ERYTHROMYCIN PROMOTES MONOCYTE TO MACROPHAGE DIFFERENTIATION

NAOTO KEICHO[a,b], SHOJI KUDOH[c], HIDEKI YOTSUMOTO[b] and KIYOKO S. AKAGAWA*[,a]

[a]Department of Immunology, National Institute of Health,
1-23-1 Toyama, Shinjuku-ku, Tokyo 162
[b]Third Department of Internal Medicine, University of Tokyo,
7-3-1 Hongo, Bunkyo-ku, Tokyo 113
[c]Fourth Department of Internal Medicine, Nippon Medical School,
1-1-5 Sendagi, Bunkyo-ku, Tokyo 113, Japan

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Recent reports have suggested that long-term administration of erythromycin (EM) appears to ameliorate some of chronic inflammatory processes where macrophages and lymphocytes play important roles. Our study was initiated to examine the effect of EM on monocyte-macrophage lineage *in vitro*. EM ($1 \sim 100\,\mu$g/ml) significantly increased the number of adherent monocyte-derived macrophages after 7 days of culture. The combination of EM and macrophage colony stimulating factor (M-CSF) synergistically increased the number of monocyte-derived macrophages, while the combination of EM and granulocyte-macrophage colony stimulating factor exerted an additive effect. Culture with EM induced the expression of a surface antigen CD71, one of the activation markers of macrophages as compared with control cultures. The combination of EM plus M-CSF significantly enhanced H_2O_2-producing capacity of those cells as compared with M-CSF alone. A differentiation process of monocytoid THP-1 cells was also augmented by EM. These results indicate that EM promotes differentiation of human monocyte-macrophage lineage, altering their functions.

Erythromycin (EM) is a macrolide antibiotic first isolated from cultures of *Streptomyces erythreus* in 1952 and has been widely used to treat a variety of infectious diseases[1]. In the past several years, it was reported that long-term treatment with EM appears to improve chronic inflammatory processes including diffuse panbronchiolitis[2~4] and bronchial asthma[5], irrespective of its antimicrobial property. Lymphocytes and macrophages derived from circulating blood are major cellular components at sites of chronic inflammation and they play important roles on the inflammatory process. Recent observations have indicated that certain antibiotics alter the function of host cells[6,7]. EM was previously reported to suppress human lymphocyte proliferation *in vitro*[8,9], whereas its direct effect on monocyte-macrophage lineage is unclear[10]. Thus, this study was designed to determine whether EM affected monocyte-macrophage differentiation and their function.

Materials and Methods

Reagents

Erythromycin base was obtained from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). It was dissolved in ethanol at 200 mg/ml and was diluted in each experiment. Concanavalin A (Con A) and Phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma Chemical Co. (St. Louis, MO). Recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) was kindly provided by Kirin Brewery Co., Ltd. (Maebashi, Japan) and recombinant human macrophage colony stimulating factor (rhM-CSF) was donated from Morinaga Milk Industry Co., Ltd. (Tokyo, Japan). Acetylated low density lipoprotein labeled with the fluorescent probe 3,3′-dioctadecylindocarbocyanine (DiI-Ac-LDL) was

obtained from Biomedical Technologies Inc. (Stoughton, MA).

Preparation of Human Monocytes

Peripheral blood mononuclear cells (PBMC) were obtained from venous blood of normal healthy volunteers whom permission was granted from. PBMC were isolated by centrifugation on a Ficoll-Metrizoate density gradient (Lymphoprep; Nycomed, Oslo, Norway) and were suspended in RPMI 1640 medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with 10% heat-inactivated fetal calf serum (FCS; Z. L. Bockneck Laboratories Inc., Ontario, Canada), 2 mM L-glutamine, 100 μg/ml streptomycin and 100 U/ml penicillin. FCS was shown to contain 0.003 ng of LPS per ml by the *Limulus* amebocyte lysate test. PBMC were placed into monocyte-isolating plates (MSP-P; Japan Immunoresearch Laboratories. Co., Ltd., Takasaki, Japan) and incubated for 2 hours at 37°C in a humidified 5% CO_2 atmosphere and adherent cells were collected by gentle pipetting. More than 95% of the cells were judged to be monocytes by morphology and nonspecific esterase staining.

Cell Lines

THP-1 cell line, derived from a patient with monocytic leukemia[11], was obtained from the American Type Culture Collection (Rockville, MD) *via* the Japanese Cancer Research Resources Bank (Tokyo, Japan) and maintained in the culture medium described above.

