International Immunology, Vol. 17, No. 9, pp. 1201–1212
doi:10.1093/intimm/dxh297

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CCR7-mediated c-Jun N-terminal kinase activation regulates cell migration in mature dendritic cells

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Keywords: chemokine, JNK, MAPK, signal transduction

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Abstract

c-Jun N-terminal kinase (JNK) is generally thought to be involved in inflammation, proliferation and apoptosis. However, functional role(s) of this molecule in dendritic cells (DCs) has not been well understood. CCR7 ligands, CCL19 and CCL21, induce not only chemotaxis but also endocytosis in mature DCs. In the present study, we examined the role of JNK for inducing chemotaxis and endocytosis in murine mature DCs. CCL19 rapidly enhanced endocytosis of mature DCs within a few minutes, whereas significant migration of mature DCs to this chemokine was detected 30 min or more after incubation. CCL19 significantly activated JNK in mature DCs at 15 min. CCL19 also increased interaction between phospho-JNK and phospho-mitogen-activated protein kinase kinase (MKK) 4 but not phospho-MKK7 in mature DCs, suggesting that the JNK activation is mediated via MKK4. Blocking of this JNK activation significantly inhibited the CCL19-induced migration of mature DCs. Blocking of Rho-associated kinase also inhibited the CCL19-induced migration without affecting the JNK activation. On the other hand, the inhibition of either JNK or Rho-associated kinase showed no significant effects on CCL19-induced endocytosis by mature DCs. These findings suggest that CCL19 activates JNK via a Rho-independent pathway, thereby inducing migration of mature DCs, whereas the JNK activation is dispensable for the CCL19-induced endocytosis. It seems that at least two different pathways, JNK pathway and Rho-associated kinase pathway, are involved in the CCR7-mediated migration of mature DCs. Thus, we demonstrate herein a novel role of JNK for regulating chemokine-induced DC migration.

Introduction

Chemokines constitute a family of low-molecular weight proteins. There are two main groups, the CXC chemokines and the CC chemokines, which differ from one another in their structural properties and chromosomal location. Most chemokines possess four conserved cysteines in specific positions. Chemokines exert their effects by interacting with seven-transmembrane receptors coupled with G proteins (1–3). Chemokines regulate leukocyte trafficking by inducing chemotaxis and adhesion, and systemically organize the suitable distribution of various leukocytes, which results in an efficient induction of immune responses against various pathogens (4, 5). However, the chemokine receptor-mediated signal pathways involved in chemotaxis and adhesion has not been fully understood.

Dendritic cells (DCs) are potent antigen-presenting cells that play a major role in the regulation of immune responses to a variety of antigens (6–9). DCs developmentally regulate the expression of chemokine receptors to facilitate their migration from the peripheral tissues to the T cell area of regional lymph nodes (LNs) (5, 10). This process is critical for interaction between DCs and T cells in the T cell area, which initiates adaptive immunity (1, 3, 11). High densities of CCR7, a receptor for two CC chemokines, CCL19 (EBV-induced receptor ligand chemokine) and CCL21 (secondary lymphoid tissue chemokine), are expressed on mature, but not immature DCs (12–14). Both CCL19 and CCL21 are constitutively expressed at high levels in the T cell area of LNs and spleen, and induce migration of mature DCs into these lymphoid tissues (1, 3). Mature DCs fail to migrate into the T cell area in CCR7-deficient mice, correlating with severely abolished adaptive immune responses (11). Thus, the interaction between CCR7 and CCL19/CCL21 is an essential process controlling the migration

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Received 19 April 2005, *accepted* 16 July 2005

Transmitting editor: R. Medzhitov

Advance Access publication 18 July 2005

of mature DCs into the regional LNs. Recently, we have demonstrated that, in addition to the chemotaxis, CCR7 ligands enhance dendritic extension and endocytic activity of mature DCs (15, 16). Thus, it seems that CCR7 can regulate multiple signaling pathways in DCs. However, the signal transduction system regulating the CCR7-mediated multiple functions remains to be elucidated.

Signal transduction via mitogen-activated protein kinases (MAPKs) plays an important role in cellular responses including growth factor-induced cell proliferation, differentiation and survival. There are at least three distinct MAPK signaling pathways in mammals, including the extracellular signal-regulated kinases (ERKs), the c-Jun N-terminal kinases (JNKs) and the p38 MAPK (17). It has been reported that extracellular stimuli such as tumor necrosis factor (TNF)-α and LPS activate ERK, JNK and p38 MAPK in DCs (18–20). The roles of ERK and p38 MAPK activation for regulating DC functions have been well documented; ERK activation is involved in cell survival and negative regulation of the phenotypic maturation and IL-12 production of DCs (21, 22); on the contrary, p38 MAPK activation positively regulates the phenotypic maturation and IL-12 production of DCs (23–25). On the other hand, the role of JNK activation for regulating DC functions is just starting to be characterized and not fully understood.

In the present study, we examined the role of JNK in CCR7 ligand-induced chemotaxis and endocytosis using murine DCs. We demonstrate herein a novel role of JNK in regulation of chemokine-induced DC migration.

Methods

Reagents

Murine recombinant granulocyte macrophage colony-stimulating factor (GM-CSF) was purchased from PeproTech (London, UK). Recombinant murine CCL19 and CCL21 were obtained from R&D Systems (Minneapolis, MN, USA). FITC-dextran (Dex.) (molecular weight 40 000, anionic) was purchased from Molecular Probes (Eugene, OR, USA). Iscove's modified Dulbecco's medium (IMDM), pertussis toxin (PTX), LPS from *Escherichia coli* 055:B5 and *Clostridium difficile* toxin B (Toxin B), Y-27632, PD98059, SP600125, c-Jun N-terminal kinases inhibitor-1 (JNKI-1), negative control peptide (NC) of JNKI-1 and wortmannin were obtained from Calbiochem (La Jolla, CA, USA). LY294002 was purchased from Cayman Chemical (Ann Arbor, MI, USA). SP600125 was purchased from BIOMOL Reseach Laboratories Inc. (Plymouth Meeting, PA, USA).

Antibodies

Anti-Akt antibody, anti-phospho-Akt (Thr308) antibody, anti-phospho-c-Jun (Ser63) II antibody, anti-phospho-mitogen-activated protein kinase kinase (MKK) 4 (Thr261) antibody, anti-phospho-MKK7 (Ser271/Thr275) antibody, anti-phospho-p38 MAPK (Thr180/Tyr182) antibody, anti-phospho-p44/p42 MAPK (Thr202/Tyr204) antibody, anti-SAPK/JNK antibody, anti-phospho-SAPK/JNK (Thr183/Tyr185) (G9) mAb and HRP-conjugated anti-rabbit IgG antibody were purchased from Cell Signaling Technology (Beverly, MA, USA). Isotype-matched control IgG (mouse $IgG_{1\kappa}$) for the anti-phospho-SAPK/JNK mAb was obtained from PharMingen (La Jolla, CA, USA). HRP-conjugated goat anti-mouse IgG_1 antibody was obtained from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA, USA).

Culture media

Culture medium used in the present study was IMDM supplemented with 100 IU ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, 600 μg ml^{-1} L-glutamine and 50 μM 2-mercaptoethanol. Fibroblast supernatants from NIH/3T3 cells were collected from confluent cultures with IMDM containing 10% heat-inactivated FCS.

DC culture

The DC line (BC1) was generated from BALB/c mouse spleen as previously described (21, 26). BC1 cells were cultured and expanded in R1 medium, IMDM containing 10% FCS, 30% culture supernatants from NIH/3T3, 10 ng ml^{-1} mouse recombinant GM-CSF and 50 μM 2-mercaptoethanol. In the present study, unstimulated BC1 cells were used as immature DCs and BC1 cells treated with 5 μg ml^{-1} LPS for 24 h were used as mature DCs (16, 21).

Spleen-derived dendritic cells (SDDCs) were generated by culturing C57BL/6 (B6) splenocytes in R1 medium for 14 days as previously described (15, 16, 27). After the culture, $CD11c^+$ cells were positively selected using anti-CD11c (N418) MicroBeads and MACS columns (Miltenyi Biotec, Bergisch Gladbach, Germany). The selected cells, >95% $CD11c^+$ DCs in purity, were used as SDDCs. SDDCs treated with 5 μg ml^{-1} LPS for 24 h were used as mature SDDCs.

Endocytosis

Mature BC1 cells or SDDCs (1×10^5) were incubated in R1 medium buffered with 20 mM HEPES at 37°C. Thereafter, endocytic reaction was conducted as previously described (16). The endocytic tracer and chemokine were added concurrently, prior to a 0.5- to 5-min incubation. FITC-Dex. was added to a final concentration of 1 mg ml^{-1}. CCL19 or CCL21 was added to a final concentration of 2 nM (18.8 ng ml^{-1}), since this concentration of each chemokine showed a maximum effect on endocytosis (data not shown). Endocytosis of the tracer was halted at the indicated time points by rapid cooling of the cells on ice. The cells were then washed and stained with propidium iodide to exclude dead cells. The fluorescence intensity of the cells was analyzed by flow cytometry on EPICS® XL (Coulter Co., Miami, FL, USA). Incubation of cells with the endocytosis tracer on ice was used as a background control. The mean fluorescence intensity (MFI) resulting from the subtraction of background control from each experimental sample represents the amount of incorporated tracer.

