts in culture, changes were observed in the methylation of specific genes that control embryonic growth and development (Khosla, S., *et al.,* 2001). Methylation of promoters is the method that evolution has chosen to silence genes, and this has enormous practical consequences. It is mandatory to identify which signal transduction pathways must be activated to induce human ES cell differentiation along a particular pathway. This includes understanding ligand-receptor interactions and the intracellular components of the signaling system, as well as identifying the genes that are activated or inactivated during differentiation of specific cell types (Khosla *et al.,* 2001).

## Adult stem cells

The ability of the human body to repair and replace the cells and tissues of some organs is often self-evident (as in the case of the skin). The ancient Greeks knew the property of self-repair of the liver as portrayed in the myth of Prometheus. Prometheus, having stolen the secret of fire, was condemned by the Gods to have part of his liver eaten by an eagle every day and then have it renewed during the night.

Stem cells are capable of renewing themselves and they can generate various cell types. Today, there is evidence that stem cells are present in

more tissues and organs than once thought, and that these cells are capable of developing into more kinds of cells than previously imagined. Efforts are now underway to harness potential adult stem cells and take advantage of this newly discovered capability, with the goal of devising new and more effective treatments for a range of diseases and disabilities.

**What is an adult stem cell?**

Adult stem cells share at least two characteristics with all stem cells. 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 shapes and functions. Typically, stem cells generate an intermediate cell type or types before they achieve their fully differentiated state. The intermediate cell is called a "precursor" or "progenitor" cell. Precursor cells in fetal or adult tissues are partly differentiated cells that divide and give rise to fully differentiated cells. Such cells are usually regarded as "committed" to differentiating along a particular cellular development pathway, although this characteristic may not be as definitive as previously thought (Stem cells, 2001; Robey, 2000).

Adult stem cells are nevertheless rare. Their primary functions are to maintain the steady state functioning of a tissue and, within limitations, to replace cells that die either naturally or from injury or disease (Holtzer, 1978; Leblond, 1964). It is estimated that one in 10,000 to 15,000 cells in the bone marrow is a hematopoietic stem cell (HSC) (Slack, 2000; Weissman, 2000). Furthermore, adult stem cells are dispersed in tissues throughout the mature animal and behave very differently, depending on their local environment. HSCs are constantly being generated in the bone marrow where they differentiate into mature types of blood cells (Domen. and Weissman, 1999) They circulate in peripheral blood (Zhao *et al.,* 2003). By contrast, gastrointestinal tract stem cells, including those of the stomach, small and large intestine, are stationary, and are physically separated from the mature cell types they generate. Gut epithelial stem cells (or precursors) occur at the bases of crypts - deep invaginations between the mature, differentiated epithelial cells that line the lumen of the intestine. These epithelial crypt cells divide fairly often (every 12 hours in the normal human gut), but they remain part of the stationary group of cells they generate (Slack, 2000).

The list of adult tissues reported to contain stem cells is growing and it includes bone marrow, peripheral blood, certain areas of brain and spinal cord, dental pulp, blood vessels, skeletal muscle, epithelia of the skin and digestive system, cornea, retina, liver, and pancreas. Recent studies report that adult stem cells are also clonogenic (Zhao *et al.,* 2003; Chagraoui *et al.,* 2003). In other words, a single adult stem cell should be able to generate a line of genetically identical cells, which then gives rise to all the appropriate, differentiated cell types of the tissue in which it resides. Again, this property is difficult to demonstrate *in vivo*. Instead, scientists have shown either that a stem cell is clonogenic *in vitro*, or that a purified population of candidate stem cells can repopulate the tissue.

An adult stem cell should also be able to give rise to fully differentiated cells that have mature phenotypes, are fully integrated into the tissue and are capable of specialized functions that are appropriate to the tissue. The term phenotype refers to all the observable characteristics of a cell or organism. This includes its morphology, its interactions with other cells and the non-cellular environment, (the interaction proteins of the extracellular matrix appear on the cell surface as surface markers) and the cell's behavior (e.g., secretion, contraction, synaptic transmission).

Several studies have demonstrated that the differentiated cells derived from adult stem cells are truly functional, and as these cells become integrated into the differentiated tissue *in vivo* they interact appropriately with neighboring cells. This concept is important, encompassing the regenerating and self-renewal capabilities of the adult stem cell (Chandross and Mezey, 2001; Zhao *et al.,* 2003; Chagraoui *et al.,* 2003).

**The distribution of adult stem cells**

The adult stem cells found in many organs replace dying, aged or injured cells and maintain the integrity of the tissue. Progenitor cells can give rise to committed specific cells. Current excitement after discovery of stem cells in many adult tissues is fueling claims that progenitor cells in those tissues are stem cells. Thus, a wave of

reports has found endothelial progenitor cells, skeletal muscle stem cells and epithelial precursor cells of the skin and the digestive system. Reports of progenitor or stem cells in the pancreas and liver are raising hopes for a substitute for whole liver transplantation (Stem cells, 2001; Chandross and Mezey, 2001; Zhao *et al.,* 2003; Chagraoui *et al.,* 2003).

**The adult stem cell is a pluripotent cell**

The stem cells in adult tissues have been shown to generate not only the specialized cell types of that tissue, but also cell types of a tissue different from the one in which they normally reside. Various studies have shown that hematopoietic stem cells (whose origin is the mesoderm) may be able to generate both skeletal muscle (mesodermal origin) but also neurons that derive from the ectoderm. Such observations have triggered a flurry of reports that stem cells derived from one adult tissue can change their appearance and commitment, and assume characteristics similar to those of differentiated cells from other tissues.

Hematopoietic stem cells are pluripotent stem cells (Chandross and Mezey, 2001; Zhao *et al.,* 2003; Chagraoui *et al.,* 2003; Wagers *et al.,* 2002; Griffith and Naughton, 2002). These cells, or their mature progeny, can be used to study differentiation processes, identify and test lineage-specific drugs, or replace tissues damaged by a disease. HSCs are therefore a very important resource. The use of PSCs derived from human fetuses, umbilical cords, or embryonic tissues raises ethical and legal questions and poses a risk of transmitting infections. PSCs may also be ineffective because of immune rejection. The simplest way to circumvent all these problems is to exploit autologous stem cells, preferably those from a readily accessible tissue. The bone marrow contains cells that maintain the ability to differentiate into mature cells belonging to distinct cell lineages (Griffith and Naughton, 2002). A recent study has indicated that bone marrow mesenchymal PSC can be expanded *in vitro*, and after transplantation differentiate *in vivo* into cells belonging to distinct lineages (Jiang *et al.,* 2002). Other studies have raised the possibility that such mature cells may result from fusion of stem cells with mature resident tissue cells (Terada *et al.,* 2002; Ying *et al.,* 2002).

