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■ Brief Communication ■

# Anti-inflammatory Effect of 9-cis Retinoic Acid on the Human Mast Cell Line, HMC-1

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Mast cells play important roles in immune-related diseases, in particular, allergic diseases. Although 9-cis retinoic acid (9CRA) has been known as an immune regulator, its function in mast cells is not characterized well. In a previous paper, we demonstrated that 9CRA differentially decreases both CCR2 expression and the MCP-1-induced chemotactic activity of the human mast cell line, HMC-1 cells. In the present study, we examined the effects of 9CRA on the migration and expressions of inflammatory cytokines in HMC-1 cells. It was found that 9CRA significantly inhibited the migration of HMC-1 cells in response to stem cell factor ($P<0.01$), and it had no effect on the mRNA and protein expression of c-kit, a receptor binding to SCF. We further investigated the alternation of inflammatory cytokine expression and identified that 9CRA blocked the mRNA and protein expressions of Th2 cytokines such as interleukin (IL)-4 and IL-5. Taken together, our results demonstrate that 9CRA blocks SCF-induced cell movement and the protein secretion of IL-4 and IL-5, and this indicates that 9CRA may have anti-inflammatory effects on mast cells.

**Key Words:** Retinoic acid, Mast cells, Inflammation

Retinoic acid (RA) is a lipophilic molecule and one of vitamin A or retinal derivatives. It includes two active stereo-isomers, 9-cis retinoic acid (9CRA) and all-trans retinoic acid (ATRA). There are two major receptors binding to RA isomers, the retinoid X receptor (RXR) and the retinoic acid receptor (RAR). 9CRA binds RXR and 9CRA, or ATRA binds RAR (Pemrick et al., 1994; Chambon, 1996). These receptors exist as several isomers (RARα, RARβ, RARγ, RXRα, RXRβ and RXRγ) (Giguere, 1994). RA carried out essential roles in a variety of immunological responses; these include cell growth, differentiation and apoptosis, and leucocyte activation (Stephensen, 2001).

Mast cells are matured from hemopoietic stem cells, and they are known as potent regulators in allergic inflammation (Williams et al., 2000; Theoharides et al., 2004). Stem cell factor (SCF), a ligand to the c-kit receptor, has been known as an activator of differentiation, and the migration of mast cells and the release of SCF-induced Th2 cytokines in mast cells also caused the pathogenesis of allergic diseases (Shakoory et al., 2004; Okayama Y et al., 2006; Frossi et al., 2007).

In the present study, we used the human mast cell line, HMC-1 (the kind gift of Dr. Butterfield), grown in Iscove's medium supplemented with 10% heat-inactivated FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml). RT-PCR was performed to measure the relative quantities of IL-4, IL-5, and c-kit mRNAs in untreated or 9CRA-treated cells. The primer sequences were as follows: IL-4, 5'- GGA CAC AAG TGC GAT ATC ACC -3' and 5'- ATT TCT CTC TCA TGA TCG TC -3'; IL-5, 5'- GAA ATT CCC ACA AGT GCA TTG G -3' and 5'- CTT TCT ATT ATC CAC TCG GTG TTC -3'; c-kit, 5'- AAA GGA GAT CTG TGA GAA TAG GCT C -3' and 5'- AGC TCC CAA AGA AAA ATC CCA TAG G -3'; GAPDH, 5'-ACC ACA GTC CAT GCC ATC AC-3' and 5'-TCC ACC ACC CTG TTG CTG TA-3'. Data are expressed as means ± S.E.M. Statistical differences were analyzed by using paired t-test for a two-group comparison. The SPSS statistical software package (Version 10.0, Chicago, IL) was used for statistical analysis. A significant value is defined as $P<0.01$.

