J. Clin. Exp. Hematopathol
Vol. 45, No. 2, Dec 2005

Original Article

Capacity of Monocytes from Patients with Chronic Myelomonocytic Leukemia to Differentiate into Macrophages and Multinucleated Giant Cells

Eriko Shoda[1,2], Eiji Kawano[3], Katsunori Aoki[2], and Kiyoko S. Akagawa[1]

Monocytes from patients with chronic myelomonocytic leukemia (CMML-Mo) strongly expressed CD14 and CD2, but not CD16. Macrophages (MΦ) generated from CMML-Mo expressed CD32, but not CD16. The expression levels of CD64 were significantly reduced from normal MΦ. While CMML-Mo-derived MΦ phagocytosed sensitized SRBC, these cells exhibited a similar defect as CMML-Mo in the ability to produce O_2^-. CMML-Mo could generate dendritic cells (DC), but not multinucleated giant cells (MGC), suggesting that CMML-Mo are different from normal Mo in cell surface marker expression, O_2^- production, and the ability to differentiate into MΦ and MGC, but not DC.
Key words CMML, monocytes, macrophages

INTRODUCTION

Normal monocytes (Mo) can differentiate into macrophages (MΦ), osteoclast-like multinucleated giant cells (MGC), and dendritic cells (DC) in differing cytokine environments of macrophage colony-stimulating factor (M-CSF) alone, M-CSF and interleukin 4 (IL-4), and granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4, respectively[1]. Chronic myelomonocytic leukemia (CMML) is a myeloproliferative disorder exhibiting peripheral blood monocytosis. Although CMML-Mo are known to be able to differentiate into DC[2], there is no report examining if CMML-Mo can differentiate into MΦ or MGC. In this study, we investigate the ability of CMML-Mo to differentiate into MΦ and MGC following treatment with M-CSF and M-CSF+IL-4, respectively.

MATERIALS AND METHODS

Patients : CMML samples were obtained from four patients, whose clinical characteristics are detailed in Table 1. All samples were acquired after obtaining informed consent with the approval of the local ethics committee.

Preparation and culture of Mo : Mo were isolated from PBMCs obtained from CMML patients using a magnetic cell separation system (MACS ; Miltenyi Biotec, Bergisch Gladbach, Germany) with anti-CD14 mAb-coated microbeads as described[3]. $CD14^+$ Mo were cultured for seven days at a density of 5×10^5/2 ml in RPMI1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS) in the 6-well plates (Falcon ; Becton, Dickinson and Company, Franklin Lakes, NJ) at 37℃ in an atmosphere of 5% CO_2 in air. Cultures were treated with M-CSF, GM-CSF+IL-4 or M-CSF+IL-4 at the following concentrations : 500 U/ml GM-CSF (Schering-Plough Japan, Osaka, Japan), 1×10^4 U/ml M-CSF (Morinaga Milk Industry Co., Ltd., Tokyo, Japan), and 100 U/ml IL-4 (GENZYME TECHNE, Minneapolis, MN).

Phenotypic analysis : Expression of cell surface markers was assessed using fluorescein (FITC)- and phycoerythrin (PE)-conjugated mouse mAbs specific for CD2, CD13, CD14, CD16, CD33, HLA-DR (BD, San Jose, CA), CD32, CD64 (BD Pharmingen, San Diego, CA), and CD1a (Beckman Coulter, Inc., Florida, MA), as well as isotype control mouse MoAbs (BD). To block nonspecific Fc-receptor-mediated binding of mAbs, cells were pre-incubated for 30 min at 0℃ in normal goat serum (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) before staining. Stained cells were analyzed using a FACS Calibur cytometer (BD) equipped with Cell Quest software (BD).