To show that the adult stem cells can generate other cell types requires them to be tracked in their new environment, either *in vitro* or *in vivo*. The usual method of overcoming the problem posed by having to extract cells from their environment, label them *in vitro*, and reinject them is to employ an animal that has been genetically engineered to express a molecular tag in all its cells.

Many experiments on stem cell plasticity, i.e. the possibility that stem cells from one tissue may be able to give rise to cell types of a completely different tissue, have been performed with bone marrow derived cells that are of mesodermal derivation. Bone marrow stem cells may be differentiated into mesodermally derived tissue, (as myocyte of muscle, cardiomyocyte or hepatocyte (Ferrari *et al.,* 1998; Gussoni *et al.,* 1999; Kocher *et al.,* 2001; Orlic *et al.,* 2001; Alison *et al.,* 2000; Lagasse *et al.,* 2000; Theise *et al.,* 2000). In the experiments reported to date, adult stem cells may assume the characteristics of cells that have developed not only from the same primary germ layer, but also from a different germ layer.

When unspecialized stem cells give rise to specialized cells, the process is called differentiation**.** We are just beginning to understand the signals inside and outside cells that trigger stem cell differentiation. The internal signals are controlled by a cellular gene. These are interspersed across long strands of DNA, and carry coded instructions for all the structures and functions of a cell. The external signals for cell differentiation include chemicals secreted by other cells, physical contacts with neighboring cells, and molecules in the microenvironment (Stem cells, 2001)**.** Adult stem cells typically generate the cell types of the tissue in which they reside. A blood-forming adult stem cell in the bone marrow, for example, normally gives rise to the cell types found in peripheral blood cells such as red blood cells, white blood cells and platelets. Until recently it had been thought that a blood-forming cell in the bone marrow, thereafter called a hematopoietic stem cell, could only generate cells that are subsequently found in the circulation (Chandross and Mezey, 2001; Zhao *et al.,* 2003; Domen and Weissman, 1999; Chagraoui *et al.* 2003; Theise *et al.,* 2000). However, a number of experiments over the last few years have raised the possibility that stem cells from one tissue may be able

to give rise to cell types of a completely different tissue. Examples of such a phenomenon, known as plasticity, include blood cells becoming neurons**,** liver cells that can be made to produce insulin, and hematopoietic stem cells that can develop into heart muscle. Exploring the possibility of using adult stem cells for cell-based therapies has become a very active area of investigation by researchers (Stem cells, 20011; Chandross and Mezey, 2001; Zhao *et al.,* 2003; Domen. and Weissman, 1999; Chagraoui *et al.* 2003; Theise *et al.,* 2000).

**Stem cells in the bone marrow and blood**

The notion that the bone marrow contains stem cells is not of course new. One population of bone marrow cells, the HSCs, is responsible for forming all of the types of blood cells in the body, and HSCs were recognized as stem cells more than 40 years ago (Becker *et al.,* 1963; Till and McCullough, 1961). Bone marrow stromal cells - a mixed cell population that generates bone, cartilage, fat, fibrous connective tissue - and the reticular network that supports blood cell formation were described shortly after the discovery of HSCs (Friedenstein *et al.,* 1966; Friedenstein *et al.,* 1970; Owen, 1988). The mesenchymal stem cells of the bone marrow also give rise to these tissues and may constitute the same population of cells as the bone marrow stromal cells (Pittenger and Marshak, 2001). Recently, a population of progenitor cells that differentiates into endothelial cells, a type of cell that lines the blood vessels, was isolated from circulating blood (Asahara *et al.,* 1997) and identified as originating in bone marrow (Shi *et al.,* 1998). Whether these endothelial progenitor cells, which resemble the angioblasts giving rise to blood vessels during embryonic development, represent a *bona fide* population of adult bone marrow stem cells remains uncertain. Thus, the bone marrow appears possibly to contain three or more stem cell populations, including hematopoietic stem cells, stromal cells, and endothelial progenitor cells. One population, called pericytes, may be closely related to bone marrow stromal cells although their origin remains elusive (Bianco *et al.,* 2001). The second population of blood-borne stem cells, which occurs in all four species of animals tested (guinea pigs, mice, rabbits, and humans) resembles stromal cells in that these cells can generate bone and fat (Kuznetsov *et al.,* 2001).

**Hematopoietic stem cells**

The shortest surviving of all the cell types in the body appear to be white blood cells and certain kinds of epithelial cells. Platelets, once activated, rapidly die, and their replenishment must therefore be prompt or the organism will succumb to the first small wound. The life of an animal depends on the ability of these and other blood cells to be continuously renewed. This replenishment process occurs largely in the bone marrow, where HSCs reside, divide and differentiate into all the blood cell types. Both HSCs and differentiated blood cells cycle from the bone marrow to the blood and back again, under the influence of a barrage of secreted factors that regulate cell proliferation, differentiation and migration (Draper *et al.,* 2004; Becker *et al.,* 1963). HSCs can restore hematopoiesis in mice that have been subjected to lethal doses of radiation to destroy their hematopoietic systems. This test, the rescue of lethally irradiated mice, has become a standard by which other candidate stem cells are measured. It shows, beyond question, that HSCs can regenerate an entire tissue system, in this case the blood (Becker *et al.,* 1963; Till and McCullough 1961). HSCs were first proven to be blood-forming stem cells in a series of experiments in mice, and similar blood-forming stem cells occur in humans. HSCs are defined by their ability to self-renew and to give rise to all the kinds of blood cells in the body. This means that a single HSC is capable of regenerating the entire hematopoietic system, although this has been demonstrated only a few times in mice (Osawa *et al.,* 1996).

