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Stem Cell Plasticity and Blood and Marrow Transplantation: A Clinical Strategy

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Abstract The newly described phenomenon of stem cell plasticity raises interesting biological questions and offers exciting opportunities in clinical application. This review uses the well-established practice of blood and marrow transplantation as a paradigm to explore the clinical consequences of this finding. Recently proposed non-myeloablative conditioning regimens have shown that mixed donor-host hematolymphoid chimerism can be established with relatively low toxicity in both animal studies and human trials. Hematopoietic growth factor treatment of transplanted patients can mobilize a large number of donor stem cells to migrate from marrow to non-hematopoietic organs. We propose that these advances, in conjunction with the developmental plasticity of stem cells, can constitute components of a clinical strategy to use blood and marrow transplantation as a platform to treat systemic diseases involving non-hematopoietic tissues. J. Cell. Biochem. Suppl. 38: 96–103, 2002.

Key words: stem cell plasticity; transplantation; conditioning; growth factor; mobilization

Most somatic tissues and organs are believed to arise from tissue-specific stem cells capable of both self-renewal and differentiation into the target tissues. Unlike totipotential embryonic stem cells, adult stem cells are generally thought to be restricted in their developmental capacities to a single tissue. Recent research, however, has demonstrated a surprising degree of plasticity in the developmental potential of adult stem cells, thereby calling into question the dogma that these stem cells differentiate solely along a unidirectional pathway into mature, tissue-restricted cells. This unanticipated phenomenon of stem cell plasticity has generated a great deal of scientific interest and has been extensively reviewed [Blau et al., 2001].

In this prospect, we will use the well-established practice of blood and marrow transplantation (BMT) as a paradigm to explore the clinical consequences of adult stem cell plasticity. BMT is the only stem cell therapy currently in routine clinical use. The current practice of BMT has been established after years of painstaking systematic investigations using animal studies and clinical trials [Thomas, 1999] and the procedure is highly successful in the treatment of a wide spectrum of diseases involving the hematolymphoid system.

Several features of the hematolymphoid system contribute to the success of BMT as a clinical procedure. Hematopoietic stem cells reside mostly in the bone marrow, a renewable and easily accessible source of donor stem cells. Harvest of these stem cells, either via bone marrow aspiration or peripheral blood leukapheresis to obtain mobilized marrow stem cells, poses relatively little insult to the donor. The donor stem cells can be transplanted simply by intravenous infusion into the recipient. The transplanted cells then circulate throughout the body and home to the bone marrow, where they subsequently engraft. To make room for the new stem cells, endogenous bone marrow of the recipient can be completely eliminated (i.e., ablated) by conditioning regimens using chemotherapy or irradiation. Regeneration of hematopoietic tissues that follows such myeloablation leads to donor stem cell proliferation and rapid reconstitution of the entire hematolymphoid system.

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Most of these characteristics are not shared by stem cells of other organ systems, such as muscle, liver, and brain. A strategy of systemic cellular therapy similar to BMT cannot, therefore, be readily developed in treatment of diseases involving non-hematopoietic tissues. The recent description of the developmental plasticity of stem cells raises the question whether BMT itself can be used as the basis for development of such a strategy.

STEM CELL PLASTICITY

Many of the recent studies that describe the phenomenon of stem cell plasticity made use of the murine BMT model, precisely because of the advantages of the BMT approach outlined above. In these experiments, mice were irradiated and then infused with donor marrow cells. After repopulation of the recipient hematolymphoid system by donor cells, the animals were analyzed for the presence of donor-derived cells in various non-hematolymphoid tissues (Fig. 1). Differentiated cells appropriate to the target organs were found, for instance, in the muscle [Ferrari et al., 1998; Bittner et al., 1999; Gussoni et al., 1999]; myocardium [Bittner et al., 1999; Jackson et al., 2001]; brain [Eglitis and Mezey, 1997; Brazelton et al., 2000; Mezey et al., 2000; Nakano et al., 2001]; liver [Petersen et al., 1999; Lagasse et al., 2000; Theise et al., 2000a]; and epithelium in lung, gastrointestinal tract, and skin [Krause et al., 2001]. Examination of liver biopsy specimens of human patients who had previously received BMT also revealed the presence of donor-derived hepatocytes and cholangiocytes [Alison et al., 2000; Theise et al., 2000b].

