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Effects of chemokine (C–C motif) ligand 1 on microglial function

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ABSTRACT

Microglia, which constitute the resident macrophages of the central nervous system (CNS), are generally considered as the primary immune cells in the brain and spinal cord. Microglial cells respond to various factors which are produced following nerve injury of multiple aetiologies and contribute to the development of neuronal disease. Chemokine (C–C motif) ligand 1 (CCL-1), a well-characterized chemokine secreted by activated T cells, has been shown to play an important role in neuropathic pain induced by nerve injury and is also produced in various cell types in the CNS, especially in dorsal root ganglia (DRG). However, the role of CCL-1 in the CNS and the effects on microglia remains unclear. Here we showed the multiple effects of CCL-1 on microglia. We first showed that CCR-8, a specific receptor for CCL-1, was expressed on primary cultured microglia, as well as on astrocytes and neurons, and was upregulated in the presence of CCL-1. CCL-1 at concentration of 1 ng/ml induced chemotaxis, increased motility at a higher concentration (100 ng/ml), and increased proliferation and phagocytosis of cultured microglia. CCL-1 also activated microglia morphologically, promoted mRNA levels for brain-derived neurotrophic factor (BDNF) and IL-6, and increased the release of nitrite from microglia. These indicate that CCL-1 has a role as a mediator in neuron-glia interaction, which may contribute to the development of neurological diseases, especially in neuropathic pain.

1. Introduction

CCL-1, also known as thymus-derived chemotactic agent 3 (TCA-3), is secreted by activated macrophages, T-cell, mast cells and endothelial cells, and has important role as the chemoattractant for neutrophils and monocytes [1]. Although effects of CCL-1 on the peripheral immune cell are well characterized, the role of CCL-1 in the CNS remains unclear.

We have previously discovered that CCL-1 was produced mainly in the DRG and spinal cord after peripheral nerve injury and contributed to the development of neuropathic pain [2]. We also showed that the expression of the specific CCL-1 receptor CCR-8 was up-regulated in the ipsilateral superficial dorsal horn after spinal nerve ligation, not only in neurons but also in microglia and astrocytes. Microglial cells are generally considered as the primary immune cells in the CNS [3,4], and play important roles in the development of neuropathic pain [5]. One of the main functions of microglia in those pathological conditions is that they act as a source and a target of chemokine actions, also in an auto/paracrine fashion. Chemokines including CCL-2 (monocyte chemoattractant protein 1), CCL-21, or CX3CL1 (fractalkine) have been shown to serve as signals from endangered neurons to microglia [6]. Though microglial expression at transcriptional and/or protein level has been documented for many chemokine receptors [7], the functional role of CCL-1/CCR-8 has not been reported yet.

In the present study, we demonstrated that CCL-1 stimulated microglial migration, proliferation and phagocytosis, and release of BDNF and IL-6 in vitro, which may contribute to the neuropathic pain due to nerve injury.

2. Materials and methods

The study was approved by the Animal Research Committee of Kyushu University.

Abbreviations: BDNF, brain-derived neurotrophic factor; bp, base pair; CCL-1, chemokine (C–C motif) ligand 1; CCL-2, chemokine (C–C motif) ligand 2; CCL-21, chemokine (C–C motif) ligand 21; CCR-8, chemokine (C–C motif) receptor 8; cDNA, complementary DNA; CNS, central nervous system; CX3CL-1, chemokine (CX3C motif) ligand 1; DAPI, 4′,6′-diamidino-2-phenylindole dihydrochloride; DMEM, dulbecco's modified eagle medium; DRG, dorsal root ganglia; FBS, fetal bovine serum; GABA, γ-amino butyric acid; GFAP, glial fibrillary acid protein; Iba1, ionized calcium-binding adapter molecule 1; IL-1β, interleukin 1β; IL-6, interleukin 6; MAP2, microtubule-associated protein 2; mRNA, messenger RNA; NO, nitric oxide; NR2B, NMDA receptor 2B; PBS, phosphate buffered saline; RT-PCR, reverse transcription polymerase chain reaction; TCA-3, thymus-derived chemotactic agent 3; TNF-α, tumour necrosis factor-α.

