

# Stem cells for all seasons? Experimental and clinical issues

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Stem cells, by definition, are capable of total or partial differentiation as well as self-renewal. The most primitive stem cells, in many people's opinion, are those derived from the blastocyst inner cell mass<sup>1</sup>. Such embryonic germ cells can be maintained in culture for a long time if an appropriate cytokine, leukaemia inhibitory factor, is present in the culture medium. They remain pluripotent; but, if the cytokine is removed, they can be modulated to produce various cell lines, from blood islands to neurons and epithelia<sup>2</sup>. These cells therefore meet the criteria of being able to divide symmetrically to expand their number and to divide asymmetrically in order to self-renew and give rise to a differentiated progeny<sup>3</sup>. However, there is no evidence that blastocyst cells self-renew *in vivo* and obviously they are not working over the entire lifespan of the organism, as stem cells should. Therefore they cannot be regarded as true stem cells, while among so-called germ-line stem cells (oocytes and sperm-producing cells) only male spermatogonial cells are present in mammals over the lifetime: oocytes are produced in a finite number near the time of birth. I confine this review to cells that can self-renew for the entire lifespan of the organism.

## SOMATIC STEM CELLS

During embryonic development pluripotential stem cells become progressively more restricted, giving rise to stem cells with narrower commitments. Somatic stem cells appear relatively late in development: haemopoietic stem cells are seen first in the yolk sac, then in the paraortic region, later in fetal liver, finally in spleen and bone marrow. In irradiated embryos, repopulating stem cells tend to increase successively through the same sites (yolk sac, liver, marrow)<sup>5</sup>. Adult stem cells are often situated in 'niches' where they are available to start a differentiation pathway; the environment of such niches seems able to exert a critical influence on their biochemical and developmental potential. For instance, when adult haemopoietic stem cells from mouse bone marrow are

injected into the inner mass of the mouse blastocyst, they express fetal rather than adult haemoglobin<sup>6</sup>.

## NEURAL STEM CELLS

Evidence for the presence of adult stem cells in the central nervous system dates back to the 1960s, when postnatal neurogenesis was found in rat and guinea-pig hippocampus<sup>7</sup>. Further research revealed neural stem cells in other regions such as the forebrain ventricular wall: in the subventricular zone, facing the lumen, a layer of proliferating subependymal cells may represent either a stem cell compartment or a stream of progenitor cells in transit<sup>8,9</sup>. It is therefore possible that such cells serve as an endogenous source for new neurons and glia in the adult mammalian forebrain. A potential for neuron generation also seems to exist in the striatum, thalamus and hypothalamus<sup>10</sup>.

What is the function of these persistent stem cells in the adult brain? A distinct possibility, so far overlooked, is slow and continuous replacement of neural cells: sphere-forming cells, a population of neural precursors produced at various stages of embryo development, may persist as a 'leftover supply' in the brain and other tissues until the necessity arises to proliferate and differentiate<sup>11</sup>. Very recently, Rietze *et al.*<sup>12</sup> reported that stem cells purified from mouse adult brain appeared capable of generating either neural or non-neural cells. It has long been known that neural stem cells immortalized in culture can be induced to yield both neurons and glial cells<sup>13</sup>. Clearly, therefore, central nervous system stem cells, preserved in culture, offer a source of replenishment for depleted tissues<sup>14</sup>. We should also note the use of fetal cells which have undergone initial neural differentiation for treatment of Parkinson's disease. This method, however, requires so much fetal tissue that it is impracticable on a large scale; therefore, for this purpose too, the focus is on stem cells in culture<sup>15</sup>.

## HAEMOPOIETIC STEM CELLS

Many classes of haemopoietic stem cells have been identified, capable of giving rise to particular sets of blood cells: among the earliest to be proposed were the so-called CFU-S (colony-forming units spleen), capable of repopulating the entire haemopoietic system in lethally irradiated mice. An analogous class of cells in human beings, including

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a subset of long-term self-renewing cells, has been shown able to reconstitute haemopoiesis in patients who have received lethal doses of radiation or chemotherapy<sup>16,17</sup>.

