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The regulatory role of Hyper-IL-6 in the differentiation of myeloid and erythroid progenitors derived from human cord blood

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Abstract

This study was designed to investigate the regulatory role of soluble interleukin-6 receptor (sIL-6R) and interleukin-6 (IL-6) fusion protein (Hyper-IL-6) in the differentiation of human myeloid and erythroid progenitors by a serum-free liquid suspension culture system, using the human cord blood-derived $CD34^{+}CD38^{-}$ cells as a target. We found that Hyper-IL-6 promoted the generation of $CD15^{+}$ granulocytic and $CD14^{+}$ monocytic cells and suppressed that of $CD14^{-}CD1a^{+}$ dendritic cells from $CD36^{-}CD15^{-}CD14^{-}CD1a^{-}IL\text{-}6R^{+}$ myeloid progenitors. Conversely, $CD34^{+}CD38^{-}$ cell-derived early erythroid progenitors were negative for IL-6R expression. Hyper-IL-6 potentiated the generation of $CD36^{+}glycophorinA^{high}$ mature erythroid cells from the $IL\text{-}6R^{-}$ early erythroid progenitors. Our results indicate that Hyper-IL-6 augments the generation of $CD15^{+}$ granulocytic, $CD14^{+}$ monocytic and $CD36^{+}glycophorinA^{high}$ cell and suppresses that of $CD14^{-}CD1a^{+}$ dendritic cells.
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1. Introduction

Interleukin-6 (IL-6) is a pleiotropic cytokine with a broad range of biologic activities, such as support of hematopoiesis and regulation of immune responses [1–3]. IL-6 executes its biological activities by binding not only to the membrane-bound IL-6 receptor but also to the soluble IL-6 receptor (sIL-6R) and forms a sIL-6R and IL-6 complex [4]. The sIL-6R/IL-6 complex then associates with the gp130 molecule and consequently initiates the gp130 signaling in both $IL\text{-}6R^{+}$ and $IL\text{-}6R^{-}$ hematopoietic progenitors [5–9]. Since the gp130, a common signal transducer for IL-6 family of cytokines, is ubiquitously expressed in hematopoietic cells [10] and the expression of membrane-bound IL-6 receptor (mIL-6R), a binding subunit of IL-6R, in human $CD34^{+}$ cells appear predominantly restricted to myeloid progenitors [6,11], the sIL-6R and IL-6 complex plays an important role that dominant over the IL-6 in the regulation of human hematopoiesis [12].

The present study was designed to examine the role of sIL-6R/IL-6 complex in the regulation of human hematopoiesis. Specifically, we determined whether sIL-6R/IL-6 complex influences the differentiation of myeloid and erythroid progenitors. A recombinant sIL-6R/IL-6 fusion protein, Hyper-IL-6, which was engineered to efficiently stimulate gp130 was employed as the substitute of sIL-6R/IL-6 complex in this study [13]. It had been reported that Hyper-IL-6, a fusion protein of sIL-6R and IL-6 linked by a flexible peptide chain, turned out to be fully active at lower concentration than the combination of unlinked IL-6 and IL-6R because of its lower off-rate in vivo [14]. Here we report that Hyper-IL-6 enhanced the generation of granulocytic and monocytic cell from $IL\text{-}6R^{+}$ myeloid progenitors and that of mature erythroid cells from $IL\text{-}6R^{-}$ early erythroid progenitors,and counteracted the generation of dendritic cells from myeloid progenitors. These data

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provide some new information about the role of sIL-6R/IL-6 complex in the regulation of human hematopoiesis.

2. Materials and methods

2.1. Isolation of $CD34^{+}CD38^{-}$ cells

Human cord blood samples were obtained at normal full-term delivery according to institutional guidelines after informed consent. Isolation of $CD34^{+}CD38^{-}$ cells were performed as followings: Briefly, mononuclear cells were enriched for $CD34^{+}$ cells using CD34 immunomagnetic beads (MACS; Miltenyi Biotec, Auburn, CA, USA), according to the instructions supplied by the manufacturer. After staining the enriched cells with fluorescein isothiocyanate (FITC)-conjugated anti-CD38 antibody and phycoerythrin (PE)-conjugated anti-CD34 antibody (Becton Dickinson, San Jose, CA, USA), the $CD34^{+}CD38^{-}$ cells were sorted on a FACSVantage (Becton Dickinson Immunocytometry Systems, Mountain View, CA, USA).

