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Ex vivo virotherapy with myxoma virus does not impair hematopoietic stem and progenitor cells

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Abstract

Background. Relapsing disease is a major challenge after hematopoietic cell transplantation for hematological malignancies. Myxoma virus (MYXV) is an oncolytic virus that can target and eliminate contaminating cancer cells from auto-transplant grafts. The aims of this study were to examine the impact of MYXV on normal hematopoietic stem and progenitor cells and define the optimal treatment conditions for *ex vivo* virotherapy. *Methods.* Bone marrow (BM) and mobilized peripheral blood stem cells (mPBSCs) from patients with hematologic malignancies were treated with MYXV at various time, temperature and incubation media conditions. Treated BM cells from healthy normal donors were evaluated using flow cytometry for MYXV infection, long-term culture-initiating cell (LTC-IC) assay and colony-forming cell (CFC) assay. *Results.* MYXV initiated infection in up to 45% of antigen-presenting monocytes, B cells and natural killer cells; however, these infections were uniformly aborted in >95% of all cells. Fresh graft sources showed higher levels of MYXV infection initiation than cryopreserved specimens, but in all cases less than 10% of $CD34^+$ cells could be infected after *ex vivo* MYXV treatment. MYXV did not impair LTC-IC colony numbers compared with mock treatment. CFC colony types and numbers were also not impaired by MYXV treatment. MYXV incubation time, temperature or culture media did not significantly change the percentage of infected cells, LTC-IC colony formation or CFC colony formation. *Conclusions.* Human hematopoietic cells are non-permissive for MYXV. Human hematopoietic stem and progenitor cells were not infected and thus unaffected by MYXV *ex vivo* treatment.

Key Words: *bone marrow, mobilized peripheral stem cell blood, myxoma virus, purging, virotherapy*

Introduction

A major challenge in treating patients with hematologic malignancies, such as multiple myeloma, is disease relapse even despite high-dose chemotherapy and autologous stem cell transplantation (ASCT) [1–3]. Relapse disease occurs due to persistence of minimal residual disease from two potential sources [1]: contaminating cancer cells in the ASCT graft and [2] protective niches in the transplant recipient that resist conditioning chemotherapy [4–7]. To address the first of these, an *ex vivo* strategy that selectively removes or "purges" any contaminating cancer cells prior to transplantation may decrease the rate of relapse observed clinically.

Ideal purging strategies should specifically target contaminating cancer cells in the ASCT graft yet spare the normal hematopoietic stem and progenitor cells (HSPCs), which are required for reconstitution of hematopoiesis. In addition, the ideal purging strategy should be applied quickly, so that the transplant process is not unduly delayed.

Oncolytic viruses may meet the criteria as ideal purging agents for ASCT [8]. We have demonstrated that myxoma virus (MYXV), an oncolytic poxvirus, selectively targets human leukemia, myeloma and lymphoma cells while sparing normal HSPCs [6,9,10].

Although MYXV selectively purged human cancer cells while sparing normal human hematopoietic cells in animal models of disease, the preclinical safety of MYXV with respect to HSPC must be critically examined before proceeding to the transplantation clinic.

Methods

Cell sources

The University of Florida Institutional Review Board (IRB) approved this study. HSPCs were collected by bone marrow aspiration and granulocyte colony-stimulating factor (G-CSF) mobilization from patients

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with hematologic malignancies. Whole bone marrow (BM) and mobilized peripheral blood stem cells (mPBSCs) from patients with hematologic malignancies were obtained from patients treated at UF Health Shands Hospital under UF IRB-01 approval. BM from healthy donors was obtained from Lonza. Peripheral blood mononuclear cells (PBMCs) were obtained from healthy donors (LifeSouth). Cryopreserved cells were thawed according to the protocol reported by Fry et al. [11], with some modifications. Briefly, aliquots of each cryopreserved graft source were thawed rapidly in a water bath at 37°C. Three times volume of 1X-phosphate-buffered saline (PBS) supplemented 20% fetal bovine serum (FBS) was added dropwise, mixed gently and followed by centrifugation at 1000 RPM for 5 min. Supernatants were carefully aspirated and cell pellets were re-suspended in the indicated growth media.

