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Ex vivo expansion of mafosfamide-purged PBPC products

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Background

Multiple studies have demonstrated that 'purging' of autografts with 4-hydroperoxycyclophosphamide (4HC) or the related compound mafosfamide (Mf), to eradicate residual leukemia, produces the best results associated with autologous blood and marrow transplantation for AML. However, 4HC purging results in prolonged aplasia. Therefore, we evaluated the potential of ex vivo *expansion of Mf-treated CD34$^+$ cells from mobilized PBPC.*

Methods

CD34$^+$ cells were isolated from PBPC products and treated with 30 μg/mL Mf. The Mf-treated CD34$^+$ cells were washed and cultured for 14 days in StemLine II-defined media containing recombinant human (rh) SCF, G-CSF and thrombopoietin (Tpo).

Results

Treatment with Mf resulted in 90% killing of progenitor cells (GM-CFC) but maintenance of SCID-repopulating cells (SRC). Ex vivo *culture of the Mf-treated CD34$^+$ cells resulted in decreased cell numbers (10–20% of the starting cell dose) during the first week. Nevertheless, in the second week of culture the total cell numbers expanded to approximately 20-fold above starting cell numbers and progenitor cells returned to approximately pre-treatment levels.*

Discussion

These studies demonstrate the potential of ex vivo *culture to expand both total cell numbers and progenitor cells following treatment of PBPC CD34$^+$ cells with Mf. Clinical studies are currently being initiated to evaluate the engraftment potential of these purged and expanded products.*

Keywords

ex vivo *expansion, leukemia, purging.*

Introduction

Many patients with AML who are treated with ABMT relapse, in part because of residual disease in the graft. A considerable body of evidence from both *in vitro* and clinical studies suggests that autograft purging with derivatives of the cytotoxic agent CY, namely 4-hydroperoxycyclophosphamide (4HC) or mafosfamide (Mf), results in a substantial (4–6 log) reduction of leukemic cells [1–8]. Comparative studies from registries suggest that patients with AML who receive 4HC- or Mf-purged autografts have a lower relapse rate and better survival than patients receiving unpurged autografts [3,4]. Normal long-term reconstituting hematopoietic stem cells (HSC) appear to be more resistant to killing by CY and its derivatives than leukemic cells, even when high doses of the drugs are used [9,10]. This is in part mediated by increased intracellular levels of aldehyde dehydrogenase (ALDH), an enzyme that is involved in the breakdown of the toxic metabolites of CY [11–14].

A major drawback to autograft purging with 4HC or Mf is that it delays engraftment by 1–3 weeks. Although HSC responsible for long-term engraftment appear resistant to 4HC and Mf, because of high levels of ALDH, hematopoietic progenitors responsible for rapid early engraftment

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are sensitive because they do not express high levels of ALDH [12–14]. However, this delay in engraftment observed with the infusion of 4HC-treated BM grafts has not resulted in an increase in graft failure or other transplant-related mortality [3,4].

Several clinical studies have demonstrated the potential of *ex vivo* expansion of autologous PBPC products to shorten the time to neutrophil recovery following high-dose chemotherapy [15–18]. We propose that *ex vivo* expansion of 4HC- or Mf-purged hematopoietic grafts could shorten the period of aplasia and thereby decrease the length of hospitalization and number of blood product transfusions. In this study we present pre-clinical data demonstrating the expansion of Mf-treated $CD34^+$ cells and define a clinically relevant *ex vivo* expansion culture methodology.

Methods

PBPC products

PBPC products comprised excess cells that were to be discarded after 20×10^6 $CD34^+$ cells/kg had been processed for autologous transplantation. All patients gave informed consent for research use of these products under a Johns Hopkins Institutional Review Board-approved protocol.

$CD34^+$ cell selection, Mf treatment and *ex vivo* expansion

PBPC products were selected for $CD34^+$ cells using a SuperMACS (Miltenyi Biotech, Cologne, Germany) selection device according to the manufacturer's recommended procedure. Selection resulted in products with 85% or greater CD34 purity. The $CD34^+$ cells were pelleted and resuspended in PBS^+ 1% HSA to a concentration of 5×10^6 cells/mL and Mf (Baxter, Oncology GmbH, Halle, Germany) added to give a final concentration of 10–40 μg/mL. The cells were incubated for 30 min at 37°C, then chilled PBS plus 1% HSA added. The cells were washed three times with PBS plus 1% HSA and then set up in *ex vivo* expansion culture. Initial experiments were performed in 100-mL Teflon culture bags [American Fluoroseal Corp. (AFC), Gaithersburg, MD, USA] containing 25 mL StemLine™ II hematopoietic expansion media (Sigma Aldrich, St Louis, MO, USA) and 100 ng/mL each of recombinant human (rh)SCF (Amgen Inc., Thousand Oaks, CA, USA), rhG-CSF (Amgen Inc.) and rh thrombopoietin (Tpo) (Kirin Breweries, Tokyo, Japan). The bags were incubated at 37°C in 5% CO_2 for 7 days and then an additional 25 mL of media and growth factors added and the cultures incubated for a further 7 days. Subsequent large-scale cultures were performed in 1-L Teflon bags (AFC) seeded with 80×10^6 cells at 200 000 cells/mL on day 0 and containing 400 mL of StemLine™ II media plus rhSCF, rhG-CSF and rhTpo (100 ng/mL). After 7 days of culture an additional 400 mL of media and growth factors were added.

Functional assays

Several assays were performed to evaluate the levels of mature cells (cells counts and flow analysis), mature progenitor cells (GM-CFC), primitive progenitor cells (HPP-CFC) and stem cells (NOD/SCID engraftment).

Progenitor cell assays

Cells were plated in 1-mL methylcellulose-based assays in rhSCF, rhIL-3, rhIL-6, rhG-CSF and rhGM-CSF (Stem Cell Technologies Inc., Vancouver, Canada). $CD34^+$ cells and Mf-treated $CD34^+$ cells were plated at 2000 and 500 cells/plate, while 14-day expanded cells were plated at 100 000 and 10 000 cells/plate. Cultures were incubated for 14 days and GM-CFC colonies identified as colonies of 50 or more translucent cells using a dissecting microscope at 20× magnification. After scoring at day 14, the cultures were incubated for an additional 14 days (total of 28 days incubation) and scored for HPP-CFC, defined as compact large colonies (diameter > 0.5mm) that contained more than 50 000 cells/colony.

Flow cytometric analysis

Cells were analyzed for phenotypic expression of surface proteins specific for subpopulations of hematopoietic cells. Cells were stained with MAb conjugated to either PE or FITC, and included anti-CD34–PE and anti-CD45–FITC (Becton Dickinson, San Jose, CA, USA). Approximately 1.0×10^6 cells prior to culture or cells were removed during expansion culture, washed once in PBS/1% HSA, conjugated Ab added, and the cells were incubated a 4°C in the dark for 20 min. Following one wash with PBS/1% HSA, cells were analyzed by flow cytometry (FACS Calibur; Becton Dickinson). Non-specific binding was determined by staining an aliquot of cells with an isotype control for both the PE and FITC fluorescence. A minimum of 50 000 cells was collected in a listmode file format.