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2.1. Cell culture

Mouse microglial cells were isolated from the mixed cultures of cerebrocortical and spinal cord from postnatal days 1–3 ddY mice (Kyudo, Tosu, Japan), as described previously [8,9]. In brief, tissue was trypsinized for 3 min and dissociated with a fire-polished pipette. Mixed glial cells were cultured for 9–12 days in Dulbecco's Modified Eagle Medium (DMEM; Nissui, Tokyo, Japan) supplemented with 10% Hyclone fetal bovine serum (FBS; Hyclone Labratories, UT, USA), 2 mM L-glutamine, 0.2% D-glucose, 5 μg/ml insulin, 0.37% $NaHCO_3$, 100 U/ml penicillin, 100 μg/ml streptomycin at 37 °C in a 10% CO_2, with medium changes every 3 days. Microglial cells were then separated from the underlying astrocytic layer by gently shaking the flask for 2 h at 37 °C in a shaker-incubator (120 rpm). After unattached cells were removed, microglial cells were isolated as strongly adhering cells. The purity of microglia was >98%, which was evaluated by staining with Iba1 (ionized calcium-binding adapter molecule-1), a marker for microglia/macrophage. Primary cultured neurons from the cerebral cortex were obtained from embryonic day 14–16 (E14–16) ddY mice as described previously [10]. Briefly, neurons were cultured at 37 °C in a 10% CO_2 incubator for 5–7 days with neurobasal medium (GIBCO, NY, USA) containing 2% B27 supplement (GIBCO) and 0.5 mM L-glutamine (GIBCO).

2.2. Immunocytochemical analysis

Primary cultured cells were fixed with 4% paraformaldehyde for 30 min at room temperature and permeabilized with 0.1% Triton X-100 in phosphate buffered saline (PBS; 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 2.7 mM KCl, 136.9 mM NaCl, pH 7.4) for 5 min, followed by treating with blocking solution (Block Ace; Dainippon Pharmaceutical, Japan) for 30 min at room temperature. Cells were incubated with primary antibodies: Iba1 (1:2000, Wako, Japan), CD11b (1:500, Serotec, UK), CCR-8 (1:500, Enzo, PA, USA), Ki67 (1:1000, Abcam, UK), NeuN (1:200, Millipore, CA, USA), MAP2 (1:1000, SIGMA, MO, USA), GFAP (glial fibrillary acid protein, 1:800, Millipore), phalloidin (mouse anti-Texas Red-conjugated phalloidin, 2 U/ml, Invitrogen, CA, USA) overnight at 4 °C. The cells were washed with PBS, and then incubated for 3 h at room temperature with secondary antibody (IgG-conjugated Alexa Fluor™ 488 or 568 or 593, 1:1000, Molecular Probes, OR, USA). The cells were washed with PBS and treated with DAPI (300 nM, Molecular Probes) for 10 min, then washed with PBS. Slides were coverslipped with permafluor aqueous mounting medium. The sections were analyzed using a confocal laser scanning microscope (LSM510META, Carl Zeiss, Germany).

2.3. Real time reverse transcription (RT) polymerase chain reaction (PCR)

Cultured microglia cells were plated in 60 mm dishes (10^6 cells/dish) and incubated for 24 h and collected after treatment of CCL-1 (R&D systems, MN, USA). They were subjected to total RNA extraction according to the protocol of the manufacture and purified with QIAamp RNA Blood Mini (Qiagen, Valencia, CA, USA). The amount of total RNA concentration was measured using Smart Spec™ 3000 (Bio Rad, Tokyo, Japan). Total RNA (175 ng) was converted to cDNA by reverse transcription, using random 9 mer (Takara, Otsu, Japan) and RNA PCR kit (Takara). The forward and reverse primers and the estimated product size were as follows: CCL-1 mRNA (5′-TTCCCCTGAAGTTTATCCAGTGTT-3′; 5′-TGAACCCACGTTTTGTTAGTTGAG-3′), 124 bp; CCR-8 mRNA (5′-ACGTCACGATGACCGACTACTAC-3′; 5′-GAGACCACCTTACACATCGCAG-3′), 301 bp; β-actin mRNA (5′-TTGCTGACAGGATGCAGAAGGAG-3′; 5′-GTGGACAGTGAGGCCAGGAT-3′), 127 bp; IL-10 mRNA (5′-GTCATCGATTTCTCCCCTGTG-3′; 5′-CCTTGTAGACACCTTGGTCTTGG-3′), 93 bp; CCL-2 mRNA (5′-CGGAACCAAATGAGATCAGAA-3′; TGTGGAAAAGGTAGTGGATGC-3′), 26 bp; TNF-α mRNA (5′-CCACCACGCTCTTCTGTCTAC-3′, 5′-TGGGCTACAGGCTTGTCACT-3′) 848 bp; IL-1β mRNA (5′-CTCCATGAGCTTTGTACAAGG-3′; 5′-TGCTGATGTACCAGTTGGGG-3′) 245 bp; IL-6 mRNA (5′-ACACTCCTTAGTCCTCGGCCA-3′; 5′-CACGATTTCCCAGAGAACATGTG-3′) 129 bp; BDNF mRNA (5′-TGCAGGGGCATAGACAAAAGG-3′; 5′-CTTATGAATCGCCAGCCAATTCTC-3′), 110 bp. All primers were purchased from Sigma Aldrich Japan (Tokyo, Japan). PCR amplification was undertaken for Thunderbird Sybr qPCR Mix (Toyobo, Osaka, Japan) in Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems Japan, Tokyo, Japan). Each reaction volume consisted of 12.5 μl Thunderbird Sybr qPCR Mix, 0.05 μl 50×ROX reference dye, 1 μl mix of forward and reverse primers (0.3 μM each), and 11.45 μl RNAase free water containing cDNA (17.5 ng). PCR was done by 15 s denaturation at 95 °C, and annealing/extending at 60 °C for 40 cycles. Each mRNA expression level was normalized by β-actin. The mRNA expression was calculated relative to β-actin using the $\Delta\Delta C_T$ algorithm.