Colony assays

For the colony-forming cell (CFC) assay, mononuclear cells (MNCs) derived from healthy BM samples (n = 3) were incubated with vMyx-green fluorescent protein (GFP) [12] at multiplicity of infection (MOI) of 10 for 3 h at different temperatures, including 4°C, room temperature (20°C–25°C) and 37°C, and different media such as Plasma-lyte A + 10% anticoagulant citrate dextrose solution A (ACDA), Plasma-lyte A + 10% heparin sodium (Wockhardt) and M199 (Gibco) + 15% heparin sodium (Wockhardt). Cells were then assayed for normal hematopoietic progenitor cell differentiation using an *in vitro* methylcellulose-based CFC assay (MethoCult H4435 Enriched, StemCell Technologies), as per manufacturer's instructions.

For long-term culture-initiating cell (LTC-IC) assay, fresh or post-thaw BM samples (n = 3) were mock-treated or vMyx-GFP-treated (MOI of 10) at room temperature (20°C–25°C) for 1–3 h. LTC-IC assay (Stem Cell Technologies) was performed according to manufacturer's instructions. In brief, cells were seeded onto established stromal cell feeders of irradiated (8000 cGy) human fibroblasts (M2-10B4, StemCell Technologies). Mock-treated BM cells were used as positive controls. Mock- or MYXV-treated cells were suspended in triplicate in myeloid long-term culture medium for primitive human hematopoietic cells (MyeloCult H5100; StemCell Technologies) containing 1×10^6 mol/L freshly dissolved hydrocortisone (in alpha modification of eagle's medium (aMEM) according to manufacturer's protocol; StemCell Technologies). Following 6 weeks of culture at 37°C with weekly half media exchanges, the contents of each well were harvested and plated into methycellulose (MethoCult GF+ H4435; StemCell Technologies). The hematopoietic stem cell content was determined by enumerating the number and variety of colonies present in the methylcellulose at 14 days of culture.

MYXV treatment and culture conditions of hematopoietic graft sources

BM or mPBSCs suspended in plain Iscove's Modified Dulbecco's (IMDM) medium or Plasma-lyte A supplemented with ACDA were incubated with vMyx-GFP at MOI of 10 for 1 h at room temperature to allow virus adsorption. An MOI of 10 was selected to ensure that hematopoietic cells would be exposed to an abundance of virus for infection. After this, cells were incubated for either 2 or 24 h at 37°C 5% CO_2 to allow for virus infection. For both time points, cells were suspended in either complete IMDM (supplemented with 10% fetal bovine serum [FBS], 2 mmol/L L-glucosamine and 100 U/mL penicillin-streptomycin) or Plasma-lyte A + 10% ACDA for 2 h. Mock-treated cells were used as controls and subjected to the same incubation conditions as the MYXV-treated cells.

Immunophenotyping analysis using flow cytometry

To determine the levels of MYXV infection of different lineage subsets, 1×10^6 cells were suspended in 100 μL of staining buffer followed by staining with monoclonal antibodies coupled with allophycocyanin fluorochrome (APC) or with phycoerythrin fluorochrome (PE; BD Biosciences) and against HLA-ABC, CD45, CD34 CD14, CD19, CD3, CD15, CD235a. The 7-Aminoactinomycin D (7-AAD) viability dye (Life Technologies) was used to discriminate live from dead cells. Cells were incubated with the antibodies for 1 h at 4°C, washed and fixed with paraformaldehyde. Unless otherwise indicated, all cell frequencies and the percentages of MYXV infection (GFP^+) refer to 7AAD-negative (viable) cells only. Immunophenotyping analysis was performed using flow

Figure 1. MYXV treatment of fresh and cryopreserved BM in various incubation conditions. MYXV treatment of fresh or cryopreserved BM samples from patients with hematologic malignancies was performed using a vMyx-GFP construct (MOI of 10) at 37°C. Incubation was carried out in either IMDM or Plasma-lyte A + 10% ACDA culture media for 2 h or 24 h. At the indicated time points, cells were collected and stained with monoclonal antibodies against cell lineages including CD45, HLA-ABC, CD19, CD3, CD34, CD14, CD15 and CD235a. Virus infection of these cell lineages was quantified using flow cytometry. (A and B) Representative two-dimensional plots showing the percentage of MYXV infection (GFP^+) for 2 h in IMDM and in Plasma-lyte A + 10% ACDA, respectively. (C–J) The percentage of MYXV infection (GFP^+) of fresh versus cryopreserved BM samples, for 2 h or 24 h, in IMDM or in Plasma-lyte A + 10% ACDA are shown. Values are mean ± SEM of at least four independent experiments with three independent specimens.