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Cell therapy for the treatment of accidental radiation overexposure

D THIERRY, PhD, J M BERTHO, PhD, A CHAPEL, PhD and P GOURMELON, MD

DRPH, Institut de Radioprotection et de Sûreté Nucléaire (IRSN), PO Box 17, 92262 Fontenay-aux-Roses, France

Abstract. Irradiation kills cells directly or indirectly, and the basic issue is to replace them. To do so, the cells required to heal the injured compartment can be obtained from a non- (or less) injured part of the body or from an allogeneic source. Because the number of cells necessary to treat an injury can be very high, the cells have to be expanded *ex vivo*. Owing to their transdifferentiation or plasticity property, cells from some tissues could even possibly repopulate another system. Human bone marrow contains two cell compartments: the haematopoietic cell compartment, and the stromal cell compartment that comprises mesenchymal stem cells (MSCs). Both compartments could be used for cell therapy. We will focus on four specific questions. Is it possible to use *ex vivo* expansion of haematopoietic cells to treat bone marrow aplasia? Are human MSCs homing in the injured tissues following irradiation? Is it possible to separate MSC haematopoietic support from MSC homing ability following irradiation? Is it possible to combine haematopoietic cells and MSCs to treat a radiation-induced multi-organ failure syndrome? To study these points, we have developed an irradiated NOD/SCID mouse model for human cell transplantation, and a non-human primate model for large field irradiation. Our results suggest that cell therapy could be a valuable approach for the treatment of multiple organ failure.

Introduction

Acute radiation sickness following an accidental radiological or nuclear exposure comprises multi-organ failure. Depending on the energy, dose, dose rate and geometry of the exposure, bone marrow aplasia may be combined with gastrointestinal syndrome, skin burns, muscle radiolysis, lung injury and/or central nervous system failure, among other conditions. Until now, each of the main aspects of these disorders has been handled separately as a single disease. Bone marrow transplantation or administration of haematopoietic growth factor have been used with mitigated success to treat radiation-induced bone marrow aplasia, the first organ failure to appear following a total body irradiation owing to the high radiation sensitivity of the haematopoietic system [1]. No therapeutic strategy has proven successful for other tissues.

Irradiation kills cells directly or indirectly, and the basic issue is to replace them. The aim of cell therapy is to use normal cells to rebuild an impaired tissue or to correct a biological defect of a patient. Owing to new scientific knowledge and biotechnological developments, only recently has it become apparent that a graft of cells processed *ex vivo* may rescue not only the haematopoietic compartment but also other organs.

To do so, the cells necessary to heal the injured compartment are obtained from a non- (or less) injured part of the body or from an allogeneic source. Because the number of cells necessary to treat an injury can be very high and because stem cells form a small percentage of the total cellularity, the cells have to be expanded *ex vivo* and injected taking into account the need for immature (stem cells and progenitors) or differentiated cells. Owing to their transdifferentiation or plasticity properties, cells from certain tissues could possibly even repopulate another system. Furthermore, to be of therapeutic use, the cells produced must have normal function and regulation. If this is possible, cell therapy could be a valuable approach for the treatment of acute radiation syndrome. In this article, we shall focus on the use of bone marrow cells for cell therapy of radiation-induced multiple organ failure syndrome.

Human bone marrow contains two cell compartments: the haematopoietic cell compartment, and the stromal cell compartment that comprises mesenchymal stem cells (MSCs) [2, 3]. Both compartments could be used for cell therapy.

Ex vivo expansion of haematopoietic stem cells, precursors and differentiated cells is a new approach of growth factor therapy that may be of interest for the treatment of patients with irradiation-induced bone marrow aplasia. These studies aim to expand the pool of progenitors and stem cells for transplantation and/or to expand differentiated cells for transfusion. Maturing cells expanded *ex vivo* could be used to limit the early risks linked to aplasia (infections related to granulocytopenia, bleeding associated with thrombocytopenia), whereas expanded immature cells could hasten haematopoietic recovery. This is made possible owing to the development of techniques allowing selection of a population of haematopoietic progenitors and stem cells from the blood (with stimulation by growth factors prior to stem cell harvesting) or bone marrow. The approach is interesting for the treatment of patients with radiation-induced aplasia, either if the cells necessary for *ex vivo* expansion have been banked before the radiation accident, or if such cells can be found in the blood or marrow and harvested in sufficient quantity after the accidental irradiation. Such cells may be available in the blood after various types of irradiation, as suggested by results on haematopoietic progenitors in the peripheral blood

