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INTERLEUKIN-4 ACTS AS A POTENT STIMULATOR FOR EXPRESSION OF MONOCYTE CHEMOATTRACTANT JE/MCP-1 IN MOUSE PERITONEAL MACROPHAGES

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The recruitment of monocyte/macrophages to inflammatory sites is one of the important events in inflammatory reactions. We show herein that interleukin-4(IL-4) acts as a potent stimulator for expression of monocyte chemoattractant JE/MCP-1 in mouse peritoneal macrophages. IL-4 induced the JE/MCP-1 gene expression in dose and time dependent fashion. Run-on assay suggests that IL-4 stimulates the JE/MCP-1 gene expression at transcriptional level. Monocyte chemotactic activity was detected in culture medium of the cytokine-treated cells. The chemotactic activity in the culture supernatant was completely neutralized by anti-JE/MCP-1 antiserum.

Interleukin-4(IL-4) have originally been identified as B cell Stimulatory Factor-I (BSF-I)(1). However, interestingly many recent studies(2-4) have demonstrated that the cytokine is a potent regulatory one of monocyte/macrophage lineage cells. For example, IL-4 was able to increase expression of major histocompatibility(MHC) class II antigen and surface adhesion molecules on human monocyte(2). And it also

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Abbreviations used in this paper: IL-4, interleukin-4; MCP-1, monocyte chemoattractant protein-1; FMLP, formyl-methyonyl-leucine-phenylalanine.

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enhances Ia antigen expression(3) and stimulates the tumoricidal activity of mouse bone marrow and peritoneal macrophages(4).

Although the mouse JE/MCP-1 gene has been cloned from fibroblasts as platelet derived growth factor(PDGF)-inducible gene(5), Rollins *et al.* have demonstrated that its product has cytokine-like properties(6,7). Furthermore, they have evidenced from analysis of its nucleotide sequences that the JE/MCP-1 is the murine homolog of the human monocyte chemoattractant factor MCP-1(8). Therefore, the JE/MCP-1 has now been widely recognized as a monocyte-specific chemoattractant, but not for neutrophils. Thus, the JE/MCP-1 may play an important role in attracting monocytes/macrophages to the sites of inflammation or injury of the tissues. In fact, recent studies(9,10) have suggested that the JE/MCP-1 is contributed to the recruitment of monocyte/macrophages in rheumatoid arthritis and atherosclerosis. Because IL-4 was produced by helper T lymphocytes and/or mast cells which were found at inflammation sites, it is possible that this cytokine might act as a potent regulator in inflammatory responses. In these views, it is very interest to explore whether IL-4 is able to induce the JE/MCP-1 expression in macrophages.

Therefore, in the present study, we examine the capability of IL-4 as an inducer of the JE/MCP-1 expression in macrophages, and show that the cytokine is a potent stimulator for the JE/MCP-1 expression in the cells.

Materials and Methods

Reagents: Brewer's thioglycollate broth was obtained from Difco Laboratories(Detroit, MI), RPMI 1640 medium was from Nissui Pharmaceutical Co., Ltd.(Tokyo, Japan). Fetal bovine serum(FBS) was from Flow Lab.(McLean, VA), and polymyxin B was from Sigma Chem.(St.Louis, MO). Recombinant mouse IL-4 (1.5 μg/ml, with a specific activity of 2 x 10^7 U/mg) and anti-mouse IL-4 antibody were purchased from Genzyme (Cambridge, MA). Anti-mouse JE/MCP-1 rabbit antiserum was kindly provided by Dr. B.J.Rollins (Dana Farber Cancer Institute, Boston, MA).

Preparation of mouse peritoneal macrophages: BALB/c mice, 6 to 8 weeks of age, were injected intraperitoneally with 3 ml of thioglycollate medium. Peritoneal macrophages were prepared from the mice as

described previously(11). The purity of the prepared macrophages was more than 95% by evaluation of their phagocytic activity.

Preparation of total RNA and Northern blotting analysis: The peritoneal macrophages prepared from peritoneal exudate cells($1X10^7$cells) were cultured in 90 mm plastic plates with RPMI 1640 containing 5% FBS. Then the cells were washed, and incubated for 24 hr in serum-free α-MEM, and then treated with test samples at various concentrations. At selected times, total RNA was extracted by the guanidine isothiocyanate procedure (12). Northern blotting analysis was assayed as described previously(13) β-actin was used as an internal standard for quantification of total mRNA on each lane of the gel.

Nuclear trascriptional(run-on) assay: The peritoneal macrophages ($5X10^7$cells) were treated for 60 min with or without IL-4(100U/ml), and their nuclei were isolated as described previously(14). Transcriptional activity was assayed as previously(13). β-actin was used as an internal standard.

