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■ Brief Communication ■

Monocyte Chemoattractant Protein-1 (MCP-1)/CCL2 Induces the Chemotactic Activity of Human Eosinophils

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Eosinophil is an improtant leukocyte in the development of various inflammatory diseases. Monocyte chemoattractant protein-1 (MCP-1) acts as a key regulator on monocyte movement, and activation of T cells and NK cells. However, the role of MCP-1 in eosinophils remains to be solved. In the present study, we examined the effect of MCP-1 on eosinophil migration, using human eosinophilic EoL-1 cells as an in vitro model of eosinophils. The surface expression of CCR2 in EoL-1 cells was little detected but MCP-1 strongly induced EoL-1 cell migration in a dose-dependent manner. Increased chemotactic activity due to MCP-1 was blocked by pertussis toxin, a G_i/G_o protein inhibitor and U73122, a phospholipase C (PLC) inhibitor. These results suggest that MCP-1 activates G_i/G_o protein and PLC and this signal pathway is involved in eosinphil movement. This finding supports the elucidation of pathogenic mechanism of eosinophilic inflammation such as asthma and atopic dermatitis.

Key Words: Eosinophilis, MCP-1, G_i/G_o protein, Phospholipase C

Eosinophils are main effector cells in allergic disorders, including atopic dermatitis and allergic asthma (Rothenberg, 1998; Gleich, 2000). After allergen or pathogen stimulation, the cells move into the inflamed tissue and secrete the granules. Migration and activation of eosinophils function as important processes in the pathogenesis of the diseases. Eotaxin/CCL11 binding to CC chemokine receptor 3 (CCR-3) is known as the most powerful CC chemokine in the activation and movement of eosinophils, because the cells express high CCR3 expression, compared to other CC chemokine receptors (Bisset and Schmid-Grendelmeier, 2005). Recently, several study groups reported that MIP-1α/CCL3 and RANTES/CCL5 have regulatory functions on eosinophil movement (Tillie-Leblond et al., 2000; Ying et al., 2001).

Monocyte chemoattractant protein (MCP-1) plays important role in immune response and the associated diseases. After binding to CCR2, MCP-1 activates monocytes, memory/activated T cells, NK cells and mast cells (Baggiolini et al., 1997; Moser et al., 2004). MCP-1 is involved in vascular diseases such as atherosclerosis and allergic diseases (Romagnani, 2002; Charo and Taubman, 2004). Although MCP-1 inhibits myeloprogenitor cells, the precise role of MCP-1 in eosinophils has not been understood well (Broxmeyer, 2001).

Human EoL-1 cells were obtained from the Ricken Cell Bank (Tsukuba, Japan) and were maintained in RPMI 1640 supplemented with 10% heat-inactivated FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml). We performed a flow cytometry for detection of CCR2 surface expression using a FACSort cytofluorimeter (Becton Dickinson, Franklin Lakes, NJ). Ten thousand events were collected for each experiment. To evaluate the chemotactic activity of EoL-1 cells induced by MCP-1, we performed a chemotaxis assay using a 48-well microchamber (Neuroprobe, Gaithersburg, MD). A polyvinylpyrrolidone-free membrane (Neuroprobe) with 5 μm pores membrane was used in this experiment.