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after therapeutic irradiation [4]. They may also be induced to emerge from the bone marrow to the blood through growth factor mobilisation. This approach has numerous advantages. *Ex vivo* expansion could be set up as an autologous process (although it can be used in allogeneic settings such as cord blood expansion), thus avoiding the specific problems of allogeneic cell use (graft-versus-host disease or graft rejection and the side effects of immune suppressive treatment, as well as alloimmunisations bound to numerous transfusions). Some of the growth factors have restricted use *in vivo* owing to their toxic side effects, although their effects on haematopoiesis could be useful. *Ex vivo* experiments could allow their use without adverse reactions and at high doses, as they are not used in the organism. The fact that the cells are kept out of the organism protects them from the indirect consequences of the accidental irradiation such as inflammation or water loss. By adjusting the conditions of expansion, it is possible to modulate the treatment to produce a specific mature cell type (granulocytes for treatment of infection, platelets to treat haemorrhage) or only immature cells for transplantation. Finally, *ex vivo* manipulation of the cells is, in most experiments, a necessary step for the transfer of a gene of therapeutic interest.

The therapeutic potential of bone marrow-derived MSCs has recently been brought into the spotlight of many fields of research. Not only are these cells able to migrate to injured or defective tissues of different origin, but their progenies are also able to acquire a different phenotype in accordance with the tissues to which they home [5]. MSCs are able to give rise to multiple mesodermal tissue types, including bone, cartilage, tendon, muscles, cardiomyocytes, fat, brain [6], and a marrow stromal connective tissue that supports the differentiation of haematopoietic stem cells (HSCs). Furthermore, it has been shown that MSCs of bone marrow origin could hasten haematopoietic recovery when infused simultaneously with HSCs in non-human primates [7] and in humans [8–10]. Altogether, the therapeutic potential associated with these properties appears significant and may have many applications. One such application is the repair of injured tissues arising from the side effects of radiation treatments or accidental radiation exposure.

Cell therapy using bone marrow cells to treat radiation injuries

Here we present some approaches to the numerous questions that it is necessary to solve in order to be able to use cell therapy for the treatment of multiple organ failure following irradiation.

All experimental procedures involving animals were approved by the Animal Care Committee of the Institut de Radioprotection et de Sûreté Nucléaire (IRSN) and conformed to the French regulations for animal experimentation (Ministry of Agriculture Act No. 87-848, 19 October 1987, modified May 2001).

Is it possible to use ex vivo expansion of haematopoietic cells to treat bone marrow aplasia?

