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Original Article

CCR7 Ligands Induce Endocytosis and the Formation of Actin-Filled Ruffles in Mature Dendritic Cells

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We have previously demonstrated that the CCR7 ligands, CCL19 and CCL21, rapidly induce vigorous endocytosis in mature murine dendritic cells (DCs). The mechanisms underlying the enhanced endocytosis, however, have remained elusive. In the present study, we examined effects of CCL19 and CCL21 on the organization of the actin cytoskeleton in murine DCs. These chemokines considerably increased the intracellular level of fibrous actin and induced the formation of large petal-like ruffles of actin filaments in mature DCs. In addition, a robust network of interconnected ruffles of actin filaments was assembled at the periphery of the cells after stimulation with the chemokines. The actin-related protein (Arp) 2/3 complex has been implicated in the formation of fibrous actin structures. During cytoskeletal reorganization, Arp2/3 complexes co-localized with the interconnected ruffles at the cell periphery. Thus, the Arp2/3 complex seems to be involved in CCR7 ligand-induced actin reorganization in mature DCs. The time course of the CCL19-induced actin-reorganization corresponded to that of the endocytic activity. In the presence of cytochalasin D, an actin polymerization inhibitor, CCL19 failed to induce endocytosis and the interconnected ruffles did not form in mature DCs. Thus, CCR7-induced actin assembly may be part of the endocytic process in mature DCs.

Key words CCR7, dendritic cells, actin filaments, endocytosis

INTRODUCTION

Chemokines are a family of low-molecular-weight proteins that typically contain four conserved cysteine residues. The two main types of chemokines, the CXC chemokines and the CC chemokines, differ in their structural properties and in their chromosomal locations[1]. These signaling molecules exert their effects via seven-transmembrane, G protein-coupled receptors that are present in the membrane of the target cell[1]. Chemokines play a prominent role in leukocyte trafficking. By regulating chemotaxis and cell adhesion, chemokines systemically organize the distribution of the various leukocytes to induce efficient immune responses against various pathogens[2,3]. The chemokine receptor-mediated signal pathways involved in chemotaxis and cell adhesion, however, are not fully understood.

In most eukaryotic cells, dynamic assembly of cortical actin is essential for membrane protrusions, cell crawling, and intracellular vesicle trafficking. The actin-related protein (Arp) 2/3 complex has been implicated in the formation of fibrous (F)-actin structures including lamellipodia[4], podosomes[5], and phagocytic uptake structures[6]. Additionally, the Arp2/3 complex initiates the actin-based motility of intracellular parasites such as *Listeria monocytogenes* and *Shigella flexneri*[7,8]. Recently, it has been reported that the Arp2/3 complex localizes to the interconnected ruffles of actin filaments in macrophages[9]. Although, it appears that the Arp2/3 complex is involved in the formation of the ruffles, no reports have thus far demonstrated chemokine-induced recruitment of the Arp2/3 complex or that the interconnected ruffles are formed via this complex.

Dendritic cells (DCs) are potent antigen-presenting cells that play a major role in the regulation of immune responses to a variety of antigens[10-12]. The developmental regulation of the expression of chemokine receptors by DCs facilitates the migration of these cells from the peripheral tissues to the T-cell area of regional lymph nodes[13,14]. This process is critical for the efficient initiation of adaptive immunity, which is controlled by the interaction between DCs and T cells in the T-cell area. A high density of CCR7 is found on mature, but not immature, DCs. CCL19 (Epstein-Barr virus-induced receptor ligand chemokine) and CCL21 (secondary lymphoid tissue chemokine) are constitutively expressed at high levels in the T-cell areas of lymph nodes and the spleen[2,15], and induce mature DCs to migrate into these lymphoid tissues[13,14]. Moreover, mature DCs fail to migrate into the T-cell area of

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the regional lymph nodes in CCR7-deficient mice[16]; this behavior correlates with severely compromised adaptive immune responses. Thus, the interaction between CCR7 and CCL19/CCL21 is an essential process that controls the migration of mature DCs into the regional lymph nodes. Recently, we have demonstrated that, in addition to chemotaxis, CCR7 ligands also enhance dendritic extension and the endocytic activity of mature DCs[17,18]. Thus, it seems that CCR7 regulates multiple signaling pathways in DCs. The precise mechanisms underlying the multiple functions of CCR7, however, are unclear.

In the present study, we examined the effects of CCR7 ligands on the organization of the actin cytoskeleton in murine DCs. These chemokines induced network formation by the interconnected ruffles of actin filaments in the cell cortices of mature DCs within a few minutes. Furthermore, it seems that this CCR7 ligand-induced network formation is involved in endocytosis in mature DCs.

MATERIALS AND METHODS

Reagents

Recombinant murine granulocyte-macrophage colony-stimulating factor (GM-CSF) was purchased from PeproTech (London, UK). Recombinant murine chemokines were obtained from Genzyme R & D Systems (Minneapolis, MN). Alexa Fluor 488- or 546-labeled phalloidin, Alexa Fluor 488-labeled DNase I, and fluorescein isothiocyanate (FITC)-labeled dextran were acquired from Molecular Probes (Eugene, OR). Rabbit polyclonal antibodies (Abs) raised against a p34-Arc peptide (EKKEMKTITGKTFS, amino acids 286-298) or an Arp3 peptide (YEEIGP-SIVRHNPVFGVMS, amino acids 400-418) were purchased from Upstate Biotechnology (Charlottesville, VA). The immunogen peptides were obtained from Greiner Bio-One (Ulm, Germany). Control rabbit IgG and goat IgG were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Alexa Fluor 546-conjugated goat anti-rabbit IgG and FITC-conjugated dextran (40,000 m.w.) were acquired from Molecular Probes. Lipopolysaccharide (LPS) from *Escherichia coli* 055 : B5, cytochalasin D, and Iscove's modified Dulbecco's medium (IMDM) were purchased from Sigma Chemical Co. (St Louis, MO).

Culture media

The culture medium used in the present study was IMDM supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin, and 600 μg/ml L-glutamine. Fibroblast supernatants were collected from confluent NIH/3T3 cell cultures grown in IMDM containing 10% heat-inactivated fetal calf serum (10% FCS-IMDM).

DC culture

A DC line (BC1 cells) was generated from BALB/c mouse spleen using Winzler's method as previously described[19,20]. BC1 cells were cultured and expanded in R1 medium (IMDM containing 10% FCS, 30% NIH/3T3 cultured supernatant, 10 ng/ml mouse recombinant GM-CSF, and 50 μM 2-mercaptoethanol). In the present study, BC1 cells treated with 5 μg/ml LPS for 24 h were used as mature DCs[18].

