Angiogenic activity of human CC chemokine CCL15 in vitro and in vivo

Jungsu Hwang[a], Chan Woo Kim[b], Kyung-No Son[a], Kyu Yeon Han[b], Kyung Hee Lee[c], Hynda K. Kleinman[d], Jesang Ko[e], Doe Sun Na[e], Byoung S. Kwon[f], Yong Song Gho[b,*], Jiyoung Kim[a,f,*]

[a]*Graduate School of Biotechnology and Institute of Life Sciences and Resources, Kyung Hee University, Yongin 449-701, South Korea*
[b]*Department of Life Science, Pohang University of Science and Technology, Pohang 790-784, South Korea*
[c]*Department of Oncology, Graduate School of East–West Medical Science, Kyung Hee University, Yongin 449-701, South Korea*
[d]*Craniofacial Developmental Biology and Regeneration Branch, National Institute of Dental and Craniofacial Research, NIH, Bethesda, MD 20892, USA*
[e]*Department of Biochemistry, College of Medicine, University of Ulsan, Seoul 138-736, South Korea*
[f]*Immunomodulation Research Center, University of Ulsan, Ulsan 680-749, South Korea*

Received 6 April 2004; revised 26 May 2004; accepted 5 June 2004

Available online 20 June 2004

Edited by Masayuki Miyasaka

Abstract CCL15 is a novel human CC chemokine and exerts its biological activities on immune cells through CCR1 and CCR3. Because a number of chemokines induce angiogenesis and endothelial cells express CCR1 and CCR3, we investigated the angiogenic activity of CCL15. Both CCL15(1-92) and N-terminal truncated CCL15(25-92) stimulate the chemotactic endothelial cell migration and differentiation, but CCL15(25-92) is at least 100-fold more potent than CCL15(1-92). Treatment with pertussis toxin (PTX), with anti-CCR1, or with anti-CCR3 antibody inhibits the CCL15(25-92)-induced endothelial cell migration. CCL15(25-92) also stimulates sprouting of vessels from aortic rings and mediates angiogenesis in the chick chorioallantoic membrane assay. Our findings demonstrate that CCL15(25-92) has in vitro and in vivo angiogenic activity, and suggest roles of the chemokine in angiogenesis.

Keywords: Chemokine; CCL15; Endothelial cells; Angiogenesis; CCR1; CCR3

1. Introduction

Chemokines play central roles in diverse biochemical and physiological events including regulation of leukocyte trafficking, immunity, and hematopoiesis [1,2]. Chemokines are subdivided into four distinct groups based on the arrangement of the two conserved N-terminal cysteine residues: CXC (α), CC (β), C (γ), and CX_3C (δ). CCL15, also named Leukotactin-1 (Lkn-1), MIP-1δ, MIP-5, HCC-2, and NCC-3, is a novel human CC chemokine [3]. Mature CCL15 is composed of predicted 92 amino acid residue and has long amino acid residues preceding the first cysteine at the NH_2 terminus [4,5]. It has been reported that N-terminal deletion upto 28 amino acids of CCL15 increased the agonistic potency on CCR1 and CCR3 [6]. Although their biochemical and biological activities are different, all the intact and truncated forms bind to both CCR1 and CCR3, and induce chemotaxis and calcium flux [4,6]. The biological significance of truncated forms of CCL15 is not known yet.

In addition to their functions in leukocyte trafficking and activation, growing evidence suggests that members of the chemokine family regulate angiogenesis. A number of CXC chemokines has been reported to either induce endothelial cell migration and/or proliferation in vitro and neovascularization in vivo or to act as angiostatic molecules [7,8]. Among the CC chemokine family reported to date, CCL1 (I-309), CCL2 (MCP-1), CCL11 (eotaxin), and CCL16 (NCC-4) have been proven to play a direct role in angiogenesis [9–12]. Although CCL15 exerts important roles in inflammation, its role in neovascularization has not been examined. Here, we demonstrate that CCL15(25-92) has more potent chemotactic activity for the endothelial cell than the intact form of CCL15 and has in vitro and in vivo angiogenic activity.