Determination of the Number of Adherent Cells

PBMC (2×10^5 per well in 200 μl) were cultured with or without Con A (2 μg/ml) in the absence or presence of EM (100 μg/ml) for 3 ~ 7 days. Human monocytes (5×10^4 per well in 200 μl) were cultured in the absence or presence of EM (1 ~ 100 μg/ml) for 7 days in 96-well flat-bottom tissue culture plates (Falcon No. 3072; Becton Dickinson Labware, Lincoln Park, NJ). To examine the effect of combining EM with CSFs, human monocytes (2×10^4 per well in 200 μl) were cultured in the absence or presence of EM (100 μg/ml) plus M-CSF (0 ~ 10,000 U/ml) or GM-CSF (0 ~ 50 U/ml) for 7 days. The percentage of viable adherent cells were determined by trypan blue dye exclusion test. The number of adherent monocyte-derived macrophages was determined by the method of NAKAGAWARA and NATHAN[12]: In brief, immediately after removing the culture medium by gentle aspiration, 50 μl of 1.0% (w/v) cetyltrimethyl ammonium bromide (Cetavlon; Wako Pure Chemical Industries, Ltd. Osaka, Japan) in 0.1 M citric acid with 0.05% (w/v) naphthol blue black (Sigma Chemical Co.) was added to each well. After a few minutes at room temperature, needed to ensure that all non-nuclear debris of adherent cells disappeared, stained nuclei were counted by using TATAI hemocytometer (Kayagaki Irikakogyo Co., Ltd., Tokyo, Japan).

THP-1 cells (2×10^5 per well in 1 ml) were placed into 24-well tissue culture clusters (Coster, Cambridge, MA) and cultured with PMA (1 ~ 2 ng/ml) or EM (100 μg/ml) or both for 4 days. Non-adherent cells were removed by washing twice with phosphate buffered saline (PBS) and then adherent cells were collected by vigorous pipetting. The number of cells was counted by a hemocytometer.

Analysis of Cell Surface Markers

For the surface marker study, human monocytes (5×10^4) were put on each coverslip, three of which were placed into each well of 6-well plates. A total of 2 ml of the culture medium in the absence or presence of EM (100 μg/ml) or M-CSF (5,000 U/ml) was added to each well and they were incubated for 7 days. Staining procedure is as follows: After the remaining medium was removed, each coverslip was preincubated with normal rabbit serum at 0°C for 30 minutes to block the nonspecific binding of monoclonal antibodies. Then, a total of 40 μl of an appropriate dilution of anti-Leu-M3 (CD14), anti-HLA-DR (Becton Dickinson Mountain View, CA) or OKT9 (CD71) (Ortho diagnostic systems, Raritan, NJ) was spotted on each coverslip, which was held at 0°C for 30 minutes followed by washing twice with cold PBS. A total of 40 μl of an appropriate dilution of FITC-conjugated goat anti-mouse IgG F $(ab')_2$ fragment (Cooper Biomedical Inc., Malverm, PA) was spotted on the coverslip, which was held again at 0°C for 30 minutes. After being washed again, the coverslips were stored in wells contaning PBS with 1% paraformaldehyde until evaluated with a fluorescence microscope.

THP-1 cells (2×10^5/ml) were cultured with PMA (1 ~ 2 ng/ml) or EM (100 μg/ml) or both for 4 days

and were harvested by vigorous pipetting and the whole cells were analyzed by indirect immunofluorescence. After preincubation with normal rabbit serum at 0°C for 30 minutes, these cells were stained with Mac-1 (Hybritech Inc., San Diego, CA) followed by FITC-conjugated goat anti-rat IgG $F(ab')_2$ fragment (Cooper Biomedical Inc.) or with anti-HLA-DR followed by FITC-conjugated goat anti-mouse IgG $F(ab')_2$ fragment and were analyzed by flow cytometry (FACStar, Becton Dickinson).