Phagocytosis of MΦ : MΦ derived from CMML-Mo in RPMI1640/10% FCS were incubated at 37℃ with 0.2% latex particles (average diameter 1 μm, Dow Chemical Co., Indianapolis, IN, USA) for 4 hrs or with 0.4% sensitized sheep red blood cells (EA) for 1 hr, then washed three times in PBS

Received : Jul 1, 2005
Revised : Aug 15, 2005
Accepted : Aug 16, 2005
[1] Department of Immunology, National Institute of Infectious Diseases, Tokyo Japan,
[2] Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, Japan
[3] Nippon Kayaku Company, Tokyo, Japan
Address correspondence and reprint request to Kiyoko S. Akagawa, Department of lmmunology, National Institute of Intectious Diseases, Toyama 1-23-1, Shinjyuku-ku, Tokyo 162-8640, Japan

without Ca^{++} or Mg^{++}.

Superoxide generation assay : To measure the amount of superoxide anion (O_2^-) generated by cells stimulated with phorbol myristate acetate (PMA, 100 ng/ml), we used lucigenin-dependent SOD-inhibitable chemiluminescence[4]. The chemiluminescence response, measured every 10 min using a Berthold LB96P luminometer, was expressed as relative light units (RLU).

RESULTS AND DISCUSSION

Almost all the Mo obtained from four CMML patients expressed CD14, as well as HLA-DR, CD13, and CD33, as demonstrated by previous studies[2]. The majority of the Mo derived from the four CMML patients, as well as normal-Mo, expressed CD32 and CD64 (Table 1). Consistent previous studies[5,6], approximately 22% (10-30%) and 31% (16-39%) of normal-Mo expressed CD16 and CD2, respectively. In contrast, CMML-Mo did not express significant levels of CD16 (0-3%), while increased cell numbers strongly expressed CD2 (59-99%) (Table 1, Fig. 1). The absence of CD16 expression by CMML-Mo correlates well with previous reports[2]. Lack of CD16 expression and strong expression of CD2 by CMML-Mo suggest that CMML-$CD14^+$Mo are less mature than normal-$CD14^+$Mo.

Next, we examined the ability of CMML-Mo to differentiate into MΦ following M-CSF treatment. Culture of CMML-Mo in M-CSF induced the morphologic changes characteristic of Mo to MΦ differentiation, including an increase in size and adherence. The resulting morphology of CMML-Mo-derived MΦ (CMML-MΦ) resembled that of normal Mo-derived MΦ (normal-MΦ) (Fig. 2). CMML-MΦ exhibited normal phagocytosis of latex particles and the Fc-receptor-mediated internalization of EA (Table 1). As seen for normal MΦ, the majority of CMML-MΦ expressed CD32 ; surface expression of CD64 by CMML-MΦ, however, was lower than that seen by normal MΦ (Table 1, Fig. 3). CMML-MΦ did not express significant levels of CD16, which is expressed strongly by normal-MΦ[7,8] (Table 1, Fig. 3). Previous studies have shown that the expression of CD16 by Mo and MΦ is enhanced by IL-10[7] ; IL-10 is produced by Mo cultured in M-CSF[9]. Thus, the possibility exists that CMML-Mo are defective in IL-10 production following M-CSF stimulation. We do not currently understand, however, the mechanisms underlying the lack of CD16 expression by CMML-Mo and CMML-MΦ.

The production of reactive oxygen intermediates, such as O_2^-, by Mo/MΦ plays a fundamental role in innate immune responses. These substances possess the ability to kill microbial pathogens within phagolysosomes. We therefore ex-

Table 1. Patient's characteristics at the time of obtaining of blood samples and immunophenotypic characteristics of normal and CMML Mo, and normal and CMML Mo-derived MΦ

Patient	Normal	No. 1	No. 2	No. 3	No. 4
Age/ Sex		63/ M	77/ F	66/ M	70/ M
Karyotype		46, XY	46, XX	46, XY	46, XY del(1)(p34), der(5;17)(p10;q10), +8
Counts of Mo ($\times 10^9$/l)		4.4	9.3	2.8	9.6
$CD14^+CD16^+$Mo (%)	22 (10〜30)	0	3	1	1
$CD14^+CD2^+$Mo (%)	31 (16〜39) (n= 5)	59	100	94	86
MΦCD14 (%)	96	93	82	81	99
CD16 (%)	85	8	16	8	1
CD32 (%)	100	80	86	87	95
CD64 (%)	87 (n= 5)	14	51	54	33
O_2^-(RLU) Mo	558 (273〜730)	14	117	239	174
MΦ	76 (33〜99) (n= 3)	9	9	8	31

Percentage shows positive cells. O_2^- was showed RLU in peak value.