Spleen-derived DCs (SDDCs) were generated by culturing C57BL/6 splenocytes in R1 medium for 14 days as previously described[17,18,21]. SDDCs treated with 5 μg/ml LPS for 24 h were used as mature SDDCs.

Microscopy

Cells (1.5×10^5) suspended in 10% FCS-IMDM buffered with 20 mM HEPES were seeded on a polylysine-coated chamber slide (8 well glass slide ; Nalge Nunc International, Naperville, IL). The cells were preincubated for 20 to 30 min at 37℃ and then treated with chemokines. To evaluate the effects of cytochalasin D, the cells were preincubated with this inhibitor for 1 h in R1 medium buffered with 20 mM HEPES before they were treated with chemokines. All chemokines were added at 100 ng/ml. The treatments were stopped at the indicated time points by rapidly cooling the cells on ice. The cells were then fixed in 4% paraformaldehyde for 10 min on ice, and additionally for 30 min at room temperature. The cells were washed with washing buffer (phosphate-buffered saline (PBS) containing 1% FCS and 0.1% NaN_3) and then permeabilized for 3 min at room temperature with saponin buffer (0.1% saponin in washing buffer). Intracellular F-actin and globular (G)-actin were stained with Alexa Fluor 546-conjugated phalloidin and Alexa Fluor 488-conjugated DNase I, respectively. The cells were washed with saponin buffer and the fluorescence intensity of the cells was analyzed using a confocal laser-scanning microscope (BX61-2CHAr-HeNeG-SP, FV500, Ver3.1, Olympus, Tokyo, Japan). In some experiments, we reconstructed the extended-focus view (Z stack image) from consecutive optical sections taken at 2-μm intervals through the full thickness of the cells[22,23].

To label the Arp2/3 complex, permeabilized cells fixed on glass slides were pretreated with blocking buffer (saponin buffer containing 1 mg/ml goat IgG and 5 μg/ml anti-FcγIII/II monoclonal Abs) and then incubated with anti-p34-Arc (an Arp2/3 complex subunit) Abs or anti-Arp3 Abs. The cells were washed and then incubated with Alexa Fluor 546-conjugated goat anti-rabbit IgG and Alexa Fluor 488-conjugated phalloidin. The cells were washed before the fluorescence intensity of the cells was analyzed by confocal laser-scanning microscopy. Control rabbit IgG did not label

these cells. Detectable binding of the anti-p34-Arc Abs or the anti-Arp3 Abs was completely blocked by pretreatment with the respective immunogen peptide.

Flow cytometric analysis of F-actin labeled cells

Cells suspended in 10% FCS-IMDM buffered with 20 mM HEPES were placed into a polypropylene tube and incubated at 37℃ for 20 min with agitation. The cells were stimulated with CCL19 (100 ng/ml) for 1 min at 37℃ with agitation. The CCL19 treatments were stopped by rapidly cooling the cells on ice, which was immediately followed by the addition of a three-fold volume of PBS containing 4% formaldehyde. The cells were fixed in the paraformaldehyde solution for 10 min on ice, and additionally for 40 min at room temperature. These cells were permeabilized for 3 min at room temperature with saponin buffer. The cells were incubated with Alexa Fluor 488-conjugated phalloidin in saponin buffer for 20 min and then washed with saponin buffer. The fluorescence intensity of the cells was analyzed on an EPICS® XL flow cytometer (Coulter Co., Miami, FL).

Endocytosis

Cells in R1 medium buffered with 20 mM HEPES were pretreated with cytochalasin D (1 μM) for 1 h. Thereafter, the experiments were conducted in the presence of this inhibitor as previously described[18]. After, the endocytic tracer and chemokine were added concurrently, the cells were subject to 0.5 to 15 min incubations. FITC-dextran was added to a final concentration of 1 mg/ml. CCL19 was added to a final concentration of 100 ng/ml. Endocytosis of the tracer was halted at the indicated time points by rapidly cooling the cells on ice. The cells were then washed with ice-cold washing buffer and stained with propidium iodide to exclude dead cells. The fluorescence intensity of the cells was analyzed on an EPICS® XL flow cytometer. Cells that were incubated with the endocytic tracer on ice were used as a background control. The mean fluorescence intensity (MFI) resulting from the subtraction of a background control sample from each experimental sample gave a measure of the amount of incorporated tracer.

Statistical analyses

The Student's *t*-test was used to identify significant differences between data sets. Differences between the groups were considered to be significant when $p<0.05$.

RESULTS

CCR7 ligand-induced network formation by the interconnected ruffles of actin filaments in mature DCs

We previously established a murine DC line (BC1 cells) from BALB/c splenocytes[19,20]. Unstimulated BC1 cells physiologically and functionally behave like immature DCs. Following treatment with LPS for 24 h, BC1 cells exhibit a mature DC phenotype[17,18,21,24-27]. Using a similar *in vitro* differentiation system, a number of important findings about DCs have been reported and verified[28-31].

In the present study, we examined the effects of CCR7 ligands on the organization of the actin cytoskeleton in BC1 cells. Phalloidin and DNase I specifically bind to F-actin (polymerized actin) and G-actin (actin monomers), respectively, and are commonly used to label the actin cytoskeleton. Immature and mature BC1 cells were treated with CCL19 or CCL21, and intracellular F-actin and G-actin were labeled. Neither CCL19 nor CCL21 significantly affected the actin cytoskeletal organization in immature BC1 cells (data not shown). In contrast, these chemokines induced a dramatic reorganization of the actin cytoskeleton in mature BC1 cells (Fig. 1). In the absence of these chemokines, a large percentage of the F-actin in mature BC1 cells appeared flecked and a few short filaments were observed (Fig. 1A). Both CCL19 and CCL21 considerably increased the intracellular level of F-actin (red) and induced the assembly of large interconnected petal-like ruffles in mature BC1 cells (Fig. 1B and C). We did not detect any significant differences in the effects of CCL19 and CCL21 on actin cytoskeletal organization. A robust network of interconnected ruffles was observed at the periphery of CCL19-treated mature BC1 cells (Fig. 1E and G). The most pronounce effects of the CCR7 ligands were observed at chemokine concentrations of 20 to 100 ng/ml (data not shown). CCL3, a ligand for CCR5, which is expressed on immature but not mature DCs[14], did not induce any significant alterations in the actin cytoskeletal organization of either immature or mature BC1 cells (data not shown). A CCR7 ligand-induced actin network was also detected in mature SDDCs (Fig. 1H and I).