It is unknown if this observed stem cell plasticity is the result of transdifferentiation of hematopoietic or mesenchymal stem cells, their dedifferentiation and subsequent redifferentiation along a different pathway, or simply the presence of certain more primitive, uncommitted, or totipotential stem cells "hiding" in the marrow [Orkin, 2000]. While the biology underlying this phenomenon needs to be further elucidated, the aggregation of published data has convincingly shown that certain rare marrow-derived cells can circulate in the body, home to target organs at a distance from the marrow, respond to local environmental cues, and develop into specialized non-hematopoietic tissues.

These results suggest that we can use the developmental plasticity of marrow-derived stem cells in combination with BMT as a platform to treat certain diseases involving non-hematopoietic tissues. Some diseases potentially treatable by this approach include congenital muscular dystrophy involving muscle cells; osteogenesis imperfecta involving bone cells; hereditary tyrosinemia involving liver cells; and lysosomal storage disease involving brain cells. In order to do that, we have to address several issues: what type of stem cells should one use in such a procedure; how should one prepare the patients for the procedure; and how can one enhance its therapeutic efficacy?

WHAT TYPE OF STEM CELLS TO USE?

The populations of marrow stem cells that might differentiate into mature, non-hematopoietic cells in the different tissues, have been characterized to varying degrees in the

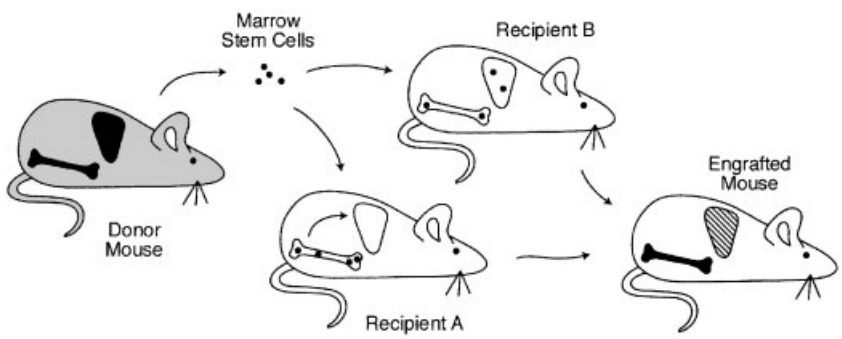

Fig. 1. Donor mouse marrow stem cells transplanted into an irradiated mouse (Recipient A) first repopulate the marrow and later migrate to a non-hematopoietic organ (illustrated in the figure as the liver). Alternatively, the transplanted stem cells may home to both the marrow and liver of the mouse (Recipient B) and engraft both organs at the same time. The engrafted mouse may have full donor cell engraftment in the marrow (solid) but only partial engraftment in the liver (hatched).

published reports. On one extreme, Gussoni et al. [1999], used purified marrow cells that were selected for the differential efflux properties of a vital dye, a well-characterized phenotype of hematopoietic stem cells (HSC) [Zhou et al., 2001], to show that they can differentiate into muscle cells. Lagasse et al. [2000] used highly purified HSC selected by immunophenotyping to show that they can transdifferentiate into hepatocytes. Krause et al. [2001] used marrow-derived stem cells functionally selected by a two-day homing protocol and demonstrated that they possess an extensive differentiation capacity. On the other extreme, Mezey et al. [2000] and Brazelton et al. [2000] both used unfractionated bone marrow cells to demonstrate the neuronal developmental fate of marrow-derived stem cells. A unified picture has yet to emerge as to whether various populations of marrow stem cells might contribute differently to the developmental plasticity phenomenon observed in the target tissues. It is important to further define the phenotype of these populations and characterize their developmental potential, preferably in a clonogenic fashion. Only then can rigorous experimentation proceed to determine the molecular and biological basis of stem cell plasticity and to establish laboratory protocols to expand these stem cells for clinical use.