We first examined CCR2 protein expression in EoL-1

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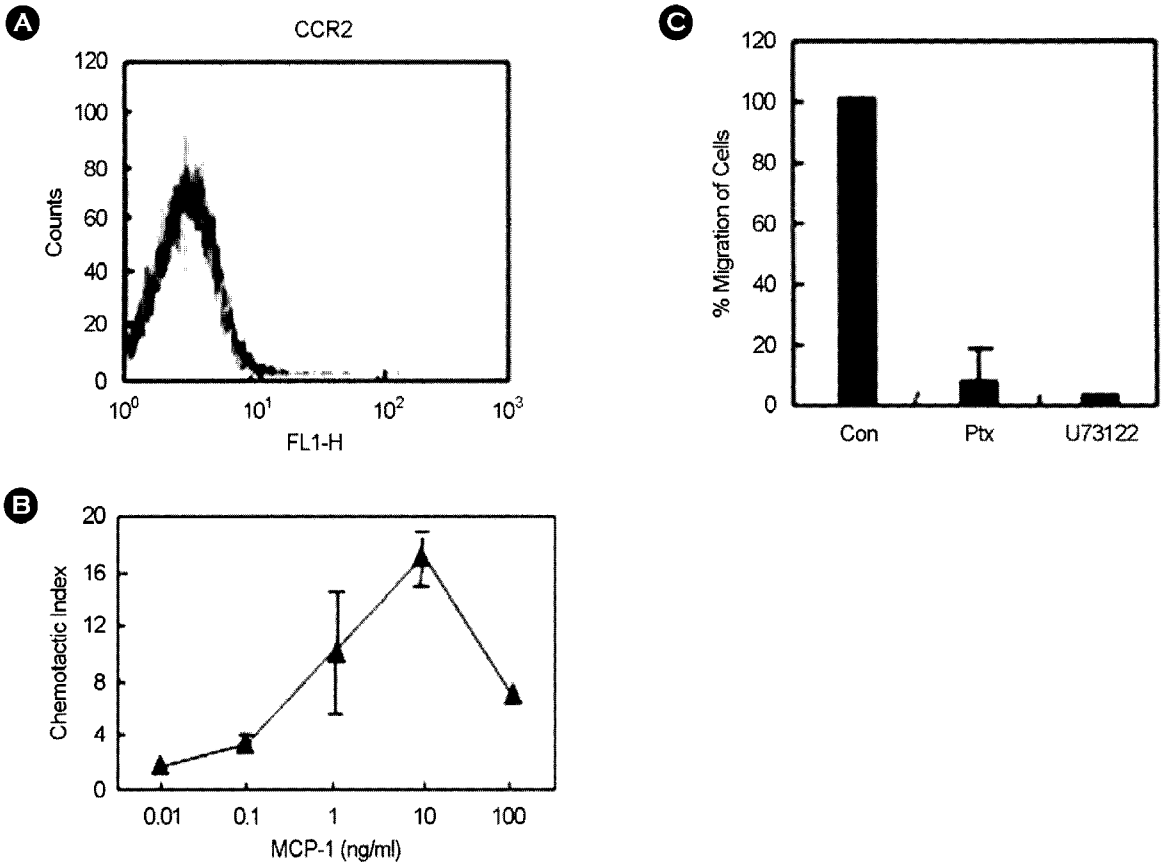

Fig. 1. MCP-1 induces EoL-1 cell migration through G_i/G_o protein and PLC. **(A)** EoL-1 cells were harvested and analyzed by fluorescence-activated cells sorter using anti-CCR2 (thick line). Baseline fluorescence was obtained by incubating with normal mouse IgG (thin line). **(B)** EoL-1 cells were applied to the indicated concentrations of MCP-1 in 48-well microchamber and were allowed to migrate for 90 min. Filters were stained with Diff-Quick. The number of cells that migrated was counted microscopically in two randomly selected fields per well. The chemotactic index (CI) was calculated from the number of cells migrating to MCP-1 divided by that migrating to buffer control. A single experiment includes six replicate measurements and data are expressed as mean CI ± SD of two separate experiments. **(C)** EoL-1 cells were pre-treated in the absence (Con) or presence of 100 ng/ml pertussis toxin (Ptx) and 10 μM U73122. The cells were applied to 10 ng/ml MCP-1 in a 48-well microchamber and were allowed to migrate for 90 min. Data are expressed as means ± SD of two independent experiments, and presented in relation to the negative control, which was set at 100%.

cells by performing flow cytometry. As shown in Fig. 1A, CCR2 was little detected on the cell surface. Although CCR2 expression is very low, we investigated whether MCP-1 induces EoL-1 cell migration. MCP-1 strongly increased the chemotactic activity of EoL-1 cells in a dose-dependent manner (Fig. 1B). These results indicate that MCP-1 has a strong affinity to CCR2, in line with our previous report (Kim et al., 2004). To further understand how MCP-1 triggers the movement of EoL-1 cells, we evaluated the EoL-1 cell migration after pre-treatment with signal protein-specific inhibitors. Since CCR2 is a seven G protein-coupled receptor, EoL-1 cells were treated with pertussis toxin, a G_i/G_o protein inhibitor before the stimulation of MCP-1. The number of the cells migrated by MCP-1 decreased to 7±9.9% of the number that chemoattracted in the absence of pertussis toxin (Fig. 1C). In addition, U73122, a phospholipase C (PLC) inhibitor has an inhibitory effect on EoL-1 cell migration, comparable to the effect of pertussis toxin. We previously reported that CC chemokine receptor, a G protein-coupled receptor, is associated with PLC (Kim et al., 2003; Kim et al., 2005). These data indicate that MCP-1 mediates chemotaxis signal via a pertussis toxin-sensitive G_i/G_o protein and PLC in EoL-1 cells. We are currently under investigation in a downstream signal mechanism after activation of G_i/G_o protein and PLC. In conclusion, we demonstrate that MCP-1 induces eosinophil migration, and this study may help elucidate the cause of eosinophil-associated inflammation.

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