To evaluate the total amount of F-actin and G-actin in mature BC1 cells, the intracellular cytoskeleton was labeled with Alexa Fluor 488-labeled phalloidin and the MFI was determined by flow cytometry (Fig. 2). Treatment of mature BC1 cells with CCL19 for 1 min approximately doubled the level of intracellular F-actin ($p<0.005$). The labeling of F-actin by Alexa Fluor 488-conjugated phalloidin was completely inhibited by pretreatment of the mature BC1 cells with a 100-fold amount of unlabeled phalloidin (data not shown).

We next examined the time course of the CCL19-induced reorganization of the actin cytoskeleton in mature BC1 cells. Reorganization of the actin cytoskeleton was de-

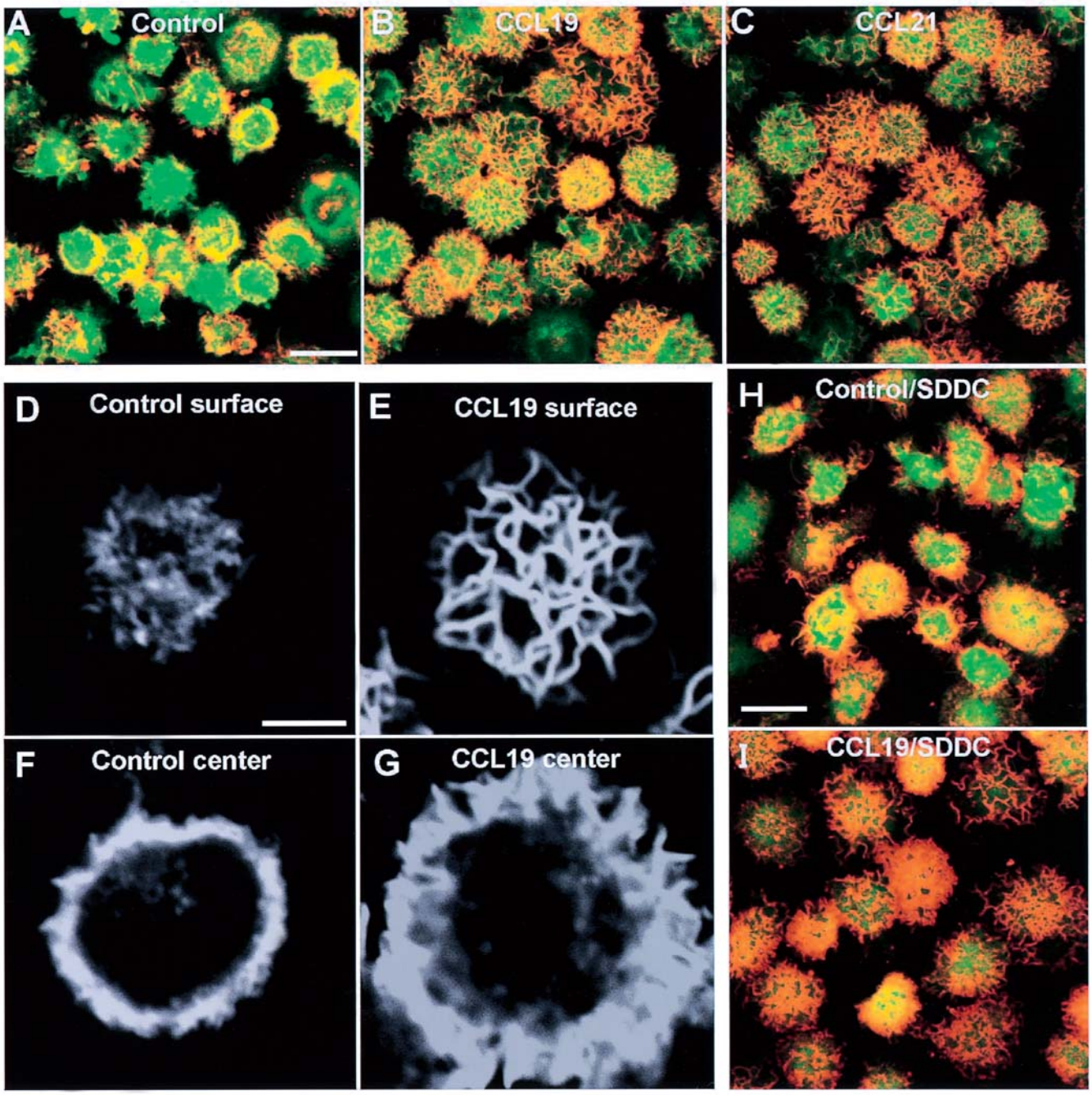

Fig. 1. CCR7 ligand-induced reorganization of the actin cytoskeleton in mature DCs. BC1 cells or SDDCs treated with LPS for 24 h were used as mature DCs. Mature BC1 cells were treated with medium alone (control) (A, D, F), CCL19 (B, E, G), or CCL21 (C) for 1 min. Mature SDDCs were treated with medium alone (H) or CCL19 (I) for 1 min. Intracellular F-actin and G-actin were labeled with Alexa Fluor 546-conjugated phalloidin (red) and Alexa Fluor 488-conjugated DNase I (green), respectively. Morphological analysis of the actin cytoskeleton was performed on a confocal laser-scanning microscope. The extended-focus view is shown in A, B, C, H, and I, whereas D and E are optical slices at the cell surface and F and G are optical slices through the center of the cells. Data represent at least four independent experiments. Bar=20 μm (A-C, H, I) or 5 μm (D-G).

tected in mature BC1 cells 30 sec after the onset of CCL19 treatment (Fig. 3). The formation of interconnected ruffles reached a peak after 1 to 2 min before dissipating after 5 to 15 min. CCL21 produced a similar time course of actin cytoskeletal reorganization (data not shown).