Ironically however, in clinical practice, knowledge of the exact phenotype of the plastic stem cells may not be so crucial. The CD34 marker present on human HSC was the first surface antigen identified that correlates with stem cell potential [Berenson et al., 1988]. This marker is now routinely used in clinical transplantation to quantify the stem cell content of a graft [Siena et al., 2000]. Despite all this, there is still a raging controversy about whether true HSC are CD34 positive [Goodell, 1999; Gao et al., 2001]. Most successful transplantation protocols use unmanipulated bone marrow or mobilized peripheral blood progenitor cells, and only a limited number of studies using highly purified HSC have been reported [Negrin et al., 2000]. For most clinical purposes, we might only need an operational (or functional) definition of the stem cell phenotype in order to proceed with further experimentation and clinical trials.

Most of these published reports focus on the in vivo plastic developmental changes of stem cells. It will be important to determine if such a change can be induced by in vitro manipulation, as that result will influence what stem cells can be used as the source material for transplantation. Several reports have demonstrated in vitro plastic developmental changes in adult precursors isolated from specialized tissues. Stem cells derived from adult rodent skin can differentiate in culture to produce neurons, glia, smooth muscle cells, and adipocytes [Toma et al., 2001]. Nestin-positive stem cells isolated from adult pancreatic islets can acquire a hepatic phenotype ex vivo [Zulewski et al., 2001]. Treatment with dexamethasone induces C/EBPβ expression in cultured pancreatic cells and provokes their transdifferentiation into hepatocytes [Shen et al., 2000]. Expression of the transcriptional repressor *msx1* induces dedifferentiation of cultured myotubes into primitive, multipotential mesenchymal precursors [Odelberg et al., 2000]. Purified neural stem cells cocultured with the C2C12 myoblast cell line rapidly differentiate in vitro into myocytes [Galli et al., 2000; Rietze et al., 2001]. Rat bone marrow stromal cells cultured with 5-azacytidine form myotubules in vitro and improve cardiac function when injected into scar tissues of experimentally damaged hearts [Tomita et al., 1999]. Unlike HSC, which are notoriously difficult to expand in culture, some of these other stem cells can proliferate and be propagated under laboratory conditions, even though their subsequent developmental potentials have not been rigorously tested. This might offer an opportunity to obtain homogenous populations of expanded stem cells that can be used in BMT in conjunction with HSC infusion.

OATH OF HIPPOCRATES: TO DO NO HARM

Standard BMT results have shown that pretransplantation myeloablative conditioning of the recipient is a necessary condition for donor cells to engraft. Though effective, these conditioning regimens are highly toxic to the end organs of the recipient. The profound pancytopenia associated with marrow ablation also poses threats of serious and often fatal infections to the patient. These regimen-related toxicities have generally limited the use of BMT to patients with malignancy and other life-threatening illnesses. Any attempt to extend BMT treatment to non-malignant disorders that are not acutely life-threatening needs to reduce the therapy-related toxicities associated with the conditioning regimens.