Over the years, many combinations of surface markers have been used to identify, isolate, and purify HSCs derived from bone marrow and blood. Undifferentiated HSCs and hematopoietic progenitor cells express c-kit, CD34 and H-2K. These cells usually lack the lineage marker Lin, or express it at very low levels ($Lin^{-/low}$). For transplant purposes, cells that are $CD34^{+}$ $Thy1^{+}$ $Lin^{-}$ are most likely to contain stem cells and result in engraftment.

Two kinds of HSCs have been defined: long-term HSCs proliferate for the lifetime of an animal; short-term HSCs proliferate for a limited time,

possibly a few months in young adult mice. An estimated 8 to 10% of long-term HSCs enter the cell cycle and divide each day. Long-term HSCs have high levels of telomerase activity. Telomerase is an enzyme that helps maintain the length of the ends of chromosomes, called telomeres, by adding on nucleotides. Active telomerase is a characteristic of undifferentiated dividing cells and cancer cells. Differentiated human somatic cells do not show telomerase activity. In adult humans, HSCs are found in the bone marrow, blood, liver and spleen, but are extremely rare in these tissues.

Short-term HSCs differentiate into lymphoid and myeloid precursors, the classes of precursors for the two major lineages of blood cells. Lymphoid precursors differentiate into T cells, B cells and natural killer cells. The mechanisms and pathways that lead to their differentiation are still being investigated (Akashi *et al.,* 1999a; Akashi *et al.*, 1999b). Myeloid precursors differentiate into monocytes and macrophages, neutrophils, eosinophils, basophils, megakaryocytes, and erythrocytes (Akashi *et al.,* 2000). *In vivo*, bone marrow HSCs differentiate into mature specialized blood cells that cycle constantly from the bone marrow to the blood and *vice versa* (Domen. and Weissman, 1999). A recent study showed that short-term HSCs are a heterogeneous population that differs significantly in its ability to self-renew and repopulate the hematopoietic system (Hunt *et al.,* 1987).

Scientists have for many years been frustrated in their attempts to induce HSC to proliferate *in vitro,* despite using many substrates including those intended to mimic conditions in the stroma. HSCs proliferate readily *in vivo*, but they usually differentiate or die *in vitro* (Domen and Weissman, 1999). Thus, much of the research on HSCs has been focused on understanding which factors, cell-cell interactions, and cell-matrix interactions control their proliferation and differentiation *in vivo*, with the hope that similar conditions could be replicated *in vitro*. Many of the soluble factors that regulate HSC differentiation *in vivo* are cytokines, which are made by different cell types and are then concentrated in the bone marrow by the extracellular matrix of stromal cells at the sites of blood formation (Hunt *et al.*, 1987; Whitlock *et al.*, 1987). Two of the most-studied cytokines are granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) (Petersen *et al.,* 1999); Roberts *et al.,* 1988). Also important to HSC proliferation and differentiation are the interactions of the cells with adhesion molecules in the extracellular matrix of the bone marrow stroma (Roy and Verfaillie, 1999; Verfaillie, 1998; Zandstra *et al.,* 2000).

**Bone marrow stromal cells**

Bone marrow (BM) stromal cells have long been recognized to play an important role in the differentiation of mature blood cells from HSCs. In addition to providing the physical environment in which HSCs differentiate, BM stromal cells generate cartilage, bone and fat. Whether stromal cells are best classified as stem cells or progenitor cells for these tissues is still in question. Whether BM stromal cells and so-called mesenchymal stem cells are the same population is also uncertain (Pittenger and Marshak, 2001).

Bone marrow stromal cells have many features that distinguish them from HSCs. The two cell types are easy to separate *in vitro*. When bone marrow is dissociated, and the mixture of cells it contains is plated at low density, the stromal cells adhere to the surface of the culture dish and the HSCs do not. Given specific *in vitro* conditions, BM stromal cells form colonies from a single cell called the colony forming unit-F (CFU-F). These colonies may then differentiate as adipocytes or myelosupportive stroma presenting a clonal assay that indicates the stem cell-like nature of stromal cells. Unlike HSCs, which do not divide *in vitro* or proliferate only to a limited extent, BM stromal cells can proliferate for up to 35 population doublings *in vitro* (Bruder *et al.,* 1997). They grow rapidly under the influence of such mitogens as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), and insulin-like growth factor-1 (IGF-1) (Bianco *et al.,* 2001).

Panels of markers used to identify the cells recognize receptors for certain cytokines (interleukin-1, 3, 4, 6, and 7) and receptors for proteins in the extracellular matrix (ICAM-1 and 2, VCAM-1, the alpha-1, 2, and 3 integrins, and the beta-1, 2, 3 and 4 integrins) (Bianco *et al.,* 2001). Despite the use of these markers and another stromal cell marker called Stro-1, the origin and

specific identity of stromal cells remained elusive. Like HSCs, BM stromal cells arise from embryonic mesoderm during development, although no specific precursor or stem cell for stromal cells has been isolated and identified. One theory about the origin of stromal cells is that a common kind of progenitor cell - perhaps a primordial endothelial cell that lines embryonic blood vessels - gives rise to both HSCs and to the mesodermal precursors. The latter may then differentiate into myogenic precursors. These are the satellite cells that are thought to function as stem cells in skeletal muscle and the BM stromal cells (Bianco and Cossu, 1999).

*In vivo*, the differentiation of stromal cells into fat and bone is not straightforward. Bone marrow adipocytes and myelosupportive stromal cells, both of which are derived from BM stromal cells, may be regarded as interchangeable phenotypes (Bianco and Cossu, 1999; Bianco *et al.,* 1999). Adipocytes do not develop until postnatal life, as the bones enlarge and the marrow space increases to accommodate enhanced hematopoiesis. When the skeleton stops growing and the mass of HSCs decreases in a normal age-dependent fashion, BM stromal cells differentiate into adipocytes which fill the extra space. New bone formation is obviously greater during skeletal growth, although bone turns over throughout life. Bone forming cells are osteoblasts, but their relationship to BM stromal cells is not clear. New trabecular bone, which is the inner region of bone next to the marrow, could develop through the action of BM stromal cells. However, the outside surface of bone also turns over, as does bone next to the Haversian system (small canals that form concentric rings within bone). Neither of these surfaces is in contact with BM stromal cells (Bianco and Cossu, 1999; Bianco *et al.,* 1999).