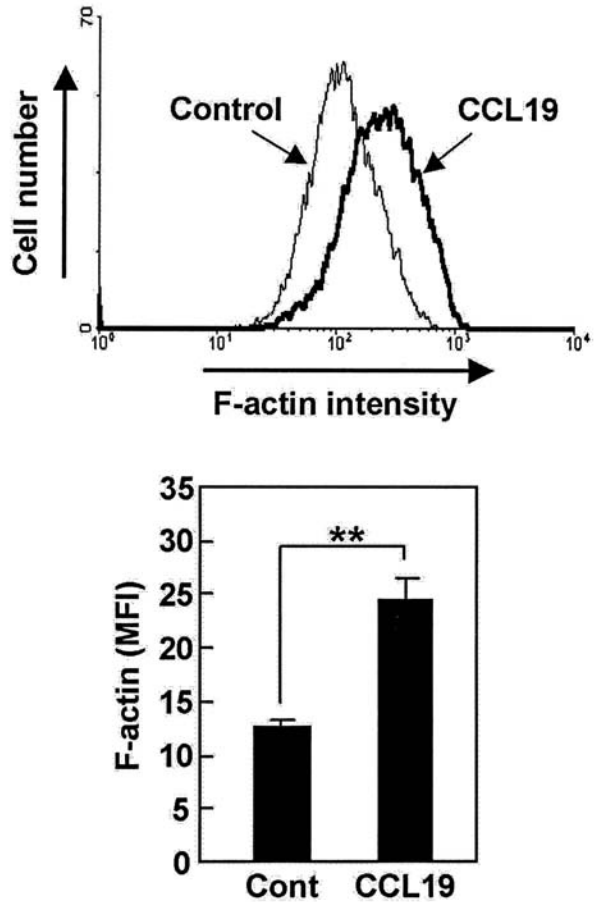

Fig. 2. Flow cytometric analysis of F-actin levels in mature DCs. Mature BC1 cells were treated with medium alone (Control) or CCL19 for 1 min. Intracellular F-actin was labeled with Alexa Fluor 488-conjugated phalloidin. Fluorescence intensity of the cells was analyzed by flow cytometry. Each column represents the mean ± SEM of four independent experiments (**, $p<0.005$).

CCL19-induced recruitment of the Arp2/3 complex in mature DCs

The Arp2/3 complex is a multifunctional actin organizer. To examine the involvement of the Arp2/3 complex in the CCL19-induced network formation of interconnected ruffles, we analyzed the intracellular distribution of the Arp2/3 complex after chemokine treatment of mature BC1 cells using a confocal microscope. After, mature BC1 cells were treated with CCL19 for 1 min, the intracellular Arp2/3 complex and F-actin were labeled with anti-p34-Arc Abs and Alexa Fluor 488-conjugated phalloidin, respectively. Arp2/3 complexes were uniformly distributed in mature BC1 cells that were treated with medium alone (Fig. 4A and F). In CCL19-treated cells, the Arp2/3 complex was concentrated around the cell cortex (Fig. 4B and G). These Arp2/3 complexes co-localized with the interconnected ruffles of actin filaments in CCL19-treated mature BC1 cells (Fig. 4D, E, G, and H). Pretreatment with the immunogen peptide completely abolished the anti-p34-Arc Ab labeling of the Arp2/3 complexes (data not shown). Similar results were obtained using anti-Arp 3 Abs (data not shown).

The effect of cytochalasin D on CCL19-induced formation of the actin network and endocytosis in mature DCs

Cytochalasin D is an actin polymerization inhibitor, which acts by capping the barbed (plus) end of actin filaments[32-34]. We examined the effects of cytochalasin D on the CCL19-induced reorganization of the actin cytoskeleton in mature BC1 cells. As described previously, CCL19 induced interconnected ruffles of actin filaments in mature BC1 cells (Fig. 5B and E). Cytochalasin D completely blocked the CCL19-induced reorganization of the actin cytoskeleton (Fig. 5C and F).

We next examined whether or not the inhibition of actin assembly by cytochalasin D influenced endocytosis in mature BC1 cells treated with CCL19. CCL19 dramatically increased FITC-dextran uptake by mature BC1 cells within a few minutes (Fig. 5G). The MFI level of the FITC-labeled cells reached a peak 2 to 5 min after treatment before decreasing 15 min after the cytokines were applied. Cytochalasin D completely blocked CCL19-induced endocytosis (Fig. 5G). Similar results were obtained with mature SDDCs (data not shown).

DISCUSSION

Recently, we have demonstrated that CCR7 ligands induce receptor-mediated endocytosis in mature DCs within a few minutes[18]. These findings contrast with the time course of CCR7 ligand-induced chemotaxis. CCR7 ligands induce the migration of mature DCs after incubations of 30 minutes or more[17]. Although endocytosis and chemotaxis may naturally follow different time courses, it appears as if CCR7 ligands have a complex role in the function of mature DCs during a pathogen infection. Pathogens and inflammatory cytokines cause immature DCs at the site of infection to mature and express CCR7. These mature DCs in turn receive signals via CCR7 and capture foreign antigens in the infected site. Thereafter, CCR7-mediated signaling gradually induces the migration of mature DCs to lymphatics and the T-cell areas of draining lymph nodes. In this way, mature DCs efficiently present pathogen antigens that have been captured at the site of the infection to T cells in the T-cell area.

In the present study, we have shown that mature DCs, when stimulated through CCR7 by CCL19 or CCL21, assembled actin filaments in large petal-like ruffles. In addition, a robust network of interconnected ruffles formed at the cell