Our improved understanding of the immune responses in transplantation have resulted in the development of new clinical strategies that aim at reducing the need for highly toxic myeloablative treatment of the patients while maintaining the effectiveness of the procedure. It is now recognized that allogeneic T-cell activation requires two signals, one from ligation of the T-cell receptors and another from engagement of certain co-stimulatory receptors, such as those in the B7-CTLA4/CD28 pathway or the CD40-CD40L pathway [Harlan and Kirk, 1999]. Ligation of the T-cell receptors without simultaneously activating a co-stimulatory pathway might lead to induction of anergy or immunologic tolerance [Appleman et al., 2001]. Reagents that specifically block the co-stimulatory pathways can anergize donor and host immune cells in transplantation and lower the incidence of complications such as graft rejection (host immune response against donor cells) and graft-versus-host disease (donor cell immune response against host). This strategy has been successfully exploited to facilitate BMT across major histocompatibility barriers [Guinan et al., 1999]. New evidence also challenges the concept that myeloablative conditioning is absolutely needed to create marrow space for the incoming graft. It now appears that if sufficient immunosuppression can be given to prevent host immune cells from rejecting the donor cells, engraftment can take place. Increasing the number of transplanted donor cells also help overcome barriers to engraftment, presumably by inducing tolerance. Basing their research on these principles, several groups have designed low-toxicity, non-myeloablative but immunosuppressive conditioning regimens that allow host and donor hematolymphoid cells to coexist in a state of mixed chimerism.

Using pretransplantation low-dose irradiation, treatment of host cells with an anti-CD40L monoclonal antibody and high-dose donor marrow cell infusion, Quesenberry et al. [2001] successfully established stable mixed hematopoietic chimerism with a high level of donor cell contribution in both syngeneic and allogeneic mouse transplantation models [Stewart et al., 1998]. By the same method, this group also showed that marrow-derived osteogenic precursors can engraft and become competent osteoblasts in non-ablated recipient mice [Nilsson et al., 1999]. Storb et al. [1999] used low-dose irradiation and CTLA4 pathway blockage to successfully establish stable mixed chimerism in dogs without the need of pretransplantation myeloablation. They also utilized this conditioning regimen clinically in transplantation patients with good results [McSweeney et al., 2001]. Wekerle et al. [2000] blocked both co-stimulatory pathways with antibodies and used high-dose marrow cell transplantation to achieve persistent mixed hematopoietic chimeras in mice, also without the need for prior cytoreductive treatment. Similar successes have been reported by several other groups using slightly different protocols, showing the general validity of the non-myeloablative approach in BMT [Slavin et al., 1998; Bachar-Lustig et al., 1999; Durham et al., 2000; Fuchimoto et al., 2000; Li et al., 2001].

The establishment of stable mixed hematolymphoid chimerism in the transplantation recipient after non-myeloablative conditioning creates a situation in which the endogenous immune cells of the host and those derived from the donor are reciprocally tolerant. Maintenance of this state of mutual donor-host tolerance does not require the continuous use of immunosuppressive drugs, and it has been shown to result in markedly decreased incidence of graft-versus-host phenomenon [Wekerle et al., 2000; Kunisaki et al., 2001; Quesenberry et al., 2001]. In many cases, the transplanted recipient can even accept skin grafts from the host, indicating effective systemic induction of donor-specific immune tolerance. This phenomenon is particularly relevant if we are considering the use of BMT in cellular or gene therapy of genetic diseases. For such a therapy to work, the transplanted cells by design will have to carry and express genes coding for defective or deficient proteins in the recipient. These new proteins might be recognized as foreign and non-self by the residual immune cells in the transplantation recipient, thus triggering an immune response and subsequent rejection of the transplanted cells. This unwanted immune response can be modulated by both non-specific immunosuppressive agents (e.g., calcineurin inhibitors like cyclosporin A) or co-stimulatory pathway blockers [Qian et al., 2000; Rossi et al., 2001], but the establishment of a mixed chimeric state might provide a more powerful way to induce tolerance to the foreign proteins introduced in cellular or gene therapy.