We have set up a high dose total body irradiation (TBI) non-human primate model to study the therapeutic potential of *ex vivo* expansion of autologous progenitors and differentiating haematopoietic cells. We studied two *ex vivo* expansion protocols for bone marrow cells. The first protocol consisted of a 7-day culture in the presence of stem cell factor, FMS-like tyrosine kinase 3 (Flt-3) ligand, thrombopoietin, and interleukin-3 (IL-3) and IL-6, which induced preferentially the expansion of immature haematopoietic cells (3.1 ± 1.4, 10.0 ± 5.1, 2.2 ± 1.9 and 1.0 ± 0.3 fold expansion for mononuclear cells (MNC), granulocyte–macrophage colony-forming units (CFU-GM), erythroid burst-forming unit (BFU-E) and long-term culture initiating cells (LTC-IC), respectively). The second protocol used the same cytokine combination supplemented with granulocyte colony-stimulating factor (G-CSF) with an increased duration of culture up to 14 days, which induced mainly the production of mature haematopoietic cells (17.2 ± 11.7 fold expansion for MNC and no detectable BFU-E and LTC-IC), although expansion of CFU-GM (13.7 ± 18.8 fold) and CD34+ cells (5.2 ± 1.4 fold) was also observed. Results also showed the presence of MSCs and cells from the lymphoid and megakaryocytic lineages in 7-day-expanded bone marrow mononuclear cells (BMMNC). To test the ability of *ex vivo* expanded cells to sustain haematopoietic recovery after radiation-induced aplasia, seven non-human primates were irradiated at a supralethal dose of 8 Gy and received the product of either 7-day (24 h after irradiation) or 14-day (8 days after irradiation) expanded bone marrow cells or unmanipulated bone marrow transplantation (Figure 1). Results showed that the 7-day *ex vivo* expanded BMMNC shortened the period and severity of pancytopenia and improved white blood cell recovery (Figure 2), whilst the 14-day *ex vivo* expanded BMMNC mainly produced a transfusion-like effect over 8 days, followed by haematopoietic recovery. These results suggest that 7-day *ex vivo* expanded BMMNC may be highly efficient in the treatment of radiation-induced aplasia [11]. We are currently developing a two-step irradiation mimicking the often heterogeneous conditions of radiation accidents. In this protocol, HSCs are sampled following a 3 Gy TBI, a foreleg is then protected and a second 5 Gy irradiation of the rest of the body is performed. The cells are expanded *ex vivo* and injected into the animal 7 days post irradiation. Preliminary data show that animals receiving *ex vivo* expanded HSC have a better haematopoietic recovery than controls treated with comparable quantities of unmanipulated marrow cells. Taken together, these elements will allow the feasibility of *ex vivo* expansion of haematopoietic cells for the treatment of accidental irradiation-induced aplasia to be established.

Are human MSCs homing in the injured tissues following irradiation?

Stem cell plasticity has increasingly been reported in a wide range of adult tissues. MSCs might proliferate and differentiate in a site-specific manner. We hypothesised that radiation-induced tissue injuries might play a role in the recruitment of MSCs for tissue repair. To determine the factors involved in MSC recruitment, migration and homing kinetics, human MSCs were injected following global or local (abdomen) irradiation to an immunotolerant non-obese diabetic/severe combined

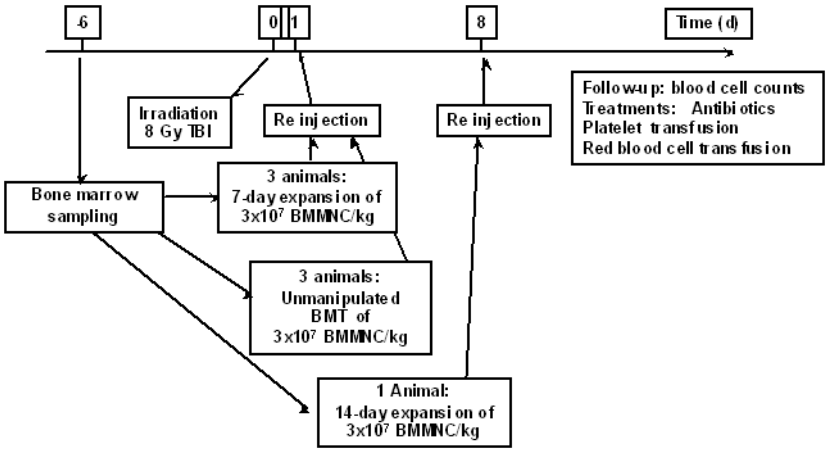

Figure 1. Experimental schedule for re-injection of expanded haematopoietic cells. TBI, total body irradiation; BMMNC, bone marrow mononuclear cells; BMT, bone marrow transplantation.

immunodeficiency (NOD/SCID) mouse model. We have tracked human MSCs using real-time polymerase chain reaction (PCR) assay that specifically amplifies human genes. Systemic delivery of human bone marrow-expanded MSCs into sublethally irradiated NOD/SCID mice recipients resulted in homing to liver, bone, bone marrow, gut, heart, kidney, brain, lung and stomach 15 days post irradiation. Human MSCs were found up to 3 months post irradiation in the gut, lung, bone marrow and heart. Following abdominal radiation exposure, human MSCs engrafted preferentially in the abdomen, *i.e.* liver, spleen, stomach, kidney and gut at 15 days. Up to 1% of the cells in the mouse tissue were of human origin. By contrast, human MSCs were scarcely detectable in other body areas and in the blood. These observations support our hypothesis that MSCs can be recruited to sites of radiation injury. In conclusion, using MSCs as a source of cells able to home in various tissues and to repair the damage induced by radiation could be an interesting strategy to limit the effects of irradiation [12]. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis and immunohistochemical staining using human tissue-specific markers is now in progress to determine the *in vivo* differentiation potential of MSCs into the cell types of the targeted organs.

