THERAPEUTIC POTENTIAL OF *EX VIVO* EXPANSION OF HAEMATOPOIETIC PRECURSORS FOR THE TREATMENT OF ACCIDENTAL IRRADIATION-INDUCED APLASIA.

T.M.A. Nguyen-Neildez[1], J. Vétillard[1], D. Thierry[1], C Parmentier[2] and J.C. Nénot[1].

[1] Institut de Protection et de Sûreté Nucléaire, Département de Protection de la santé de l'Homme et de Dosimétrie, IPSN, BP n° 6, F- 92265 Fontenay-aux-Roses Cédex, France.
[2] Institut Gustave Roussy, 39 rue Camille Desmoulins, Villejuif Cédex, France.

INTRODUCTION

Since the first radiation accidents which resulted into severe health effects into the workforce or the population, progress has been made in the fields of diagnosis, prognosis and treatment of accidentally overexposed victims. Since then, progress has also been made in the medical management of diseases such as aplasia. Because of the relative scarcity of radiation accidents, there is a need for complementary researches, in order to take profit of new techniques and medical approaches.

After whole body overexposure, the key issue is the therapeutic decision, i.e. the choice between bone marrow transplantation and other strategies. The indications of bone marrow transplantation cover only a short range of doses, provided the exposure is distributed uniformly within the body; a rare event in accidental settings. The results of the clinical trials for Granulocyte-Colony Stimulating Factor : G-CSF, Granulocyte/Macrophage-Colony Stimulating Factor : GM-CSF or Interleukin 3 : IL-3, *in vivo* and *in vitro* radiobiology experiments suggest that growth factor therapy could be of use after most accidental overexposures to evidence and to stimulate the remaining haematopoietic stem cells in order to shorten the duration of aplasia, although questions have been raised about growth factor infusion real clinical efficiency (1). The lessons learnt from the management of accidents evidenced that growth factor infusion has been mildly successful. This may be due to ill-defined administration protocols, to the rather narrow spectrum of action of the growth factors available for clinical use, but also to adverse side effects bound to the characteristics of these patients among them combined injuries and radiation induced inflammation (2). Therefore, great efforts should be devoted to researches in this field, exploring possibilities of alternative therapy such as haematopoietic stem cell and progenitor transfusion after their expansion.

Ex vivo expansion of haematopoietic precursor, stem cells and differentiated cells is a new approach of growth factor therapy, which may be of interest for the treatment of patients with accidental radiation-induced aplasia. These studies aim to expand the pool of progenitors and stem cells for transplantation or to expand differentiated cells (mainly granulocytes but also megakaryocytes) for transfusion (3).

This is made possible due to the development of techniques allowing the selection of a population of haematopoietic progenitors and stem cells from the blood (with stimulation by growth factors prior stem cell harvesting) or bone marrow using immature cell positive selection. The next step consisting in their culture with combination of growth factors or additional stroma cells is also under development. Autologous progenitor cells generated *ex vivo* has been recently used with some success for reconstitution of haematopoiesis after high-dose chemotherapy (4).

EXPERIMENTS

Our aim is to investigate the feasibility of expansion of the pool of progenitors and differentiated cells (mainly myeloid) for transfusion purposes.

The first step is the selection of a population of haematopoietic progenitors and stem cells from the blood (PBSC from multiple myeloma patients collected following Endoxan plus G-CSF- mobilisation) or bone marrow using CD 34+ cell positive selection (through immunoaffinity or immunomagnetic bead techniques 70-90% purity of the CD 34+ cell population are achieved).

Depending on the culture conditions, the selected human CD 34+ cell samples are then cultured at various cell densities, with recombinant growth factor combinations to promote expansion and differentiation (liquid cultures 10^3 and up to 50 10^3 cells/ml, G-CSF 10 to 1000ng/ml in conjunction with SCF, Interleukine-1, 3 and 6, 10 to 100 ng/ml) expansion of 88.3 +/- 32 fold could be obtained in one week of cultures for differentiating cells. Committed progenitors which have a longer survival *in vivo* and a high proliferation potential are also expanded 49+/-15 fold (assayed through secondary culture techniques). Expansion of 1625+/-615 fold could be obtained in 2 weeks of cultures (Fig 1,2 and 3). However simultaneous production of mature and progenitor cells was not observed.

High concentration of G-CSF and other growth factor are required to expand maturing cells whereas low G-CSF and high SCF are required to expand myeloid progenitors. Expansion of differentiating cells resulted in the loss of CD 34 expression amongst the cultured cells. The best growth factor combinations assayed allowed a good reproducibility of the experiments. For cultures up to 4 weeks, the harvested cells exhibit a fully-developed granulocyte phenotype.

Our results suggest that, in theory, it is possible to culture from the blood or bone marrow the cells able to proliferate and differentiate, in order to produce a sufficient quantity of cells to cover the transfusion needs of an accidentally irradiated victim through an aplasia episode (5).

The qualitative aspects of *ex vivo* expansion have to be studied in details. To be of therapeutic use the cells produced must retain normal mature cell functions. Our results suggest that the expanded cells have a close to normal chemotactic ability as measured through assay on PVP free polycarbonate filters 48 wells microchemotaxis chamber using fMLP as attractant (43+/-4 vs 68+/-5 for controls). The cells also retain a part of their capacity to generate superoxyde anions after two weeks of culture (measured through chemoluminescence tests). Cytological studies evidenced that *ex vivo* expansion resulted in a combination of cells of various degrees of differentiation in one to two weeks of cultures. For cultures up to 4 weeks, the harvested cells exhibit a fully-developed granulocyte phenotype (6).