**Adult stem cells in other tissues**

It is often difficult to distinguish adult, tissue-specific stem cells from progenitor cells. With that caveat in mind, the following summary identifies reports of stem cells in other adult tissues. Hematopoietic stem cells from bone marrow are the most studied, and already used in clinical applications to restore various blood and immune components to the bone marrow via transplantation; but there are at least two other populations of adult stem cells that have been identified from bone marrow and blood. Several populations of adult stem cells have been identified in the brain and particularly within the hippocampus, but their function there is unknown. Proliferation and differentiation of brain stem cells are influenced by various growth factors. There are reports of adult stem cells in several other tissues (muscle, blood, and fat) that demonstrate plasticity. Few research reports on the plasticity of adult stem cells have, however, included clonality studies, and there is therefore limited evidence that a single adult stem cell or genetically identical line of adult stem cells can demonstrate plasticity.

**Stem cells in the liver**

The status of stem cells in the adult pancreas and liver is still controversial. Both tissues arise from the endoderm during embryonic development. A recent study has indicated that a single precursor cell derived from the embryonic endoderm may generate both the ventral pancreas and the liver (Deutsch *et al.,* 2001). In adult mammals both the pancreas and the liver contain multiple kinds of differentiated cells that may be repopulated or regenerated by multiple types of stem cells.

The identity of stem cells that can repopulate the liver of adult mammals is also a matter of debate. Recent studies in rodents indicate that HSCs (derived from mesoderm) may be able to home to the liver after it is damaged and demonstrate plasticity by differentiating into hepatocytes (usually derived from the endoderm) (Theise *et al.,* 2000; Petersen *et al.,* 1999; Lagasse *et al.,* 2000). The question remains whether cells from the bone marrow normally generate hepatocytes *in vivo*. It is not known whether this kind of plasticity occurs in the absence of severe damage to the liver, or whether HSCs from the bone marrow generate oval cells of the liver (Crosby. and Strain, 2001). These oval cells may arise from the portal tracts in liver and may give rise either to hepatocytes or to the epithelium of the bile ducts (Stem cells, 20011; Draper *et al.,* 2004; Dabeva *et al.,* 1993; Lazaro *et al.,* 1998). Indeed, when a lesion occurs that requires only a limited supply of new cells, hepatocytes themselves are responsible for the well-known regenerative capacity of liver.

The extraordinary potential of adult stem cells to regenerate tissues and organs has led to a heated debate in many countries on ES research, in particular on the use of these progenitor cells in cell transplantation for the reversal of liver failure (Gupta *et al.,* 1999) or in bioartificial organ development (Strain and Neuberger, 2002).
Nowadays liver cell therapies including liver cell transplantation and bio- artificial livers (Gupta *et al.,* 1999) are being developed as alternatives to whole liver transplantation for patients with severe liver dysfunction (Chowdhury *et al.,* 1991; Strom *et al.,* 1997; Fox *et al.,* 1998; Muraca *et al.,* 2002; Sokal *et al.,* 2003). However the use of human hepatocytes in biomedical research has to date been hampered by the restricted accessibility to suitable tissue samples and by the very limited hepatocyte capability to expand the cell population *in vitro*. On the other hand, the use of PSCs from human fetuses, umbilical cords or embryonal tissues derived from in vitro fertilized eggs not only raises ethical and legal questions mainly concerning the risk of transmitting infections but may also prove ineffective because of immune rejection. Patients transplanted for hepatitis C virus or non-A, non-B liver disease are at high risk of hepatitis C virus reinfection (Marzano *et al.,* 1994). Therefore the use of liver stem cells in cell therapy for infected patients is limited and it must take into account the problem of reinfection. Recently, genetically engineered cells for inhibiting HCV replication were described and this approach may represent a new direction to follow for the development of effective cell therapy for HCV infection (Prabhu *et al.,* 2004).

**Human peripheral blood monocytes as pluripotent stem cells**

Huberman and coworkers have identified, characterized, cultured and propagated a previously unknown subset of human peripheral blood monocytes that act as pluripotent stem cells (Zhao *et al.,* 2003). These cells, which display a fibroblast-like morphology and exhibit monocyte and hematopoietic stem cell markers including CD14, CD34, and CD45, can be induced to differentiate into macrophages, T lymphocytes, epithelial cells, endothelial cells, neuronal cells and hepatocytes. The pluripotent nature of these adult PSC was deduced on the basis that the combined absolute number of mature cells belonging to the different induced lineages far exceeded the available starting cells in the macrophage cultures. Furthermore, the progeny of single macrophages were induced to express differentiated traits belonging to five distinct cell lineages (Draper *et al.,* 2004). Other investigators, using a mouse model and engrafting a single CD34-positive bone marrow cell per animal, concluded that these cells could also differentiate into distinct cell lineages (Krause *et al.,* 2001). Recent studies have, however, questioned the existence of such a transdifferentiation and raised the possibility that the emerging mature cells resulted from the fusion of stem cells with preexisting mature tissue cells (Terada *et al.,* 2002; Ying *et al.,* 2002). This is not, however, possible in the case of Huberman and coworkers. These authors induced cells with the mature epithelial, neuronal, endothelial, or liver cell phenotypes generated from the progeny of single cells and the cells cannot have originated from a fusion.
A number of investigators have described the culture and propagation of mesenchymal PSC from human peripheral blood or bone marrow (Jiang *et al.,* 2002; Toma *et al.,* 2002). These cells differ from macrophages in a number of ways including their failure to express CD34 and/or CD45. Also, unlike the mesenchymal stem cells (Jiang *et al.,* 2002; Toma *et al.,* 2002), the macrophage cultures grow firmly attached to the tissue culture matrices and cannot be readily removed and dispersed by standard digestion with trypsin, trypsin–EDTA or dispase solutions.
The stem cell-like macrophages are present into peripheral blood as monocytes and as such are dispersed throughout the human body. The physiological functions of these cells in the repair of tissue damage are recognized. In this context, a recent study with transplantation patients who underwent chemotherapy or radiation treatment indicated that blood preparations enriched by CD34-positive cells were able to populate different tissues and differentiate into cells belonging to distinct lineages (Korbling *et al.,* 2002). These preparations probably contained parental macrophage-like cells which expressed CD34.