Monocyte chemotactic assay: Monocyte chemotactic activity was measured in a 48-well microchamber apparatus, as described previously(15). Ficoll-Hypaque (Pharmacia LKB Japan, Tokyo, Japan) separated human peripheral blood mononuclear cells were used as the indicator cells. A polycarbonate filter (Pore size 5.0 μm, Nuclepore, Pleasanton, CA) was used to separate the cells from the lower compartment of the chamber containing the test medium. The apparatus were incubated at 37°C for 90 min in a humidified atmosphere of 5% CO_2 in air, then the filters removed, fixed in methanol, stained with Diff-Quik(Kokusai Shiyaku, Kobe, Japan). FMLP was used as positive control. Chemotactic activity is defined as the mean ± SD of total migrating monocytes per oil immersion fields in triplicate samples as described previously(16).

Results

Initially we explored whether IL-4 is able to induce JE/MCP-1 gene expression in mouse peritoneal macrophages. The cells were treated with or without IL-4 at 100 U/ml, and then their total RNA was prepared at the selected time after initiation of the culture. Thereafter, the JE/MCP-1 gene expression was analyzed by Northern blotting assay. Fig.1(A and B) shows that IL-4 induces a significant expression of the JE/MCP-1 gene at 30 min after its treatment. The high level of the JE/MCP-1 gene expression was decreased gradually as the following incubation period. And, as shown in Fig.2(A and B), the IL-4-induced JE/MCP-1 gene expression was dose-dependent.

Because many studies(17-22) have shown that early genes, such as c-fos, c-jun, IL-1β, and TNF-α, in mouse peritoneal macrophages were

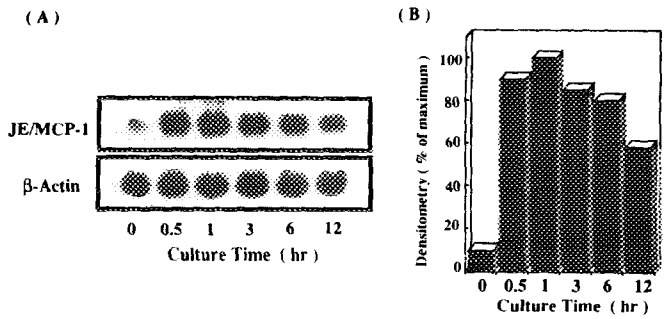


Fig.1. Kinetics of IL-4-stimulated JE/MCP-1 gene expression in mouse peritoneal macrophages. A, The cells were incubated with or without IL-4(100 U/ml), and total RNA was prepared at the indicated times after the initiation of cytokine treatment. Northern blot analysis was performed with JE/MCP-1 and β-actin cDNAs. B, JE/MCP-1 mRNA levels in panel (A) were determined by densitometric analyses and are expressed as a percentage of maximum.

markedly expressed by lipopolysaccharide, we tested using polymyxin B, a potent inhibitor of lipopolysaccharide, whether lipopolysaccharide contaminant in IL-4 preparation used contributes to the JE/MCP-1 gene expression induced by the cytokine. We observed that no the IL-4-induced JE/MCP-1 gene expression was affected by pretreatment with polymyxin B at 50 U/ml(data not shown). Therefore, lipopolysaccharide

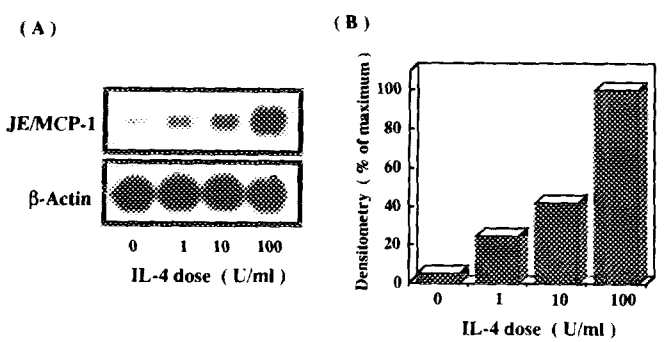
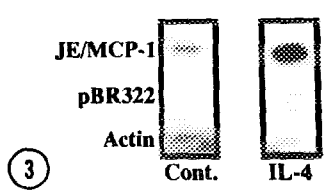


Fig.2. IL-4 stimulates in a dose-dependent fashion JE/MCP-1 gene expression in mouse peritoneal macrophages. A, The cells were incubated with or without various concentrations of IL-4, and total RNA was prepared at 1 hr after the initiation of the cytokine treatment. Northern blot analysis was performed with JE/MCP-1 and β-actin cDNAs. B, JE/MCP-1 mRNA levels in panel (A) were determined by densitometric analyses and are expressed as a percentage of maximum.

contaminant in the IL-4 preparation cannot be responsible for IL-4-induced JE/MCP-1 gene expression.