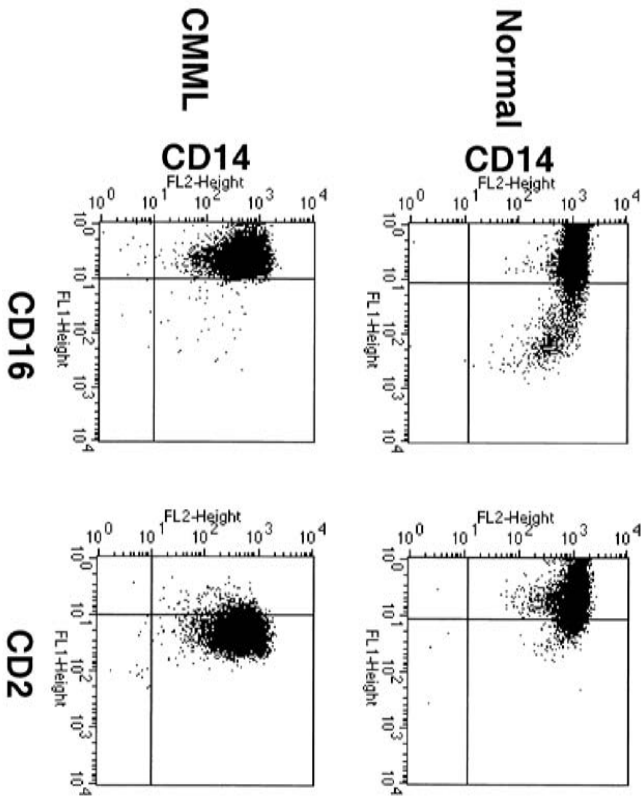

Fig. 1 Dot plot analysis of the expression of CD14, CD16, and CD2 by normal-Mo and CMML-Mo. The data shown, obtained using CMML-Mo derived from patient No. 3, are representative of CMML-Mo from the other three patients.

amined the O_2^- production by CMML-Mo and CMML-MΦ. In contrast to normal-Mo and normal-MΦ, neither CMML-Mo nor CMML-MΦ produced significant levels of O_2^- following PMA stimulation (Fig. 4). O_2^- is produced by the activation of NADPH oxidase, an enzyme consisting of six subunits ; the integral membrane proteins $p22^{phox}$ and $gp91^{phox}$, which form a heterodimeric flavocytochrome, bind four cytosolic proteins, the small GTPase Rac, $p40^{phox}$, $p47^{phox}$, and $p67^{phox}$ [10]. Preliminary western blot analysis demonstrated that the protein levels of p47 and p67 in CMML-Mo were similar to those seen in normal-Mo (data not shown), suggesting either impairment of the remaining components of NADPH oxidase or aberrant activation of the oxidase signaling pathway in CMML-Mo/ MΦ.

CMML-Mo cultured in GM-CSF and IL-4 differentiated into $CD1a^+$ DC, as reported[2] (Fig. 2). Culture of CMML-Mo in M-CSF and IL-4, however, did not generate MGC (Fig. 2). These results suggest that the signaling pathway induced by the combination of M-CSF+IL-4, but not that activated by GM-CSF+IL-4, is impaired in CMML-Mo. The precise mechanisms governing the inability of CMML-Mo to generate MGC following M-CSF+IL-4 treatment remains unclear.