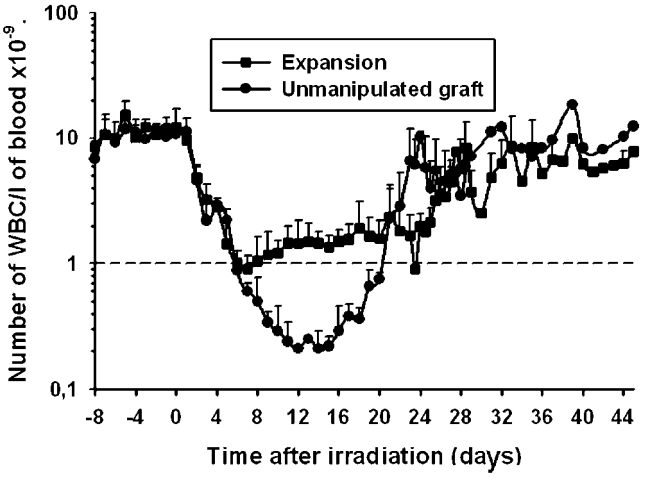

Figure 2. Effect of re-injection of 7-day *ex vivo* expanded cell on white blood cells (WBC).

Is it possible to separate MSC haematopoietic support from MSC homing ability following irradiation?

MSCs are heterogeneous and little is known about the role of MSC subsets in haematopoietic engraftment support and their homing in various tissues. Stro-1 antigen is present on fibroblast colony-forming unit (CFU-F) cells in adult human bone marrow and potentially defines a MSC precursor subpopulation [13]. The aim of the present study was to evaluate the role of *ex vivo* expanded Stro-1+ and Stro-1− MSCs on engraftment of human CD34+ cord blood cells in NOD/SCID mice. Our data showed that the levels of human haematopoietic engraftment (as assessed by the presence of CD45, CD34, CD19 and CD11b cells) in the blood, spleen and mouse bone marrow were higher when Stro-1−-derived cells were co-infused with CD34+ cells than when Stro-1+-derived cells were used.

In a second step, we investigated the homing of expanded Stro-1+ and Stro-1− cells (infused without CD34+ cells) in bone marrow, spleen, liver, brain, heart, lungs, kidneys and muscles of NOD/SCID mice. 8-week-old NOD/SCID mice received 3.5 Gy irradiation and 24 h later the cells were infused. We analysed the homing of cells by quantitative PCR of DNA of human β GLOBIN gene. Results showed that the quantity of DNA from expanded Stro-1+ cells was higher than that from expanded Stro-1− cells in spleen (8×), muscles (6×), bone marrow (2×), liver (1.5×) and kidneys (1.5×). No significant difference was observed in brain, whilst more Stro-1− than Stro-1+ cell DNA was found in the lungs (3.5×).

In conclusion, expanded Stro-1+ cells migrated better than expanded Stro-1− cells in the majority of mouse tissues. This indicated that Stro-1+ cells would potentially be a good vector to bring specific therapeutic genes into tissues. To test this hypothesis, we infused expanded Stro-1+ cells transfected with an enhanced green fluorescence protein (eGFP) gene into NOD/SCID mice. The specific eGFP DNA was found in every investigated tissue, namely bone marrow, liver, brain, heart, spleen, kidneys, muscles and lungs.

The difference between the haematopoeitic support and the homing capacities of Stro-1+ and Stro-1− cells may be of importance for clinical application of MSCs [12]: Stro-1+ cells would preferentially be used for repair of tissues other than bone marrow, whereas Stro-1− cells (or unseparated MSCs since they contained approximately 90% Stro-1− cells) would be used for haematopoietic engraftment support [14].