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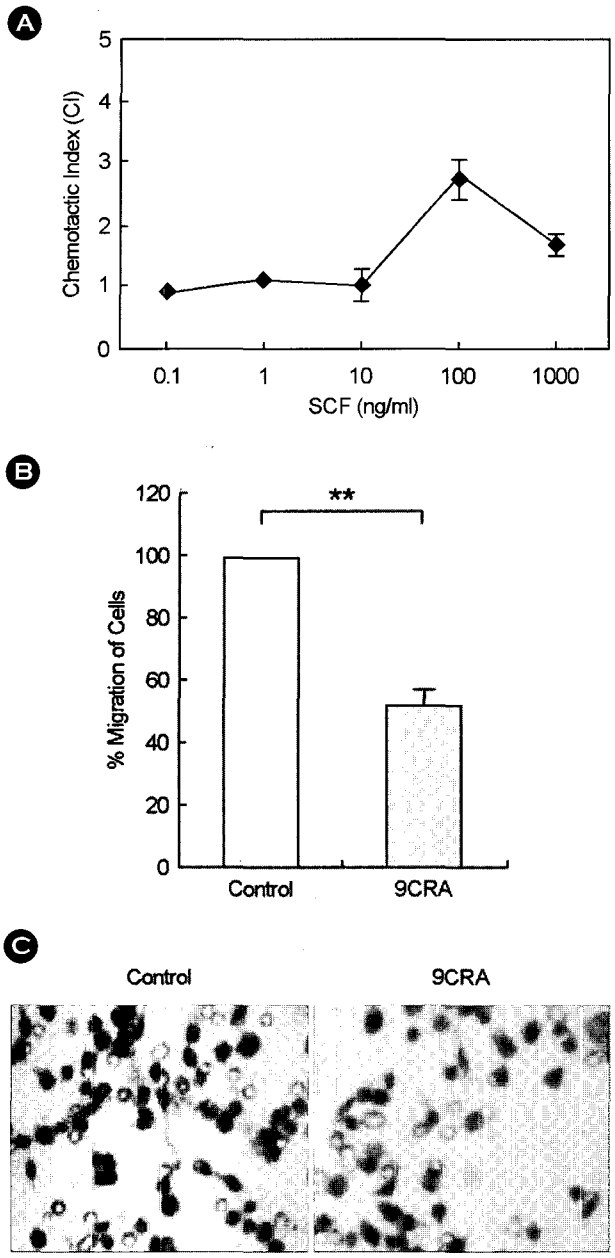


**Fig. 1.** 9CRA inhibits HMC-1 cell migration in response to SCF. **(A)** HMC-1 cells were applied to the indicated concentrations of SCF. After incubation for 5 h, filters were stained with Diff-Quick. The number of cells that migrated was counted microscopically in four randomly selected fields per well. The chemotactic index (CI) was calculated from the number of cells migrating to SCF divided by that migrating to control. Data are expressed as mean CI ± S.E.M. of three independent experiments. **(B)** HMC-1 cells are incubated in the absence (open bar) or presence of 1 μM 9CRA (closed bar) for 24 h. Chemotactic activity in response to the SCF concentration of 100 ng/ml was measured by performing chemotaxis assay. Data are expressed as [mean number of 9CRA-treated cells / number of untreated cells × 100] ± S.E.M of three independent experiments. $^{**}P<0.01$ is assessed as a significant difference between the control and the 9CRA-treated group. **(C)** Photograph of migrated cells after untreatment or 9CRA treatment.

We first examined whether or not SCF induces HMC-1 cell migration by performing cell migration assay using a 48-well microchamber (Neuroprobe, Gaithersburg, MD). The peak level of cell migration reached the SCF concentration of 100 ng/ml, and the migration curve appeared with a typical bell shape (Fig. 1A). 9CRA significantly inhibited SCF-elicited chemotaxis ($P<0.01$) (Fig. 1B & 1C). Although this has been debated in the role of 9CRA in inflammation, including anti-inflammatory or pro-inflammatory effect, we identified the suppressive effect of 9CRA on mast cell migration, which is in agreement with the results of the anti-inflammatory effect of 9CRA on mast cell migration in response to MCP-1 in a previous work (Ko et al., 2006).