A HOME AWAY FROM HOME

An important parameter that determines if plastic development of marrow-derived stem cells in non-hematopoietic tissues may be of any clinical significance, is the degree of donor cells engraftment in these sites. The results are highly variable in the published animal studies. Gussoni et al. [1999] detected 1%–5% donor cells in the muscle fibers of the recipients, twelve weeks after BMT, with one animal having a 10% contribution. Mezey et al. [2000] found that only 0.3%–2.3% of neurons in the transplanted mice were derived from donor marrow cells. Several possible reasons might explain the low engraftment rate of the donor cells in these tissues in many studies. The marrow-derived stem cells might not home to the non-hematopoietic target tissues efficiently. Those that have arrived at the target tissues may not express the necessary receptors for them to respond to the local environmental signals for differentiation into non-hematopoietic cells. The probability of marrow-derived stem cells transdifferentiating could be intrinsically low.

One conclusion that can clearly be drawn from the published data is that active tissue regeneration at the target non-hematopoietic organ is required for the marrow-derived stem cells to engraft there. For example, normal mice that were transplanted with marrow cells show no donor cell contribution to the muscle, whereas there is demonstrable engraftment in mice with muscular dystrophy and continuing muscle regeneration [Bittner et al., 1999]. Petersen et al. [1999] and Lagasse et al. [2000] detected hepatocytes developed from donor marrow cells only in the context of induced liver damage and subsequent regeneration. Similar to these animal models, many human diseases exhibit extensive tissue regeneration in the diseased organs that may favor engraftment of donor stem cells. In Duchenne muscular dystrophy, for instance, segmental necrosis of muscle fibers and subsequent regeneration of this segment is a cardinal feature seen in a muscle biopsy. In hereditary tyrosinemia, the diseased liver may be completely replaced with regenerating nodules, a result of the hepatocellular damage caused by toxic metabolites of tyrosine catabolism. In other diseases, such as osteogenesis imperfecta and lysosomal storage disease, it is not clear if extensive tissue regeneration occurs in the diseased organs. Nevertheless, most of these diseases can be diagnosed in early postnatal period, when the diseased organs undergo rapid growth. That may also provide the necessary condition for transplanted cells to engraft in these organs.

In order to increase the level of donor cell contribution in the diseased organs to bring about clinical benefits, one can envision using the following strategy. As indicated in transplantation experiments using non-ablated syngeneic mice [Stewart et al., 1998], donor cell engraftment efficiency depends largely on the ratio of donor and host stem cells numbers at the site of tissue regeneration. Increasing the number of donor cells that home to the target sites might increase the level of donor cell engraftment. In a transplantation recipient with mixed hematopoietic donor-host chimerism, this goal can be achieved by mobilizing donor stem cells from the marrow into circulation by treatment of the recipient with specific cytokines. Once released into the blood stream, the mobilized donor stem cells will circulate until they reach the target organs. The growth signals generated by tissue regeneration at these organs will stimulate survival, growth, and differentiation of these donor stem cells at those sites. Compared with the defective endogenous stem cells at the target organs, the healthy donor stem cells might even have selective growth advantages and contribute disproportionately to the function of the organs, thus ameliorating the severity of the disease [Overturf et al., 1996].

Marrow stem cell mobilization by this approach is a routinely performed clinical procedure, usually in the setting of harvest of stem cells from transplantation donors [To et al., 1997]. Donors are given doses of a hematopoietic growth factor, e.g., granulocyte-colony stimulating factor (G-CSF), before the harvesting of peripheral blood stem cells by leukapheresis. Such mobilization schemes usually result in an increase of the stem cell content in the peripheral circulation by at least 40 fold [Begley et al., 1997; Seong et al., 1997]. Marrow stem cell mobilization has also been examined in animal models. Splenectomized mice given a combination of G-CSF and stem cell factor (SCF), for instance, can have a 250-fold increase of pluripotent HSC in the peripheral circulation [Bodine et al., 1994]. Other ways to mobilize marrow stem cells into circulation include the use of blocking antibodies against adhesion

molecules present on these cells [Christ et al., 2001; Papayannopoulou et al., 2001].