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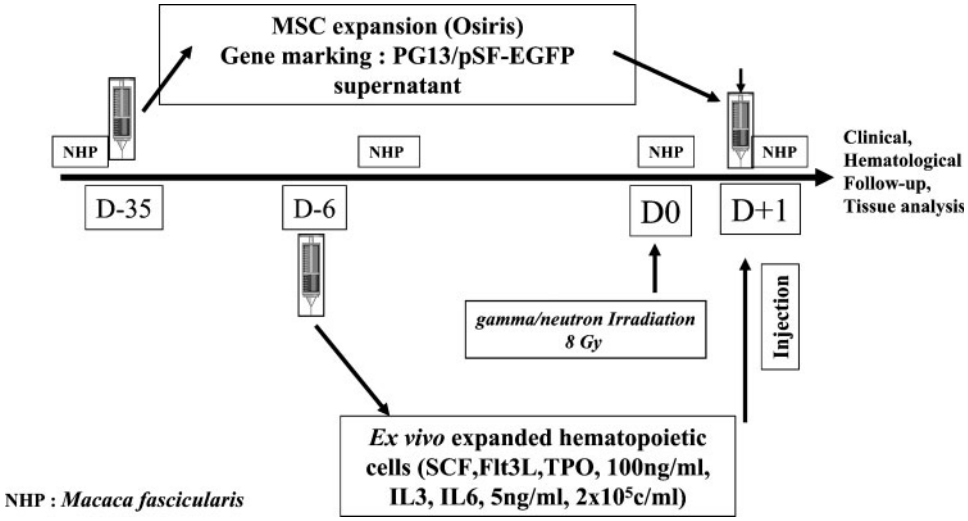

Figure 3. Mesenchymal stem cell (MSC)/haematopoietic stem cell (HSC) infusion: experimental design.

Is it possible to combine HSCs and MSCs to treat a radiation-induced multi-organ failure syndrome?

We hypothesised that using MSCs as a source of cells able to home to various tissues and to repair the widespread damage induced by irradiation might be an interesting therapeutic strategy. We therefore investigated the potential of combining *ex vivo* expansion of HSCs with *ex vivo* expanded MSCs for the treatment of acute radiation syndrome in a non-human primate model (Figure 3). HSCs and MSCs were taken from the bone marrow and expanded. A sample of MSCs was transduced with the gene encoding for eGFP to track them in the organism. The animals received TBI at 8 Gy from a neutron/γ source, thus resulting in heterogeneous exposure (Figure 4). Depending on the neutron/γ ratio, an acute radiation sickness of varying severity, but of the same nature, could be produced. Three animals received expanded HSCs alone, one animal received expanded MSCs alone, two animals received unmanipulated bone marrow, and three animals received expanded HSCs and MSCs. Our results showed that both therapeutic objectives were reached. The presence of MSCs in the injured tissues as well as a better haematopoietic recovery for the surviving animals treated with expanded cells than for animals treated with unmanipulated bone marrow cells were seen. This current study demonstrates that, following a very severe multi-organ injury involving neutron/γ irradiation, transplanted MSCs can be found in numerous injured tissues up to 81 days post transplant. In some organs, the grafted MSCs represent a large proportion of the assayed samples (up to 7% for jejunum, taking into account that only a fraction of MSCs are eGFP-labelled). Our report is the first evidence of the long-term ability of MSCs to home in numerous tissues of a primate as a response to a severe radiation injury. Our results might suggest a MSC distribution kinetic as a function of the degree of severity of the lesions (and the state of regeneration of damaged tissues). The kinetics of MSC distribution in organs might be explained by specific migration of MSCs to tissues in the process of regeneration rather than to necrotic tissues. Our data support this hypothesis, since tissues receiving the higher level of neutron radiation exhibited less homing of MSCs than other tissues. For the animal receiving MSCs alone, eGFP-labelled MSC were limited to those tissues receiving the highest radiation doses. For the animal irradiated with a neutron/γ ratio of 1:1 receiving MSCs and HSCs, MSCs were mostly found in the less severely irradiated tissues, whereas the inverse was observed for the animal receiving fewer neutrons (Figure 5). This discrepancy might indicate that MSC homing is related to the dose of irradiation and the time post transplant. These findings may indicate the contribution of MSCs to tissue repair at 2 weeks and after more than 2 months. The presence of eGFP-labelled cells in the CFU-F assays from the bone marrow of an animal receiving expanded HSCs and MSCs suggests that the transplanted MSCs may directly contribute to the restoration of bone marrow functions [15].