2.4. Microchemotaxis assay

Microglial chemotactic migration was tested with 48-well microchemotaxis Boyden chamber (Neuroprobe, MD, USA) [11]. Upper and lower wells were separated by polycarbonate filter (8 μm pore size; Poretics, CA, USA). Primary cultured microglial cells (200×10^4 cells/ml) in 50 μl of serum-free DMEM were added to the upper wells, and the lower wells contained the same medium with or without (control) CCL-1 and other drugs if mentioned. The chamber was incubated at 37 °C and 10% CO_2 for 90 min. Cells remaining on the upper surface of the membrane were removed by wiping, and migrated cells were subjected to Diff-Quik stain kit (Sysmex Corp., Kobe, Japan). Rate of microglial migration was calculated by counting cells in four random fields of each well.

2.5. Motility experiment

Experiments were performed as described [9]. Cultured microglial cells were seeded on glass-bottom dishes (Matsunami, Osaka, Japan) at a density of 4×10^4 cells/dish in serum-free DMEM. Motility of microglia under controlled temperature (37 °C) and gas (10% CO_2/90% humidity) was monitored with a time-lapse videomicroscopy system (Nikon Instec, Fukuoka, Japan). The video camera (Nikon inverted microscope, TE-2000-E) was controlled by Luminavision software (MITANI, Osaka, Japan). Images were acquired at 1 min intervals for 1 h in the presence or absence (control) of drugs, and stored on a computer and analyzed by Dipp-Motion 2D (DITECT, Tokyo, Japan). Serum-free DMEM was used as control.

2.6. Phagocytosis assay

Microglial cells seeded on the slide glass (4×10^4 cells/dish) were pre-incubated with 4 μg/ml fluorescent zymosan beads (Zymosan A (*S. cerevisiae*) BioParticles, Alexa Fluor 488 conjugate, Invitrogen, UK) in serum-free DMEM for 1 h, then incubated with CCL-1 with or without other drugs for another 1 h. Beads-treated microglial cells were fixed with 4% paraformaldehyde, treated with anti-Iba1 (1:2000, overnight), then incubated with Alexa Fluor 568-conjugated goat anti-rabbit IgG (1:1000). Series of Z-stack images (every 0.6 μm) were examined with an All-in-One fluorescence microscope (BIOREVO BZ-9000; Keyence, Osaka, Japan) for 3D-analysis of each phagocytic cell.

2.7. Quantification of Ki67 or CCR-8 positive microglia

For cultured microglia, the number of CD11b- and Ki67-positive cells ($CD11b^+$ and $Ki67^+$, respectively) was counted in ten areas of 450 μm × 450 μm, and the total number of $CD11b^+$ cells in the same area was obtained for each group. The ratio of $Ki67^+$/$CD11b^+$ microglial cells was normalized to $CD11b^+$cells. Using the same method, the number of Iba1- and CCR-8-positive cells were counted in ten areas of 150 μm × 150 μm.

2.8. Measurement of nitrite production

The nitrite accumulated in the culture medium was measured as an indicator of NO production using the Griess reagent kit (Promega, Madison, WI, USA). Cell culture media were centrifuged at 4 °C for 5 min and the supernatant was subjected to measurement of nitrite.

2.9. Statistical analysis

All data are presented as mean ± SEM. The statistical analyses of the results were evaluated by using two-tailed Student's unpaired *t*-test, one-way ANOVA followed by Dunnett's test or two-way ANOVA followed by Tukey–Kramer test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Expression of CCR-8 in microglia, astrocytes and neurons

We first examined the expression of CCR-8, a specific receptor for CCL-1, in primary cultured microglia, astrocytes and neurons. Cultured cells were co-stained with CCR-8 and several glial or neuronal markers. We identified CCR-8 expression in microglia (Fig. 1A), astrocytes (Fig. 1B) and neurons (Fig. 1C and D). We further confirmed expression of CCR-8 mRNA in cultured microglia derived from cortex and the spinal cord, showing the similar expression level (Fig. 1E). The spleen was used as a positive control. The ratio of CCR-8-positive microglia was 31.0 ± 1.69%, which was increased significantly by application of CCL-1 (50 ng/ml) for 1 h (to 43.23 ± 2.36%, $n = 10$, Fig. 1F). The relative expression level of CCL-1 mRNA was 1129.84 ± 115.13% in neurons and 206.68 ± 10.56% in microglia compared to that of astrocytes (Fig. 1G).