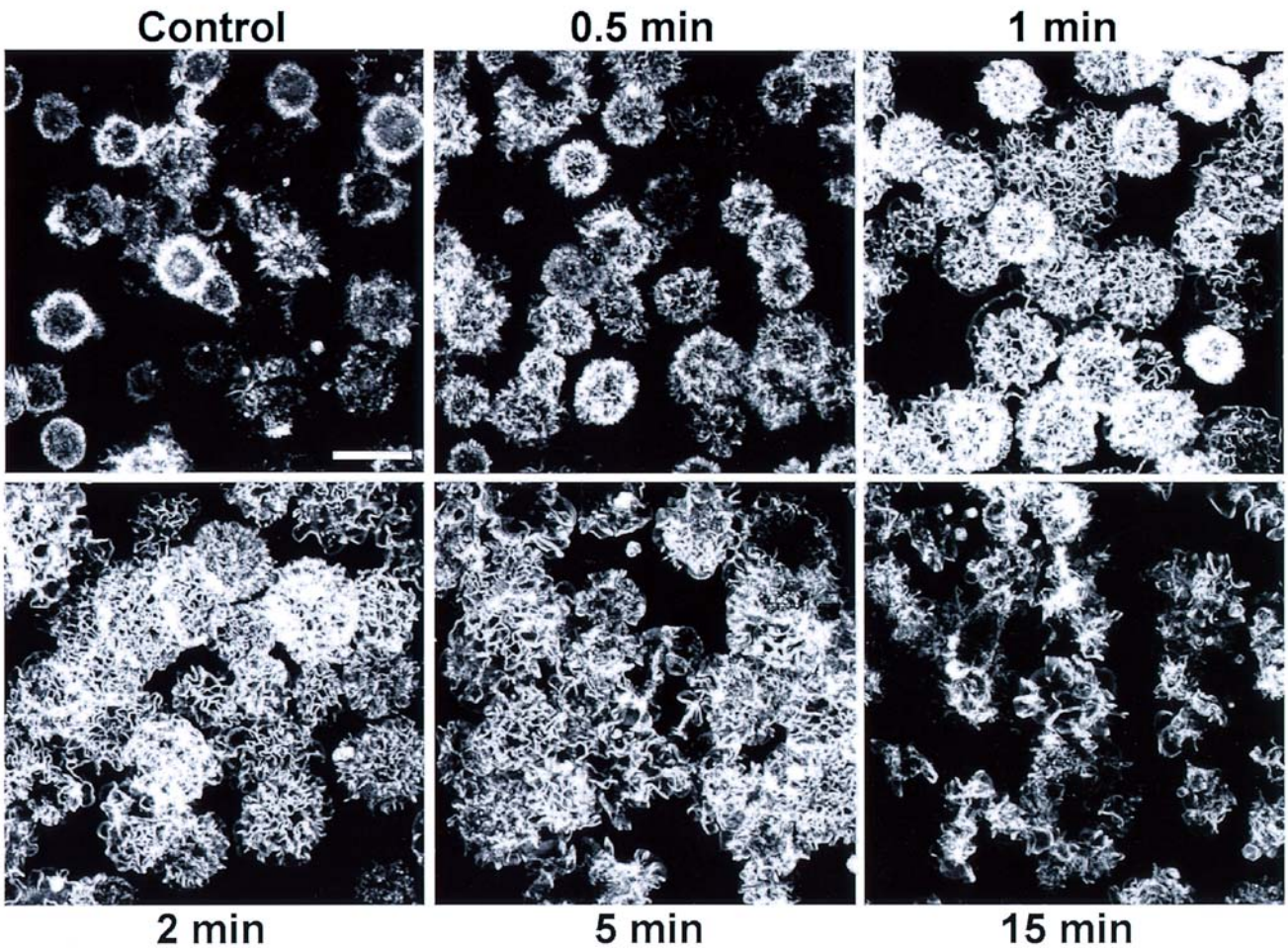

Fig. 3. The time course of CCL19-induced reorganization of the actin cytoskeleton in mature DCs. Mature BC1 cells were treated with CCL19 for 0.5, 1, 2, 5, or 15 min. Intracellular F-actin was labeled with Alexa Fluor 546-conjugated phalloidin. Morphological analysis of the actin cytoskeleton was performed on a confocal laser-scanning microscope. Data represent four independent experiments. Bar = 20 μm.

periphery in the chemokine-treated mature DCs. During the cytoskeletal reorganization, Arp2/3 complexes co-localized with the interconnected ruffles. Thus, it seems that CCR7 ligands induce the formation of the ruffle network via recruitment of the Arp2/3 complex.

Thus far, the relationship between the formation of the ruffle network and other CCR7-mediated events remains unclear. Stimulation of mature DCs via CCR7 induced vigorous endocytosis within a few minutes, whereas dendritic extension and chemotaxis have been observed 30 min or more after stimulation[17]. Thus, the time course of the actin filament organization is different than those of chemotaxis and dendritic extension, but appears to correspond to that of the endocytosis induced by the CCR7 ligands. In the presence of cytochalasin D, CCL19 induced neither the endocytosis nor the actin network formation in mature DCs. In addition, it has been suggested that ruffle formation is involved in macropinocytosis, a form of endocytosis, in macrophages and immature DCs[35,36]. These observations suggest that, in mature DCs, the CCR7-mediated network formation by interconnected ruffles is associated with endocytosis but not chemotaxis or dendritic extension. This network formation may be used as a scaffold for the concurrent endocytosis.

It has been demonstrated that the Arp2/3 complex in macrophages is involved in phagocytosis mediated via FcγR or complement receptor 3[6]. The co-localization of the interconnected ruffles with the Arp2/3 complex, however, has not been detected in the cell cortices of these macrophages during the phagocytosis. The role of the Arp2/3 complex in DC endocytosis has remained unclear. Moreover, no reports have demonstrated that chemokines induce the recruitment of the Arp2/3 complex. In the present study, we induced recruitment of the Arp2/3 complex in mature DCs following stimulation via CCR7. We therefore postulate that the recruitment of the Arp2/3 complex and the formation of large-scale actin networks play a role in CCR7 ligand-induced endocyto-