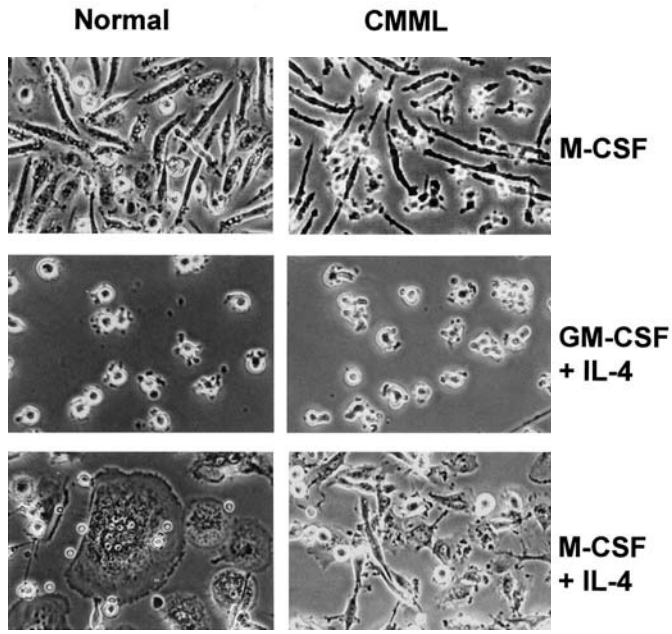

Fig. 2 Morphology of the cells generated from normal Mo and CMML-Mo following culture in M-CSF alone (upper), GM-CSF+IL-4 (middle), and M-CSF+IL-4 (lower). CMML-Mo shown were taken from patient No. 2 ; the CMML-Mo from the remaining three patients demonstrated similar results (data not shown). Phase-contrast micrographs are shown at an original magnification of × 200.

In conclusion, CMML-Mo can differentiate into MΦ following M-CSF treatment ; the MΦ generated, however, are distinct from normal Mo-derived MΦ in their expression of CD16 and capacity for O_2^- production. Although CMML-Mo are defective in their ability to differentiate into MGC following M-CSF+IL-4 treatment, these cells can differentiate into DC when given GM-CSF+IL-4. It will be interesting to use CMML-Mo to elucidate the signaling pathways involved in the differentiation of Mo into MΦ and/or MGC that require M-CSF and/or M-CSF+IL-4.

REFERENCE

1 Akagawa KS, Takasuka N, Nozaki Y, Komuro I, Azuma M, Ueda M, Naito M, Takahashi K : Generation of $CD1^+RelB^+$ dendritic cells and tartrate-resistant acid phosphatase-positive osteoclast-like multinucleated giant cells from human monocytes. Blood 88 : 4029-4039, 1996

2 Vuckovic S, Fearnley DB, Gunningham S, Spearing RL, Patton WN, Hart DN : Dendritic cells in chronic myelomonocytic leukaemia. Brit J Haematol 105 : 974-985, 1999

3 Komuro I, Keicho N, Iwamoto A, Akagawa KS : Human alveolar macrophages and granulocyte-macrophage colony-stimulating factor-induced monocyte-derived macrophages are resistant to H_2O_2 via their high basal and inducible levels of catalase activity.