To examine the effect of each inhibitor, the cells were pre-treated with PTX (100 ng ml^{-1}), Toxin B (100 ng ml^{-1}) or Y-27632 (10 μM) for 3 or 4 h or 30 min, respectively. In some experiments, the cells were pre-treated with PD98059 (30 μM), SP600125 (10 μM), LY294002 (30 μM) or wortmannin (300 nM) for 1 h. After pre-treatment with an inhibitor, the cells were incubated with the endocytic tracer for 2 min at 37°C in the

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presence of the inhibitor. Viability of the cells was unaffected by any inhibitors in the present experiments (data not shown).

Chemotaxis assay

BC1 cells or SDDCs (1.5×10^5) were added to upper wells of 5-μm-pore, polycarbonate 24-well tissue culture inserts (Coster, Cambridge, MA, USA) in 100 μl, with 600 μl chemokine or medium in the lower wells (15, 28). CCL19 or CCL21 was added to a final concentration of 2 nM, since this concentration of each chemokine showed maximum effect on chemotaxis (data not shown). Migration assays were conducted in R1 medium buffered with 20 mM HEPES for 90 min at 37°C except one shown in Fig. 3H. Thereafter, 60 μl of 30 mM EDTA was added to lower wells and further incubated for 5 min at 37°C. Migrated cells recovered from each lower well were counted using comparison to a known number of beads as an internal standard (Flow-Count™ Fluorospheres; Coulter, Miami, FL, USA) as described elsewhere (28). The percent migration was determined from numbers of the starting cells and the migrated cells.

To examine the effect of inhibitor, the cells were pre-treated with PTX (100 ng ml^{-1}), Toxin B (100 ng ml^{-1}) or Y-27632 (10 μM) for 3 or 4 h or 30 min, respectively. In the other experiments, the cells were pre-treated with PD98059 (30 μM), SP600125 (10 μM), LY294002 (30 μM) or wortmannin (300 nM) for 1 h. In some experiments for dose–response analysis, various concentrations of SP600125 or Y-27632 were used. After the inhibitor pre-treatment, the upper well including the pre-treated cells was superposed on the lower well with chemokine-containing medium. Both the upper well and the lower well contain the inhibitors throughout the assay. The chemotaxis assays were then performed for 90 min.

Adhesion assay

Adhesion assay was performed as described elsewhere with minor modifications (29, 30). Mature BC1 cells were pre-cultured with SP600125 (10 μM) for 1 h. After the inhibitor treatment, the cells (1×10^4) suspended in R1 medium containing the inhibitor and/or CCL19 (2 nM) were seeded on a fibronectin (FN)-coated chamber slide (eight-well glass slide; Nalge Nunc International, Naperville, IL, USA) set on ice. The cells were pre-incubated for 15 min on ice and then incubated for 2 min at 37°C. The slide was dipped twice in PBS to remove non-adherent cells. All adherent cells were detached by incubating with 2 mM EDTA/PBS for 5 min at 37°C and recovered. The recovered cell number was counted using comparison with a known number of beads as an internal standard (Flow-Count™ Fluorospheres) as described elsewhere (28). The proportion of adherent cells was determined from numbers of the starting cells and the adherent cells.

In vitro kinase assays

Mature BC1 cells (6×10^6) were cultured in 0.5% FCS IMDM buffered with 20 mM HEPES for 4 h at 37°C, and then treated with CCL19 (2 nM) for 0.5–60 min. Reactions were halted by rapidly cooling on ice. The cells were washed by ice-cold PBS containing 2 mM EDTA and then lysed in buffer solution containing 20 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM glycoletherdiamine tetraacetic acid, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM Na_3VO_4, 1 μg ml^{-1} leupeptin and 1 mM phenylmethanesulfonyl fluoride. The protein concentration of supernatants was determined using the Bio-Rad protein determination assay (Bio-Rad, Hercules, CA, USA). Kinase activity of ERK1/2 or JNK of the cells was determined using a commercially available kinase assay kit (Cell Signaling Technology) according to the manufacturer's protocol. The cell lysate was subjected to immunoprecipitation with immobilized anti-phospho-ERK1/2 mAb or c-Jun fusion protein beads. The complex was then subjected to kinase reaction with a substrate [Ets-like-protein-1 (Elk-1) for ERK1/2 or c-Jun for SAPK/JNK]. After the kinase reaction, the amount of phospho-Elk-1 or phospho-c-Jun was determined by western blotting. Intensity of the specific band was analyzed with Science Lab 99 Image Gauge Ver. 3.4 software (FUJI FILM, Tokyo, Japan).

Immunoblotting

Mature BC1 cells ($3–6 \times 10^6$) were incubated in 0.5% FCS IMDM buffered with 20 mM HEPES. To detect the level of each phosphorylated protein or total protein, the cell lysates were separated by SDSP (7.5% for the JNK protein or 12% for other proteins), and then blotted onto an immobilon membrane (Millipore Corp.). Phosphorylated protein or total protein was detected using a primary antibody against each protein and a HRP-conjugated second antibody with enhanced chemiluminescence system.

Immunoprecipitation

Mature BC1 cells (6×10^6) were incubated in 0.5% FCS IMDM buffered with 20 mM HEPES for 4 h at 37°C, and then treated with CCL19 (2 nM) for 10 min. The cell lysates were immunoprecipitated with anti-phospho-SAPK/JNK (Thr183/Tyr185) (G9) mAb and protein G–Sepharoses (Sigma Chemical, St Louis, MO, USA). The protein concentration of supernatants was determined using the above-described methods. The cell lysate was subjected to a similar procedure with isotype-matched control IgG, as a negative control. The immunocomplexes were washed and separated by SDSP, followed by immunoblot analysis using anti-phosphorylated MKK4 antibody or anti-phosphorylated MKK7 antibody and HRP-conjugated second antibody with enhanced chemiluminescence system.

Statistical analysis

The Student's *t*-test was used to analyze data for significant differences. *P*-values <0.05 were regarded as significant.

Results

CCL19-induced JNK activation in mature DCs

We have previously established a murine DC line, BC1 cells, from BALB/c splenocytes, according to the Winzler's method (21, 26). Unstimulated BC1 cells are physiologically and functionally typical immature DCs. Following treatment with TNF-α or LPS for 24 h, BC1 cells exhibit mature DC phenotype and functions (15, 16, 31–35). It was demonstrated that

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CCR7 ligands, CCL19 and CCL21, induced not only chemotaxis but also endocytosis in these mature BC1 cells (16). Using a similar *in vitro* differentiation system of DCs, a number of important findings have been reported and verified (21, 26, 36, 37).

In the present study, we examined a role of JNK in DC functions using these BC1 cells and SDDCs that were freshly generated by culturing mouse splenocytes. Since no reports have shown that CCR7 ligands activate JNK, we first evaluated the effect of CCL19 on JNK activity in mature DCs. Mature BC1 cells were treated with CCL19 for variable times, ranging from 30 s to 60 min. *In vitro* kinase assay for JNK activity was performed using c-Jun as a substrate for JNK. A modest but distinct band of phospho-c-Jun was detected in mature BC1 cells treated with the medium alone (Fig. 1A). At early time points, ranging from 30 s to 5 min, CCL19 exerted no influences on the JNK activity in mature BC1 cells. In contrast, 10 min after stimulation with CCL19, levels of the activated JNK increased significantly; this activity showed a peak at 15 min and then decreased from 30 to 60 min (Fig. 1A).

In addition to the kinase assay, we also analyzed the level of phospho-JNK, an active form of JNK, in mature DCs after treatment with CCL19 (Fig. 1B). The level of phospho-JNK in mature DCs increased after 10 min or more incubation with CCL19 and reached a peak at 15 min. Thus, the level of phospho-JNK in mature DCs followed almost the same time course as that in the kinase activity of JNK. On the other hand, levels of phospho-ERK and phospho-p38 MAPK, active forms of ERK and p38 MAPK, respectively, rapidly increased (at 2 min) after CCL19 treatment, and then decreased at 10–30 min. Akt is a downstream effector of phosphatidylinositol 3′-kinase (PI3K). The level of phospho-Akt, an active form of Akt, increased 2 min after the CCL19 treatment. The increased Akt activity was sustained at least for 30 min. Thus, the time course of JNK activation was delayed as compared with that of the other kinases in mature BC1 cells.