**Autologous transplantation therapy**

The ability to generate and propagate unlimited numbers of PSCs outside the body would have

a major impact on the safety, cost, and availability of stem cells for transplantation. The current approach of isolating hematopoietic stem cells from a patient's own peripheral blood places the patient at risk of a flare-up of their autoimmune disease. This is a potential consequence of repeated administration of the stem cell growth factors needed to mobilize hematopoietic stem cells from the bone marrow to the blood stream in numbers sufficient for transplantation. In addition, contamination of the purified hematopoietic stem cells with the patient's mature autoreactive T and B cells could affect the success of the treatment in some patients. Propagation of pure cell lines in the laboratory would avoid these potential drawbacks and increase the numbers of stem cells available to each patient, thus shortening the at-risk interval before full immune reconstitution (Stem cells, 2001).

The current treatments for many autoimmune diseases include the systemic use of anti-inflammatory drugs and potent immunosuppressive and immunomodulatory agents (i.e., steroids and inhibitor proteins that block the action of inflammatory cytokines). Despite their profound effect on immune responses, however, these therapies are unable to induce clinically significant remissions in certain patients.

In recent years, researchers have contemplated the use of stem cells to treat autoimmune disorders (Stem cells, 2001). The immune-mediated injury in autoimmune diseases can be organ-specific, such as type 1 diabetes which is the consequence of the destruction of the pancreatic beta islet cells or multiple sclerosis which results from the breakdown of the myelin covering of nerves. These autoimmune diseases are amenable to treatments involving the repair or replacement of damaged or destroyed cells or tissue.

An ability *in vitro* to expand and differentiate PSCs from an easily accessible source such as peripheral blood should offer a real possibility of autologous transplantation therapy. For example, *in vitro* generated PSCs could be used to replenish immune cells that have been eradicated by cancer therapy or to replace neuronal tissue damaged during spinal cord injury, stroke, dementia (including Alzheimer's syndrome), or Parkinson's disease or to treat heart diseases or to reverse severe damage to the liver. The ability to expand autologous PSCs *in vitro* before transplantation should yield a large number of stem cells (Zhao *et al.,* 2003) and these ought to be more effective and asseriate procedures more versatile than current transplantations which do not include such an expansion of the patient's own stem cells.

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## REFERENCES

AKASHI, K., KONDO, M., CHESHIER, S., SHIZURU, J., GANDY, K., DOMEN, J., MEBIUS, R., TRAVER, D., AND WEISSMAN, I.L. (1999a). Lymphoid development from stem cells and the common lymphocyte progenitors. *Cold Spring Harb. Symp. Quant. Biol.* **64**, 1-12.

AKASHI, K., TRAVER, D., KONDO, M., AND WEISSMAN, I.L. (1999b). Lymphoid development from hematopoietic stem cells. *Int. J. Hematol.* **69**, 217-226.

AKASHI, K., TRAVER, D., MIYAMOTO, T., AND WEISSMAN, I.L. (2000). A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature*. **404**, 193-197.

ALISON, M.R., POULSOM, R., JEFFERY, R., DHILLON, A.P., QUAGLIA, A., JACOB, J., NOVELLI, M., PRENTICE, G., WILLIAMSON, J., AND WRIGHT, N.A. (2000). Hepatocytes from non-hepatic adult stem cells. *Nature*. **406**, 257.

ANDREWS, P.W., DAMJANOV, I., SIMON, D., BANTING, G.S., CARLIN, C., DRACOPOLI, N.C., AND FOGH, J. (1984). Pluripotent embryonal carcinoma clones derived from the human teratocarcinoma cell line Tera-2. Differentiation *in vivo* and *in vitro*. *Lab. Invest.* **50**, 147-162.

ANDREWS, P.W. (1988). Human teratocarcinomas. *Biochim. Biophys. Acta.* **948**, 17-36.

ANDREWS, P.W. (1998). Teratocarcinomas and human embryology: pluripotent human EC cell lines. Review article. *APMIS*. **106**, 158-67.

ASAHARA, T., MUROHARA, T., SULLIVAN, A., SILVER, M., VAN DER ZEE R., LI, T., WITZENBICHLER, B., SCHATTEMAN, G., AND ISNER, J.M. (1997). Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. **275**, 964-967.

BAIN, G., KITCHENS, D., YAO, M., HUETTNER, J.E., AND GOTTLIEB, D.I. (1995). Embryonic stem cells express neuronal properties *in vitro*. *Dev. Biol.* **168**, 342-357.

BECKER, A.J., MCCULLOUGH, E.A., AND TILL, J.E. (1963). Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature*. **197**, 452-454.

Bianco, P. and Cossu, G. (1999). Uno, nessuno e centomila: searching for the identity of mesodermal progenitors. *Exp. Cell Res.* **251**, 257-263.

Bianco, P., Riminucci, M., Kuznetsov, S., and Robey, P.G. (1999). Multipotential cells in the bone marrow stroma: regulation in the context of organ physiology. Crit. Rev. Eukaryotic. *Gene Expr.* **9**, 159-173.

Bianco, P., Riminucci, M., Gronthos, S., and Robey, P.G. (2001). Bone marrow stromal stem cells: nature, biology, and potential applications. *Stem Cells*. **19**, 180-192.

Bongso, A., Fong, C.Y., Ng, S.C., and Ratnam, S.S. (1994a). Blastocyst transfer in human *in vitro*: fertilization; the use of embryo co-culture. *Cell Biol. Int*. **18**, 1181-1189.

Bongso, A., Fong, C.Y., Ng, S.C., and Ratnam, S. (1994b). Isolation and culture of inner cell mass cells from human blastocysts. *Hum. Reprod.* **9**, 2110-2117.

Bongso, A., Fong, C.Y., Ng, S.C., and Ratnam, S.S. (1995). Co-culture techniques for blastocyst transfer and embryonic stem cell production. *Asst. Reprod. Rev.* **5**, 106-114.