2. Materials and methods

2.1. Materials

Recombinant CCL15 proteins, bFGF, anti-CCL15, anti-CCR1, and anti-CCR3 antibodies were from R&D Systems (Minneapolis, MN). Matrigel and rat tail type I collagen were from Collaborative Biomedical Products (Bedford, MA). Thermonox disks were from Nunc (Naperville, IL). Protein A-Sepharose and PTX were from Amersham Pharmacia Biotech (Uppsala, Sweden) and BIOMOL Research Laboratories (Plymouth Meeting, PA), respectively. Human fibrosarcoma HT1080 conditioned medium was prepared as described previously [13].

2.2. Preparation of immuno-depleted and heat-inactivated CCL15

For immuno-depletion, a mixture of either CCL15(25-92) (0.5 μg) or bFGF (0.5 μg) and 10 μg of either anti-CCL15 monoclonal antibody or control isotype-matched mouse IgG_1 in 0.25 ml of RPMI 1640 containing 0.1% BSA was incubated for 2 h and then incubated with 1:1 slurry of Protein A-Sepharose beads (0.2 ml) overnight at 4 °C. Then, the supernate was aliquoted and stored at −80 °C until use. For heat-inactivation, CCL15(25-92) (10 μg/ml in PBS) was boiled for 10 min, placed on ice, and then stored at −80 °C until use.

2.3. Cell migration assay

Human umbilical vein endothelial cells (HUVECs) and immortalized human microvascular endothelial cells (HMEC-1s) and HUVECs

* Corresponding authors. Fax: +82-54-279-8611 (Y.S. Gho); +82-31-203-4969 (J. Kim).
E-mail addresses: ysgho@postech.ac.kr (Y.S. Gho), jkim@khu.ac.kr (J. Kim).

0014-5793/$22.00 © 2004 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2004.06.023

were grown as described previously [14,15]. Cell migration assays and checkerboard assays were performed in 48-well microchemotaxis chambers (Neuro Probe, Inc., Cabin John, MD) [16]. The bottom chamber was loaded with 30 000 cells, and polyester membrane (Neuro Probe, Inc.) was laid over the cells. The microchamber was then inverted and incubated at 37 °C for 2 h. The upper wells were then loaded with RPMI 1640 containing 0.1% BSA and CCL15. The chamber was reincubated at 37 °C for 2 h, and the filters were fixed and stained using Diff-Quick (Baxter Healthcare Corp., McGraw Park, IL). Each condition was studied in triplicate wells, and each experiment was performed three times. Checkerboard assays were carried out as described above, except various amounts of CCL15 were placed in the top and/or bottom wells.

2.4. Endothelial cell proliferation assay

The proliferation assay of endothelial cells was performed by BM 5-bromo-2′-deoxy-uridine (BrdU) labeling and Detection Kit from Roche (Indianspolis, IN). HUVEC cells (1×10^4) were seeded in each well of a gelatin-coated 96-well plates for 24 h at 37 °C. After removing medium, cells were starved for 6 h and then added RPMI 1640 containing 5% FBS and CCL15 at various concentration for 18 h at 37 °C. The bFGF (5 ng/ml) were used as positive control. The each well was added BrdU labeling solution, washed and fixed precooled ethanol fixative at −20 °C and then added nuclease solution, following anti-BrdU-POD. The DNA that integrated BrdU was quantitated by relative luminescence (RLU) of each well using Wallac Victor^2 1420 Multilabel counter (Perkin Elmer, Norwalk, CT).

2.5. Capillary-like tube formation assay

Tube formation assay was performed as described previously [16]. Briefly, HUVECs (40 000 cells) in 0.4 ml of serum-free RPMI 1640 medium with varying concentrations of CCL15 were added to each well coated with 1:1 mixture of RPMI 1640 and Matrigel. bFGF was added at a concentration of 5 ng/ml as a positive control. After 3 h incubation, two randomly chosen fields (20×) from each sample were photographed and total tube areas were analyzed by the Scion Image program.