At a less primitive level there are oligopotent stem cells that can produce either a common lymphoid progenitor or a common myeloid precursor, which in turn may give rise to an erythroid or megakaryocytic or granulo-monocytic precursor. Furthermore, so-called colony-forming cells can produce colonies of a restricted type, such as BFU-Es and CFU-Es for cells of the erythroid lineage, CFU-GMs for granulocytes and monocytes, and CFU-MKs for megakaryocytes<sup>11</sup>. A stem cell acting as a lymphoid progenitor has also been identified<sup>19</sup>.

The potential of such haemopoietic stem cells has been exploited clinically, not only in blood neoplastic disorders but also in genetic diseases of the immune system<sup>20</sup> and now treatment of solid tumours<sup>21</sup> (see later).

### STEM CELL PLASTICITY

Adult stem cells can be highly versatile, capable of modulation from one type to another. Neural stem cells, for instance, can transform under selective stimulation into blood cells of various sorts<sup>22</sup>, and haemopoietic stem cells injected into irradiated hosts have yielded glial cells<sup>25</sup> and hepatic oval cells<sup>24</sup> among others. Human mesenchymal stem cells have differentiated in culture into adipocytes, chondrocytes and osteocytes<sup>25</sup>. This wide spectrum of possible differentiation illustrates the impressive potential of early stem cells taken out of their microenvironment. If one wishes to achieve differentiation away from the expected lineage, how crucial is culture and how long must it continue to allow dedifferentiation and a subsequent shift of lineage? Lengthy passage in culture does not seem essential: for instance, marrow stromal cells grown for only five days and injected into the forebrain of neonatal mice were able to differentiate into mature astrocytes, and possibly into neurons as well<sup>26</sup>.

Haemopoietic stem cells can now be purified to such an extent that single bone-marrow-derived cells are available. Seemingly, one such cell can not only achieve long-term haemopoietic repopulation of an irradiated host but also differentiate into non-haemopoietic elements such as epithelial cells of liver, lung and gastrointestinal tract<sup>27</sup>.

### CLINICAL OBSTACLES TO STEM CELL TRANSPLANTATION

#### Established methods

Transplantation of human haemopoietic stem cells, obtained from various sources, is now established practice for certain disorders<sup>28</sup>. To obtain sufficient quantities, these cells are usually exposed *in vitro* to a cocktail of cytokines<sup>29</sup>.

How frequent are such early clonogenic cells in blood or blood-forming cells? The exact number is still uncertain, but a comparison of bone marrow, peripheral blood and cord blood showed cord blood to be the richest source and peripheral blood the poorest<sup>30</sup>. Cord blood clonogenic cells expand rapidly *in vitro* and are already used in treatment of genetic disorders of haemopoiesis and leukaemia<sup>31,32</sup>.

It seems important to avoid early depletion of the stem cell pool in culture and attention has been focused on the action of negative regulators of haemopoiesis, such as macrophage inflammatory protein 1-alpha (MIP-1-alpha) and tumour necrosis factor alpha<sup>33</sup>. MIP-1 alpha and leukaemia inhibitory factor protect the repopulating ability of purified haemopoietic stem cells, and preliminary clinical trials to test tolerance to MIP-1-alpha are in progress<sup>34</sup>.

A new approach is to use a preparative regimen which though not totally myeloablative allows engraftment of transplanted allogeneic cells. This can result in a pronounced graft-versus-tumour effect, and early results are encouraging. 10 of 19 patients with metastatic renal cell carcinoma showed a clear response<sup>35</sup>.

### Experimental protocols

As regards the central nervous system, a widely used model in animals is the lesioned striatum, in which cells at different stages of maturation have been inserted<sup>36</sup>. Virtually immortal human nervous stem cell lines are available, and when implanted into the striatum of immunodeficient mice were detectable in the host parenchyma up to a year after grafting<sup>14</sup>. Implantation of dopamine-producing cells has been tried not only in mice but also in patients with Parkinson's disease<sup>37</sup>; transplants of embryo-derived cells as well as adult neural stem cells have also been successfully performed in laboratory models of demyelinating diseases such as multiple sclerosis<sup>38,39</sup>.