Assay for H_2O_2 Production

The cellular release of H_2O_2 was detected by the semi-automated micro-assay reported by HARPE and NATHAN[13]. In brief, human monocytes (5×10^4 per well in 100 μl) were cultured in 96-well flat-bottom tissue culture plates in the presence of M-CSF (5,000 U/ml) alone or in the presence of M-CSF plus EM (100 μg/ml) for 7 days. Then the culture medium was removed from the culture plates and the adherent cells were rinsed with PBS before the assay mixture (100 μl) was dispensed into the wells. The assay mixture was prepared before use from stock solutions and consisted of 30 μM scopoletin (Sigma), 1 mM NaN_3, 1 purpurogallin unit/ml horseradish peroxidase (Sigma), in Krebs-Ringer phosphate buffer (145 mM NaCl, 4.86 mM KCl, 0.54 mM $CaCl_2$, 1.22 mM $MgSO_4$, 5.7 mM sodium phosphate) with 5.5 mM glucose. PMA (100 ng/ml), zymosan (1 mg/ml), or heat-inactivated staphylococcus aureus (1 mg/ml) was used as stimulus. Immediately after the addition of the stimuli, the plate was placed in a fluorometer (Titertek Fluoroskan II, Flow Laboratories Inc., McLean, VA) and the fluorescence was recorded for each well (0 minute). The plate was maintained at 37°C and after 60 minutes the fluorescence in each well was again measured. H_2O_2 release was calculated from the loss of fluorescence, using the formula:

$$\text{nmol } H_2O_2 \text{ released} = \{(E_0 - W)/(C_0 - W) - (E_{60} - W)/(C_{60} - W)\} \times S$$

where E_0 is the initial fluorescence reading for the well; E_{60} is the fluorescence reading at 60 minutes; W is the fluorescence recorded in an empty well; C_0 and C_{60} are the mean fluorescence readings in the cell-free control wells at 0 and 60 minutes respectively; and S is the amount of scopletin, 3 nanomoles added to each well at the start of the assay.

Measurement of DNA Synthesis

THP-1 cells (2×10^5/ml) were cultured in 96-well microplates with PMA or EM or both for 48 hours. Each culture was pulsed with 0.5 μCi of [^{3}H]thymidine (New England Nuclear, Boston, MA) per well for the final 18 hours of incubation before being harvested onto glass fiber filters. The radioactivity collected was assayed with a liquid scientillation counter.

Phagocytosis of Latex Beads

THP-1 cells (2×10^5/ml) were cultured with PMA or EM or both for 4 days. For the test of phagocytic activity, each culture was added with 0.2% (w/v) polystyrene latex particles (average diameter 1 μm, Dow Chemical Co., Indianapolis, IN) and incubated at 37°C for 4 hours and then the cells were harvested by vigorous pipetting and the whole cells were collected. After washing, the percentage of cells ingesting latex particles was determined in a hemocytometer by counting at least 300 viable cells[14].

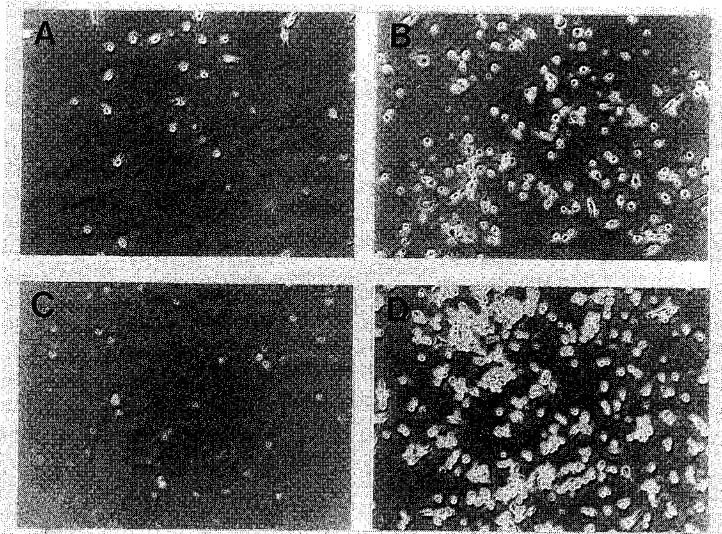
Uptake of Acetylated LDL

THP-1 cells (2×10^5/ml) were cultured with PMA or EM or both for 4 days. For labeling, each culture was added with DiI-Ac-LDL (10 μg/ml in the culture medium) and incubated at 37°C for 4 hours and then the cells were harvested by vigorous pipetting and the whole cells were collected. After washing, the percentage of cells labeled with DiI-Ac-LDL was determined by flow cytometry (FACStar, Becton Dickinson)[15].

