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Bone morphogenetic protein (BMP)-7 but not BMP-2 and BMP-4 improves maintenance of primitive peripheral blood-derived hematopoietic progenitor cells (HPC) cultured in serum-free medium supplemented with early acting cytokines

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Abstract

BMPs regulate the developmental program of hematopoiesis. We demonstrate an increased expression of the BMP receptors Ia and II on cultured $CD34^+$ cells and examine the impact of BMP-2, -4 and -7 on postnatal HPC cultured with stem cell factor, flt3-ligand and interleukin-3 (SF3). The addition of BMP-2 at 5, 25 and 50 ng/m to serum-free medium with SF3 yielded a 1.4- to 1.2-fold increase of $CD34^+$ cells after seven days, but no effect on CFC or LTC-IC was observed. BMP-4 at 25 ng/ml induced a 2.9-fold expansion of colony-forming cells (CFC) within 1 week followed by a decrease to pre-culture values on day 14. The number of long-term culture initiating cells (LTC-IC) decreased by the factor 40 from day 0 to day 14. BMP-7 at 5–50 ng/ml had not effect on the expansion of $CD34^+$ cells and CFC, but improved at 5 ng/ml the survival of LTC-IC significantly as compared to SF3 alone. In summary, BMP-2, -4 and -7 have no effect on the proliferation of $CD34^+$ cells and CFC cultured with serum-free medium and SF3. However, BMP-7 but not BMP-2 and BMP-4 prevents the loss of primitive hematopoietic progenitor cells cultured in SFM plus SF3.

Keywords: Hematopoiesis; Hematopoietic progenitor cells; Bone morphogenetic protein; Cell differentiation; Cell proliferation

1. Introduction

Bone morphogenetic proteins belong to the transforming growth factor beta superfamily and influence multiple organ systems like bone, neural and renal tissue during different developmental stages [1,2]. Recent studies indicate that BMPs also regulate the developmental program of human hematopoietic stem cells [3–5]: zebra fish BMP-2 and BMP-7 mutants have shown a lack of ventral mesoderm development and a complete absence of blood cells [6], and during embryogenesis, BMP-4 induces formation of the hematopoietic mesoderm [7]. The role of BMPs in the control of the differentiation of postnatal hematopoietic progenitor cells has not been extensively explored. The type I BMP receptors activin-like kinase (ALK)-3 and ALK-6 are expressed in primitive lineage-depleted (lin^-) $CD34^+/CD38^-$ cells isolated from human cord blood (CB) and bone marrow (BM) as well as their downstream transducer SMAD-1 [4]. BMP-2 when implanted subcutaneously induces a hematopoietic environment including clonogenic precursors of both erythroid and myeloid lineages and progenitors competent to regenerate splenic lymphopoiesis [8]. Treatment of purified CB-derived $CD34^+/CD38^-$ with BMP-2 or -7 at high concentrations inhibited proliferation yet maintained the primitive $CD34^+/38^-$ phenotype. In contrast, low concentrations of BMP-4-induced proliferation and differentiation of $CD34^+/CD38^-$ cells, whereas at higher concentrations BMP-4 extended the

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length of time that repopulation capacity could be maintained in ex-vivo culture [4]. A second study demonstrated that BMP-4 in conjunction with granulocyte–macrophage colony-stimulating factor and erythropoietin increased the number of both erythroid (CFC-E) and granulocyte/macrophage colonies (CFC-GM) in cultures of BM-derived $CD34^+$ cells [9]. A third study found no effect of BMP-4 on the number of CFU-E formed in vitro by BM-derived $CD34^+$ cells [10]. Thus, functional data on the successful ex-vivo expansion of postnatal hematopoietic progenitor cell are scarce and data on peripheral blood-derived cells are lacking.

In our experiments, we sought to investigate the impact of BMP-2, BMP-4 and BMP-7 alone and in combination on the ex-vivo expansion and the functional properties of lin^- hematopoietic progenitor cells obtained from the peripheral blood of granulocyte colony-stimulating factor (G-CSF) treated healthy donors.

First, the presence of the BMP-Ia and the BMP-II receptor on the target cells was confirmed by polymerase chain reaction (PCR) of RNA from sorted $CD34^+$ cells. Then, the maximum proliferation of $CD34^+$ cells stimulated with BMP-2, or BMP-7 at different concentrations in addition to SFM supplemented with stem cell factor (SCF), flt3-ligand (FL) and interleukin (IL)-3 was determined in a bulk culture. To assess the functional potential of the cells before and after 7 days of culture, we used the formation of colonies of hematopoietic cells in semisolid media and the long-term culture on murine stroma cells to detect and enumerate early human hematopoietic progenitor cells referred to as LTC-IC [11].