Next, we explored using Run-on assay whether the IL-4-induced JE/MCP-1 expression in the macrophages was resulted from transcriptional stimulation. We observed that IL-4 stimulates the JE/MCP-1 gene expression at transcriptional level(Fig.3).

Finally, we tested for the presence of JE/MCP-1 gene product in culture supernatant of the IL-4-treated macrophages. The cells were treated for 24 hr with IL-4 at 100 U/ml, and then monocyte chemotactic activity and the presence of the JE/MCP-1 in the culture supernatant were assessed. A marked monocyte chemotactic activity was observed in the culture supernatant, and the marked activity was neutralized completely by



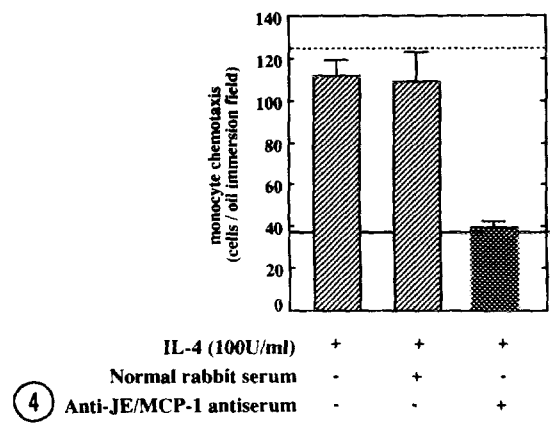


Fig.3. Run on assay of JE/MCP-1 gene in IL-4-treated peritoneal macrophages. The cells were incubated for 30 min with or without IL-4(100 U/ml), and then their nuclei were incubated for 30 min in the presence of 5'-[α-^{32}P]UTP, after which the RNA was isolated. Run on assay was performed with JE/MCP-1 and β-actin cDNAs. pBR322, the vector plasmid, was used as a negative control(Cont).

Fig.4.Monocyte chemotactic activity in culture supernatant of IL-4-treated mouse peritoneal macrophages is neutralized by anti-JE/MCP-1 antiserum. The cells were incubated with or without IL-4(100 U/ml) for 24 hr. After the incubation, the culture supernatant was prepared and treated for 90 min at 37°C with anti-mouseJE/MCP-1 antiserum(1:1000) or normal rabbit serum and then applied to the monocyte chemotactic assay. Chemotactic activity is defined as the mean ± SD of total migrating monocyte per oil immersion fields in triplicate samples. The solid line represents the control level. The dashed line represents the activity of 10^{-8}M FMLP.

anti-JE/MCP-1 antiserum(Fig.4). We also observed that the IL-4-stimulated monocyte chemotactic activity was abolished completely by anti-mouse IL-4 antibody(data not shown).

Discussion

The present study shows that IL-4 is a potent inducer of the JE/MCP-1 expression in mouse peritoneal macrophages. To our knowledge, this is the first demonstration that IL-4 may be involved in inflammatory reaction throughout monocyte specific chemoattractant JE/MCP-1 by macrophages. In contrast, since IL-4 inhibited the expression of inflammatory cytokines, such as interleukin-1 and tumor necrosis factor-α, in activated human monocytes(23,24), IL-4 may play a functional role as an anti-inflammatory cytokine. Together with our present study, these studies have suggested that IL-4 may play an important role as a negative and positive regulator of inflammatory cytokine expression in macrophages.

The functional role of IL-4 for monocyte recruitment to inflammatory sites have not been well defined. However, Rollins *et al.*(25) have recently demonstrated that IL-4 induced MCP-1 expression in endothelial cells. Therefore, when circulating monocytes will be migrated across endothelieum, IL-4 may play a functional role via the cytokine-induced MCP-1 by endothelial cells. Our present study suggests a possible involvement of IL-4 in attraction of monocytes migrated from endothelial tissue to the inflammation site. Therefore, the presence of IL-4 in inflammation site may be very important for monocyte recruitment to inflammatory sites.

Although several studies have demonstrated that both JE/MCP-1 and KC, a neutrophil chemoattractant, genes were coexpressed in mouse peritoneal macrophages and fibroblasts treated with lipopolysaccharide and interleukin-1(19-22), interestingly we have observed that IL-4 was unable to induce the KC expression in mouse peritoneal macrophages

(Unpublished data). Standiford *et al.* have shown that IL-4 inhibited IL-8 expression in activated human monocyte(26). Therefore, these results suggest a possibility that IL-4 involved in the selective accumulation of monocytes to inflammatory sites, but not of neutrophils.

Further studies will be required to demonstrate the regulatory mechanism(s) of IL-4 for expression of inflammatory cytokines in inflammatory responses.

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