DISCUSSION

Important research is necessary to adapt the *ex vivo* expansion of haematopoietic precursor stem cells and differentiated cells for the treatment of radiation-induced aplasia. Some of the growth factors (among which Stem Cell Factor : SCF, interleukine-1 and 6) have restricted use *in vivo* due to their toxic side effects although their effects on haematopoiesis could be useful. *Ex vivo* experiments could allow their use without adverse reaction. Furthermore, while proliferating, *ex vivo* expanded cells are not submitted to the effects of haematopoietic inhibitors (such as Tumor Necrosis Factor TNF alfa) which may be generated in the body following an irradiation. They are also independent from the clinical problems of the patient (such as burns and dehydration) which could delay haematopoietic recovery. It has been suggested after the Brazil accident (where a victim experienced a late hypoplasia) that, when internal exposure is involved, the use of growth factors *in vivo* would stimulate haematopoiesis-induced progenitors or stem cells to progress in the cell cycle, while the cells are irradiated. The combination of haematopoietic growth factors inducing mitosis and simultaneous prolonged radiation exposure might result in the depletion of the stem cell pool. *Ex vivo* expansion may allow the use of haematopoietic growth factors independently of these problems.

This approach could be of interest for the treatment of radiation induced aplasia if the cells necessary for *ex vivo* expansion could be found in the blood or in the marrow and harvested in sufficient quantity and that it is possible to culture them. Such cells could be available in the blood after various types of irradiation as suggested by results on haematopoietic progenitors in the peripheral blood after therapeutic irradiations. Data analysis from the past accidental exposure evidenced also that unhomogenous exposure is very frequent among victims of radiation injury (7), suggesting that in numerous accidents a bone marrow territory may still contain cells which could be used for expansion. Furthermore, preliminary results on *in vitro* irradiated human CD 34+ cells suggest that *ex vivo* expansion remains feasible for cells irradiated up to 2 gy without real loss of expanding ability.

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Figure 1 : Progenitor cell ex vivo expansion within 7 days

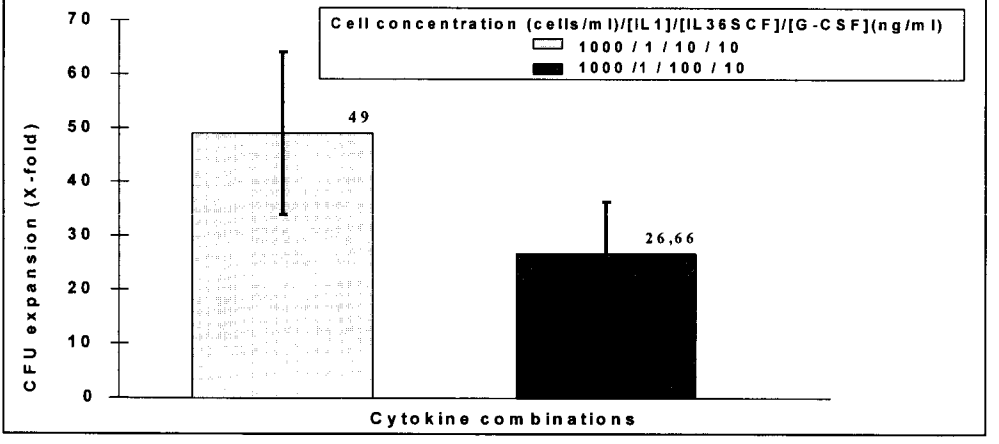

Figure 2 : Mature cell ex vivo expansion within 7 days

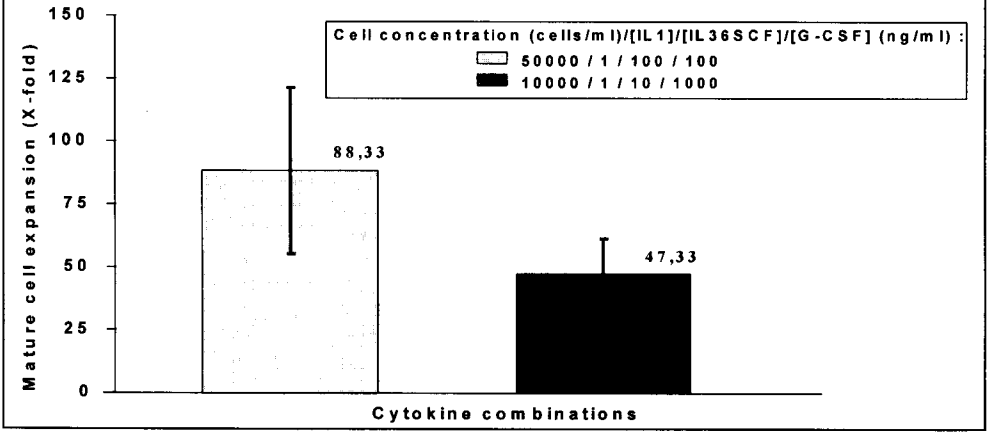

Figure 3 : Mature cell ex vivo expansion within 14 days