3.2. Effects of CCL-1 on microglial chemotaxis and motility

The addition of CCL-1 to the lower well of the Boyden chamber increased microglial migration, with more cells on the lower membrane surface compared to that of control (Fig. 2A). CCL-1 (1.0, 5.0, 10.0, 50.0, 100.0 ng/ml) induced a concentration-dependent increase in microglial chemotaxis (424.8 ± 13.9%, 569.8 ± 23.6%, 878.8 ± 30.0%, 925.9 ± 50.0%, 892.8 ± 43.3%, respectively; $n = 24$) (Fig. 2B). Next, we measured the total migration distance of the

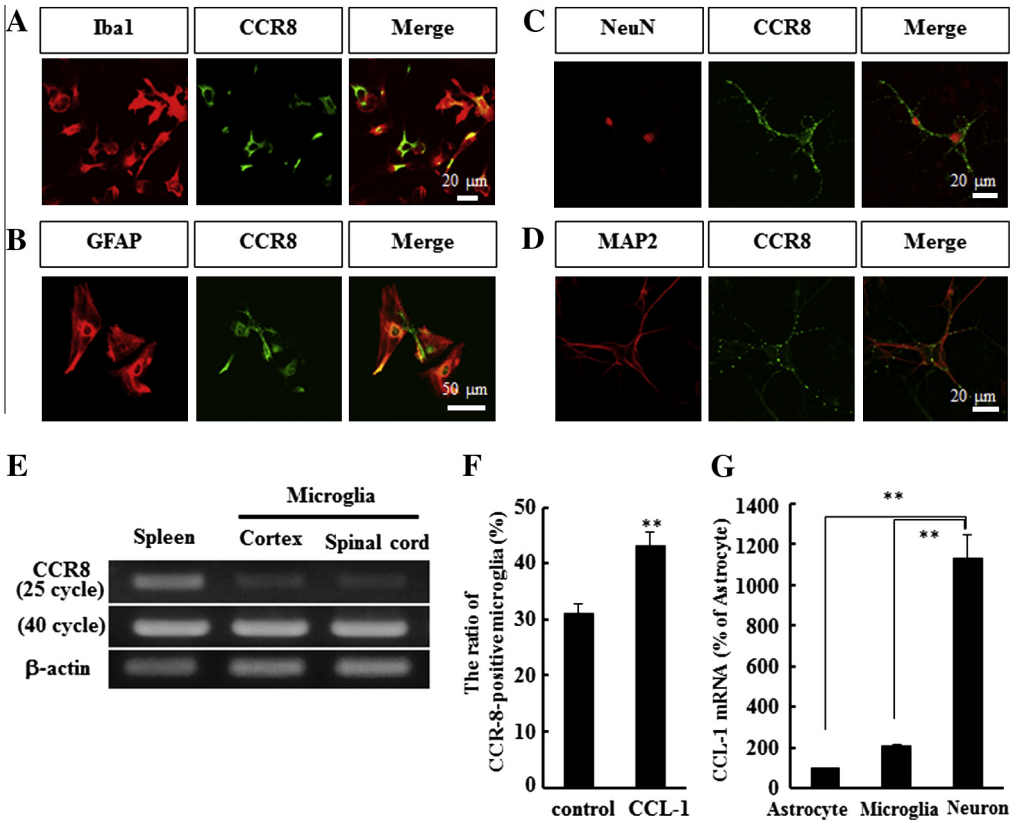

Fig. 1. Expression of CCR-8 in microglia, astrocytes and neurons. (A) Immunostaining for Iba1 (red) and CCR-8 (green) in primary cultured miroglia. Scale bar = 20 μm. (B) Immunostaining for GFAP (red) and CCR-8 (green) in primary cultured astrocytes. Scale bar = 50 μm. (C) Immunostaining for NeuN (red) and CCR-8 (green) in primary cultured neurons. Scale bar = 20 μm. (D) Immunostaining for MAP2 (red) and CCR-8 (green) in primary cultured neurons. Scale bar = 20 μm. (E) CCR-8 and β-actin mRNA expression in primary microglia derived from the cortex and the spinal cord by RT-PCR analysis. Spleen is positive control. (F) The ratio of $CCR\text{-}8^+/Iba1^+$ cells normalized by $Iba1^+$ cells in cultured microglia with or without (control) application of CCL-1 (50 ng/ml) for 1 h. Data represent mean ± SE. ($n = 10$) $^{**}P < 0.01$. (two-tailed Student's unpaired *t*-test). (G) Relative expression of CCL-1 mRNA in astrocytes, microglia and neurons (% of astrocytes). Data represent mean ± SE ($n = 4$). $^{**}P < 0.01$ vs. astrocytes (one-way ANOVA followed by Tukey–Kramer tests). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