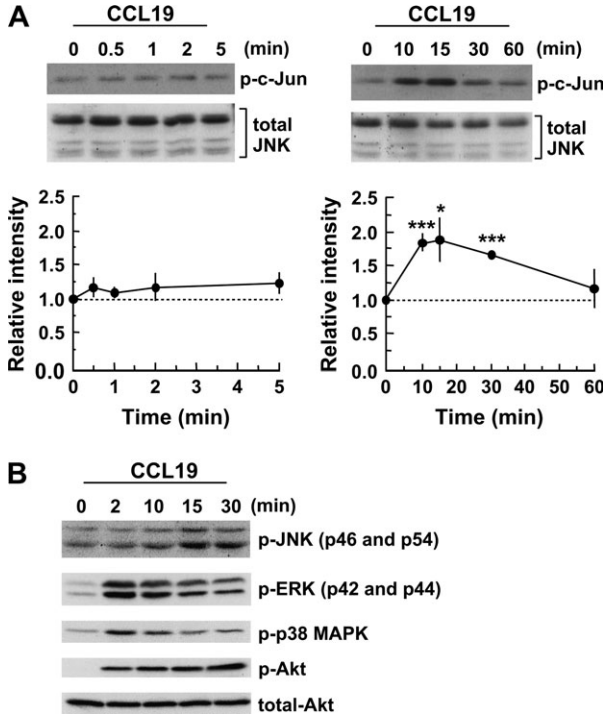

Fig. 1. CCL19-induced activation of various intracellular signaling pathways in mature BC1 cells. Mature BC1 cells (3–6 × 10^6) were treated with CCL19 (2 nM) for the indicated times (0.5–60 min). (A) Kinase activity of JNK in mature BC1 cells. Kinase activity of JNK in the cell lysates was evaluated by *in vitro* kinase assay with glutathione-S-transferase-c-Jun as the substrate. Amounts of phosphorylated-c-Jun (p-c-Jun) and total JNK protein levels in the cell lysates were determined by western blotting. A representative blotting is shown (A, upper). Data are expressed as -fold increase relative to representing the unstimulated levels (A, lower). Each symbol shows the mean ± SE of five independent experiments. Statistical significance was calculated by the Student's *t*-test (*$P < 0.05$, ***$P < 0.001$). (B) Levels of active form of JNK, ERK, p38 MAPK and Akt in mature DCs. Phospho-JNK (p-JNK), phospho-ERK (p-ERK), phospho-p38 MAPK (p-p38 MAPK), phospho-Akt (p-Akt) and total Akt in the cell lysates were determined by western blotting. The data are representative of two independent experiments.

Time course of CCR7 ligand-induced endocytosis and chemotaxis of mature DCs

We then examined and compared the time course of CCR7 ligand-induced endocytosis and that of chemotaxis of mature BC1 cells. In agreement with our previous study (16), CCL19 markedly increased endocytosis of FITC-Dex. by mature BC1 cells within a few min, while significant cell migration to CCL19 was detected at later time (Fig. 2A and B). In the absence of chemokines, mature BC1 cells exhibited negligible endocytosis. After CCL19 treatment, MFI of mature BC1 cells internalizing FITC-Dex. significantly increased from 30 s to 2 min (Fig. 2A). The CCL19-induced endocytosis by mature BC1 cells was also detected using confocal microscopy (data not shown) (16). On the other hand, significant migration of mature BC1 cells to CCL19 was detected after 30 min or more of incubation (Fig. 2B). The number of migrated cells

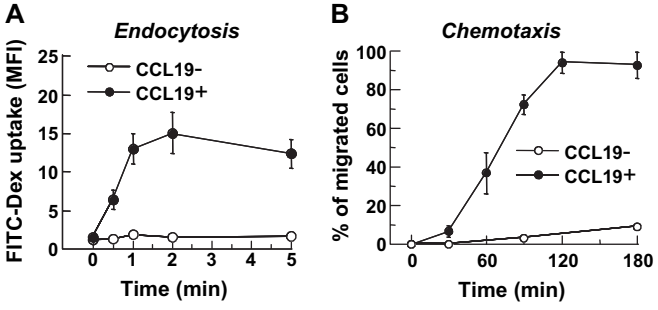

Fig. 2. Time course of CCL19-induced endocytosis and chemotaxis of mature BC1 cells. (A) CCL19-induced endocytosis. Mature DCs were incubated with FITC-Dex. (1 mg ml^{-1}) in the presence or absence of CCL19 (2 nM) for the indicated times (0.5–5 min). MFI of FITC-Dex. endocytosed is shown. (B) CCL19-induced chemotaxis. Mature BC1 cells were seeded on the upper well and CCL19 (2 nM) was added to the lower well. Chemotaxis assay was then performed for the indicated times (30–180 min). Numbers of migrated cells into the lower well are expressed as the percentage of input cells in the upper well at the start time of the chemotaxis assay. Each symbol represents the mean ± SE of four (A) or three (B) independent experiments.

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reached a peak at 120 min after the incubation with CCL19. In the absence of chemokines, mature BC1 cells exhibited negligible migration.

Effects of specific inhibitors for MAPK and PI3K on CCL19-induced endocytosis and chemotaxis of mature DCs

Figure 1 shows that CCL19 activates MAPK and PI3K/Akt pathways in mature DCs. We next examined effects of specific inhibitors of MAPK and PI3K on CCL19-induced endocytosis and chemotaxis in mature BC1 cells. SP600125, a specific inhibitor of JNK, decreased the level of phospho-JNK in the CCL19-treated mature BC1 cells in a dose-dependent manner (Fig. 3A); SP600125 at 10 μM significantly decreased the level to the constitutive level in unstimulated cells; SP600125 at 20 μM decreased the level to below the constitutive level. When functional influences of SP600125 were analyzed, CCL19-induced migration was inhibited by SP600125 in a dose-dependent manner (Fig. 3B). This inhibitory effect of SP600125 reached a maximum at 10 μM. Since 10 μM SP600125 showed no significant effects on the cell viability, while this chemical at 20 μM slightly but significantly affected the viability (data not shown), we used 10 μM SP600125 in subsequent experiments. This dose of SP600125 considerably reduced the CCL19-induced increase in JNK activity (Fig. 3C) and significantly inhibited the chemotaxis (Fig. 3D). In contrast, SP600125 showed no significant effects on CCL19-induced endocytosis (Fig. 3D). Similarly, SP600125 abrogated CCL21-induced chemotaxis but not endocytosis in mature BC1 cells (data not shown). Thus, JNK pathway appeared to be involved in CCR7-mediated migration but not endocytosis in mature DCs.

It has been reported that stimulation of CCR7 or CXCR4 results in activation of ERK and PI3K in T cells (38). However, it remains unclear whether these activations are related to the chemotaxis or not. We then analyzed influences of ERK and PI3K activation on DC functions using specific inhibitors. PD98059 (30 μM), a specific inhibitor of ERK pathway, completely inhibited CCR7-mediated increase of ERK activity in mature BC1 cells (Fig. 3E). However, PD98059 showed no significant effects on CCL19-induced endocytosis and chemotaxis (Fig. 3D). A similar result was obtained with wortmannin, a specific inhibitor of PI3K. Wortmannin (300 nM) completely blocked CCL19-induced activation of Akt, a downstream effector of PI3K, in mature BC1 cells (Fig. 3F), but this chemical affected neither CCL19-induced endocytosis nor chemotaxis of mature BC1 cells (Fig. 3D). LY294002 (30 μM), another inhibitor of PI3K, also significantly inhibited CCL19-induced Akt activation (Fig. 3F), while showing no significant effects on either endocytosis or chemotaxis (Fig. 3D). Thus, it seemed that CCR7-mediated activation of ERK or PI3K/Akt pathway was not involved in induction of endocytosis and chemotaxis in mature DCs.

Chemokines induce rapid integrin-dependent adhesion to adhesion molecules such as intercellular adhesion molecule (ICAM)-1 and extracellular matrix such as FN (29, 30). We then analyzed effects of SP600125 on CCR7-mediated rapid adhesion of mature BC1 cells to FN. CCL19 triggered significant adhesion of the cells to FN after 2 min of incubation (Fig. 3G), which is in agreement with the previous study (29). However, SP600125 showed no significant effects on the CCR7-mediated adhesion of mature BC1 cells.

Functional analyses of BC1 cells were performed in R1 medium containing GM-CSF and fibroblast supernatants, since this conditioned medium well maintained the cell viability. However, a possibility that these growth factors affected the CCR7-mediated cell migration under the influence of activated JNK remained. Thus, we examined the effect of JNK blocking on CCR7-mediated migration of mature BC1 cells in the absence of GM-CSF and fibroblast supernatants. Both CCL19 and CCL21 markedly induced the migration of mature BC1 cells without these growth factors and the CCR7-mediated migration was significantly inhibited by SP600125 (Fig. 3H).

We also examined the effect of SP600125 on functions of SDDCs that were freshly generated from splenocytes. SP600125 significantly reduced CCL19-induced chemotaxis of mature SDDCs to 38% of the control (Fig. 3I). By contrast, no significant influences of SP600125 were detected on the CCL19-induced endocytosis. Similarly, SP600125 abrogated CCL21-induced chemotaxis but not endocytosis in mature SDDCs (data not shown). These findings were consistent with prior observations with the BC1 cell line.

Effect of a JNK inhibitory peptide on CCL19-induced endocytosis and chemotaxis of mature DCs

A cellular regulator of JNK pathway has been identified and termed c-Jun-interacting protein 1 (JIP1). It has been reported that JNK, MKK7 and mixed lineage kinases (MLK) bind to separate sites on JIP1 (39). Further, a novel JNK inhibitor, JNKI-1, has recently been developed by linking the JNK-binding motif of JIP1 to the HIV-TAT transporter sequence (40, 41). JNKI-1 is a cell-penetrating peptide and selectively blocks the access of JNK to c-Jun and other substrates by a competitive mechanism. Then, we examined the effect of JNKI-1 on CCL19-induced endocytosis and chemotaxis in mature BC1 cells. JNKI-1 significantly reduced the CCL19-induced chemotaxis to 52% of the control [NC of JNKI-1] (Fig. 3J). In contrast, this peptide exhibited no significant influence on the CCL19-induced endocytosis by mature BC1 cells.