Lately, further evidence of the surprising potential of adult neural stem cells, pointing to a 'repertoire' of differentiation very close to that of embryonic cells<sup>12,40</sup>, has provided further stimulus to work in this area<sup>41</sup>. Preliminary clinical trials are contemplated on replacement of damaged cartilage or repair of injured tendons. Some groups are trying to grow neural stem cells for transplantation into the brain or spinal cord of patients with severe central nervous system damage<sup>14,42,43</sup>; modulation by growth factors of cells derived from human microspheres (the early aggregates of neural progenitors) could provide the almost unlimited supply of enriched non-genetically-transformed neurons required for transplantation studies<sup>44</sup>.

Stem cell transplantation and modulation has potential applications in various other clinical conditions. The turning point has been the recognition and identification of stem

cells in several different tissues, with a plasticity that allows transdifferentiation: bone marrow cells, for instance, may be capable of yielding epidermal cells, skeletal muscle cells and even hepatic oval cells (precursors of mature liver cells)<sup>24</sup>.

When stem cells are introduced in a new 'niche', they are believed to undergo a process of reprogramming and differentiation in response to signals promoted from the new micro-environment<sup>45</sup>. The nature of such signals, however, remains obscure. It is noteworthy that stem cells from different tissues, such as bone marrow and muscle exposed to a given culture environment, yield cells with similar properties. We already know that areas of muscle regeneration show an influx of progenitor cells from other sources, and in immunodeficient mice transplanted marrow-derived cells can migrate into such areas, differentiate and give rise to muscle fibres<sup>47</sup>. This suggests a possible means of treatment for degenerative disorders such as muscular dystrophies. Another observation of great interest is that bone marrow stem cells can lead to myocardial regeneration in mice with experimentally induced infarction<sup>48</sup>; attempts to isolate the responsible cells are in progress.

Another ready source of adult stem cells with high proliferation potential is the skin. Such cells may prove useful not only for repair of skin lesions but also, after reprogramming, for transplantation into other tissues or organs<sup>49</sup>.

### A QUESTION OF IDENTITY

The finding of high plasticity in adult stem cells demands revision of the stem-cell concept<sup>50</sup>. When the identity of cells capable of repairing damaged tissues is scrutinized, we see that not all stem cells are equivalent; in the central nervous system, for example, most neural stem cells are regionally and temporally restricted, although it is possible to find and isolate rare stem cells which are capable of producing diverse cells types<sup>51</sup>.

In future, therefore, it will probably be necessary to use stem cells from many different sources, for the repair of damaged tissues and organs. The main limiting factor at present is the poverty of information on signals in host tissue that promote optimal homing and activation of transplanted cells. A promising development, in this context, is a very recent approach whereby a synthetic matrix and controlled-release microparticles are assembled with the progenitor cells before transplantation, thus creating an artificial microenvironment: within such 'neo-tissue' each microparticle is designed to supply agents that promote certain aspects of cell function<sup>52</sup>. This whole area of research is showing a welcome surge of activity<sup>53</sup>.