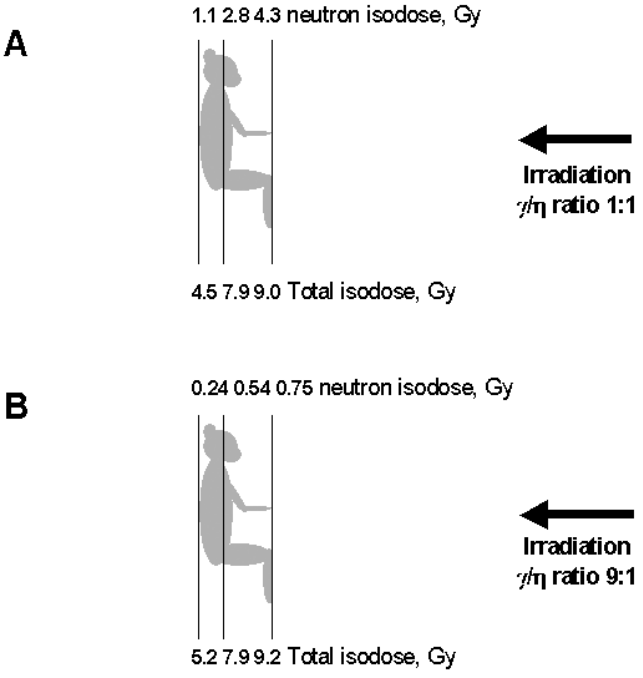

Figure 4. Physical dosimetry.

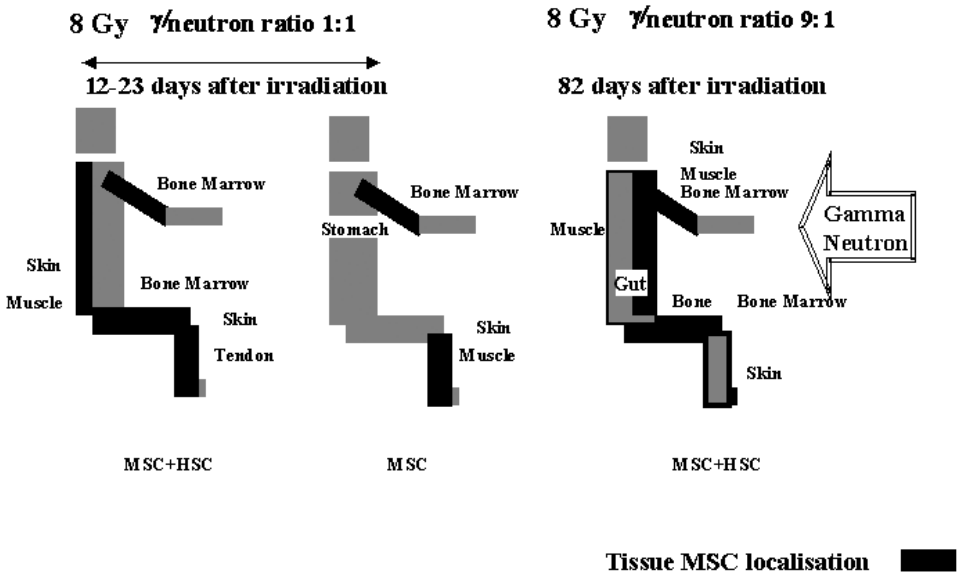

Figure 5. Mesenchymal stem cell (MSC) localisation. HSC, haematopoietic stem cell.

Conclusion

Cell therapy is a promising approach for the treatment of accidental irradiation-induced multiple organ failure. However, numerous problems need to be solved before this will become a common clinical practice. The type of cell to use and the culture conditions may be important, as it is possible to induce some differentiation *in vitro*, which may be beneficial to treat a specific injured tissue. The quantity of cells and the time of injection also require a lot of attention, as it is possible that cell injection could be efficient only if a specific niche is available in the tissue. A better knowledge of the immunology of the studied cells is required to be able to perform allogeneic transplant. Finally, the plasticity of the cells used for cell therapy must be studied in detail, as the phenomenon is still poorly understood in human tissue [16].

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