CCL19-induced interaction between JNK and MKK4 in mature DCs

Our results indicate that CCL19 activates JNK pathway and thereby induces migration of mature DCs. We then examined the protein–protein interactions between JNK and MKK4 or MKK7 that are upstream kinases of JNK, following stimulation of mature DCs with CCL19. Mature BC1 cells were treated with CCL19 for 10 min and phospho-JNK in the cell lysate was immunoprecipitated by anti-phospho-JNK mAb. Then, the levels of phospho-MKK4 or phospho-MKK7 in the immunocomplexes were evaluated by western blotting. A distinct band of phospho-MKK4 was detected in untreated cells, indicating constitutive interaction between phospho-JNK and phospho-MKK4 (Fig. 4A). Stimulation of BC1 cells with CCL19 markedly increased the band intensity of phospho-MKK4. In contrast, CCL19 showed negligible effect on amounts of phospho-MKK7 bound to phospho-JNK, although constitutive

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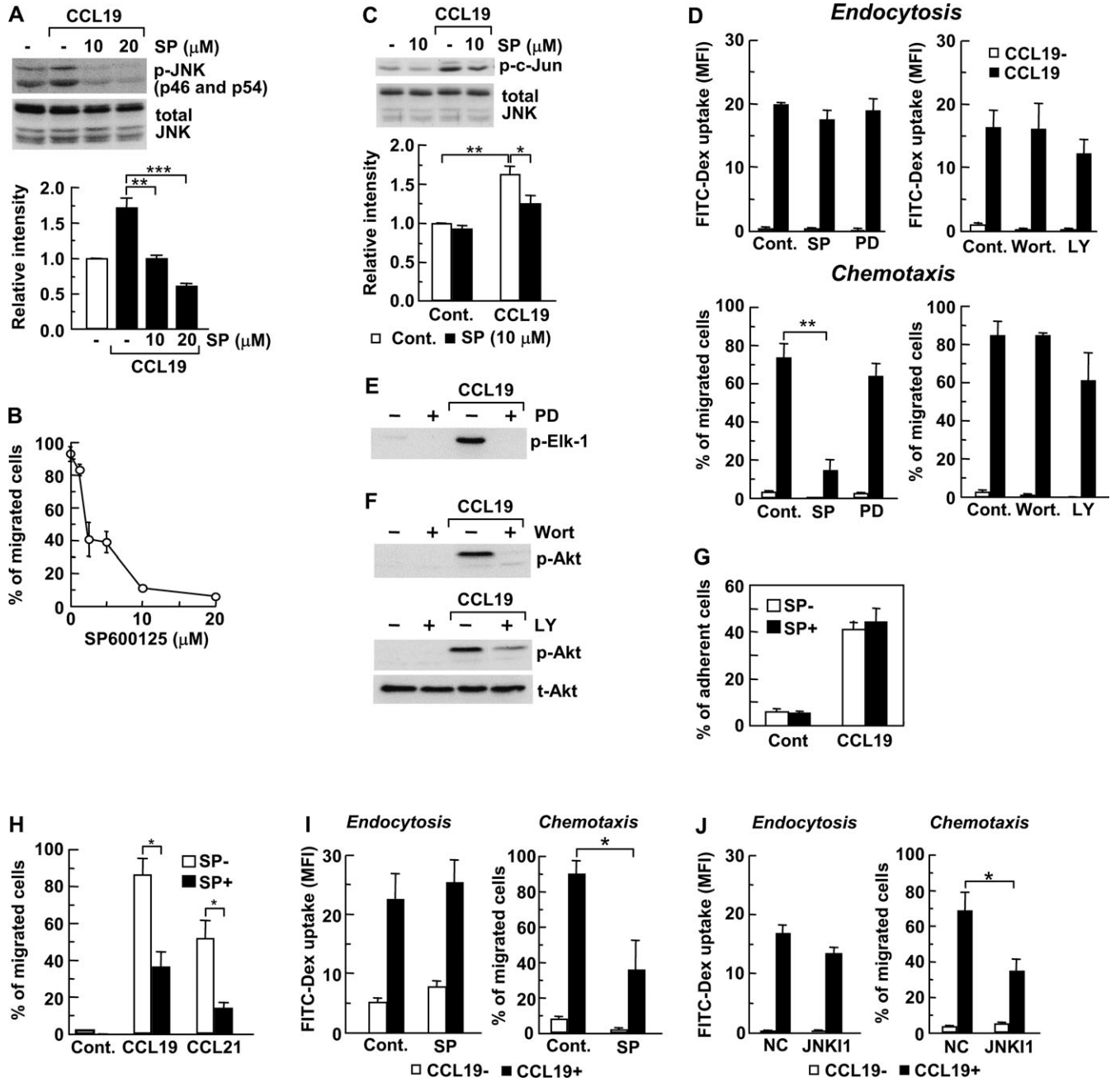

Fig. 3. Role of JNK activation in CCR7-mediated migration of mature DCs. (A) Western blotting of activated JNK (p-JNK) in mature BC1 cells. Mature BC1 cells were pre-treated with SP600125 (SP) at 10 or 20 μM for 1 h. After the pre-treatment, the cells were stimulated with CCL19 (2 nM) for 15 min and the cell lysates were subjected to the western blotting of p-JNK or total JNK. A representative immunoblot of three independent experiments is shown (upper). The relative intensity of the specific band of p-JNK is shown (lower). Data represent the mean relative intensity ± SE of three independent experiments. (B) Dose–response analysis of the effect of SP on CCL19-induced chemotaxis of mature BC1 cells. Mature BC1 cells were pre-treated with SP at 1.25–20 μM for 1 h and then chemotaxis assay was performed for 90 min in the presence of SP. (C) Kinase assay for JNK activity in mature BC1 cells. Mature BC1 cells were pre-treated with SP (10 μM) for 1 h. After the pre-treatment, the cells were stimulated with CCL19 (2 nM) for 15 min and the cell lysates were subjected to the *in vitro* kinase assay with glutathione-S-transferase-c-Jun as the substrate of activated JNK. The levels of phosphorylated c-Jun (p-c-Jun) and total JNK protein levels in the whole cell lysates (total JNK) were determined by immunoblotting. A representative immunoblot from five independent experiments is shown (upper). The relative intensity of the specific band of p-c-Jun is shown (lower). Data represent the mean relative intensity ± SE of five independent experiments. (D) Effects of various inhibitors for MAPK and PI3K on CCL19-induced endocytosis and chemotaxis of mature BC1 cells. Mature BC1 cells were pre-treated with PD98059 (PD) at 30 μM, SP at 10 μM, LY294002 (LY) at 30 μM or wortmannin (Wort.) at 300 nM for 1 h. After the pre-treatment, the cells were subjected to endocytosis assay (upper) or chemotaxis assay (lower). In the endocytosis assay, the cells were incubated with FITC-Dex. (1 mg ml^{-1}) in the presence of CCL19 (2 nM) for 2 min and then the MFI of the cells was analyzed by flow cytometry. In the chemotaxis assay, the cells were seeded on upper wells and CCL19 (2 nM) was added to lower wells. Numbers of migrated cells into the lower well after 90 min were analyzed by flow cytometry and expressed as the percentage of input cells in the upper well at the start time of chemotaxis assay. Each column represents the mean ± SE of three

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interaction of these proteins appeared to be present (Fig. 4B). These findings suggest that JNK is activated by MKK4 but not by MKK7 following the CCR7-mediated stimulation in mature DCs.

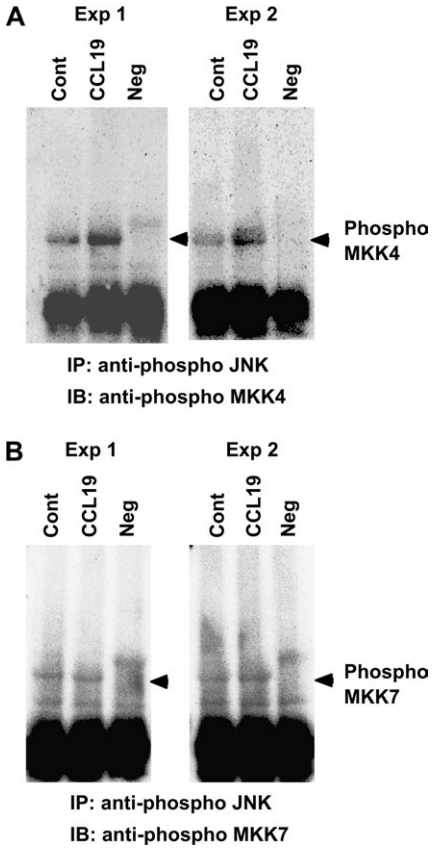

Fig. 4. Interaction of JNK and MKK4 in CCL19-treated mature DCs. Mature BC1 cells were untreated (Cont.) or treated with CCL19 (2 nM) for 10 min. The cell lysates were subjected to immunoprecipitation (IP) with an anti-phospho-SAPK/JNK (Thr183/Tyr185) mAb and protein G–Sepharoses. As a negative control (Neg), cell lysate from CCL19-treated cells was immunoprecipitated with isotype-matched control IgG. Phosphorylated MKK4 or MKK7 in the immunocomplexes was detected by immunoblotting (IB) with an anti-phospho-MKK4 mAb (A) or an anti-phospho-MKK7 mAb (B). Two independent experiments (Exp. 1 and Exp. 2) are shown.