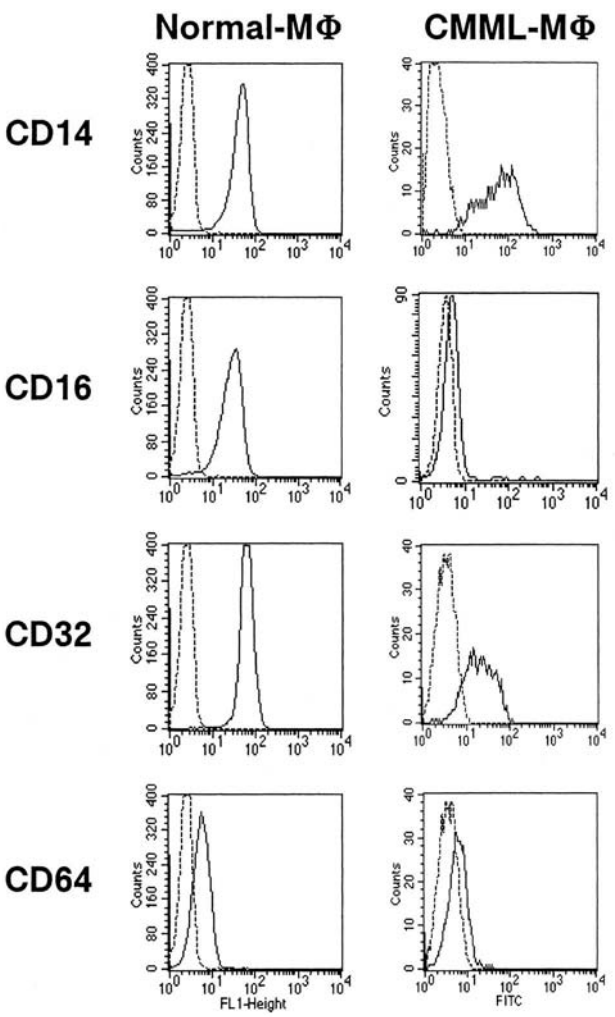

Fig. 3 Phenotypic analysis of MΦ generated from normal-Mo (left side) and CMML-Mo (right side) by M-CSF stimulation. The dotted line represents the isotype control. The data shown, detailing the results of CMML-Mo-derived MΦ from patient No. 1, were representative of all CMML patients.

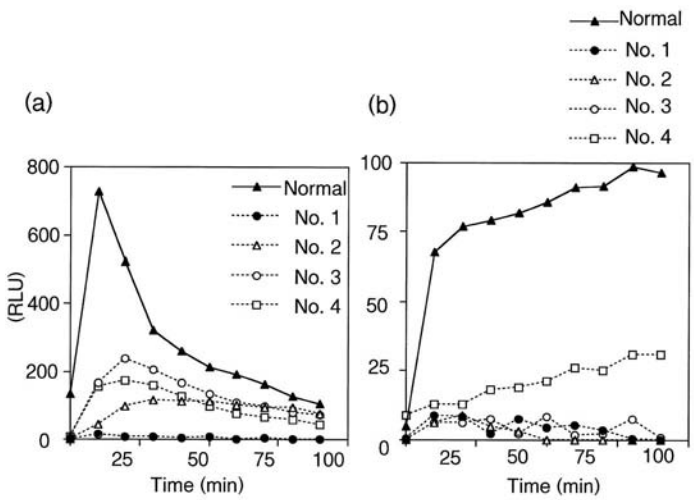

Fig. 4 PMA-stimulated O_2^- production by normal-Mo and CMML-Mo (a) and normal-MΦ and CMML-MΦ (b). Data for the normal-Mo and normal-MΦ are representative of cells from three normal subjects.

J Biol Chem 276 : 24360-24364, 2001

4 Yu D, Imajoh-Ohmi S, Akagawa K, Kanegasaki S : Suppression of superoxide-generating ability during differentiation of monocytes to dendritic cells. J Biochem 119 : 23-28, 1996

5 Passlick B, Flieger D, Ziegler-Heitbrock HW : Identification and characterization of a novel monocyte subpopulation in human peripheral blood. Blood 74 : 2527-2534, 1989

6 Crawford K, Gabuzda D, Pantazopoulos V, Xu J, Clement C, Reinherz E, Alper CA : Circulating $CD2^+$ monocytes are dendritic cells. J Immunol 163 : 5920-5928, 1999

7 Hashimoto SI, Komuro I, Yamada M, Akagawa KS : IL-10 inhibits granulocyte-macrophage colony-stimulating factor-dependent human monocyte survival at the early stage of the culture and inhibits the generation of macrophages. J Immunol 167 : 3619-3625, 2001

8 Young DA, Lowe LD, Clark SC : Comparison of the effects of IL-3, granulocyte-macrophage colony-stimulating factor, and macrophage colony-stimulating factor in supporting monocyte differentiation in culture. Analysis of macrophage antibody-dependent cellular cytotoxicity. J Immunol 145 : 607-615, 1990

9 Mochida-Nishimura K, Akagawa KS, Rich EA : Interleukin-10 contributes development of macrophage suppressor activities by macrophage colony-stimulating factor, but not by granulocyte-macrophage colony-stimulating factor. Cell Immunol 214 : 81-88, 2001

10 Groemping Y, Lapouge K, Smerdon SJ, Rittinger K : Molecular basis of phosphorylation-induced activation of the NADPH oxidase. Cell 113 : 343-355, 2003