2.2. Recombinant factors

Recombinant human stem cell factor (SCF), thrombopoietin (TPO), IL-3, granulocyte–macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), erythropoietin (EPO), and IL-6 were purchased from R&D Systems (Minneapolis, MN, USA). Hyper-IL-6 was a gift from Dr. Ohishi (Mie University Graduate School of Medicine, Tsu, Mie, Japan) (Hyper-IL-6 was genetically engineered by linking the Ala (333) of IL-6R residues with Ala of IL-6, expressed in the Pichia pastoris and purified from the yeast supernatants by anion-exchange chromatography) [14]. Recombinant flt3 ligand (Flt3L) was purchased from R&D Systems (Minneapolis, MN, USA). Cytokines were used at the following concentrations: SCF, 50 ng/ml; TPO, 20 ng/ml; IL-3, 10 ng/ml; GM-CSF, 10 ng/ml; G-CSF, 10 ng/ml; EPO, 2 U/ml; IL-6, 10 ng/ml; Hyper-IL-6, 100 ng/ml; and Flt3L, 50 ng/ml.

2.3. Cell culture

Suspension cultures were performed using serum-free medium (StemSpan; Stem Cell Technologies, Vancouver, BC, Canada) containing 50 U/ml penicillin, 50 μg/ml streptomycin, and designated cytokines.Viable cell numbers were counted by trypan blue dye exclusion methods.

For semisolid colony assay, cells were plated in 35-mm culture dishes containing 1 ml of α modification of Eagle's medium (β-MEM; ICN Biomedicals, Aurora, OH, USA) supplemented with 30% fetal bovine serum (HyClone Laboratories, Logan, UT, USA), 1% deionized fraction V bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO, USA), 1.3% methylcellulose (Shinetsu Kagaku, Tokyo, Japan), 2 mM L-glutamine (Gibco-BRL, Gaithersburg, MD, USA), 50 U/ml penicillin, 50 μg/ml streptomycin, 5×10^{-5} M 2-mercaptoethanol (2-ME; Sigma-Aldrich), SCF, Flt3L, TPO, GM-CSF, G-CSF, and EPO. Colonies were scored by in situ observation of the plates on an inverted microscope. On day 14 of culture, mixed colonies, erythroid bursts, and colonies consisting of granulocytes and/or macrophages were scored as CFU-Mix, BFU-E, and CFU-GM, respectively. The number of CFU-E was counted on day 7.

2.4. Flow cytometric analysis

Immunofluorescent staining was performed, according to the instructions supplied by the manufacturer of the monoclonal antibodies. The following murine monoclonal antibodies (mAbs) were used: anti-CD14-FITC, anti-HLA-DR-FITC, anti-CD11c-PE, anti-CD14-PE, anti-glycophorin (GPA)-PE, anti-CD14- allophyocyanin (APC) (Becton Dickinson); anti-CD15-FITC, anti-CD36-FITC, anti-CD40-FITC, anti-CD80-FITC, anti-CD86-FITC, anti-CD11b-PE, anti-GPA-APC, biotinylated anti-IL-6R (BD Pharmingen); anti-CD1a-FITC, and anti-CD1a-PE (Coulter, Miami, FL, USA). Streptavidin–PE conjugate (BD Pharmingen) was used as a secondary antibody. Mouse IgG_1-FITC, IgG_{2a}-FITC, IgG_1-PE (Becton Dickinson), IgM-FITC, IgG_{2b}-FITC, IgG_{2b}-PE, or IgG_{2b}-APC (BD Pharmingen) served as an isotype control. IL-6R expression was analyzed by staining with biotinylated anti-IL-6R mAb and designated mAbs, followed by streptavidin-PE conjugate. Flow cytometric analysis was performed using FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems). Dead cells were excluded by propidium iodide or 7-amino-actinomycin-D (BD Pharmingen) staining.

3. Results

3.1. Hyper-IL-6 promotes the generation of $CD15^{+}$ granulocytic and $CD14^{+}$ monocytic cells and suppressed the differentiation of $CD14^{-}CD1a^{+}$ myeloid dendritic cells

In the first set of experiments, we investigated whether IL-6R is expressed on myeloid-committed progenitors. $CD34^{+}CD38^{-}$ cells were incubated for 7 days with SCF, Flt3L, TPO, and IL-3 (4GFs) and then cultured cells were immunomagnetically depleted of $CD36^{+}$ erythroid cells. The resultant cells were analyzed for the expression of IL-6R versus CD15, CD14, or CD1a. While almost all cells positive for CD15, CD14, or CD1a expressed IL-6R, the cell fraction lacking the expression of CD15, CD14, and CD1a could be divided into $IL\text{-}6R^{+}$ and $IL\text{-}6R^{-}$ cell populations (Fig. 1a, left column). Since progenitor cells are supposed to be negative for CD15, CD14, and CD1a, we sorted the $CD15^{-}CD14^{-}CD1a^{-}IL\text{-}6R^{+}$ and $CD15^{-}CD14^{-}CD1a^{-}IL\text{-}6R^{-}$ cell fractions, plated them in semisolid cultures supported by 4GFs, GM-CSF, G-CSF, and EPO, and determined the type of colonies detected in cultures of each cell fraction (Fig. 1a, right column). The $IL\text{-}6R^{+}$ cell fraction gave rise exclusively to CFU-GM. The majority of col-

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