Effects of PTX, Toxin B or Y-27632 on CCL19-induced endocytosis and chemotaxis of mature DCs

PTX specifically binds to Gi proteins and inhibits chemokine-induced cell migration (2, 14, 42). We examined the effect of PTX on CCL19-induced endocytosis and chemotaxis in mature BC1 cells. CCL19 again markedly induced endocytosis and chemotaxis of mature BC1 cells at 2 and 90 min, respectively (Fig. 5A). PTX completely inhibited both the CCL19-induced endocytosis and chemotaxis, suggesting that these functions were mediated via Gi proteins coupled with CCR7.

Rho family proteins are thought to be involved in endocytosis by immature DCs and macrophages (43–46). We have recently demonstrated that CCL19-induced endocytosis can be completely blocked by Toxin B, an inhibitor of the Rho family proteins, Rho, Rac and Cdc42 (16). However, this process was not abrogated by Y-27632 that specifically inhibits Rho-associated kinase. We then compared the effect of Toxin B and that of Y-27632 on endocytosis or chemotaxis of CCL19-treated mature BC1 cells. Toxin B markedly reduced both the CCL19-induced endocytosis and chemotaxis to 18 and 33% of the control, respectively (Fig. 5B). In contrast, Y-27632 significantly reduced the CCL19-induced chemotaxis to 39% of the control, but rather slightly enhanced the CCL19-induced endocytosis (Fig. 5C).

Effects of Y-27632 and Toxin B on CCL19-induced JNK activation in mature DCs

Our prior findings suggested that both JNK pathway and Rho-associated kinase pathway were involved in CCR7-mediated induction of chemotaxis in mature DCs (Figs 3– 5). To examine whether Rho pathway participates in CCL19-mediated JNK activation, we then analyzed the effect of Y-27632 on the JNK activation in mature DCs. Y-27632 exerted no significant influences on the JNK activity in either CCL19-treated or -untreated mature BC1 cells (Fig. 6A).

Recently, we have demonstrated that CCL19 markedly enhances Cdc42 and Rac activities in mature BC1 cells (16). We thus examined the effect of Toxin B on the JNK activity in mature DCs. No influences of Toxin B were detected on the CCL19-induced JNK activation at all (Fig. 6B). Thus, Cdc42 and Rac activation appeared not to be involved in JNK activation by CCL19 in mature DCs.

independent experiments. (E) Kinase assay of ERK activity in mature BC1 cells. Mature BC1 cells were pre-treated with PD (30 μM) for 1 h. After the pre-treatment, the cells were stimulated with CCL19 (2 nM) for 2 min and the cell lysates were subjected to the *in vitro* kinase assay with Elk-1 fusion protein. The levels of phosphorylated Elk-1 (p-Elk-1) were determined by immunoblotting. Data are representative of two independent experiments. (F) Western blotting of activated Akt (p-Akt) in mature BC1 cells. Mature BC1 cells were pre-treated with Wort. (300 nM) or LY (30 μM) for 1 h. After the pre-treatment, the cells were stimulated with CCL19 (2 nM) for 2 min and the cell lysates were subjected to western blotting of p-Akt or total Akt (t-Akt). Data are representative of two independent experiments. (G) Effects of SP on CCL19-induced adhesion of mature BC1 cells to FN. Mature BC1 cells were pre-treated with SP (10 μM) and then stimulated with CCL19 (2 nM) for 2 min on a FN-coated chamber slide. The proportion of adherent cells was determined from numbers of the starting cells and the adherent cells. Each column represents the mean ± SE of three independent experiments. (H) CCR7 ligand-induced migration in the absences of GM-CSF and fibroblast supernatants. Mature BC1 cells were pre-treated with SP (10 μM) for 1 h and the chemotaxis assay was conducted in 2% FCS IMDM buffered with 20 mM HEPES for 90 min at 37°C. Each column represents the mean ± SE of three independent experiments. (I) Endocytosis and chemotaxis of mature SDDCs. Mature SDDCs were pre-treated with SP (10 μM) for 1 hand then chemotaxis assay was performed for 90 min in the presence of SP. Each column represents the mean ± SE of four independent experiments. (J) Effects of JNKI-1 peptide on CCL19-induced endocytosis and chemotaxis in mature BC1 cells. Mature BC1 cells were pre-treated with JNKI-1 at 25 μM or NC at 25 μM for 1 h. After the pre-treatment, endocytosis assay or chemotaxis assay was performed. Each column represents the mean ± SE of five (endocytosis) or six (chemotaxis) independent experiments. In these experiments (A, C, D, G, H, I and J), statistical significance was calculated by the Student's *t*-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.005$).

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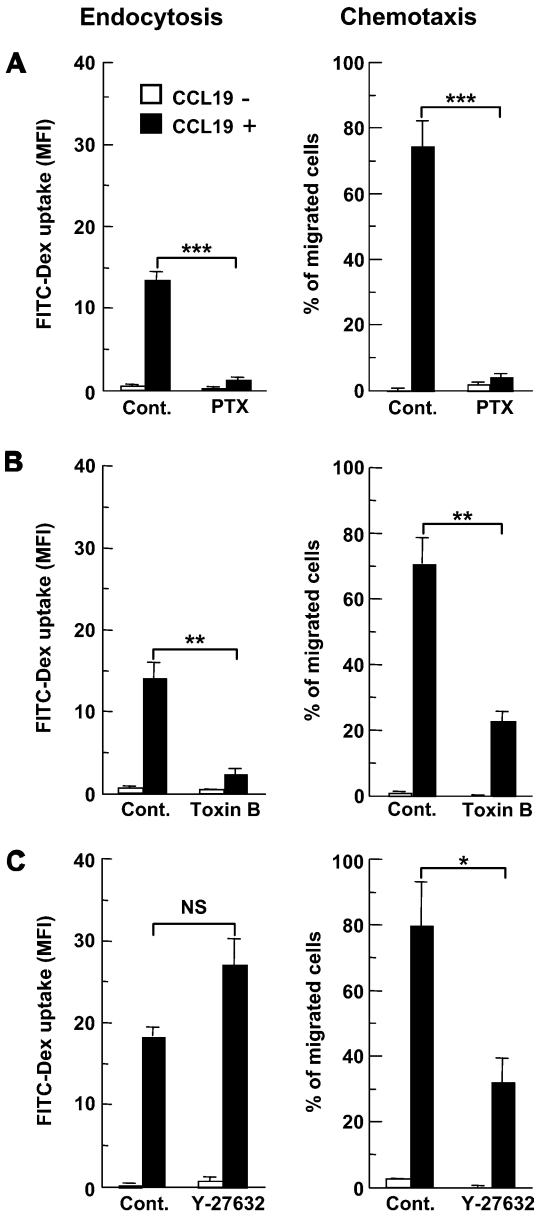

Fig. 5. Effects of PTX, Toxin B or Y-27632 on CCL19-induced endocytosis and chemotaxis in mature DCs. Mature BC1 cells were treated with PTX (100 ng ml^{-1}) (A), Toxin B (100 ng ml^{-1}) (B) or Y-27632 (10 μM) (C) for 3 and 4 h or 30 min, respectively. Endocytosis assay (left panel): after the pre-treatment, endocytosis assay was performed as in Fig. 3(D). Chemotaxis assay (right panel): the upper well including the pre-treated cells superposed with the lower well in the presence of CCL19 (2 nM). The chemotaxis assay was then performed as in Fig. 3(D). Each column represents the mean ± SE of three (PTX and Toxin B) or four (Y-27632) independent experiments. Statistical significance was calculated by the Student's *t*-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Effect of simultaneous treatment of mature DCs with SP600125 and Y-27632 on CCL19-induced chemotaxis

We showed that either SP600125 or Y-27632 significantly inhibited CCL19-induced chemotaxis of mature BC1 cells. Figure 7(A) shows effects of various doses of Y-27632 on the chemotaxis. Y-27632 inhibited CCL19-induced migration of mature BC1 cells in a dose-dependent manner, and the inhibitory effect reached a maximum at 10 μM (Fig. 7A). However, the inhibition of DC migration by Y-27632 was not complete. We then evaluated effect of the simultaneous treatment of mature DCs with SP600125 and Y-27632 on the CCL19-induced chemotaxis (Fig. 7B). Treatment of mature BC1 cells with both Y-27632 and SP600125 almost completely abolished the CCL19-induced chemotaxis.

