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Research paper

Human monocytes differentiate into macrophages under the influence of human KPB-M15 conditioned medium

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Abstract

Culture medium conditioned by the L929 murine fibroblast cell line contains macrophage colony-stimulating factor (M-CSF), providing an alternative to recombinant M-CSF for in vitro generation of murine macrophages. No such alternative has been described for in vitro studies requiring human macrophages. We tested the differentiation of human blood monocytes into mature macrophages by culturing in media conditioned by the human KPB-M15 cell line, which produces M-CSF and interleukin 6 (IL-6). The phenotypes of macrophages cultured in KPB-M15 conditioned media and recombinant M-CSF were compared by examining viability, expression of cell surface markers, phagocytic/pinocytic activity, and cytokine/chemokine secretion in response to bacterial lipopolysaccharide (LPS). In conditioned medium, monocytes differentiated into a homogeneous population of large cells that exhibited higher expression of CD14 and the macrophage mannose receptor (CD206) than did M-CSF-cultured cells. Cells matured in KPB-M15 conditioned medium exhibited macrophage morphology, were phagocytic, and were activated in response to LPS. These data demonstrate that KPB-M15 conditioned medium can be used to differentiate human blood monocytes into macrophages in vitro.

Keywords: KPB-M15 conditioned medium; Human monocyte differentiation; Human macrophages

Abbreviations: BDCA, blood dendritic cell antibody; CFU, colony forming unit; CM, conditioned medium; DC, dendritic cells; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; FITC, fluorescein isothiocyanate; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN-γ, interferon-gamma; IL, interleukin; LPS, lipopolysaccharide; M-CSF, macrophage colony-stimulating factor; MFU, mean fluorescence units; MR (CD206), mannose receptor; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PE, phycoerythrin; PerCP, peridinin chlorophyll protein; RH, recombinant human; SCGF, stem cell growth factor; SD, standard deviation; SEM, standard error of the mean; Na-azide, sodium azide; TNF-α, tumor necrosis factor-alpha; CXCL1 (GRO-α), growth related oncogene; CCL5 (RANTES), regulated on activation normal T cell expressed and secreted; CCL3 (MIP-1α), macrophage inflammatory protein-1 alpha; CCL2 (MCP-1), macrophage chemoattractant protein-1.

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1. Introduction

Blood monocytes represent a large pool of circulating precursor cells that can differentiate into macrophages or dendritic cells, cells that are differentially specialized to capture (and kill) microbes, and to present antigens to induce and modulate immune responses (Erickson-Miller et al., 1990; Steinman, 1991). Differentiation of monocytes to macrophages is induced by macrophage colony-stimulating factor (M-CSF), a heavily glycosylated, disulfide-linked homodimer produced by monocytes, macrophages, fibroblasts and endothelial cells (Geissler et al., 1989; Erickson-Miller et al., 1990).

M-CSF was cloned and produced as a recombinant protein in 1985, enabling culture of human monocytes to macrophages in vitro (Kawasaki et al., 1985). Recombinant human M-CSF (rhM-CSF) differentiates monocytes into CD14+ macrophages, while granulocyte-macrophage colony-stimulating factor (GM-CSF) with interleukin-4 differentiates monocytes into DCs (Romani et al., 1994; Sallusto and Lanzavecchia, 1994). Recent studies have shown that optimal rhM-CSF-induced differentiation involves the autocrine activity of secreted interleukin 6 (IL-6), which upregulates the expression of functional M-CSF receptors on monocytes and enhances macrophage cytotoxicity, superoxide production, phagocytosis, chemotaxis, and secondary cytokine secretion (Akira, 1997). The interplay between IL-6 and M-CSF regulates monocyte differentiation into macrophages and inhibits DC differentiation from GM-CSF/IL-4-treated monocytes (Mitani et al., 2000).

The human myeloid cell line KPB-M15, which was originally established from a patient with chronic myeloid leukemia in blast crisis, constitutively secretes high concentrations of stem cell growth factor (SCGF), M-CSF and IL-6 (Kamamoto et al., 1986; Hiraoka et al., 1987, 1989, 1997, 1998). Previous studies showed that this cell line does not produce IL-1α, IL1-β, IL-3, IL-4, G-CSF or GM-CSF, nor does it express mRNA for IL-11, leukemia inhibitory factor, stem cell factor or *flk-2/flt3* ligand (Hiraoka et al., 1998). We hypothesized that medium conditioned by KPB-M15 cells (KPB-M15-CM) may serve as an optimal in vitro environment for macrophage differentiation from human peripheral blood monocytes. Results of this study demonstrate that KPB-M15-CM can serve as a culture medium for differentiating monocytes in vitro.

2. Materials and methods

2.1. Preparation of conditioned medium and cell culture

KPB-M15 cells were grown in Dulbecco's Modified Eagle Medium (Gibco, Grand Island, NY) supplemented with 10% heat-inactivated (56 °C for 30 min) fetal calf serum (SeraCare Lifesciences, Oceanside, CA), 0.3 mg/mL L-glutamine (Gibco) and 200 U/mL penicillin/streptomycin solution (Gibco). KPB-M15 cells, plated at a concentration of 1×10^6 cells/mL, were cultured for 4 days in the above medium; conditioned medium was filtered and stored frozen at −80 °C.

Healthy human volunteers provided blood after informed consent, under a protocol that was approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center. Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation of heparinized blood over a Ficoll–Hypaque density gradient at 800 × g for 30 min. Isolated PBMC were washed three times in sterile phosphate-buffered saline with EDTA (PBS/EDTA, Gibco), and resuspended to a concentration of 2×10^6 cells/mL in complete medium [Iscove's Modified Dulbecco's Medium (Gibco) supplemented with 10% heat-inactivated human AB serum (Sigma, St. Louis, MO), 0.3 mg/mL L-glutamine and 200 U/mL penicillin/streptomycin solution]. Monocytes were isolated by adherence using Falcon Primaria™ coated (24-well) tissue culture plates (Becton Dickinson, Franklin Lakes, NJ). The cells were allowed to attach to the plates for 3 h at 37 °C (+5% CO_2) and non-adherent cells were removed by shaking the plates (3000 RPM for 2 min) and washing the wells twice with PBS/EDTA. The remaining adherent cells were resuspended in 1.5 mL of complete medium supplemented with an additional 10% autologous human serum and 40 ng/mL rhM-CSF (R&D Systems, Minneapolis, MN) or with 50% KPB-M15-CM. Cells cultured in complete medium (plus an additional 10% autologous serum) served as negative controls. Cultures were maintained in a humidified