2.6. Rat aortic ring assay

Rat aortic ring assay was described previously [17]. Thoracic and abdominal aortas were obtained from male rats of 6 week age. Plates (48-well) were coated with 150 μl of Matrigel, and then Matrigel was gelled at 37 °C incubator. The rings of sectioned aortas were placed into the wells and sealed with 50 μl of Matrigel. CCL15 was added to the well in 200 μl of human endothelial serum free medium (Gibco-BRL). Heat-treated CCL15, bFGF, and medium alone were assayed as controls. After 6 days, aortas were fixed and stained with Diff-Quick. The assay was scored from 0 (least positive) to 4 (most positive) in a double blinded manner.

2.7. Chick chorioallantoic membrane assay

To investigate the in vivo angiogenic activity of CCL15, the modified chorioallantoic membrane (CAM) assay was carried out as described previously [16]. Briefly, 10 μl of test samples in type I collagen were applied onto Thermonox disks and polymerized at room temperature. The discs were loaded onto the CAM of 10-day-old embryos. After 70 ± 4 h incubation at 37 °C, the area around the loaded disk was photographed with a Nikon digital camera and the number of newly formed vessels was counted by two observers in a double blinded manner.

3. Results

3.1. CCL15 stimulates human endothelial cell migration

The migratory activities of two different forms of CCL15 on human endothelial cells were tested using Boyden chamber migration assays. Our results demonstrated that both forms of CCL15 induced endothelial cell migration in a dose-dependent manner and that CCL15(1-92), the mature form of CCL15 was less potent than that of CCL15(25-92) (Fig. 1(A) and (B)), indicating that truncation of N-terminal region of CCL15 re-

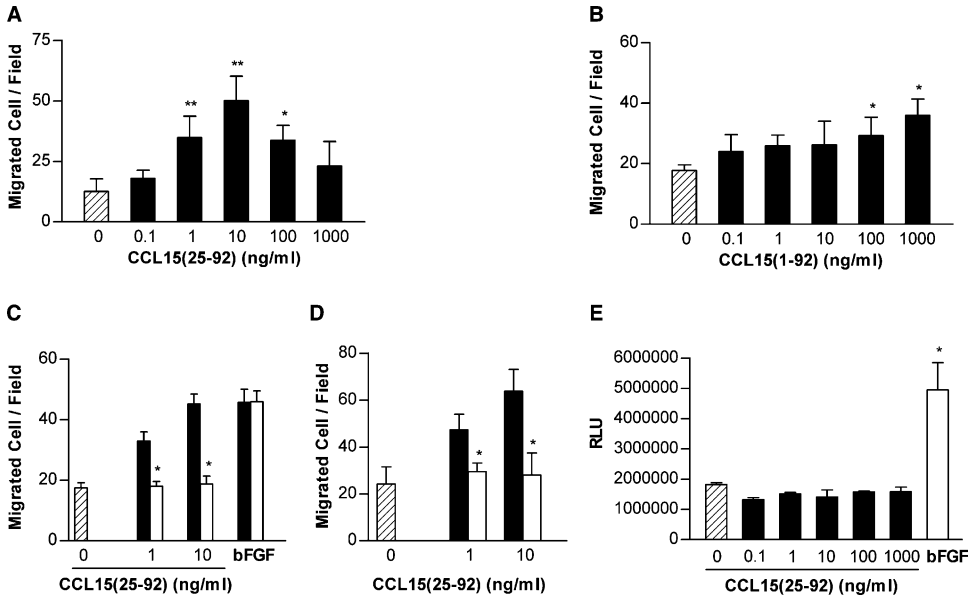

Fig. 1. CCL15 stimulates migration of human endothelial cells. (A) CCL15(25-92) significantly induces migration of HUVEC over migration in the presence of medium alone. *, $P < 0.05$; **, $P < 0.01$ versus medium alone. (B) CCL15(1-92) also induces migration of HUVEC in a dose-dependent manner. *, $P < 0.05$ versus medium alone. (C) Samples immuno-depleted with anti-CCL15 antibody (□) show significantly reduced migratory activity compared to that of isotype-matched mouse IgG treated samples (■), but bFGF (5 ng/ml) absorbed with anti-CCL15 antibody was as active as that absorbed with the control IgG. *, $P < 0.01$ versus mouse IgG treated CCL15(25-92). (D) Heat-treated CCL15(25-92) (□) shows significantly reduced migratory activity compared to that of untreated CCL15(25-92) (■). *, $P < 0.05$ versus untreated CCL15(25-92). (E) HUVEC proliferation at different concentrations of CCL15 was compared with that observed in the presence of medium alone (negative control, ▨) or bFGF (5 ng/ml, □), which were used as positive controls. *, $P < 0.01$ versus basal proliferation in the presence of medium alone. The data are expressed as mean values ± S.D. from quadruplicates and are representative of at least three experiments.