Bongso, A. (1996). Behaviour of human embryos *in vitro* in the first 14 days: blastocyst transfer and embryonic stem cell production. *Clin. Sci.* (Colch.) **91**, 248-249.

Brook, F.A. and Gardner, R.L. (1997). The origin and efficient derivation of embryonic stem cells in the mouse. *Proc. Natl. Acad. Sci. U. S. A.* **94**, 5709-5712.

Bruder, S.P., Jaiswal, N., and Haynesworth, S.E. (1997). Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation. *J. Cell. Biochem.* **64**, 278-294.

Brustle, O., Jones, K.N., Learish, R.D., Karram, K., Choudhary, K., Wiestler, O.D., Duncan, I.D., and McKay, R.D. (1999). Embryonic stem cell-derived glial precursors: a source of myelinating transplants. *Science*. **285**, 754-756.

Buttery, L.D., Bourne, S., Xynos, J.D., Wood, H., Hughes, F.J., Hughes, S.P., Episkopou, V., and Polak, J.M. (2001). Differentiation of osteoblasts and *in vitro* bone formation from murine embryonic stem cells. *Tissue Eng.* **7**, 89-99.

Call, L.M., Moore, C.S., Stetten, G., and Gearhart, J.D. (2000). A cre-lox recombination system for the targeted integration of circular yeast artificial chromosomes into embryonic stem cells. *Hum. Mol. Genet*. **9**, 1745–1751

Chagraoui, J., Lepage-Noll, A., Anjo, A., Uzan, G. and Charbord, P. (2003). Fetal liver stroma consists of cells in epithelial-to-mesenchymal transition. *Blood*. **101**, 2973-2982

Chandross, K.J. and Mezey, E. (2001). Plasticity of adult bone marrow stem cells. Mattson, M.P. and Van Zant, G. eds. (Greenwich, CT: JAI Press).

Chowdhury. J.R., Grossman, M., Gupta, S., Chowdhury, N.R., Baker, J.R., and Wilson, J.M. (1991). Long-term improvement of hypercholesterolemia after ex vivo gene therapy in LDLR-deficient rabbits. *Science* **254**, 1802-1805.

Crosby, H.A. and Strain, A.J. (2001). Adult liver stem cells: bone marrow, blood, or liver derived? *Gut*. **48**, 153-154.

Dabeva, M.D. and Shafritz, D.A. (1993). Activation, proliferation, and differentiation of progenitor cells into hepatocytes in the D-galactosamine model of liver regeneration. *Am. J. Pathol.* **143**, 1606–1620.

Dani, C., Smith, A.G., Dessolin, S., Leroy, P., Staccini, L., Villageois, P., Darimont, C., and Ailhaud, G. (1997). Differentiation of embryonic stem cells into adipocytes in vitro. *J. Cel. Sci.* **110**, 1279-1285.

Deutsch, G., Jung, J., Zheng, M., Lora, J., and Zaret, K.S. (2001). A bipotential precursor population for pancreas and liver within the embryonic endoderm. *Development*. **128**, 871-881.

Doetschman, T., Eistetter, H., Katz, M., Schmit, W., and Kemler, R. (1985). The *in vitro* development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium. *J. Embryol. Exp. Morph.* **87**, 27-45.

Domen, J. and Weissman, I.L. (1999). Self-renewal, differentiation or death: regulation and manipulation of hematopoietic stem cell fate. *Mol. Med. Today.* **5**, 201-208.

Draper J.S., Moore H.D., Ruban L.N., Gokhale P.J., Andrews P.W. (2004). Culture and characterization of human embryonic stem cells. *Stem Cells Dev*. **13** (4), 325-36.

Evans, M.J. and Kaufman, M.H. (1981). Establishment in culture of pluripotential cells from mouse embryos. *Nature*. **292**, 154-156.

Fairchild, P.J., Brook, F.A., Gardner, R.L., Graca, L., Strong, V., Tone, Y., Tone, M., Nolan, K.F., and Waldmann, H. (2000). Directed differentiation of dendritic cells from mouse embryonic stem cells. *Curr. Biol.* **10**, 1515–1518.

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

Fox, I.J., Chowdhury, J.R., Kaufman,S.S., Goertzen, T.C., Chowdhury, N.R., Warkentin, P.I., Dorko, K., Sauter, B.V., and Strom, S.C. (1998). Treatment of the Crigler-Najjaa syndrome type I with hepatocyte transplantation. *N. Engl. J. Med.* **338**, 1422-1426.

Fraichard, A., Chassande, O., Bilbaut, G., Dehay, C., Savatier, P., and Samarut, J. (1995). *In vitro* differentiation of embryonic stem cells into glial cells and functional neurons. *J. Cell Sci.* **108**, 3181-3188.

Friedenstein, A.J., Piatetzky-Shapiro, I.I., and

PETRAKOVA, K.V. (1966). Osteogenesis in transplants of bone marrow cells. *J. Embryol. Exp. Morphol.* **16**, 381-390.

FRIEDENSTEIN, A.J., CHAILAKHJAN, R.K., AND LALYKINA, K.S. (1970). The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. *Cell Tissue Kinet.* **3**, 393-403.

GRIFFITH, L. G., AND NAUGHTON, G. *(*2002). Tissue engineering-current challenges and expanding opportunities. *Science.* **295**, 1009-1014.

GUPTA, S., GORLA G.R., AND IRANI, A.N. (1999). Hepatocytes transplantation : emerging insights into mechanisms of liver repopulation and their relevance to potential therapies. *J. Hepatol.* **30**, 162-170.

GUSSONI, E., SONEOKA, Y., STRICKLAND, C.D., BUZNEY, E.A., KHAN, M.K., FLINT, A.F., KUNKEL, L.M., AND MULLIGAN, R.C. (1999). Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature*. **401**, 390-394.

HOLTZER, H. (1978). Cell lineages, stem cells and the 'quantal' cell cycle concept. In: Stem cells and tissue homeostasis. Eds: B.I. Lord, C.S. Potten, and R.J. Cole. (Cambridge, New York: Cambridge University Press). 1-28.

HUNT, P., ROBERTSON, D., WEISS, D., RENNICK, D., LEE, F., AND WITTE, O.N. (1987). A single bone marrow-derived stromal cell type supports the *in vitro* growth of early lymphoid and myeloid cells. *Cell.* **48**, 997-1007.