Statistical Analysis

We repeated each type of experiment at least three times and confirmed that similar data were obtained respectively. The results, obtained in triplicate, are expressed as mean values $\pm$ SD. The statistical significance of differences was determined by STUDENT's t test.

Fig. 1. Phase contrast appearance of adherent PBMC after 7 days of incubation.



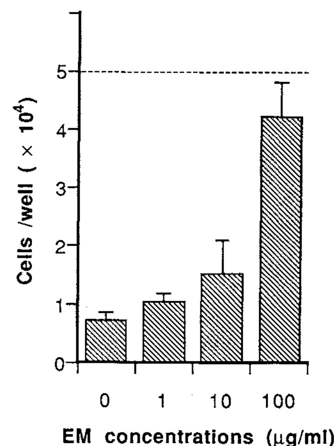
A. 10% FCS-RPMI 1640 medium alone, B. medium with EM (100 μg/ml), C. medium with Con A (2 μg/ml), D. medium with Con A (2 μg/ml) plus EM (100 μg/ml). Non-adherent cells were removed by gentle washing and the remaining adherent cells were shown in the photograph.

Results

EM Increases the Number of Adherent Monocyte-derived Macrophages

We previously observed that the proliferation of PBMC stimulated with Con A for 72 hours was slightly suppressed by EM[9]. When PBMC were cultured for more than 3 days, the proportion of adherent cells derived from cultured PBMC with or without Con A obviously increased in the presence of EM (Fig. 1). These cells were monocyte-derived macrophages morphologically and more than 95% of these adherent cells were alive by dye exclusion test (data not shown). This finding prompted us to investigate the effect of EM on monocyte-macrophage lineage.

Fig. 2. Effect of EM on the number of monocyte-derived macrophages.



The adherent monocytes were cultured in the absence or presence of EM (1 ~ 100 μg/ml) for 7 days and the number of adherent monocyte-derived macrophages was counted. The dashed line represents the cell number (5×10^4/well) at the time of addition of EM (day 0). Error bars are SD.

Human monocytes were cultured for 7 days and the number of adherent monocyte-derived macrophages was counted. As shown in Fig. 2, the number of adherent cells increased in the presence of EM in a dose-dependent manner. This effect was significant from 1 μg/ml of the drug ($P<0.01$); the percent of control response was 140% at EM 1 μg/ml and 570% at EM 100 μg/ml. The diluent of stock solution, 0.05% of ethanol in EM 100 μg/ml had no effect in this system (data not shown).

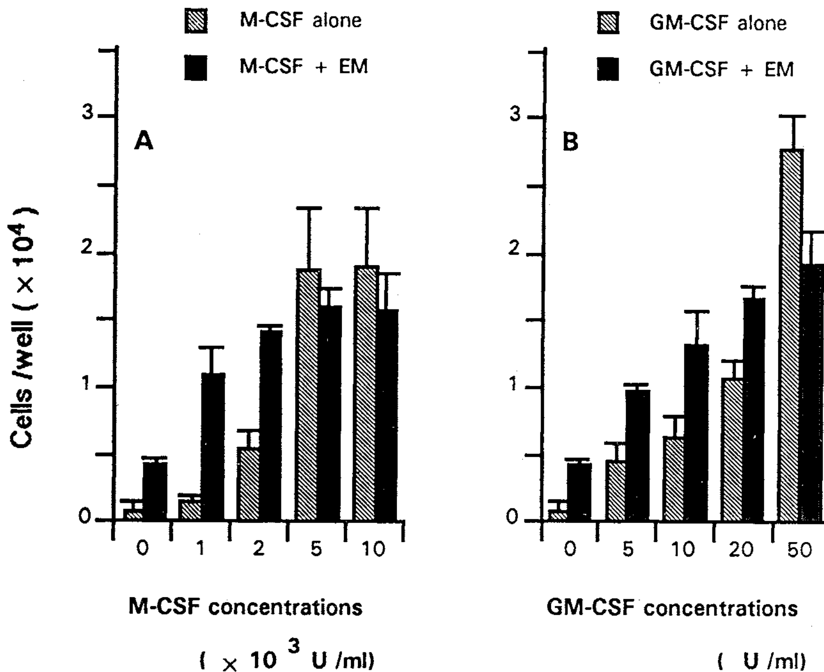
Effect of EM on the Number of Adherent Monocyte-derived Macrophages in the Presence of CSFs