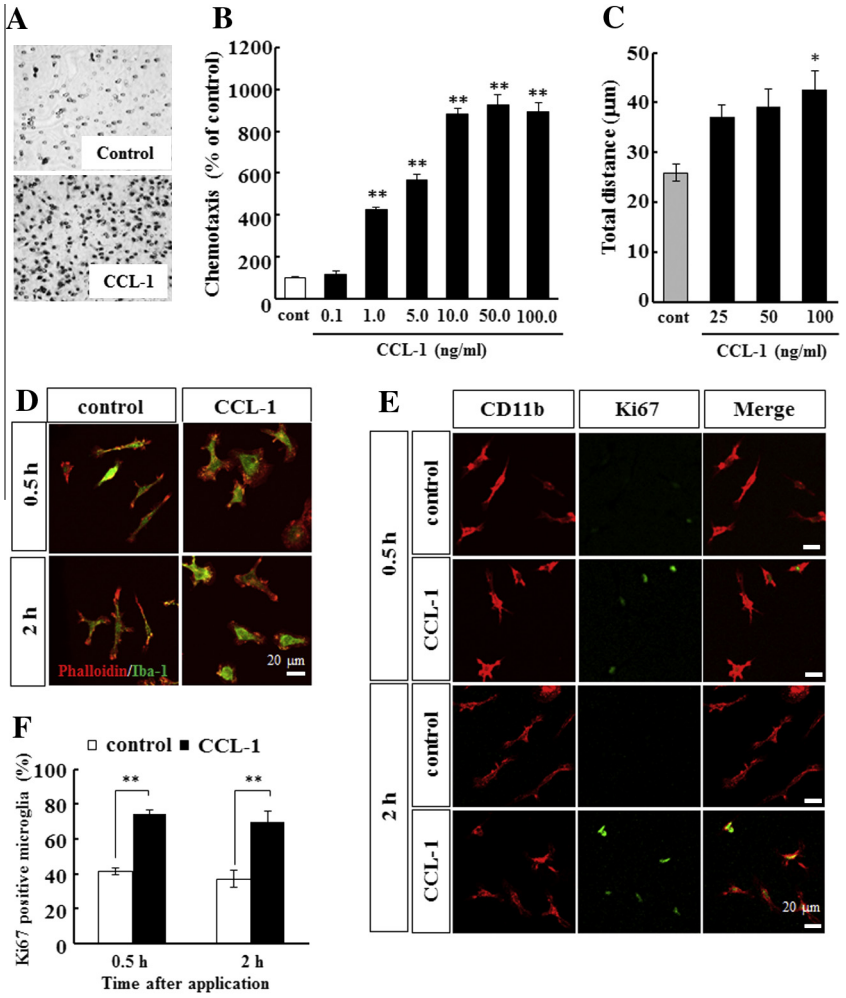

Fig. 2. Effects of CCL-1 on microglial chemotaxis, motility and proliferation. (A and B) CCL-1-induced microglial chemotaxis determined by Boyden chamber. (A) Microglial staining on the lower surface of polycarbonate filter 90 min after application of CCL-1 (50 ng/ml) or control (serum-free DMEM). (B) Quantification of microglial chemotaxis induced by CCL-1 (0.1, 1.0, 5.0, 10.0, 50.0 and 100.0 ng/ml) or control (serum-free DMEM). Data represent mean ± SE. ($n = 10$) $^{**}P < 0.01$ vs. control (one-way ANOVA followed by Dunnett's test). (C) Motility of CCL-1-stimulated microglial cells. Total distance of microglial cells stimulated by CCL-1 (100 ng/ml) or control (serum-free DMEM) for 1 h. Data represent mean ± SE. ($n = 40$) $^{*}P < 0.05$ vs. control (one-way ANOVA followed by Dunnett's test). (D) Immunostaining for phalloidin (red) and Iba1 (green) in cultured microglia 0.5 h and 2 h after application of CCL-1 (50 ng/ml). Scale bar = 20 μm. (E) Immunostaining for CD11b (red) and Ki67 (green) in cultured microglia 0.5 h and 2 h after application of CCL-1 (50 ng/ml) or control (serum-free DMEM). Scale bars = 20 μm. (F) The ratio of $Ki67^{+}/CD11b^{+}$ microglial number normalized by $CD11b^{+}$ number in cultured microglia with or without application of CCL-1 (50 ng/ml) for 0.5 and 2 h. Data represent mean ± SE ($n = 10$). $^{**}P < 0.01$ (two-way ANOVA followed by Tukey–Kramer test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

microglia after application of CCL-1 for 1 h. CCL-1 (100 ng/ml) increased the total migration distance significantly (from 42.5 ± 3.9 to 25.86 ± 1.71 μm; $n = 40$), showing that CCL-1 increased microglial motility (Fig. 2C).