2. Materials and methods

2.1. Progenitor cell preparation

Human HPC were aliquots of leukapheresis products obtained from normal individuals donating G-CSF mobilized $CD34^+$ cells for allogeneic transplantation. Approved institutional procedures including written informed consent from each donor were followed. The light density (<1.077 g/cm^3) cells were isolated from the samples by centrifugation on Ficoll–Hypaque (Pharmacia, Uppsala, Sweden) and then either cryopreserved in fetal calf serum (FCS) with 10% DMSO (Sigma) at −180 °C or immediately used. Cells from 16 individual donors were thawed as needed, and cell populations expressing mature erythroid, granulopoietic, megakaryopoietic and lymphoid markers were removed using a StemSep™ column (StemCell Technologies Inc., Vancouver, BC) resulting in a lin^- cell fraction comprising $84 \pm 10\%$ $CD34^+$ cells (mean ± standard error of the mean; median 89%, range 63–96%).

2.2. Progenitor cell assays

CFC assays were performed in methylcellulose cultures (MethoCult™ GF H4434, Stem Cell Technologies, Vancouver, BC, Canada) as described [12]. Briefly, CFU-E, CFU-GM and colony-forming units granulocyte–erythrocyte–monocyte–macrophage (CFU-GEMM) were assayed by plating the cells in Iscove's modified Dulbecco's medium containing 1% methylcellulose, 30% FCS, 1% bovine serum albumin, 3 U/ml recombinant human erythropoietin, 10^{-4} M 2-mercaptoethanol, 2 mM L-glutamine and additionally 50 ng/ml SCF, 10 ng/ml, GM-CSF and 10 ng/ml IL-3 (MethoCultTM GF H4434, StemCell Technologies). After 2 weeks, typical colonies were visually scored using an inverted microscope, and the frequency of CFU was calculated. LTC-IC were assayed using a 6 week CFC endpoint after maintenance of the cultures on murine fibroblast feeder cells engineered to produce the human cytokines SCF, G-CSF and IL-3 [11]. To determine the frequency of LTC-IC, the cells were plated under limiting dilution conditions in 96-well plates. After 6 weeks with weekly half medium changes, the cells were plated in methylcellulose using one individual 35 mm Petri dish for each well and assessed for their CFC content. Wells that contained at least one CFC were scored positive, and the frequency of LTC-IC was calculated using the L-calc® program (StemCell Technologies).

2.3. Flow cytometric analysis and cell sorting

For flow cytometric analysis, lin^- cells were incubated with anti-human CD34-APC (8G12, Becton–Dickinson, St. José, CA, USA), CD38-PE (HB-7, Becton–Dickinson) and CD45-FITC (Dianova, Hants, UK) washed twice with phosphate-buffered saline (PBS, GIBCO BRL, Invitrogen GmbH, Paisley, UK), and resuspended in PBS containing propidium iodine (Sigma, 0.5 μg/ml) to allow for dead cell discrimination. Sample analysis was performed using a FACS Calibur (Becton–Dickinson). Cell sorting was done on a FACStarPlus (Becton–Dickinson) equipped with two lasers (488 nm, UV), an automatic cell deposition unit and a sort enhancement module. Region and gates were defined in the FL-1–FL-3 and FL1–FL-2 fluorescence dot blot diagrams defining viable $CD34^+$-APC/$CD45^+$-FITC events for cell sorting.

2.4. Cell culture conditions and cytokine stimulation

After immunomagnetic enrichment of $CD34^+$ cells an aliquot of the cells was stained for flow cytometry. The remaining cells were plated in serum-free medium (CellGroTM SCGM Bio Whittaker Europe, Verviers, Belgium) supplemented with SCF (100 ng/ml; Pepro Tech, Northampton, UK), FL (100 ng/ml, Pepro Tech) and IL-3 (20 ng/ml, Pepro Tech) into Petri dishes (Greiner Bio-One Inc., Longwood, USA) at 10^5 cells per ml. BMP-4 (25 ng/ml; R&D Systems, Wiesbaden, Germany), BMP-2 or BMP-7 (both at 5, 25 or 50 ng/ml; R&D Systems) were added as required. After 7 and 14 days, the cells were harvested, counted and analyzed by flow cytometry and CFC or LTC-IC assays. In order to minimize a possible transfer