It was reported that human monocytes proliferate and differentiate in the presence of M-CSF or GM-CSF[16]. Both of CSFs are inferred to play important roles on local differentiation or proliferation of macrophages *in vivo*[17,18]. Thus, we examined the effect of combining EM with various doses of either M-CSF of GM-CSF (Fig. 3). Both of CSFs dose-dependently increased the number of adherent monocyte-derived macrophages. In this system, the number of adherent monocyte-derived macrophages in the presence of EM 100 μg/ml plus M-CSF 1,000 U/ml is much larger than the simple addition of the number in the presence of EM 100 μg/ml alone and the number in the presence of M-CSF 1,000 U/ml alone; EM synergistically increased the number of monocyte-derived macrophages in the presence of suboptimal doses of M-CSF. On the other hand, the number of adherent macrophages in the presence of EM 100 μg/ml plus GM-CSF 5 U/ml is the simple addition of the number in the presence of EM 100 μg/ml alone and the number in the presence of GM-CSF 5 U/ml alone; EM additively increased their number in the presence of suboptimal doses of GM-CSF. EM did not further increase the number of macrophages in the presence of optimal doses of M-CSF (5,000 ~ 10,000 U/ml), whereas EM rather suppressed the increase in cell number in the presence of optimal doses of GM-CSF (50 U/ml).

EM Enhances the Expression of CD71 on Monocyte-derived Macrophages

We examined whether EM affected the expression of surface antigens on macrophages. The

Fig. 3. Effect of EM on the number of monocyte-derived macrophages in the presence of M-CSF (A) or GM-CSF (B).



The adherent monocytes were cultured in the absence or presence of EM (100 μg/ml) plus various concentrations of M-CSF or GM-CSF for 7 days. A representative experiment (of at least three performed) is shown. The cell number at the time of addition of EM (day 0) was 2×10^4/well. Error bars are SD.

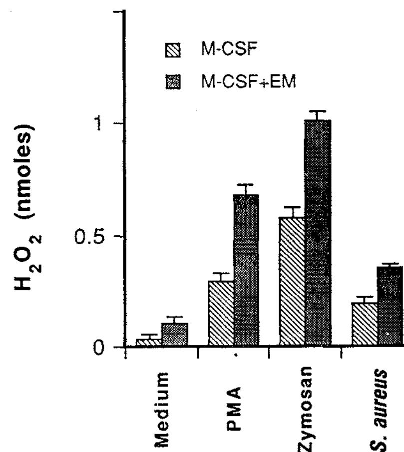
Table 1. Expression of cell surface antigens on monocytes cultured with EM or M-CSF[a].

	Percent positive[b]		
	Medium alone	EM (100 μg/ml)	M-CSF (5,000 U/ml)
CD71 (OKT9)	<1	31±8	6±2
CD14 (Leu-M3)	<1	3±4	25±18
HLA-DR	82±3	78±4	83±5

[a] Human monocytes on the coverslip were cultured in the presence of EM (100 μg/ml) or M-CSF (5,000 U/ml) for 7 days. The cells were stained with indicated antibodies as described in Materials and Methods. Percent positive cells stained with control IgG subclass antibodies was less than 1% respectively.

[b] Each value represents the mean±SD.

Fig. 4. H_2O_2 production by monocyte-derived macrophages treated with M-CSF alone or together with EM.



The adherent monocytes were cultured in the presence of M-CSF (5,000 U/ml) alone or in the presence of M-CSF plus EM (100 μg/ml) for 7 days. PMA (100 ng/ml), zymosan (1 mg/ml) or heat-inactivated staphylococcus aureus (1 mg/ml) was added. After H_2O_2 assay, the cell number was counted. The released H_2O_2 was adjusted by the cell number and was represented as the amount produced by 5×10^4 cells. Error bars are SD.

expression of CD71 (OKT9; transferrin receptor), CD14 (Leu-M3) and HLA-DR on the surface of adherent macrophages was measured (Table 1). EM definitely induced CD71 expression ($P<0.01$), whereas FCS alone or the addition of M-CSF failed to induce sufficient expression. CD14 which is expressed on most fresh monocytes was maintained to some degree in the presence of M-CSF, while it was lost after 7 days of culture on the coverslip in the presence of EM as well as in the control culture. EM unaffected the expression of HLA-DR.