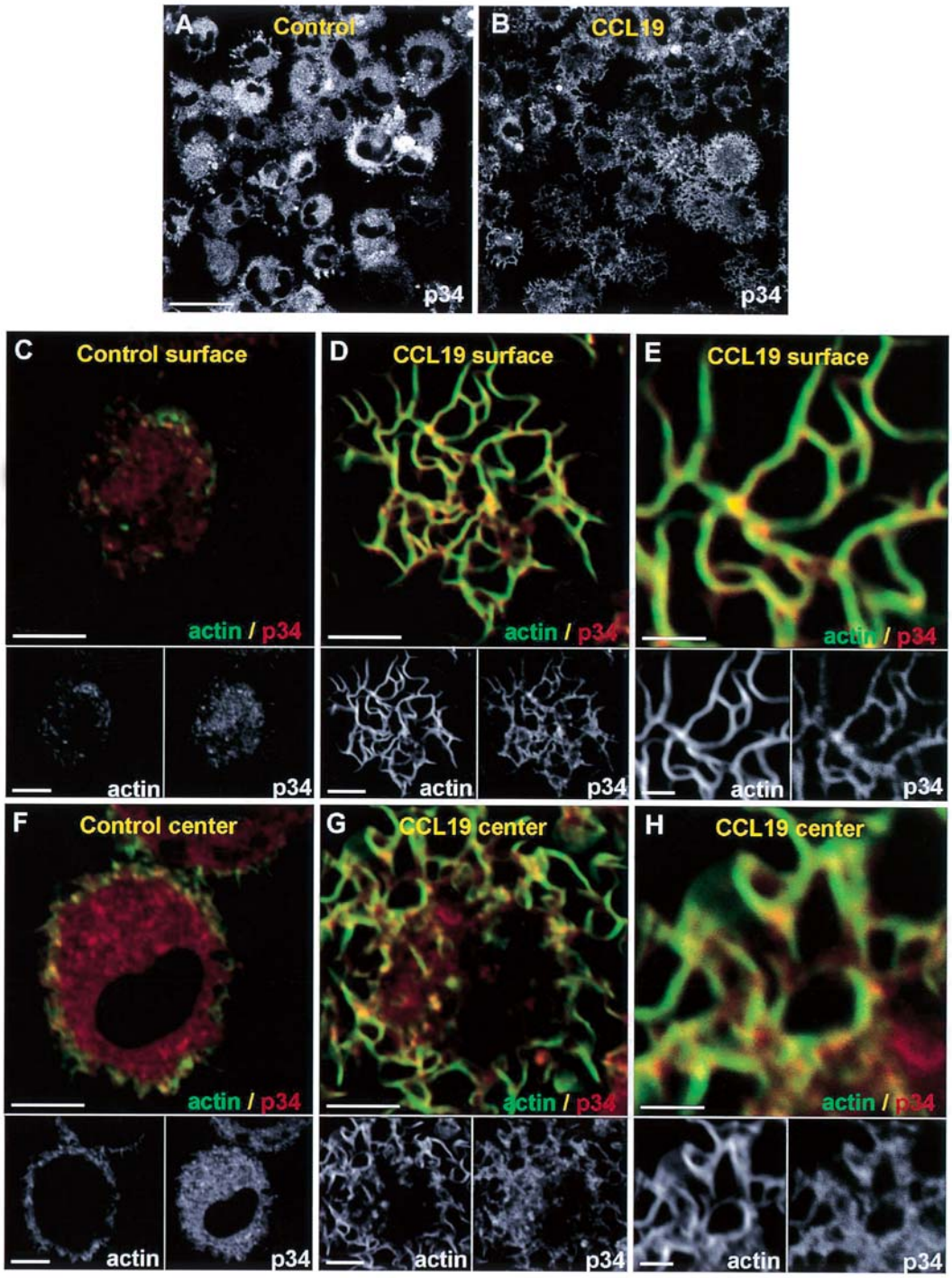

Fig. 4. The effect of CCL19 on the localization of the Arp2/3 complex in mature DCs. Mature DCs were treated with medium alone (Control) (A, C, and F) or CCL19 (B, D, G, E, and H) for 1 min. Intracellular F-actin and the Arp2/3 complexes were labeled with Alexa Fluor 488-conjugated phalloidin (green) and rabbit anti-p34 Abs (red), respectively. Morphological analysis of the actin cytoskeleton was performed on a confocal laser-scanning microscope. A, B, F, G, and H are optical sections through the center of the cells. C, D, and E are optical sections at the cell surface. Data represent at least four independent experiments. Bar=20 μm (A, B), 5 μm (C, D, F, G), or 2 μm (E, H).

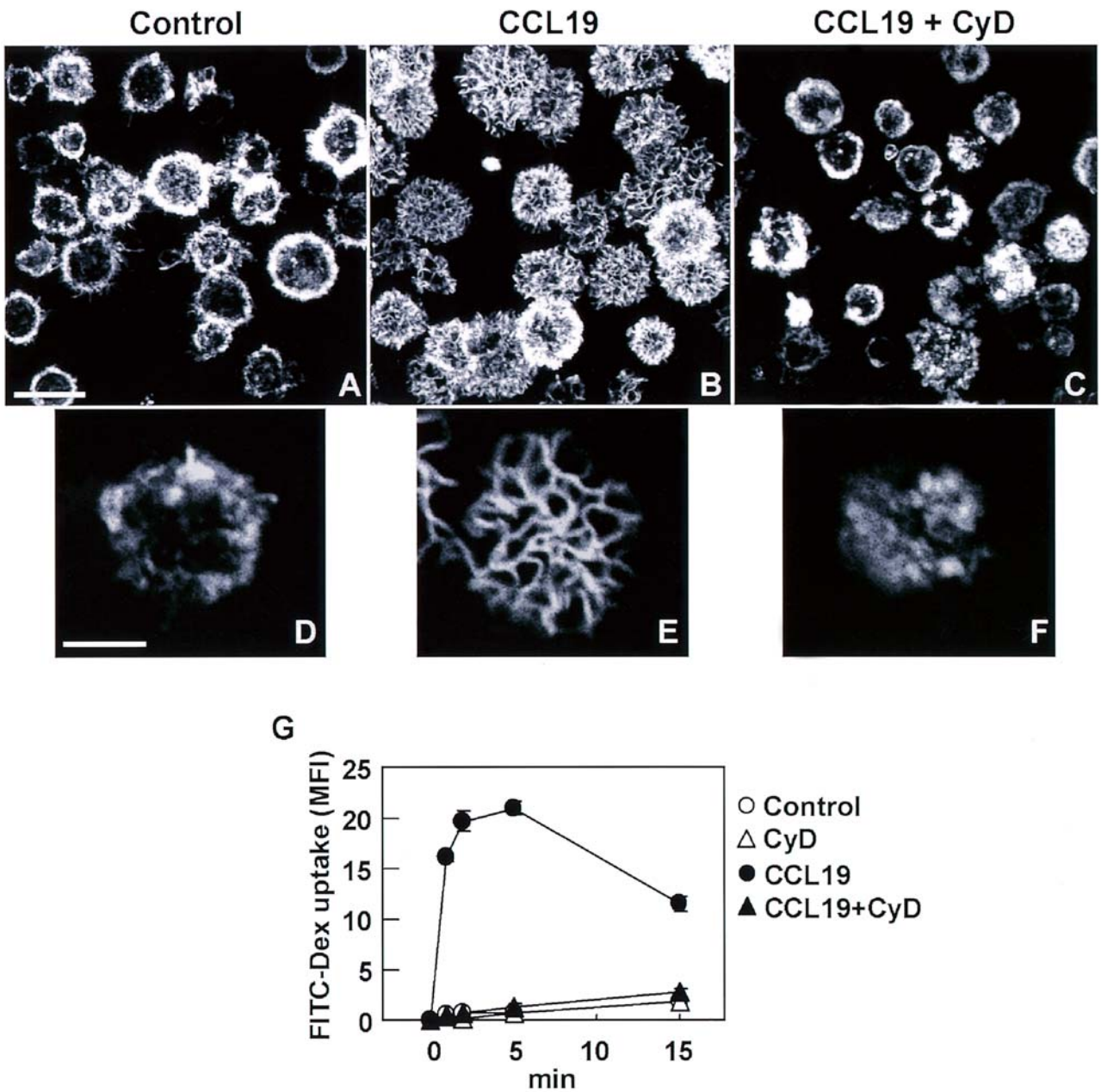

Fig. 5. The effects of cytochalasin D on CCL19-induced formation of actin networks and endocytosis in mature DCs. A-F, The effect of cytochalasin D (CyD) on the actin network. Mature BC1 cells were pretreated with CyD for 1 h, and then treated with CCL19 for 1 min in the presence of the inhibitor ; after labeling the intracellular F-actin with Alexa Fluor 546-conjugated phalloidin, morphological analyses were performed on a confocal laser-scanning microscope. A and D : untreated (Control) cells ; B and E : CCL19-treated cells ; C and F : CCL19-treated cells in the presence of CyD. Data represent four independent experiments. Scale bar=20 μm (A-C) or 5 μm (D-F) ; A-C are extended-focus views, whereas D-F are optical sections at the cells' surfaces. G, The effect of CyD on CCL19-induced endocytosis. Mature BC1 cells were pretreated with CyD for 1 h, and then incubated with FITC-dextran (Dex) and CCL19 in the presence of the inhibitor for 1, 2, 5, or 15 min. The amount of FITC-Dex in DCs was determined using flow cytometry. Each symbol represents the mean ± SE of three independent experiments.

sis in mature DCs.