A recent report describes experiments in which researchers made use of this principle and elegantly showed the power of this approach [Orlic et al., 2001]. Splenectomized mice were injected subcutaneously with SCF and G-CSF, daily for five days to mobilize marrow stem cells into circulation. The coronary arteries of the mice were then surgically ligated to induce experimental myocardial infarctions. Survival of the mice was followed and echocardiac and hemodynamic measurements were done 27 days after the surgery. The mice were then sacrificed and their myocardia examined for infarct size and evidence of tissue regeneration. The results showed that with mobilization of marrow stem cells, mortality of the mice was decreased by 68%, infarct size by 40%, ventricular cavitary dilation by 26%, and diastolic stress by 70%. This not only demonstrates that endogenous marrow stem cells can be mobilized to migrate to the heart and contribute to tissue regeneration after injury, but also such tissue repair can make a impact on the survival of the mice.

FOLLOW THE YELLOW BRICK ROAD

Basing on the discussion above, we propose that we might already have in hand the necessary components of a practical strategy to exploit the phenomenon of stem cell plasticity in the clinic. In this strategy, BMT is used as a scaffold for subsequent cellular therapy of systemic diseases that involve non-hematopoietic tissues (Fig. 2). After conditioning the patient with a low-toxicity, non-myeloablative regimen, bone marrow from a suitable donor will be transplanted into the patient to establish a mixed donor-host hematopoietic chimerism. A small number of donor stem cells might also home to the diseased, non-hematopoietic target organ that the therapy is designed to treat and establish a low level of engraftment there. The recipient would subsequently be given repeated cycles of growth factors in order to mobilize donor cells in the marrow to migrate to the target organ. The continuing tissue regeneration occurring in the diseased target organ might provide the microenvironmental signals that facilitate the mobilized donor stem cells to engraft and differentiate at the site. With each cycle of marrow stem cell mobilization by growth factors, there might be a progressively higher level of donor cell engraftment at the non-hematopoietic target organ. At the end of therapy, there might be a high enough level of donor cell engraftment in the target organ to functionally improve the patient's phenotype.

Each individual component of this strategy is experimentally testable and has been shown to function in animal models. We only need to further refine the details and design clinical trials to test the entire strategy. We need, for instance, to define the right combination of growth factors that should be used to mobilize marrow stem cells to migrate to the non-hematopoietic target organs. We need to investigate the optimal dosing levels and the appropriate intervals of growth factor administration. We need to determine if splenectomy is necessary to maximize the benefits of the stem cell mobilization. There are many important biological and clinical questions to answer as well as preclinical experiments to perform, but

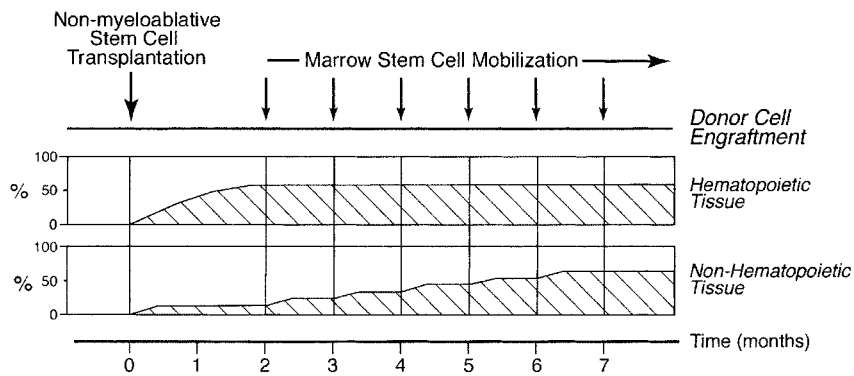

Fig. 2. A conceptual schema of systemic cellular therapy of genetic diseases of non-hematopoietic tissues that makes use of BMT and the plasticity of marrow stem cells.

definitive clinical applicability appears to be on the horizon. The therapeutic possibilities opened up by this approach are limited only by the extent of our imagination.

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