3.3. Effects of CCL-1 on microglial proliferation

To investigate the effects of CCL-1 on microglial morphology, cells exposed to CCL-1 (50 ng/ml) for 0.5 h or 2 h were stained with Iba1 (green) and phalloidin (F-actin marker, red) to visualize actin polymerization. Exposure to CCL-1 induced membrane ruffling in microglia (Fig. 2D). We also stained with CD11b (red) and Ki67 (proliferation marker, green) with or without CCL-1 (50 ng/ml) for 0.5 h and 2 h to investigate the change in proliferation (Fig. 2E). The number of Ki67-positive cells significantly increased at 0.5 h (from 41.52 ± 2.2 to 74.6 ± 2.2%; $n = 10$) and at 2 h (from 37.04 ± 5.0 to 69.7 ± 6.1%; $n = 10$) after application of CCL-1 (Fig. 2F).

3.4. Effects of CCL-1 on microglial phagocytosis

The effect of CCL-1 on microglial phagocytosis was assayed by Z-stack 3D imaging (Fig. 3A and B). Application of different concentration of CCL-1 (5–50 ng/ml) tended to increase the number of microglia phagocytizing beads (Fig. 3C), and, at 50 ng/ml significantly increased the average number of beads phagocytosed per cell (Fig. 3D).

3.5. Effects of CCL-1 on microglial neurotrophic and proinflammatory factors

Various factors produced by activated microglia were analyzed in terms of mRNA levels. BDNF mRNA significantly increased in cultured microglia at 1 and 4 h after application of 50 ng/ml CCL-1 (173.2 ± 4.3% and 175.7 ± 20.0%, respectively; $n = 3$) (Fig. 4A). IL-6 mRNA transiently increased only at 4 h (158.8 ± 16.6%; $n = 3$) after CCL-1 application (Fig. 4B). On the other hand, mRNAs for TNF-α, CCL-2, IL-1β and IL-10 did not change after CCL-1 application