EM Enhances H_2O_2 Production of Monocyte-derived Macrophages

We further examined the effect of EM on the function of maturing macrophages. We compared H_2O_2-producing capacity between those macrophages cultured with M-CSF plus EM and those cultured with M-CSF alone. Culture without CSFs failed to collect sufficient number of monocyte-derived macrophages to carry out this experiment. Monocyte-derived macrophages cultured with M-CSF plus EM produced a larger amount of H_2O_2 than those cultured with M-CSF ($P<0.02$), stimulated with PMA, zymosan or heat-inactivated staphylococcus aureus (Fig. 4). EM itself did not affect this H_2O_2 assay system, because the production of H_2O_2 was measured after EM was removed.

EM Affects Phenotypes of PMA-stimulated THP-1 Cells

Investigating this mechanism, it is useful to determine whether EM promotes differentiation of a monocytoid cell line as well as human monocytes. We found that THP-1 cell line stimulated with a differentiation inducer, PMA, behaved like native monocytes, when treated with EM (Table 2). When THP-1 cells were stimulated with a low dose of PMA (1 ng/ml), a part of THP-1 cells adhered to plastic culture dishes. When these cells were treated with the same dose of PMA plus EM (100 μg/ml), the number of adherent THP-1 cells significantly increased ($P<0.01$) and adherent THP-1 cells, like monocyte-derived macrophages, changed sticky in morphology, while no adherent cells were observed when native THP-1 cells were treated with EM alone (Fig. not shown). DNA synthesis decreased with PMA-treatment and

Table 2. Effects of EM on differentiation of THP-1 cells.

Group	The number of adherent cells[a] (× 10⁴/well)	[³H]TdR incorporation[b] (× 10⁴ cpm)	Phagocytosis of latex beads[c] (%)	Uptake Ac-LDL[d] (%)	Surface markers[e] (MFI) HLA-DR	Mac-1
Medium alone	0	14.9±3.0	3.7±1.7	<5	29.4	58.1
EM (100 μg/ml)	0	16.1±1.8	12.2±0.6	<5	35.1	61.9
PMA (1 ng/ml)	1.7±0.5	11.3±2.0	4.4±1.9	27.7	49.2	183.2
PMA (1 ng/ml)+EM	5.5±0.2	5.6±0.3	35.4±6.8	47.6	75.9	350.0
PMA (2 ng/ml)	6.4±0.6	2.2±1.0	NT[f]	54.9	72.8	435.9
PMA (2 ng/ml)+EM	12.1±1.3	0.3±0.1	NT	69.3	106.0	471.2

[a] THP-1 cells (2×10^5 per well in 1 ml) were cultured with PMA or EM or both for 4 days. The number of adherent cells was determined as described in Materials and Methods. Each value represents the mean ± SD.
[b] The cells were cultured with PMA or EM or both for 48 hours.
[c] Phagocytic activity of the cells was measured as described in Materials and Methods.
[d] The proportion of the cells labelled with Ac-LDL was determined as described in Materials and Mehods.
[e] Surface markers were examined by flow cytometry and each value represents the mean fluorescence intensity (MFI).
[f] NT; not tested.

it further decreased in the presence of EM ($P<0.02$), while [³H]thymidine incorporation to a native cell line was not reduced by EM. Phagocytosis of latex beads of native THP-1 cells appeared to be augmented by EM alone. Phagocytosis of latex beads by PMA-treated THP-1 cell was markedly augmented by addition of EM treatment ($P<0.01$). Uptake of acetylated LDL, one of the markers of mature macrophages, was augmented with PMA-treatment and further augmented together with EM. Mean fluorescence intensity of Mac-1 increased with PMA-treatment and it further increased in the presence of EM, while the expression of HLA-DR slightly increased in the presence of EM.

Discussion

In the present investigation, we have shown that EM augments the differentiation of human monocytes and a monocytoid cell line *in vitro*. EM caused functional alterations as well as phenotypical changes through monocyte-macrophage differentiation process.

EM increased the number of adherent macrophages after long-term culture of human monocytes. This effect was observed at the therapeutic concentrations of EM (0.5 ~ 2 μg/ml)[1] and was further enhanced at higher concentrations (~100 μg/ml). The similar finding was observed even when the culture medium was supplemented with human AB serum instead of FCS (data not shown). Although it is poorly understood how monocytes mature and differentiate into tissue macrophages once they leave circulation, it was reported that *in vitro* differentiation of monocytes to macrophages was supported by M-CSF or GM-CSF[16,19,20] and it is inferred that this system mimics differentiation of tissue macrophages in part. EM and suboptimal doses of M-CSF acted synergistically and increased the number of adherent macrophages. Although the concentration we used in most experiments (100 μg/ml) was higher than the therapeutic level of EM, the effect of EM was dose-dependent on the number of monocyte-derived macrophages and it was statistically significant. Considering that EM is condensed in tissue cells[10], long-term administration of EM might affect macrophages at inflammatory sites especially where M-CSF is actively produced.