ITSKOVITZ-ELDOR, J., SCHULDINER, M., KARSENTI, D., EDEN, A., YANUKA, O., AMIT, M., SOREQ, H., AND BENVENISTY, N. (2000). Differentiation of human embryonic stem cells into embryoid bodies comprising the three embryonic germ layers. *Mol. Med.* **6**, 88-95.

JIANG, Y., JAHGIRDAR, B.N., REINHADT, R.L., SCHWARTZ, R.E., KEENE, C.D., ORTIZ-GONZALES, X.R., REYES, M., LENVIK, T., BLACKSTAD, M., DU, J., ALDRICH, S., LISTBERG, A., LOW, W.C., LARGAESPADA, D.A., VERFAILLE, C.M. (2002). Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*. **418**, 1-9.

KHOSLA, S., DEAN, W., BROWN, D., REIK, W., AND FEIL, R. (2001). Culture of preimplantation mouse embryos affects fetal development and the expression of imprinted genes. *Biol. Reprod.* **64**, 918-926.

KOCHER, A.A., SCHUSTER, M.D., SZABOLCS, M.J., TAKUMA, S., BURKHOFF, D., WANG, J., HOMMA, S., EDWARDS, N.M., AND ITESCU, S. (2001). Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat. Med.* **7**, 430-436.

KORBLING, M., KATZ, R.L., KHANNA, A., RUIFROK, A.C., RONDON, G., ALBITAR, M., CHAMPLIN, R.E., AND ESTROV, Z. (2002). Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N. Engl. J. Med.* **346**, 738-746.

KRAMER, J., HEGERT, C., GUAN, K., WOBUS, A.M., MULLER, P.K., AND ROHWEDEL, J. (2000). Embryonic stem cell-derived chondrogenic differentiation *in vitro*: activation by BMP-2 and BMP-4. *Mech. Dev.* **92**, 193-205.

KRAUSE, D.S., THEISE, N.D., COLLECTOR, M.I., HENEGARIU, O., HWANG, S., GARDNER, R., NEUTZEL, S., AND SHARKIS, S.J. (2001). Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell*. **105**, 369-377.

KUZNETSOV, S.A., MANKANI, M.H., GRONTHOS, S., SATOMURA, K., BIANCO, P., AND ROBEY P.G. (2001). Circulating skeletal stem cells. *J. Cell Biol.* **153**, 1133-1140.

LAGASSE, E., CONNORS, H., AL DHALIMY, M., REITSMA, M., DOHSE, M., OSBORNE, L., WANG, X., FINEGOLD, M., WEISSMAN, I.L., AND GROMPE, M. (2000). Purified hematopoietic stem cells can differentiate into hepatocytes *in vivo*. *Nat. Med.* **6**, 1229-1234.

LAZARO, C.A., RHIM, J.A., YAMADA, Y., AND FAUSTO, N. (1998). Generation of hepatocytes from oval cell precursors in culture. *Cancer Res.* **58**, 5514-5522.

LEBLOND, C.P. (1964). Classification of cell populations on the basis of their proliferative behavior. *National Cancer Institute.* **14**, 119-150.

LEE, S.H., LUMELSKY, N., STUDER, L., AUERBACH, J.M., AND MCKAY, R.D. (2000). Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. *Nat. Biotechnol.* **18**, 675-679.

LUMELSKY, N., BLONDEL, O., LAENG, P., VELASCO, I., RAVIN, R., AND MCKAY, R. (2001). Differentiation of Embryonic Stem Cells to Insulin-Secreting Structures Similiar to Pancreatic Islets. *Science*. **292**, 1389-1394.

MARSHAK, D.R., GOTTLIEB, D., KIGER, A.A., FULLER, M.T., KUNATH, T., HOGAN, B., GARDNER, R.L., SMITH, A., KLAR, A.J.S., HENRIQUE, D., D'URSO, G., DATTA, S., HOLLIDAY, R., ASTLE, C.M., CHEN, J., HARRISON, D.E., XIE, T., SPRADLING, A., ANDREWS, P.W., PRZYBORSKI, S.A., THOMSON, J.A., KUNATH, T., STRUMPF, D., ROSSANT, J., TANAKA, S., ORKIN, S.H., MELCHERS, F., ROLINK, A., KELLER, G., PITTENGER, M.F., MARSHAK, D.R., FLAKE, A.W., PANICKER, M.M., RAO, M., WATT, F.M., GROMPE, M., FINEGOLD, M.J., KRITZIK, M.R., SARVETNICK, N., AND WINTON, D.J. (2001). Stem cell biology, Marshak, D.R., Gardner, R.L., and Gottlieb, D. eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press).

MARTIN, G.R. (1981). Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7634-7638.

MARZANO, A., SMEDILE, A., ABATE, M., OTTOBRELLI, A., BRUNETTO, M., NEGRO, F., FARCI, P., DURAZZO, M., DAVID, E., LAGGET, M., VERME, G., BONINO, F., AND PIZZETTO, M. (1994). Hepatitis type C after ortho-

topic liver transplantation: reinfection and disease recurrence. *J Hepatol.* **21**, 961-959.

Muraca M., Gerunda, G., Neri,D., Vilei, M.T., Granato, A., Feltracco, P., Meroni, M., Giron, G., and Burlina, A.B. (2002). Hepatocyte transplantation as a treatment for glycogen storage disease type 1a. *Lancet* **359**, 317-318.

Odorico, J.S., Kaufman, D.S., and Thomson, J.A. (2001). Multilineage differentiation from human embryonic stem cell lines. *Stem Cells.* **19**, 193-204.

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

Osawa, M., Hanada, K., Hamada, H., and Nakauchi, H. (1996). Long-term lymphohematopoietic reconstitution by a single CD34- low/negative hematopoietic stem cell. *Science*. **273**, 242-245.

Owen, M. (1988). Marrow derived stromal stem cells. *J. Cell Science Supp.* **10**, 63-76.

Pedersen, R.A. (1999). Embryonic stem cells for medicine. *Sci. Am.* **280**, 68-73.