sulted in the activation of chemotactic activity of CCL15 on endothelial cells. Endothelial cell migration stimulated by 10 ng/ml of CCL15(25-92) was similar to the levels observed for the positive control, bFGF at 5 ng/ml (data not shown). The maximal chemotactic responses for CCL15(25-92) and CCL15(1-92) were observed at 10 ng/ml and at 1 μg/ml, respectively. CCL15(25-92) also induced the migration of immortalized human microvascular endothelial cells, HMEC-1 with a response comparable to that observed with HUVECs (data not shown). CCL15(25-92) treated with anti-CCL15 antibody and heat-treated CCL15 was less active for HUVEC (Fig. 1(C) and (D), respectively), indicating that migration of HUVECs was induced specifically by CCL15.

We next determined whether CCL15 was stimulating endothelial cell migration by chemoattraction (directional migration) or chemokinesis (random motility). Checkerboard assays were performed with various concentration of CCL15 in the top chamber, in the bottom chamber, or in both chambers in triplicate samples at least three separate experiments. CCL15 stimulates the chemotactic cell migration of HUVECs (data not shown), indicating that CCL15 induces directional migration of HUVECs, not a random migration.

Because several angiogenic factors induce endothelial cell proliferation, we next investigated whether CCL15 has a mitogenic effect on endothelial cells using the 5-bromo-2′-deoxyuridine assay. The presence of CCL15 at concentrations upto 1 μg/ml did not show any effect on DNA synthesis by human endothelial cells (Fig. 1(E)). These results indicate that CCL15 may not act as a mitogen on endothelial cells.

3.2. CCL15 induces the chemotactic response of human endothelial cells through CCR1 and CCR3

Because several studies suggested that CCL15 displayed its biological activities via binding to CCR1 and CCR3 [4,6] and human endothelial cells expressed both CCR1 and CCR3 [11], we investigated whether CCL15 induced the chemotactic response of endothelial cells through CCR1 and CCR3 which belong to the seven-transmembrane G_i/G_0 protein-coupled receptor (GPCR) family and are sensitive to pertussis toxin (PTX). PTX efficiently blocked CCL15(25-92)-induced endothelial cell migration, but did not affect the migratory activity of either bFGF or HT1080-conditioned medium (Fig. 2(A)). These results suggest that CCL15 exerts its effect through a receptor linked to PTX-sensitive G_i/G_0 family G proteins. As shown in Fig. 2(B), treatment of the cells with anti-CCR1 or anti-CCR3 antibody alone effectively reduced CCL15(25-92)-induced HUVEC migration. CCL15-induced HUVEC migration was completely abolished by the presence of both anti-CCR1 and anti-CCR3 antibodies. These data demonstrate that both CCR1 and CCR3 are indeed functional receptors important for endothelial cell migration to CCL15.

3.3. CCL15 promotes endothelial cell differentiation

We next examined the ability of CCL15 to promote the formation of capillary-like structures by endothelial cells on basement membrane Matrigel. This assay can evaluate endo-