### REFERENCES

- 1 Thompson JA, Itskovitz-Eldor J, Shapiro SS, *et al.* Embryonic stem cells derived from human blastocysts. *Science* 1998;**282**:1142–5
- 2 Bradley A. Embryonic stem cells: proliferation and differentiation. *Curr Opin Cell Biol* 1990;**2**:1013–17
- 3 Lajtha L. Stem cell concepts. *Differentiation* 1979;**14**:23–34
- 4 Van der Kooy D, Weiss S. Why stem cells? *Science* 2000;**287**: 1439–41
- 5 Dzierzak E, Medvinsky A, De Bruijn M. Qualitative and quantitative aspects of haematopoietic cell development in the mammalian embryo. *Immunol Today* 1998;**19**:228–35
- 6 Geiger H, Sick S, Bonifer C, Muller AM. Globin gene expression is reprogrammed in chimeras generated by injecting adult hemopoietic stem cells in mouse blastocyst. *Cells* 1998;**93**:1055–5
- 7 Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rat. *J Comp Neurol* 1965;**124**: 319–35
- 8 Lois C, Alvarez-Buylla A. Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proc Natl Acad Sci USA* 1993;**90**:2074–7
- 9 Doetsch F, Garcia-Verdugo M, Alvarez-Buylla A. Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *J Neurosci* 1997;**17**: 5046–61
- 10 Pencea AN, Pragnell IB. Inhibitors of haemopoiesis and their potential clinical relevance. *Blood Rev* 1995;**9**:226–33
- 11 Scheffler B, Horn M, Blumke I, *et al.* Marrow mindedness: a perspective on neurogenesis. *Trends Neurosci* 1999;**22**:348–57
- 12 Rietze RL, Valcanis H, Brooker GF, Thomas T, Voss AK, Bartlett PF. Purification of a pluripotent neural stem cell from the adult mouse brain. *Nature* 2001;**412**:736–9
- 13 Gritti A, Parati FA, Cova L, *et al.* Multipotent stem like cells from the adult mouse brain proliferate and self-renew in response to basic fibroblast growth factor. *J Neurosci* 1996;**16**:1091–100
- 14 Vescovi AL, Parati EA, Gritti A, *et al.* Isolation and cloning of multipotential stem cells from the embryonic human CNS and establishment of transplantable human neural stem cell lines by epigenetic stimulation. *Exp Neurol* 1999;**156**:71–83
- 15 Barinaga M. Fetal neuron grafts pave the way for stem cell therapies. *Science* 2000;**287**:11–12
- 16 Keating A, Powell J, Takahashi M, Singer JW. The generation of human long-term marrow cultures from marrow depleted of 1a positive cells. *Blood* 1984;**64**:1159–62
- 17 Osawa M, Hanada K, Hamada H, Nakauchi H. Long term hemopoietic reconstitution by a single CD34-low negative hematopoietic stem cell. *Science* 1996;**273**:242–4
- 18 Eridani S, Morali F. Identification of haemopoietic stem cells. *Cytotechnology* 1993;**11**:101–6
- 19 Kondo M, Weissmann IL, Akashi K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* 1997;**91**:661–72
- 20 Thomas ED. Frontiers in bone marrow transplantation. *Blood Cells* 1991;**17**:259–67
- 21 Appelbaum FR. Haematopoietic cell transplantation as immunotherapy. *Nature* 2001;**411**:385–9
- 22 Bjornson CRR, Rietze RL, Reynolds BA, *et al.* Turning brain into blood: a haemopoietic fate adopted by adult neural stem cells *in vivo*. *Science* 1999;**283**:534–7
- 23 Eglitis MA, Mezey E. Haematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. *Proc Natl Acad Sci USA* 1997;**94**:4080–5
- 24 Petersen BE, Bowen WC, Patrene KD, *et al.* Bone marrow as a potential source of hepatic oval cells. *Science* 1999;**284**:1168–74