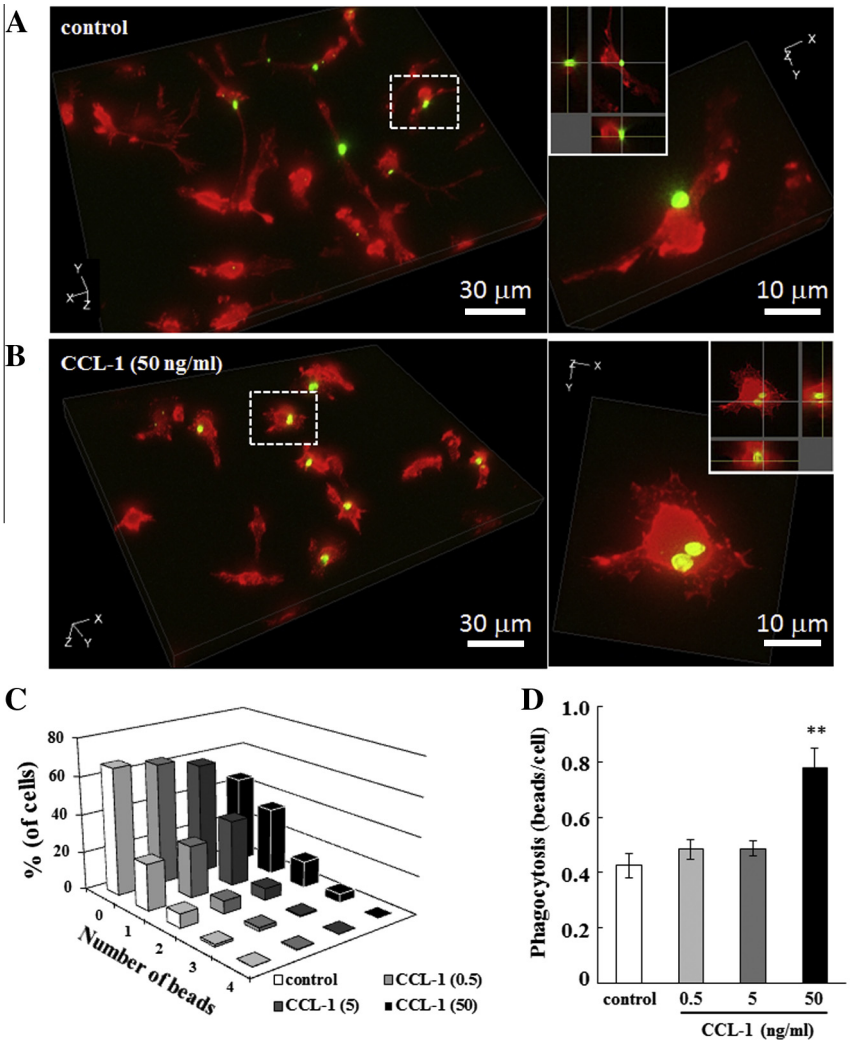

Fig. 3. Effects of CCL-1 on microglial phagocytosis. (A and B) 3D-images of microglial phagocytosis of fluorescent zymosan beads (green) after application of CCL-1 (50 ng/ml). Immunostaining for Iba1 (red) in cultured microglia in control (serum-free DMEM) (A) and 1 h after application of CCL-1 (B). (C) The distribution chart of phagocytized beads in cultured microglia 1 h after application of CCL-1. (D) The number of phagocytized beads per one cultured microglia increased significantly with 50 ng/ml CCL-1. Data show mean ± SE ($n = 10$). $^{**}P < 0.01$ vs. control (one-way ANOVA followed by Dunnett's test). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fig. 4C–F). Application of CCL-1 (50 ng/ml) also significantly increased NO release from microglia at 4, 6 and 12 h (from 1.01 ± 0.05 to 1.48 ± 0.06 μM, 1.32 ± 0.09 μM, 2.14 ± 0.04 μM, respectively; $n = 3$) (Fig. 4G).

4. Discussions

We have previously reported that expression of CCL-1 mRNA was detected in the ipsilateral DRG and spinal cord, and expression of CCR-8 protein was upregulated in the spinal cord after partial sciatic nerve ligation. Thus, we showed that CCL-1 might be one of the key mediators involved in the development of neuropathic pain following peripheral nerve injury [2].

The present study demonstrates that CCL-1, as well as other chemokines, serves as chemoattractant for microglia, and induces proliferation and phagocytosis of microglia. We further showed that CCL-1 induced expression of neurotrophic and proinflammatory factors in cultured microglia.

We confirmed that CCL-1 specific receptor, CCR-8, were expressed on primary cultured microglia (Fig. 1A), while CCL-1 mRNA was most strongly expressed in neurons (Fig. 1G). This indicates that CCL-1 works as a mediator between neuron and microglia in the CNS.

A key aspect of microglial response to damage is an increase in the microglial population at the site of injury. This is achieved mainly by proliferation and migration of the resident microglia. It has been shown that following peripheral nerve injury microglia accumulated in the dorsal horn of the spinal cord [5,12,13]. Migration of microglia is thought to be orchestrated by various chemotactic agents that are released at the site of injury. CCL-2 and CX3CL-1 have been implicated in microglial migration to the site of injury after peripheral nerve injury [14,15]. Increase in intracellular calcium mobilization and actin rearrangement as well as morphological change in microglia was also reported with CX3CL-1 [16]. Here we showed that CCL-1 induced microglial chemotactic migration, motility and proliferation (Fig. 2).

Another key function of microglia within the CNS is the clearance of neurotoxic molecules, cellular debris or microbes via phagocytosis to maintain neural networks [17]. In the present study, we showed that CCL-1 significantly increased microglial phagocytosis (Fig. 3). It is uncertain whether this microglial function plays a role in restoring transmission through the clearance of degenerating synaptic terminal or synaptic stripping [18,19]. Although we do not have direct evidence for the removal of synapses within the dorsal horn or for the clearance of cellular debris