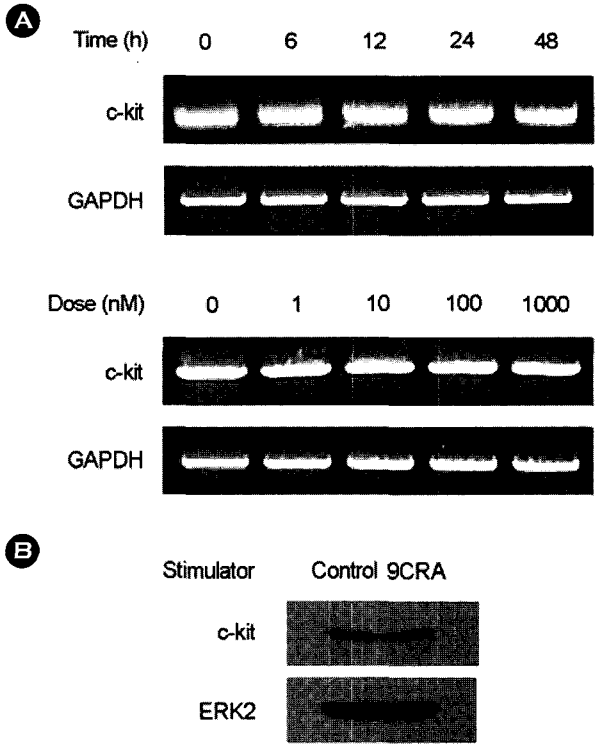


**Fig. 2.** The mRNA and protein expressions of c-kit were not altered by 9CRA in HMC-1 cells. **(A)** HMC-1 cells were starved with 0.5% serum for 24 h, and were incubated with 1 μM 9CRA for the indicated time (left panel) or the indicated concentration of 9CRA for 24 h (right panel). Total RNA was extracted from harvested HMC-1 cells. RNA levels of c-kit were measured by RT-PCR. GAPDH was used as an internal control. Data are expressed as representative of three individual experiments. **(B)** HMC-1 cells were serum starved with 0.5% serum for 24 h. The cells were incubated with 1 μM 9CRA for 24 h. The harvested cells were then lysed, and the extracted proteins were separated on 10% SDS-polyacrylamide gels (100 μg/lane) and were transferred to nitrocellulose membrane. The protein expressions of c-kit were detected by Western blotting with anti-c-kit antibodies. The membrane was stripped and reprobed with ERK2 antibodies for normalization.

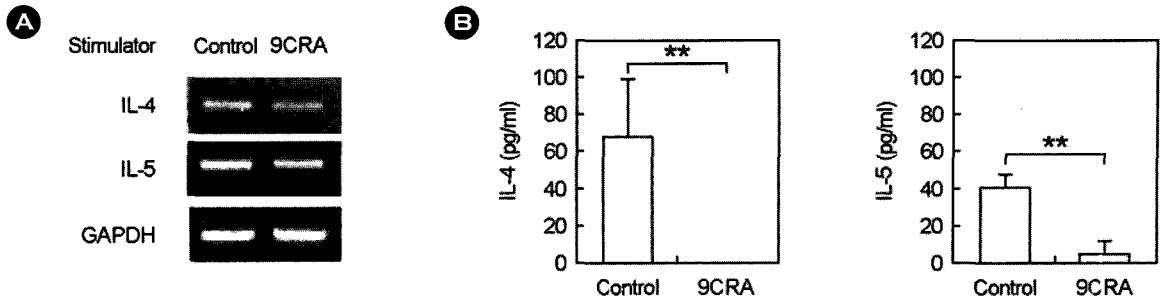


**Fig. 3.** 9CRA decreases both IL-4 and IL-5 secretion. HMC-1 cells were starved with 0.5% serum for 24 h, and were incubated with 1 μM 9CRA for 24 h. Total RNA was extracted from harvested HMC-1 cells. The mRNA levels of IL-4 and IL-5 were analyzed by RT-PCR **(A)**. The supernatant was used for measuring the protein expression of IL-4 and IL-5 by ELISA $^{**}P<0.01$ is assessed as a significant difference between the control and the 9CRA-treated group.

To investigate the precise mechanism of the decreased migration in response to SCF after 9CRA stimulation, we examined the mRNA and protein expression of c-kit by performing RT-PCR and Western blotting. The mRNA and protein levels of c-kit were not changed by 9CRA treatment (Fig. 2). These results indicate that 9CRA decreases SCF-induced migration by the regulation of other signal molecules, and not the down-regulation of c-kit expression. Since Th 2 cytokines, IL-4, and IL-5 are key proteins in allergic inflammation, we examined the alternation of both cytokines due to 9CRA. The mRNA expressions of IL-4 and IL-5 were inhibited by 9CRA, and the secretion of both proteins also showed significant decrease after 9CRA stimulation (Fig. 3). Although it has been recently reported that RA activates signaling molecules as well as gene expression, the exact mechanisms by which 9CRA regulates the anti-inflammatory function, which have been shown in our study's results, are unknown until now (Antonyak et al., 2003; Teruel et al., 2003; Ko et al., 2007). We are currently under investigation in this mechanism.

In summary, we investigated whether or not 9CRA has an anti-inflammatory or pro-inflammatory effect on mast cells and demonstrated that 9CRA functions as an anti-inflammatory stimulator in mast cells by identifying the inhibition of SCF-induced migration and the expressions of IL-4 and IL-5.

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