CCL3, a ligand for CCR5, is expressed by immature but not mature DCs[14]. CCL3 induced no significant alterations of the actin cytoskeleton in immature DCs (data not shown). Although CCL3 enhanced endocytic activity in immature DCs, the enhancement was much smaller than that seen in CCR7 ligand-treated mature DCs[18]. Thus, the poor ability of CCL3 to enhance the endocytic activity of immature DCs may be related to the absence of actin network formation in the CCL3-treated DCs.

The mammalian Wiskott-Aldrich syndrome protein (WASp) family consists of WASp, N-WASp, and WAVE-1, -2, and -3. WASp is expressed only in hematopoietic cells[33], such as monocytes, lymphocytes, and platelets, whereas N-WASp is widely expressed. WASp and N-WASp are downstream effectors of the small GTPases including Cdc42 and Rac[37-42], and are thought to activate Arp2/3 complex-mediated actin nucleation. It has been reported that co-injection of the C-terminal acidic region of WASp family proteins and a constitutively active mutant of Rac into macrophages results in the formation of interconnected actin-rich ruffles, which co-localize with Arp2/3 complexes[9]. On the other hand, we have recently demonstrated that CCL19 rapidly activates Cdc42 and Rac in mature DCs[18]. Thus, WASp activation via Cdc42 or Rac enhanced Arp2/3 complex-mediated actin nucleation in CCL19-treated mature DCs. We, however, were unable to detect a CCL19-induced interaction between WASp and the Arp2/3 complex in mature DCs (data not shown). We are currently examining the involvement of other WASp family proteins in the activation of Arp2/3 complex-mediated actin nucleation. On the other hand, no reports, thus far, have shown a CCR7-mediated interaction between the Arp2/3 complex and a WASp family protein.

Endocytosis of microorganic antigens by DCs is an essential step in antigen presentation to T cells. We showed that CCR7-mediated actin assembly was associated with rapid enhancement of endocytosis in mature DCs. Recently, it has been demonstrated that DCs actively rearrange their actin cytoskeleton in the face that contacts T cells during the formation of an immunological synapse[43,44]. This DC cytoskeletal polarization appears to be critical for efficient antigen presentation to T cells. Because CCR7 ligands are abundantly expressed in the T-cell areas of lymph nodes and the spleen[2,15], our present findings suggest that CCR7-mediated signaling may be involved in the formation of immunological synapses during antigen presentation. Thus, the elucidation of the complex pathways that control actin cytoskeletal reorganization in DCs may lead to the development of new treatments for various infectious diseases and immune disorders.

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REFERENCES

1 Mellado M, Rodriguez-Frade JM, Manes S, Martinez-A C : Chemokine signaling and functional responses : the role of receptor dimerization and TK pathway activation. Annu Rev Immunol 19 : 397-421, 2001

2 Cyster JG : Chemokines and cell migration in secondary lymphoid organs. Science 286 : 2098-2102, 1999

3 Sallusto F, Mackay CR, Lanzavecchia A : The role of chemokine receptors in primary, effector, and memory immune responses. Annu Rev Immunol 18, 593-620, 2000

4 Svitkina TM, Borisy GG : Arp2/3 complex and actin depolymerizing factor/cofilin in dendritic organization and treadmilling of actin filament array in lamellipodia. J Cell Biol 145 : 1009-1026, 1999

5 Linder S, Higgs H, Hufner K, Schwarz K, Pannicke U, Aepfelbacher M : The polarization defect of Wiskott-Aldrich syndrome macrophages is linked to dislocalization of the Arp2/3 complex. J Immunol 165 : 221-225, 2000

6 May RC, Caron E, Hall A, Machesky LM : Involvement of the Arp2/3 complex in phagocytosis mediated by FcγR or CR3. Nat Cell Biol 2 : 246-248, 2000

7 Welch MD, Iwamatsu A, Mitchison TJ : Actin polymerization is induced by Arp2/3 protein complex at the surface of *Listeria monocytogenes*. Nature 385 : 265-269, 1997

8 Welch MD, Rosenblatt J, Skoble J, Portnoy DA, Mitchison TJ : Interaction of human Arp2/3 complex and the *Listeria monocytogenes* ActA protein in actin filament nucleation. Science 281 : 105-108, 1998

9 Hüfner K, Schell B, Aepfelbacher M, Linder S : The acidic regions of WASp and N-WASP can synergize with CDC42Hs and Rac1 to induce filopodia and lamellipodia. FEBS Lett 514 : 168-174, 2002

10 Steinman RM : The dendritic cell system and its role in immunogenicity. Annu Rev Immunol 9 : 271-296, 1991

11 Hart DNJ : Dendritic cells : unique leukocyte populations which control the primary immune response. Blood 90 : 3245-3287, 1997

12 Banchereau J, Steinman RM : Dendritic cells and the control of immunity. Nature 392 : 245-251, 1998

13 Dieu MC, Vanbervliet B, Vicari A, Bridon JM, Oldham E, Ait-Yahia S, Briere F, Zlotnik A, Lebecque S, Caux C : Selective

recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. J Exp Med 188 : 373- 386, 1998

14 Sallusto F, Lanzavecchia A : Understanding dendritic cell and T-lymphocyte traffic through the analysis of chemokine receptor expression. Immunol Rev 177 : 134- 140, 2000

15 Luther SA, Tang HL, Hyman PL, Farr AG, Cyster JG : Coexpression of the chemokines ELC and SLC by T zone stromal cells and deletion of the ELC gene in the plt/plt mouse. Proc Natl Acad Sci USA 97 : 12694- 12699, 2000

16 Forster R, Schubel A, Breitfeld D, Kremmer E, Renner-Muller I, Wolf E, Lipp M : CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell 99 : 23- 33, 1999

17 Yanagawa Y, Onoé K : CCL19 induces rapid dendritic extension of murine dendritic cells. Blood 100 : 1948- 1956, 2002

18 Yanagawa Y, Onoé K : CCR7 ligands induce rapid endocytosis in mature dendritic cells with concomitant up-regulation of Cdc42 and Rac activities. Blood 101 : 4923- 4929, 2003