- 25 Pittenger MF, Mackay AM, Beck SC, *et al.* Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;**284**:143–6
- 26 Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brain. *Proc Natl Acad Sci* 1999;**96**:10 711–16
- 27 Krause DS, Theise ND, Collector MI, *et al.* Multi-organ, multi-lineage engraftment by a single bone-marrow derived stem cell. *Cell* 2001;**105**:369–77
- 28 Emerson SG. *Ex vivo* expansion of haematopoietic precursors, progenitors and stem cells: the next generation of cellular therapeutics. *Blood* 1996;**87**:3082–8
- 29 Piacibello W, Sanavio F, Garetto L, *et al.* Extensive amplification and self-renewal of human haematopoietic stem cells from cord blood. *Blood* 1997;**89**:2644–53
- 30 Eridani S, Mazza U, Massaro P, *et al.* Cytokine effect on *ex vivo* expansion of haemopoietic stem cells from different human sources. *Biotherapy* 1998;**11**:291–6
- 31 Gluckman E, Rocha V, Boyer A, *et al.* Outcome of cord blood transplantation from related and unrelated donors. *N Engl J Med* 1997;**337**:373–81
- 32 Long G, Madan B, Kurtzberg J, *et al.* Unrelated umbilical cord blood transplants in patients with hematologic malignancies and genetic disorders [Abstract]. *Blood* 1999;Nov(Suppl):Abstract No. 2544
- 33 Parker AN, Pragnell IB. Inhibitors of haemopoiesis and their potential clinical relevance. *Blood Rev* 1995;**9**:226–33
- 34 Tanosaki R, Ashihara E, *et al.* MIP-1-alpha and LIF protect the repopulating ability of purified murine haemopoietic stem cells in serum-deprived cultures stimulated with SCF and IL-3. *Ann Ist Superiore Sanita* 1999;**35**:553–62
- 35 Childs R, Chernoff A, Contentin N, *et al.* Regression of metastatic renal-cell carcinoma after non myeloablative allogeneic peripheral blood stem cell transplantation. *N Engl J Med* 2000;**343**:750–8
- 36 Sabata O, Horellu P, Vigne E, *et al.* Transplantation to the rat brain of human neural progenitors that were genetically modified using adenovirus. *Nat Genet* 1995;**9**:256–60
- 37 Olanow CW, Freeman TB, Kordower CW. Neural transplantation as a therapy for Parkinson's disease. *Adv Neurol* 1997;**74**:249–69
- 38 Brustle O, Jones KN, Learish RD, *et al.* Embryonic stem cell-derived glial precursors: a source of myelinating transplants. *Science* 1999;**285**:754–6
- 39 Zhang SC, Ge B, Duncan ID. Adult brain retains the potential to generate oligodendroglial progenitors with extensive myelination capacity. *Proc Natl Acad Sci USA* 1999;**96**:4089–94
- 40 Clarke DL, Johansson CB, Wilbertz J, *et al.* Generalized potential of adult neural stem cells. *Science* 2000;**288**:1660–3
- 41 Snyder EY, Vescovi A. The possibilities/perplexities of stem cells. *Nat Biotechnol* 2000;**18**:827–8
- 42 Eridani S. Replacement of damaged neural cells: a mirage? *J R Soc Med* 1999;**92**:502–4
- 43 Cassidy R, Frisen J. Neurobiology: stem cells on the brain. *Nature* 2001;**412**:690–1
- 44 Caldwell MA, He X, Wilkie N, *et al.* Growth factors regulate the survival and fate of cells derived from human microspheres. *Nat Biotech* 2001;**19**:475–9
- 45 Wyatt FW, Hogan BLM. Out of Eden: stem cells and their niches. *Science* 2000;**287**:1427–30
- 46 Gussoni E, Soneoka Y, Strickland CD, *et al.* Dystrophin expression in the mdx mouse restored by stem cell transplantation. *Nature* 1999;**401**:390–4
- 47 Ferrari G, Cusella Deangelis G, Coletta M, *et al.* Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 1998;**179**:1528–30
- 48 Orlic D. Bone marrow cells regenerate infarcted myocardium. *Nature* 2001;**410**:701–5
- 49 Fuchs E, Segre JA. Stem cells: a new lease of life. *Cell* 2000;**100**:143–55
- 50 Blau HM, Brazelton TR, Weinmann JM, *et al.* The evolving concept of a stem cell: entity or function? *Cell* 2001;**105**:829–41
- 51 Temple S. The development of neural stem cells. *Nature* 2001;**414**:112–17
- 52 Mahoney MJ, Saltzman WM. Transplantation of brain cells assembled around a programmable synthetic microenvironment. *Nat Biotechnol* 2001;**19**:934–9
- 53 Lovell-Badge R. The future of stem cell research *Nature* 2001;**414**:88–91