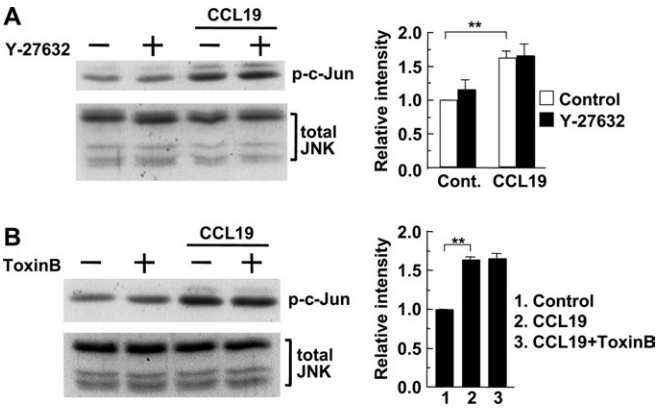

Fig. 6. Effects of Y-27632 or Toxin B on CCL19-induced JNK activation in mature DCs. Mature BC1 cells (6×10^6) were incubated in 0.5% FCS IMDM with 20 mM HEPES for 3.5 h at 37°C, and then treated with Y-27632 (10 μM) for 30 min (A). Mature DCs were incubated in 0.5% FCS IMDM with 20 mM HEPES in the presence of Toxin B (100 ng ml^{-1}) for 4 h at 37°C (B). After the pre-treatment (A and B), these cells were treated with CCL19 (2 nM) for 15 min. Cell lysates were used for the *in vitro* kinase assay with glutathione-S-transferase-c-Jun as the substrate. Representative immunoblot obtained with anti-p-c-Jun antibody is shown (left panels). Total JNK protein levels were determined by immunoblotting with anti-SAPK/JNK antibody. Results of densitometric analysis using Science Lab 99 Image Gauge are shown (right panels). Data are expressed as -fold increase relative to those representing the unstimulated levels. Each column with bar shows the mean ± SE of five (A) or three (B) independent experiments. Statistical significance was calculated by the Student's *t*-test (**$P < 0.01$).

Discussion

It is generally thought that JNK pathway is involved in inflammation, proliferation and apoptosis in mammalian cells (47). In the present study, we focused on the role of JNK in CCR7-mediated functions of mature DCs. CCL19 significantly enhanced JNK activity in mature DCs 10–30 min after stimulation and significant migration of mature DCs to this chemokine was detected after 30 min or more of incubation. Thus, CCR7-mediated migration appeared to be initiated following JNK activation. Furthermore, either SP600125 or JNKI-1, a specific inhibitor of JNK, significantly inhibited the CCR7-mediated migration of mature DCs. These findings demonstrate that CCR7-mediated JNK activation results in migration of mature DCs. Recently, it was also reported that JNK was involved in CCR7-medited migration of mature DCs in human DC system (48). On the basis of these findings, we consider that CCR7-mediated JNK activation is essential for migration of mature DCs. In contrast, CCR7-mediated endocytosis was

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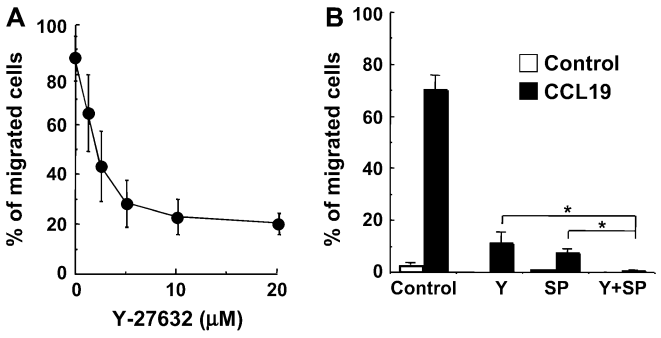

Fig. 7. Effects of simultaneous treatment of mature DCs with SP600125 (SP) and Y-27632 (Y) on CCL19-induced chemotaxis. (A) Mature BC1 cells were pre-treated with Y (1.25–20 μM) for 1 h. (B) Mature BC1 cells were pre-treated with SP (10 μM) and/or Y (10 μM) for 1 h. The chemotaxis assay was then performed and expressed as in Fig. 3(D). Each column represents the mean ± SE of three (A) or four (B) independent experiments. Statistical significance was calculated by the Student's *t*-test (*$P < 0.05$).

observed in mature DCs within a few minutes, before significant enhancement of JNK activity was seen in these DCs. In addition, specific inhibitors of JNK showed no considerable effects on the CCR7-mediated endocytosis by mature DCs. These findings demonstrate that CCR7-mediated JNK activation is not involved in endocytic machinery in mature DCs. It seems clear that CCR7-mediated signal pathway for endocytic machinery is different from the JNK-dependent pathway for chemotactic machinery.

MKK4 and MKK7 are upstream kinases of JNK (49). It has been reported that CpG DNA activates JNK following MKK4 activation in DCs (50). However, the role of MKK4 in chemokine-induced signal transduction has not been addressed so far. In the present study, CCL19 markedly increased interaction between phospho-JNK and phospho-MKK4 but not phospho-JNK and phospho-MKK7 in CCL19-treated mature DCs. Thus, CCR7-mediated JNK activation appears to be mediated via MKK4 in mature DCs.

Cell migration is a multi-step process, which can be divided into four mechanistically separate steps as follows. A migrating cell extends a lamellipodium at the front (first step). This extension is stabilized through the formation of new adhesions to the extracellular matrix (second step). The cell body is moved forward by actomyosin-mediated contraction (third step). Finally, the tail of the cell detaches from the substratum and retracts (forth step). Recently, it has been reported that JNK regulates rapid cell movement mediated via phosphorylating paxillin in fish keratocytes and rat carcinoma cells (51). Paxillin is a multi-domain protein that localizes in cultured cells, primarily to sites of cell adhesion to the extracellular matrix called focal adhesion (52). In addition, it has been reported that chemokines induce rapid integrin-dependent adhesion to adhesion molecules and extracellular matrix (29, 30). Thus, it seemed to us that JNK was involved in the adhesion step in the migration of mature DCs to CCL19. Then, we examined a role of JNK in CCR7-mediated adherence of mature DCs. It was shown that SP600125 exerted no significant effects on the CCL19-induced adhesion that took place in 2 min. It seems that JNK plays a role in the later step (s) than the adhesion step during chemotaxis of mature DCs.

Rho family proteins, Cdc42, Rac and Rho, also control cell motility (53). Rho-associated kinase, which is a downstream effector of Rho, enhances myosin light chain phosphorylation, thereby regulating actin–myosin contraction (54, 55). It is generally thought that the Rho pathway is involved in chemokine-induced migration of T cells and eosinophils (55, 56). Recently, it has been reported that Rho-associated kinase is required for CCR7-mediated polarization and chemotaxis of T lymphocytes (57). In the present study, blocking of Rho-asociated kinase by Y-27632 significantly reduced CCR7 ligand-induced chemotaxis of mature DCs. Thus, Rho pathway appeared to be generally involved in migration of leukocytes to chemokines. However, thus far we could not detect a distinct activity of Rho in CCR7 ligand-stimulated mature DCs by our assay system (data not shown). Undetectable levels of Rho activity may contribute to the CCR7-mediated migration of mature DCs.

We then examined the relationship between JNK and Rho in the signal transduction cascade in mature DCs stimulated with CCR7 ligands. Blocking of Rho pathway by Y-27632 showed no significant effects on the CCR7-mediated JNK activation (Fig. 6A). Thus, CCR7 ligands appear to activate JNK through a Rho-independent pathway. On the other hand, even in the presence of Y-27632, CCL19 induced modest but significant migration of mature DCs. This Rho-independent process was completely abrogated when JNK pathway was simultaneously blocked by SP600125 (Fig. 7B). Thus, the CCR7-mediated chemotaxis appears to be operated by the two different signal cascades mediated via JNK or Rho in mature DCs.

JNK is activated via Rho family proteins including Rho, Rac and Cdc42 in several types of cells (58–63). However, the role of Rho family proteins in the JNK activation varies depending on the cell types and assay systems. It was recently reported that neurotrophin 3 (NT3) induced Schwann cell migration through TrKC with concomitant up-regulation of JNK activity and blocking of the JNK activation by SP600125 abolished this migration (64). NT3 also activated Cdc42 and Rac, but not Rho, whereas Toxin B, a specific inhibitor of Rho GTPase proteins, inhibited both the cell migration and JNK activation. Thus, it was suggested that NT3 induced Schwann cell migration through the JNK pathway, and Cdc42 and Rac were involved in the JNK activation. In the present study, however, Toxin B significantly inhibited CCR7-mediated DC migration but not the JNK activity. Thus, Rho GTPase proteins are unlikely to be involved in CCR7-mediated JNK activation in mature DCs. It seems that the roles of Rho GTPase proteins in JNK activation are different among various cell types or various ligand–receptor systems. On the other hand, blocking of Gi proteins by PTX abrogated activation of both JNK (data not shown) and Rho family proteins, Cdc42 and Rac (16), in CCL19-treated mature DCs. Gi proteins coupled with CCR7 in mature DCs appear to activate both JNK and Rho family proteins via distinct pathways.