19 Yanagawa Y, Iijima N, Iwabuchi K, Onoé K : Activation of extracellular signal-related kinase by TNF-α controls the maturation and function of murine dendritic cells. J Leukoc Biol 71 : 125- 132, 2002

20 Winzler C, Rovere P, Rescigno M, Granucci F, Penna G, Adorini L, Zimmermann VS, Davoust J, Ricciardi-Castagnoli P : Maturation stages of mouse dendritic cells in growth factor-dependent long-term cultures. J Exp Med 185 : 317- 328, 1997

21 Iijima N, Yanagawa Y, Iwabuchi K, Onoé K : Selective regulation of CD40 expression in murine dendritic cells by thiol antioxidants. Immunology 110 : 197- 205, 2003

22 Yeh HI, Lai YJ, Lee SH, Lee YN, Ko YS, Chen SA, Severs NJ, Tsai CH : Heterogeneity of myocardial sleeve morphology and gap junctions in canine superior vena cava. Circulation 104 : 3152- 3157, 2001

23 Yeh HI, Lai YJ, Lee YN, Chen YJ, Chen YC, Chen CC, Chen SA, Lin CI, Tsai CH : Differential expression of connexin43 gap junctions in cardiomyocytes isolated from canine thoracic veins. J Histochem Cytochem 51 : 259- 266, 2003

24 Kikuchi K, Yanagawa Y, Aranami T, Iwabuchi C, Iwabuchi K, Onoé K : Tumour necrosis factor-α but not lipopolysaccharide enhances preference of murine dendritic cells for Th2 differentiation. Immunology 108 : 42- 49, 2003

25 Kikuchi K, Yanagawa Y, Iwabuchi K, Onoé K : Differential role of mitogen-activated protein kinases in CD40-mediated IL-12 production by immature and mature dendritic cells. Immunol Lett 89 : 149- 154, 2003

26 Iijima N, Yanagawa Y, Onoé , K : 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- 328, 2003

27 Pavlinkova G, Yanagawa Y, Kikuchi K, Iwabuchi K, Onoé K : Effects of histamine on functional maturation of dendritic cells. Immunobiology 207 : 315- 325, 2003

28 Villadangos JA, Cardoso M, Steptoe RJ, van Berkel D, Pooley J, Carbone FR., Shortman, K : MHC class II expression is regulated in dendritic cells independently of invariant chain degradation. Immunity 14 : 739- 749, 2001

29 Rodriguez A, Regnault A, Kleijmeer M, Ricciardi-Castagnoli P, Amigorena S : Selective transport of internalized antigens to the cytosol for MHC class I presentation in dendritic cells. Nat Cell Biol 1 : 362- 368, 1999

30 Rescigno M, Urbano M, Valzasina B, Francolini M, Rotta G, Bonasio R, Granucci F, Kraehenbuhl JP, Ricciardi-Castagnoli P : Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. Nat Immunol 2 : 361- 367, 2001

31 Granucci F, Vizzardelli C, Pavelka N, Feau S, Persico M, Virzi E, Rescigno M, Moro G, Ricciardi-Castagnoli P : Inducible IL-2 production by dendritic cells revealed by global gene expression analysis. Nat Immunol 2 : 882- 888, 2001

32 He HJ, Kole S, Kwon YK, Crow MT, Bernier M : Interaction of filamin A with the insulin receptor alters insulin-dependent activation of the mitogen-activated protein kinase pathway. J Biol Chem 278 : 27096- 27104, 2003

33 Mateo V, Brown EJ, Biron G, Rubio M, Fischer A, Deist FL, Sarfati M : Mechanisms of CD47-induced caspase-independent cell death in normal and leukemic cells : link between phosphatidylserine exposure and cytoskeleton organization. Blood 100 : 2882- 2890, 2002

34 Szaszi K, Paulsen A, Szabo EZ, Numata M, Grinstein S, Orlowski J : Clathrin-mediated endocytosis and recycling of the neuron-specific Na^+/H^+ exchanger NHE5 isoform. Regulation by phosphatidylinositol 3'-kinase and the actin cytoskeleton. J Biol Chem 277 : 42623- 42632, 2002

35 Nobes C, Marsh M : Dendritic cells : new roles for Cdc42 and Rac in antigen uptake ? Curr Biol 10 : R739- 741, 2000

36 West MA, Antoniou AN, Prescott AR, Azuma T, Kwiatkowski DJ, Watts C : Membrane ruffling, macropinocytosis and antigen presentation in the absence of gelsolin in murine dendritic cells. Eur J Immunol 29 : 3450- 3455, 1999

37 Miki H, Miura K, Takenawa T : N-WASP, a novel actin-depolymerizing protein, regulates the cortical cytoskeletal rearrangement in a PIP2-dependent manner downstream of tyrosine kinases. EMBO J 15 : 5326- 5335, 1996

38 Symons M, Derry JM, Karlak B, Jiang S, Lemahieu V, Mccormick F, Francke U, Abo A : Wiskott-Aldrich syndrome protein, a novel effector for the GTPase CDC42Hs, is implicated in actin polymerization. Cell 84 : 723- 734, 1996

39 Miki H, Sasaki T, Takai Y, Takenawa T : Induction of filopodium formation by a WASP-related actin-depolymerizing protein N-WASP. Nature 391 : 93- 96, 1998

40 Yarar D, To W, Abo A, Welch MD : The Wiskott-Aldrich syndrome protein directs actin-based motility by stimulating actin nucleation with the Arp2/3 complex. Curr Biol 9 : 555- 558, 1999

41 Castellano F, Montcourrier P, Guillemot JC, Gouin E, Machesky

L, Cossart P, Chavrier P : Inducible recruitment of Cdc42 or WASP to a cell-surface receptor triggers actin polymerization and filopodium formation. Curr Biol 9 : 351- 360, 1999.

42 Castellano F, Le Clainche C, Patin D, Carlier MF, Chavrier P : A WASp-VASP complex regulates actin polymerization at the plasma membrane. EMBO J 20 : 5603- 5614, 2001

43 Al-Alwan MM, Rowden G, Lee TD, West KA : The dendritic cell cytoskeleton is critical for the formation of the immunological synapse. J Immunol 166 : 1452- 1456, 2001

44 Al-Alwan MM, Liwski RS, Haeryfar SM, Baldridge WH, Hoskin DW, Rowden G, West KA : Cutting edge : dendritic cell actin cytoskeletal polarization during immunological synapse formation is highly antigen-dependent. J Immunol 171 : 4479- 4483, 2003