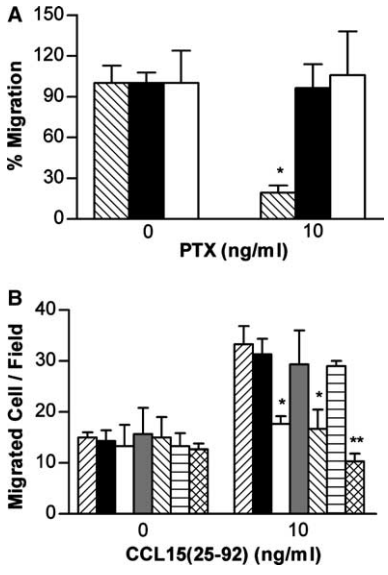

Fig. 2. CCL15 induces the chemotactic response of human endothelial cells through CCR1 and CCR3. (A) The effect of PTX on HUVEC migration induced by CCL15(25-92) (10 ng/ml, ▨), bFGF (5 ng/ml, ■) and HT1080 conditioned medium (□). Results are expressed as the percentage migration of untreated versus PTX-treated endothelial cells. *, $P < 0.05$. (B) The effect of anti-CCR1 (10 μg/ml, □), anti-CCR3 IgG (10 μg/ml, ▨), or mixture of anti-CCR1 and anti-CCR3 IgG (▦) on CCL15(25-92)-induced migration of HUVEC was investigated. Treatment of isotype-matched mouse (10 μg/ml, ■), rat IgG (10 μg/ml, ■), or mixture of mouse and rat IgG (▤) shows no effect on the CCL15-induced HUVEC migration. ▨, no treatment. *, $P < 0.05$; **, $P < 0.01$ versus control IgG treated endothelial cells. The data are mean values ± S.D. from quadruplicates and are representative of at least three experiments.

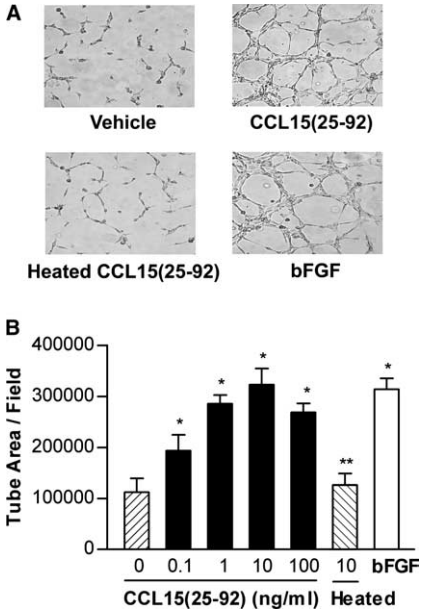

Fig. 3. CCL15 promotes endothelial cell differentiation. HUVECs were seeded on Matrigel and incubated in the presence of different concentrations of CCL15(25-92) or medium alone. bFGF was used as a positive control. After 3 h, cells were photographed using an inverted phase contrast microscope. (A) Representative photographs of vehicle, CCL15(25-92) (10 ng/ml), heated CCL15(25-92) (10 ng/ml), and bFGF (10 ng/ml). (B) Quantification of newly formed tube in the presence of different concentrations of CCL15(25-92) (■) was compared with that observed in the presence of either medium alone (▨), heated CCL15(25-92) (▧) or bFGF (□). *, $P < 0.01$ versus medium alone; **, $P < 0.01$ versus CCL15(25-92) (10 ng/ml). The data are mean values ± S.D. from triplicates and are representative of three independent experiments.

thelial cell migration and differentiation, important steps in neovascularization. CCL15 stimulated tube formation by HUVECs (Fig. 3). The presence of CCL15(25-92) at 0.1, 1, 10, and 100 ng/ml showed 1.7-, 2.5-, 2.9-, and 2.4-fold increases, respectively, in tube area over control medium alone. The activity observed with CCL15(25-92) is comparable with that of the potent angiogenic molecule bFGF (2.9-fold increase), while CCL15(25-92) pretreated with heat was not active for HUVEC (Fig. 3), indicating that tube formation of HUVECs was due to the CCL15. These results suggest that CCL15 may be angiogenic.

3.4. CCL15(25-92) induces angiogenesis

To investigate the ex vivo angiogenic activity of CCL15(25-92), we used a rat aortic ring-sprouting assay. CCL15(25-92) stimulated vessel sprouting above background levels in a dose-dependent manner, while CCL15(25-92) pretreated with heat was not affected vessel sprouting (Fig. 4).

