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Review

Delivering cellular therapies: Lessons learned from ex vivo culture and clinical applications of hematopoietic cells

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Abstract

Advances in stem cell biology and cellular therapy have led to promising treatments in a range of incurable diseases. However, it is unclear whether primitive stem cells can be delivered to damage tissue for regeneration of functional mature cells or stem cells must be stimulated to differentiate into mature cells in vitro and these cells delivered to patients. A range of other questions remains to be determined including how to formulate cellular products for in vivo delivery and how to undertake pharmacological testing of cellular products. Insights into these questions can be obtained from hematopoietic stem cells (HSC) which have been used for the past 50 years in bone marrow transplantation for regeneration of blood cells in patients undergoing high dose chemotherapy to treat cancer. The differentiation of HSC into mature blood cells is controlled by proteins called hematopoietic growth factors and these factors have been used to generate cellular products in vitro for clinical applications. This chapter will review some of the results of cellular therapies performed with HSC and the lessons that can be learned from these studies.

Keywords: HSC; Cell therapy; Ex vivo expansion; BMT; MSC; Growth factors

Contents

1. Introduction

Cellular therapy offers the potential of treatments and cures for a range of diseases including diabetes, cancer, neural and cardiac diseases. Many studies have demonstrated the potential of stem cells and mature cells to provide a therapeutic bene-

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fit, however the optimal protocols for formulation, delivery and growth factor support, remain to be defined. In this manuscript, we present the results from a number of studies performed with hematopoietic cells and discuss the implications on the general field of cellular therapy.

The bone marrow is the principal site for blood cell formation in humans. In normal adults the body produces about 2.5 billion red blood cells (RBC), 2.5 billion platelets and 10 billion granulocytes per kilogram of body weight per day [1]. The production of mature blood cells is a continual process that is the result of proliferation and differentiation of stem cells, committed progenitor cells and differentiated cells. Within these three stages, extensive expansion of cell numbers occurs through cell division. A single stem cell has been proposed to be capable of more than 50 cell divisions or doublings and has the capacity to generate up to 10^{15} cells, or sufficient cells for up to 60 years [2]. The proliferation and differentiation of hematopoietic stem and progenitor cells is controlled by a group of proteins called hematopoietic growth factors (HGFs).

The major clinical application for hematopoietic cells (HC) has been as a cellular source to support cancer patients undergoing high dose chemotherapy (HDC) with hematologic malignancies and solid organ diseases [for more details visit the website of the National Marrow Donor Program – www.marrow.org]. Bone marrow (BM) was the first source of hematopoietic cells used for transplantation [3]. Since then other sources including mobilized peripheral blood progenitor cells (PBPC) [4] and cord blood cells [5] have been utilized in different clinical settings. An optimal hematopoietic graft contains a mixture of cells including (i) committed progenitor cells that provide rapid recovery of neutrophils and platelets and (ii) hematopoietic stem cells (HSC) that provide long-term durable engraftment.

HC transplants (HCT) are used most commonly as part of the treatment of blood cancers, some solid tumors and also when the BM is damaged or diseased and cannot function normally. Since the main form of cancer treatment, chemotherapy (the use of tumor toxic drugs), as well as radiotherapy, are non-specific, they also damage normal cells including bone marrow cells. If the therapy is intense enough to destroy the bone marrow a transplant is necessary to prevent complications such as infection and bleeding that can lead to death.

2. Ex vivo expansion of hematopoietic cells

One limitation to the application of HC to various clinical settings has been the absolute number of stem cells and/or mature cells available in products and so many Investigators have evaluated the ex vivo and/or manipulation expansion of hematopoietic cells. The clinical applications include:

(1) Supplementing stem cell grafts with more mature precursors to shorten or potentially prevent chemotherapy-induced pancytopenia.
(2) Increasing the number of primitive progenitor cells to ensure hematopoietic support for multiple cycles of high-dose chemotherapy.
(3) Generating a sufficient number of stem cells from a single marrow aspirate or apheresis procedure, thus reducing the need for large-scale harvesting of marrow or multiple leukaphereses.
(4) Generating sufficient cells from a single umbilical cord blood to reconstitute an adult following high-dose chemotherapy.
(5) Purging stem cell products of contaminating tumor cells.
(6) Generating large volumes of immunologically active cells with antitumor activity to be used in immunotherapeutic regimens.
(7) Increasing the pool of stem cells which could be targets for the delivery of gene therapy.

The use of ex vivo expansion to generate mature neutrophil precursors was proposed in 1992 by Haylock et al. [6]. These authors demonstrated that a combination of hematopoeitic growth factors, including interleukins 1, 3 and 6 (IL-1, IL-3, IL-6), granulocyte macrophage colony stimulating factor (GM-CSF) and stem cell factor (SCF) could generate a 1324-fold increase in nucleated cells and a 66-fold increase in GM-CFC. The cells produced under these conditions were predominantly neutrophil precursors. The culture conditions used were static cultures and utilized CD34+ cells from mobilized PBPC products as the starting population. Several Investigators have demonstrated the requirement for CD34 selection of the starting cells for optimal expansion [7,8]. Subsequent studies were performed at a clinical scale using optimal culture conditions in teflon bags and with fully defined media appropriate for clinical applications [8]. This work utilized the growth factor cocktail comprising of SCF, G-CSF and MGDF. Other cocktails of growth factors are effective in expanding CD34+ cells, however the availability of clinical grade growth factors has been limited due to a reticence of companies to manufacture them due to cost.

3. Ex vivo expanded cells provide faster neutrophil engraftment

3.1. Ex vivo expansion of PBPC cells

Studies from three groups using similar culture conditions have reported more rapid neutrophil recovery and engraftment with ex vivo expanded PBPC [9–11]. Neutrophils are considered to have engrafted when counts reach a sustainable level of 500/μl, post medical intervention. Two groups cultured CD34+ cells in media supplemented with the growth factors rhSCF, rhG-CSF and rhMGDF at 100 ng/ml for 10 days in teflon bags (American Fluoroceal) [9,11], while the third group cultured a mononuclear fraction in the same media and growth factors [10].

Reiffers et al. [9] reported the results of a phase I/II study in myeloma patients ($N = 14$) using ex vivo expanded PBPC. A median of 4.1×10^6 CD34+ cells were expanded for 10 days after which time the cells were washed and reinfused into patients. Unexpanded PBPC were also transplanted into patients on day +1 and the patients received rhG-CSF until neutrophil engraftment was achieved. The post-transplant neutropenia was