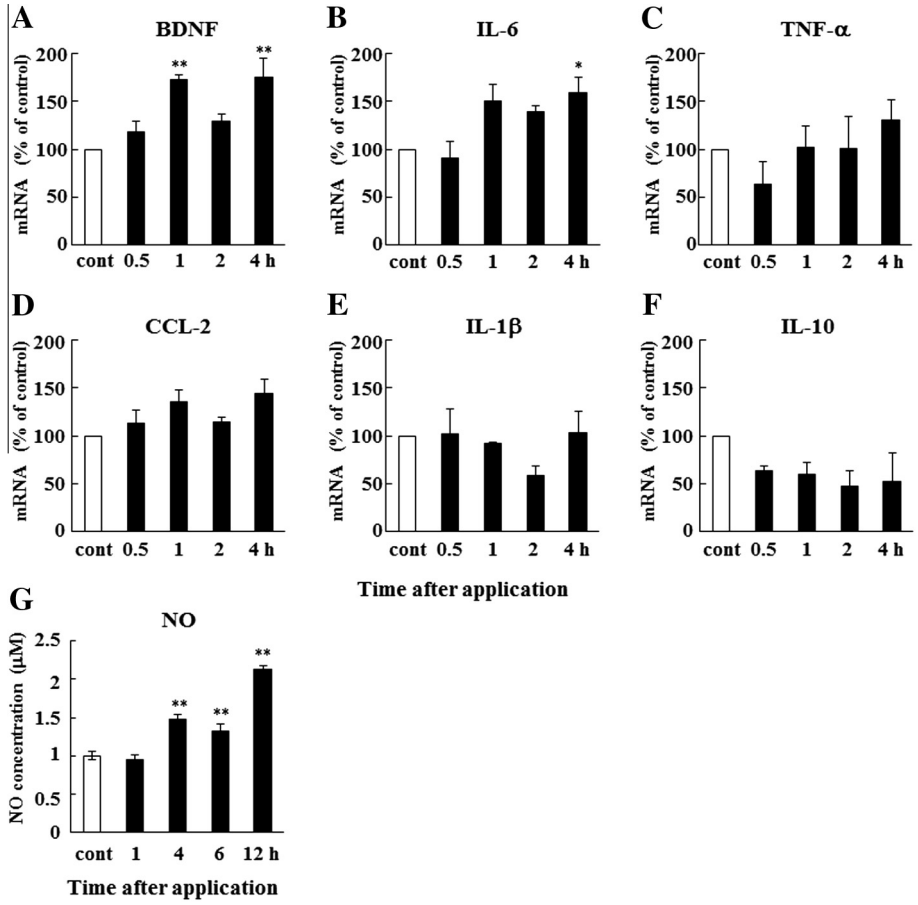

Fig. 4. Effects of CCL-1 on the production of microglial neurotrophic and proinflammatory factors. Expression of mRNA of cytokine (TNF-α, IL-6, IL-1β, IL-10), chemokine (CCL-2) and growth factors (BDNF) in cultured microglia (A–F) and the release of NO from cultured microglia (G) after application of CCL-1 (50 ng/ml). Each mRNA value was normalized to the control level. Data represent mean ± SE ($n = 3$). $^{**}P < 0.01$, $^{*}P < 0.05$: vs. control (one-way ANOVA followed by Dunnett's test).

by microglia, it is reasonable to suggest that phagocytosis induced by CCL-1 might play a role in restoring homeostasis after peripheral nerve injury.

CCL-1 also changed microglial morphology mediated by actin polymerization (Fig. 2D). Cell migration, morphological change and phagocytic activity are all regulated by reorganization of actin cytoskeleton. In particularly, characteristic morphological remodeling of migrating cell defined as a 'membrane ruffling' is induced by actin polymerization [20–22], suggesting that CCL-1 affects actin polymerization in microglia.

Once stimulated through 'danger' stimuli, microglia secrete cytokines and BDNF, which directly affect synaptic transmission through enhancing dorsal horn neuron excitability [23,24]. These cytokines released by microglia can induce central sensitization via distinct mechanism [25]. IL-6 regulates inhibitory neurotransmission by reducing the frequency of spontaneous inhibitory postsynaptic currents and the amplitude GABA- and glycine-induced currents [24]. BDNF from activated microglia induces a depolarising shift in equilibrium potential for Cl^- ions in spinal neurones, shifting the reversal potential for GABA currents and switching inhibitory transmission to excitatory transmission [23]. At the same time, BDNF contributes to the development and maintenance of neuropathic pain by activating the NR2B-containig NMDA receptors [26]. Furthermore, peripheral nerve injury-induced neuropathic pain is associated with iNOS induction and NO release in the spinal cord [27]. Our data showed that BDNF and IL-6 mRNA were significantly increased, and that NO was released, in cultured microglia after application of CCL-1 (Fig. 4). These data indicated that microglia activated by CCL-1 could be involved in maintaining neuropathic pain through the release of IL-6, BDNF and NO.

Our previous study showed that intrathecal injection of CCL-1 increased the expression of CCR-8 not only in microglia but also in astrocytes in the spinal cord [2]. In the present study, we also showed that CCR-8 is expressed in cultured astrocytes (Fig. 1B), suggesting that CCR-8 in astrocytes in the spinal cord may also contribute to the pathological events after nerve injury.

In conclusion, we showed the expression of CCR-8 in both neuron and glial cells and demonstrated the function of CCL-1/CCR-8 in cultured microglia. These indicate that CCL-1 induced by nerve injury may work as a mediator in neuron-glia interaction, which may contribute to the development of neurological diseases, especially in neuropathic pain.

Acknowledgments

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