CCR7 ligand activates the multiple signaling pathways in mature DCs (65, 66). In addition to JNK activation, we also detected CCR7-mediated activation of other MAPKs including p38 MAPK and ERK and PI3K/Akt pathway. p38 MAPK activation was detected at an early time point (2 min) after CCL19 treatment. However, p38 MAPK-specific inhibitor

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SB203580 showed no considerable effect on the CCR7-mediated p38 MAPK activation, chemotaxis and endocytosis. Thus, we are currently analyzing the effect of SB203580 on CCR7-mediated p38 MAPK activation by analyzing the phosphorylation of cAMP response element-binding protein and MAPK-activated protein kinase 2, which are downstream pathways of p38 MAPK.

Stimulation via CCR7 or CXCR4 results in the activation of ERK in T cells (38). However, the role of this kinase in the cell migration remains unclear. It has been reported that PD98059 blocks CCR7-mediated ERK activation, but does not affect CCR7-mediated migration of T cells (57). In human monocyte-derived DC system, blocking of ERK pathway reduced CCR7-mediated cell migration of TNF-α-induced mature DCs (48). On the other hand, we showed in the present study that CCL19 fully elicited endocytosis and chemotaxis of LPS-induced mature DCs in the presence of a specific inhibitor for ERK. Thus, the role of signal pathway via ERK in the CCR7-mediated endocytosis and chemotaxis appears to be different between TNF-α-induced human mature DCs and LPS-induced murine mature DCs.

Recently, Del Prete *et al.* (65) reported that PI3Kγ played a pivotal role in DC migration using PI3Kγ-deficient mice. They prepared DCs from $CD34^+$ bone marrow cells of normal and PI3Kγ-deficient mice by culturing with GM-CSF and Flt3 ligand. Migration of immature DCs toward CCL3 was almost completely abolished in PI3Kγ-deficient mice. In contrast, migration of TNF-α-induced mature DCs toward CCL19 was partially (50%) abolished in PI3Kγ-deficient mice. Thus, it seems that PI3Kγ-independent pathway also, at least in part, contributes to the CCL19-induced migration of the mature DCs. In the present study, blocking of PI3K showed no significant effects on CCL19-induced migration of LPS-induced mature BC1 cells. Furthermore, it was reported that CCL19-induced migration of human monocyte-derived DCs maturated by CD40L plus prostaglandin E2 was unaffected by blocking PI3K, even though the CCL19 activated PI3K/Akt pathway in the mature DCs (65). Thus, PI3K-independent pathway in CCL19-induced migration seems to be predominant in these two culture systems, BC1 cells and human DCs. Taken together, we consider that the role of MAPKs or PI3K may be different among the maturational conditions and/or culture systems of DCs. These points should be carefully examined in the future experiments.

Trafficking of a variety of leukocytes to suitable sites is an essential process for the establishment of immune defense system against various pathogens. Chemokines play a pivotal role in the leukocyte trafficking. It has been reported that Rho pathway is involved in the chemokine-induced cell migration (54–56). In the present study, we have demonstrated a novel role of JNK in CCR7 ligand-mediated migration of mature DCs. Thus, we consider that elucidation of the JNK function in DC migration will provide an important basis for immunomodulation of adaptive immunity initiated by DCs.

Acknowledgements

We wish to thank Mayumi Kondo for her assistance in the preparation of this manuscript. This study was supported by a Grant-in-Aid for Scientific Research (S) and a Grant-in-Aid for Young Scientists (B) from Japan Society for the Promotion of Science (JSPS) and a Grant-in-Aid for Scientific Research on Priority Areas by the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) Japan. This study was also supported by The Akiyama Foundation.

Abbreviations

DC	dendritic cell
Dex.	dextran
Elk-1	Ets-like-protein-1
ERK	extracellular signal-regulated kinase
FN	fibronectin
IMDM	Iscove's modified Dulbecco's medium
JIP1	c-Jun-interacting protein 1
JNK	c-Jun N-terminal kinase
JNKI-1	c-Jun N-terminal kinase inhibitor 1
LN	lymph node
MAPK	mitogen-activated protein kinase
MFI	mean fluorescence intensity
MKK	mitogen-activated protein kinase kinase
NC	negative control peptide
NT3	neurotrophin 3
PI3K	phosphatidylinositol 3′-kinase
PTX	pertussis toxin
SDDC	spleen-derived dendritic cell
TNF	tumor necrosis factor

References

1 Cyster, J. G. 1999. Chemokines and cell migration in secondary lymphoid organs. *Science* 286:2098.
2 Thelen, M. 2001. Dancing to the tune of chemokines. *Nat. Immunol.* 2:129.
3 Sallusto, F., Mackay, C. R. and Lanzavecchia, A. 2000. The role of chemokine receptors in primary, effector, and memory immune responses. *Annu. Rev. Immunol.* 18:593.
4 Christopherson, K., II and Hromas, R. 2001. Chemokine regulation of normal and pathologic immune responses. *Stem Cells* 19:388.
5 Sallusto, F. and Lanzavecchia, A. 2000. Understanding dendritic cell and T-lymphocyte traffic through the analysis of chemokine receptor expression. *Immunol. Rev.* 177:134.
6 Banchereau, J. and Steinman, R. M. 1998. Dendritic cells and the control of immunity. *Nature* 392:245.
7 Lane, P. J. and Brocker, T. 1999. Developmental regulation of dendritic cell function. *Curr. Opin. Immunol.* 11:308.
8 Hart, D. N. 1997. Dendritic cells: unique leukocyte populations which control the primary immune response. *Blood* 90:3245.
9 Lipscomb, M. F. and Masten, B. J. 2002. Dendritic cells: immune regulators in health and disease. *Physiol. Rev.* 82:97.
10 Sallusto, F., Schaerli, P., Loetscher, P. *et al.* 1998. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. *Eur. J. Immunol.* 28:2760.
11 Forster, R., Schubel, A., Breitfeld, D. *et al.* 1999. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* 99:23.
12 Dieu, M. C., Vanbervliet, B., Vicari, A. *et al.* 1998. Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. *J. Exp. Med.* 188:373.
13 Dieu-Nosjean, M. C., Vicari, A., Lebecque, S. and Caux, C. 1999. Regulation of dendritic cell trafficking: a process that involves the participation of selective chemokines. *J. Leukoc. Biol.* 66:252.
14 Sato, K., Kawasaki, H., Nagayama, H. *et al.* 2001. Signaling events following chemokine receptor ligation in human dendritic cells at different developmental stages. *Int. Immunol.* 13:167.
15 Yanagawa, Y. and Onoé, K. 2002. CCL19 induces rapid dendritic extension of murine dendritic cells. *Blood* 100:1948.
16 Yanagawa, Y. and Onoé, K. 2003. CCR7 ligands induce rapid endocytosis in mature dendritic cells with concomitant up-regulation of Cdc42 and Rac activities. *Blood* 101:4923.