Petersen, B.E., Bowen, W.C., Patrene, K.D., Mars, W.M., Sullivan, A.K., Murase, N., Boggs, S.S., Greenberger, J.S., and Goff, J.P. (1999). Bone marrow as a potential source of hepatic oval cells. *Science*. *284,* 1168-1170.

Pittenger, M.F. and Marshak, D.R. (2001). Mesenchymal stem cells of human adult bone marrow. Marshak, D.R., Gardner, D.K., and Gottlieb, D. eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press). 349-374.

Prabhu, R., Khalap, N., Burioni, R., Clementi, M., Garry, RF., and Dash, S. (2004). Inhibition of hepatitis C virus nonstructural protein, helicase activity, and viral replication by a recombinant human antibody clone. *Am. J. of Pathology* **165**, 1163-1173.

Reubinoff, B.E., Pera, M.F., Fong, C.Y., Trounson, A., and Bongso, A. (2000). Embryonic stem cell lines from human blastocysts: somatic differentiation *in vitro*. *Nat. Biotechnol.* **18**, 399-404.

Roberts, R., Gallagher, J., Spooncer, E., Allen, T.D., Bloomfield, F., and Dexter, T.M. (1988). Heparan sulphate bound growth factors: a mechanism for stromal cell mediated haemopoiesis. *Nature*. **332**, 376-378.

Robey, P.G. (2000). Stem cells near the century mark. *J. Clin. Invest.* **105**, 1489-1491.

Roy, V. and Verfaillie, C.M. (1999). Expression and function of cell adhesion molecules on fetal liver, cord blood and bone marrow hematopoietic progenitors: implications for anatomical localization and developmental stage specific regulation of hematopoiesis. *Exp. Hematol.* **27**, 302-312.

Shi, Q., Rafii, S., Wu, M.H., Wijelath, E.S., Yu, C., Ishida, A., Fujita, Y., Kothari, S., Mohle, R., Sauvage, L.R., Moore, M.A., Storb, R.F., and Hammond, W.P. (1998). Evidence for circulating bone marrow-derived endothelial cells. *Blood*. **92**, 362-367.

Slack, J.M. (2000). Stem cells in epithelial tissues. *Science*. **287**, 1431-1433.

Smith, A.G. (2001). Origins and properties of mouse embryonic stem cells. *Annu. Rev. Cell. Dev. Biol.*

Sokal, EM., Smet, F., Bourgois, A., Van Maldergen, L., Buts, J.P., Reding, R., Bernard Otte, J., Evrard, V., Latinne, D., Vincent, M.F., Moser, A., and Soriano, H.E. (2003). Hepatocyte transplantation in 4-year-old girl with peroxisomal biogenesis disease: technique, safety, and metabolic follow-up. *Transplantation* **76**, 735-738.

Stem Cells: Scientific Progress and Future Research Directions. Department of Health and Human Services. June 2001.

Strain, A.J., and Neuberger, J.M. (2002). A bioartificial liver- State of the Art. *Science* **295**, 005-1009.

Strom, SC., Fisher, R.A., Thompson, M., Sanyal, A.J., Cole,P.E., Ham, J.M., and Posner, M.P. (1997). Hepatocytes transplantation as a bridge to orthotopic liver transplantation in terminal liver falure. *Transplantation* **63**, 559-569.

Terada, N., Hamazaki, T., Oka, M., Hoki, M., Mastalerz, D.M., Nakano, Y., Meyer, E.M., Morel, L., Petersen, B.E., and Scott, E.W. (2002). Bone marrow cells adopt the phenotype of other cells by spontaneous cell fusion. *Nature*. **416**, 542545.

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

Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., and Jones, J.M. (1998). Embryonic stem cell lines derived from human blastocysts. *Science*. **282**, 1145-1147.

Thomson, JA., and Oderico, JS. (2000). Human embryonic stem cell and embryonic germ cell lines. *Trends Biotechnol.* **18**, 53-57.

Till, J.E. and McCullough, E.A. (1961). A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat. Res.* **14**, 213-222.

Toma, C., Pittenger, M.F., Cahill, K.S., Byrne, B.J., and Kessler, P.D. (2002). Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation*. **105**, 93-98.

Wagers, A.J., Christensen, J.L., and Weissman, I.L. (2002). Cell fate determination from stem cells. *Gene Ther.* **9**, 606612.

Weissman, I.L. (2000). Stem cells: units of development, units of regeneration, and units in evolution. *Cell*. **100**, 157-168.

Whitlock, C.A., Tidmarsh, G.F., Muller-Sieburg, C.,

and Weissman, I.L. (1987). Bone marrow stromal cell lines with lymphopoietic activity express high levels of a pre-B neo-plasia-associated molecule. *Cell*. **48**, 1009-1021.

Wiles, M.V., and Keller, G. (1991). Multiple hematopoietic lineages develop from embryonic stem (ES) cells in culture. *Development*. **111**, 259-267.

Wiles, M.V., Vauti, F., Otte, J., Fuchtbauer, E.M., Ruiz, P., Fuchtbauer, A., Arnold, H.H., Lehrach, H., Metz, T., von Melchner, H., and Wurst, W. (2000). Establishment of a gene-trap sequence tag library to generate mutant mice from embryonic stem cells. *Nat. Genet.* **24**, 13-14.

Uzan, G. (2004). Therapeutic use of stem cells. *Rev Prat*. **54** (13): 1399-403.

Yamashita, J., Itoh, H., Hirashima, M., Ogawa, M., Nishikawa, S., Yurugi, T., Naito, M., Nakao, K., and Nishikawa, S. (2000). Flk1-positive cells derived from embryonic stem cells serve as vascular progenitors. *Nature*. **408**, 92-96.

Ying, Q.-L., Nichols, J., Evans, E.P., and Smith, A.G. (2002). Changing potency by spontaneous fusion. *Nature*. **416**, 545548.

Verfaillie, C.M. (1998). Adhesion receptors as regulators of the hematopoietic process. *Blood*. **92**, 2609-2612.

Zandstra, P.W., Lauffenburger, D.A., and Eaves, C.J. (2000). A ligand-receptor signaling threshold model of stem cell differentiation control: a biologically conserved mechanism applicable to hematopoiesis. *Blood*. **96**, 1215-1222.

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