An increase in the number of adherent macrophages might reflect promotion of replication, elongation of survival or induction of differentiation. Although it is possible that proliferation of a small part of monocytes is masked by unnegligible cell death, the number of adherent macrophages failed to exceed the initial number of cultured monocytes as shown in Fig. 2. To explore whether EM simply supports monocyte survival or induces monocyte differentiation, we examined the expression of surface markers on monocyte-macrophages. CD71 (transferrin receptor), not detected on fresh monocytes, was sufficiently induced on the surface of adherent monocyte-derived macrophages in the presence of EM. CD71 was

reported to be expressed in the process of monocyte-macrophage differentiation[21,22], while EM unaffected the expression of the other two non-differentiation markers, CD14 and HLA-DR. This supported that EM augments monocyte differentiation.

It is inferred that CD71 expression plays an important role in killing microorganisms because iron obtained *via* transferrin receptor is utilized to generate one of the important oxygen intermediates, hydroxyl radical[23]. Therefore, enhancement of CD71 expression by EM raised a possibility that EM induces functional changes with macrophage activation. As expected, monocyte-derived macrophages in the presence of optimal dose of M-CSF together with EM had greater H_2O_2 producing capacity than those treated with M-CSF alone. Augmentation of H_2O_2 production was observed, regardless of stimuli (PMA, zymosan or heat-inactivated staphylococcus aureus). It might be advantageous to eliminate microbial pathogens and might reinforce host defence mechanism at sites of inflammation.

A human monocytic leukemia cell line, THP-1 is well known to be a good model for the investigation of monocyte-macrophage differentiation[24]. EM increased the number of adherent cells and decreased [^{3}H]thymidine uptake of PMA-treated THP-1 cells. Phagocytosis of latex beads and uptake of acetylated LDL, which are important indicators of macrophage function, were also augmented by EM. These results indicate that PMA-induced differentiation of THP-1 cells is augmented by EM.

Since this monocytoid cell line as well as native monocytes was affected by EM, it is unlikely that EM affects the small number of contaminating lymphocytes or lymphokines and indirectly augments normal human monocyte differentiation. One can postulate that EM influences monokine production, which might modulate their own differentiation process, because monokines such as IL-1 and TNF-α have been reported to promote their differentiation or activation[25,26]. In our preliminary study, however, the expression of mRNA of TNF-α and IL-1β was affected little after 3~48 hours of culture in the presence of EM (data not shown). Similarly, the expression of c-fms (M-CSF receptor) which regulates M-CSF-dependent differentiation[27] was unaffected by EM (data not shown). It should be further investigated whether EM indirectly promotes differentiation *via* production of other factors or EM directly acts on signal transduction system of macrophage differentiation.

EM is originally an inhibitor of protein synthesis in microorganisms and binds to a receptor on the 50S subunit of the bacterial ribosome[28]. In our preliminary study, benzylpenicillin or streptomycin did not increase in the number of macrophages. Several antibiotics acting as bacterial protein synthesis inhibitors are known to have binding potential to mitochondrial ribosome and inhibit the proliferation of rapidly replicating eukaryotic cells by interfering with mitochondrial protein synthesis[29] and some inhibitors of cell proliferation are known to act as macrophage differentiation inducers[30]. However, EM never suppressed the growth of native THP-1 cells. Therefore, the mechanism by which EM induces macrophage differentiation, seems to be different from that of such DNA synthesis inhibitors.

Several observations indicate that EM affects the physiology of other mammalian cells *in vitro*. It has been reported that EM influences neutrophil functions[10,31] and reduces mucus secretion from secretory epithelial cells[32] and promotes the bowel movement as an agonist of an enteric hormone, motilin[33,34]. It would be interesting to study whether EM exerts all these effects by acting on similar target molecules or not.

The finding that EM induces monocyte-macrophage differentiation gives us a novel concept of antibiotics which directly act on host defence mechanism *via* promotion of differentiation process of mononuclear phagocyte system. Investigation of this biological activity might lead to the development of a new class of pharmaceutic agents.

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