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17 Johnson, G. L. and Lapadat, R. 2002. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* 298:1911.
18 Rescigno, M., Martino, M., Sutherland, C. L., Gold, M. R. and Ricciardi-Castagnoli, P. 1998. Dendritic cell survival and maturation are regulated by different signaling pathways. *J. Exp. Med.* 188:2175.
19 Sato, K., Nagayama, H., Tadokoro, K., Juji, T. and Takahashi, T. A. 1999. Extracellular signal-regulated kinase, stress-activated protein kinase/c-Jun N-terminal kinase, and p38mapk are involved in IL-10-mediated selective repression of TNF-α-induced activation and maturation of human peripheral blood monocyte-derived dendritic cells. *J. Immunol.* 162:3865.
20 Ahn, S. C., Kim, G. Y., Kim, J. H. *et al.* 2004. Epigallocatechin-3-gallate, constituent of green tea, suppresses the LPS-induced phenotypic and functional maturation of murine dendritic cells through inhibition of mitogen-activated protein kinases and NF-κB. *Biochem. Biophys. Res. Commun.* 313:148.
21 Yanagawa, Y., Iijima, N., Iwabuchi, K. and Onoé, K. 2002. Activation of extracellular signal-related kinase by TNF-α controls the maturation and function of murine dendritic cells. *J. Leukoc. Biol.* 71:125.
22 Puig-Kroger, A., Relloso, M., Fernandez-Capetillo, O. *et al.* 2001. Extracellular signal-regulated protein kinase signaling pathway negatively regulates the phenotypic and functional maturation of monocyte-derived human dendritic cells. *Blood* 98:2175.
23 Aicher, A., Shu, G. L., Magaletti, D. *et al.* 1999. Differential role for p38 mitogen-activated protein kinase in regulating CD40-induced gene expression in dendritic cells and B cells. *J. Immunol.* 163:5786.
24 Ardeshna, K. M., Pizzey, A. R., Devereux, S. and Khwaja, A. 2000. The PI3 kinase, p38 SAP kinase, and NF-κB signal transduction pathways are involved in the survival and maturation of lipopolysaccharide-stimulated human monocyte-derived dendritic cells. *Blood* 96:1039.
25 Arrighi, J. F., Rebsamen, M., Rousset, F., Kindler, V. and Hauser, C. 2001. A critical role for p38 mitogen-activated protein kinase in the maturation of human blood-derived dendritic cells induced by lipopolysaccharide, TNF-α, and contact sensitizers. *J. Immunol.* 166:3837.
26 Winzler, C., Rovere, P., Rescigno, M. *et al.* 1997. Maturation stages of mouse dendritic cells in growth factor-dependent long-term cultures. *J. Exp. Med.* 185:317.
27 Granucci, F., Petralia, F., Urbano, M. *et al.* 2003. The scavenger receptor MARCO mediates cytoskeleton rearrangements in dendritic cells and microglia. *Blood* 102:2940.
28 Yanagawa, Y., Iwabuchi, K. and Onoé, K. 2001. Enhancement of stromal cell-derived factor-1α-induced chemotaxis for CD4/8 double-positive thymocytes by fibronectin and laminin in mice. *Immunology* 104:43.
29 Campbell, J. J., Hedrick, J., Zlotnik, A., Siani, M. A., Thompson, D. A. and Butcher, E. C. 1998. Chemokines and the arrest of lymphocytes rolling under flow conditions. *Science* 279:381.
30 Gasperini, S., Marchi, M., Calzetti, F. *et al.* 1999. Gene expression and production of the monokine induced by IFN-γ (MIG), IFN-inducible T cell α chemoattractant (I-TAC), and IFN-γ-inducible protein-10 (IP-10) chemokines by human neutrophils. *J. Immunol.* 162:4928.
31 Kikuchi, K., Yanagawa, Y., Aranami, T., Iwabuchi, C., Iwabuchi, K. and Onoé, K. 2003. Tumour necrosis factor-α but not lipopolysaccharide enhances preference of murine dendritic cells for Th2 differentiation. *Immunology* 108:42.
32 Iijima, N., Yanagawa, Y., Iwabuchi, K. and Onoé, K. 2003. Selective regulation of CD40 expression in murine dendritic cells by thiol antioxidants. *Immunology* 110:197.
33 Iijima, N., Yanagawa, Y. and Onoé, K. 2003. Role of early- or late-phase activation of p38 mitogen-activated protein kinase induced by tumour necrosis factor-α or 2,4-dinitrochlorobenzene during maturation of murine dendritic cells. *Immunology* 110: 322.
34 Kikuchi, K., Yanagawa, Y., Iwabuchi, K. and Onoé, K. 2003. Differential role of mitogen-activated protein kinases in CD40-mediated IL-12 production by immature and mature dendritic cells. *Immunol. Lett.* 89:149.
35 Pavlinkova, G., Yanagawa, Y., Kikuchi, K., Iwabuchi, K. and Onoé, K. 2003. Effects of histamine on functional maturation of dendritic cells. *Immunobiology* 207:315.
36 Schuurhuis, D. H., Laban, S., Toes, R. E. *et al.* 2000. Immature dendritic cells acquire CD8(+) cytotoxic T lymphocyte priming capacity upon activation by T helper cell-independent or -dependent stimuli. *J. Exp. Med.* 192:145.
37 Villadangos, J. A., Cardoso, M., Steptoe, R. J. *et al.* 2001. MHC class II expression is regulated in dendritic cells independently of invariant chain degradation. *Immunity* 14:739.
38 Tilton, B., Ho, L., Oberlin, E. *et al.* 2000. Signal transduction by CXC chemokine receptor 4. Stromal cell-derived factor 1 stimulates prolonged protein kinase B and extracellular signal-regulated kinase 2 activation in T lymphocytes. *J. Exp. Med.* 192:313.
39 Whitmarsh, A. J. and Davis, R. J. 1998. Structural organization of MAP-kinase signaling modules by scaffold proteins in yeast and mammals. *Trends Biochem. Sci.* 23:481.
40 Bonny, C., Oberson, A., Negri, S., Sauser, C. and Schorderet, D. F. 2001. Cell-permeable peptide inhibitors of JNK: novel blockers of β-cell death. *Diabetes* 50:77.
41 Wang, J., Van De Water, T. R., Bonny, C., de Ribaupierre, F., Puel, J. L. and Zine, A. 2003. A peptide inhibitor of c-Jun N-terminal kinase protects against both aminoglycoside and acoustic trauma-induced auditory hair cell death and hearing loss. *J. Neurosci.* 23:8596.
42 Itano, A. A., McSorley, S. J., Reinhardt, R. L. *et al.* 2003. Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity. *Immunity* 19:47.
43 Cox, D., Chang, P., Zhang, Q., Reddy, P. G., Bokoch, G. M. and Greenberg, S. 1997. Requirements for both Rac1 and Cdc42 in membrane ruffling and phagocytosis in leukocytes. *J. Exp. Med.* 186:1487.
44 West, M. A., Prescott, A. R., Eskelinen, E. L., Ridley, A. J. and Watts, C. 2000. Rac is required for constitutive macropinocytosis by dendritic cells but does not control its downregulation. *Curr. Biol.* 10:839.
45 Garrett, W. S., Chen, L. M., Kroschewski, R. *et al.* 2000. Developmental control of endocytosis in dendritic cells by Cdc42. *Cell* 102:325.
46 Patel, J. C., Hall, A. and Caron, E. 2002. Vav regulates activation of Rac but not Cdc42 during FcγR-mediated phagocytosis. *Mol. Biol. Cell* 13:1215.
47 Ip, Y. T. and Davis, R. J. 1998. Signal transduction by the c-Jun N-terminal kinase (JNK)–from inflammation to development. *Curr. Opin. Cell Biol.* 10:205.
48 Riol-Blanco, L., Sanchez-Sanchez, N., Torres, A. *et al.* 2005. The chemokine receptor CCR7 activates in dendritic cells two signaling modules that independently regulate chemotaxis and migratory speed. *J. Immunol.* 174:4070.
49 Dent, P., Yacoub, A., Fisher, P. B., Hagan, M. P. and Grant, S. 2003. MAPK pathways in radiation responses. *Oncogene* 22: 5885.
50 Hacker, H., Mischak, H., Miethke, T. *et al.* 1998. CpG-DNA-specific activation of antigen-presenting cells requires stress kinase activity and is preceded by non-specific endocytosis and endosomal maturation. *EMBO J.* 17:6230.
51 Huang, C., Rajfur, Z., Borchers, C., Schaller, M. D. and Jacobson, K. 2003. JNK phosphorylates paxillin and regulates cell migration. *Nature* 424:219.
52 Turner, C. E. 2000. Paxillin interactions. *J. Cell Sci.* 113:4139.
53 Ridley, A. J. 2001. Rho GTPases and cell migration. *J. Cell Sci.* 114:2713.
54 Amano, M., Fukata, Y. and Kaibuchi, K. 2000. Regulation and functions of Rho-associated kinase. *Exp. Cell Res.* 261:44.
55 Vicente-Manzanares, M., Cabrero, J. R., Rey, M. *et al.* 2002. A role for the Rho-p160 Rho coiled-coil kinase axis in the chemokine stromal cell-derived factor-1α-induced lymphocyte actomyosin and microtubular organization and chemotaxis. *J. Immunol.* 168:400.

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56 Adachi, T., Vita, R., Sannohe, S. *et al.* 2001. The functional role of rho and rho-associated coiled-coil forming protein kinase in eotaxin signaling of eosinophils. *J. Immunol.* 167:4609.
57 Bardi, G., Niggli, V. and Loetscher, P. 2003. Rho kinase is required for CCR7-mediated polarization and chemotaxis of T lymphocytes. *FEBS Lett.* 542:79.
58 Honda, T., Shimizu, K., Kawakatsu, T. *et al.* 2003. Cdc42 and Rac small G proteins activated by trans-interactions of nectins are involved in activation of c-Jun N-terminal kinase, but not in association of nectins and cadherin to form adherens junctions, in fibroblasts. *Genes Cells* 8:481.
59 Kam, A. Y., Chan, A. S. and Wong, Y. H. 2003. Rac and Cdc42-dependent regulation of c-Jun N-terminal kinases by the δ-opioid receptor. *J. Neurochem.* 84:503.
60 Yamauchi, J., Kawano, T., Nagao, M., Kaziro, Y. and Itoh, H. 2000. G(i)-dependent activation of c-Jun N-terminal kinase in human embryonal kidney 293 cells. *J. Biol. Chem.* 275:7633.
61 Minden, A., Lin, A., Claret, F. X., Abo, A. and Karin, M. 1995. Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. *Cell* 81:1147.
62 Coso, O. A., Chiariello, M., Yu, J. C. *et al.* 1995. The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. *Cell* 81:1137.
63 Gallagher, E. D., Gutowski, S., Sternweis, P. C. and Cobb, M. H. 2004. RhoA binds to the amino terminus of MEKK1 and regulates its kinase activity. *J. Biol. Chem.* 279:1872.
64 Yamauchi, J., Chan, J. R. and Shooter, E. M. 2003. Neurotrophin 3 activation of TrkC induces Schwann cell migration through the c-Jun N-terminal kinase pathway. *Proc. Natl Acad. Sci. USA* 100:14421.
65 Del Prete, A., Vermi, W., Dander, E. *et al.* 2004. Defective dendritic cell migration and activation of adaptive immunity in PI3Kγ-deficient mice. *EMBO J.* 23:3505.
66 Scandella, E., Men, Y., Legler, D. F. *et al.* 2004. CCL19/CCL21-triggered signal transduction and migration of dendritic cells requires prostaglandin E2. *Blood* 103:1595.

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