Because CCL15 stimulated migration and differentiation of endothelial cells in vitro and sprouting from explanted aorta rings ex vivo, we next examined whether CCL15(25-92) stimulated angiogenesis in vivo using the chick CAM assay. CCL15(25-92) significantly induced neovascularization from pre-existing blood vessels (Fig. 5). In association with angiogenesis, CCL15(25-92) induced an inflammatory response as indicated by an area with increased opacity on the disks (Fig. 5(A)). The presence of 0.1, 1, 10, and 100 ng of CCL15(25-92) per egg caused 1.7-, 2.1-, 2.6-, and 2.0-fold increases, respectively, in the number of newly formed blood vessels compared with that of PBS alone or with that of heat-treated CCL15 (Fig. 5(B)). The angiogenic activity observed with CCL15(25-92) was comparable to that of the angiogenic molecule bFGF. These results indicate that CCL15(25-92) is a potent angiogenic factor in vivo.

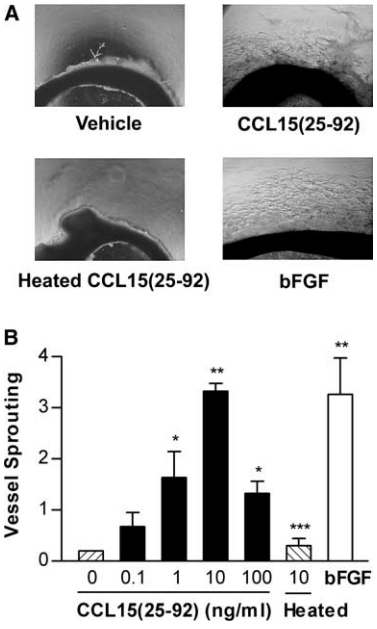

Fig. 4. CCL15 promotes sprouting from rat aortic rings on Matrigel. Rat aortas were seeded on Matrigel and incubated in the presence of different concentrations of CCL15(25-92) or medium alone. bFGF was used as a positive control. After 6 days, aortas were fixed and photographed. (A) Representative photographs of vehicle, CCL15(25-92) (10 ng/ml), heated CCL15(25-92) (10 ng/ml), and bFGF (100 ng/ml). (B) Cell sprouting in the presence of different concentrations of CCL15(25-92) (■) was compared with that observed in the presence of either medium alone (▨), heated CCL15(25-92) (▧) or 100 ng/ml bFGF (□). * $P < 0.05$; $P < 0.01$ versus medium alone; ***, $P < 0.01$ versus CCL15(25-92) (10 ng/ml). The data are mean values ± S.D. ($n = 5$) and are representative of two experiments.

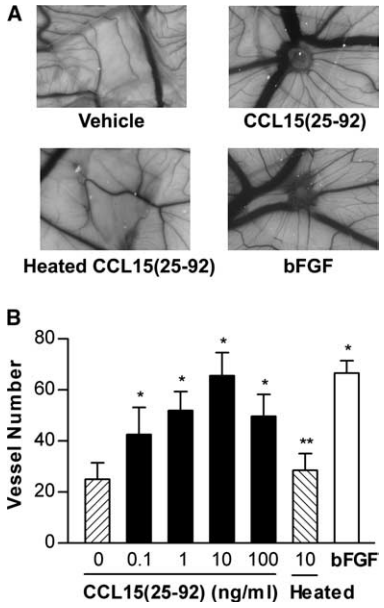

Fig. 5. CCL15 induces neovascularization in the chick CAM assay. CCL15(25-92) or bFGF was loaded on the CAMs of day 10 chick embryos. After 70 ± 4 h incubation, a fat emulsion was injected under the CAMs for better visualization of the vessels. Disks and surrounding CAMs were photographed. (A) Representative photographs of vehicle, CCL15(25-92) (10 ng/egg), heated CCL15(25-92) (10 ng/egg), and bFGF (100 ng/egg). (B) Quantification of newly formed blood vessels. *, $P < 0.01$ versus vehicle **, $P < 0.01$ versus CCL15(25-92) (10 ng/ml). Twelve to fifteen eggs were used for each data point and mean values ± S.D. are shown.

4. Discussion

In this report, we demonstrate that CCL15 has in vitro and in vivo angiogenic activity. CCL15(25-92) induced the migration of human endothelial cells, such as HUVEC and HMEC-1 that are of two different tissue origins. Although CCL15(1-92) induced endothelial cell migration, its chemotactic activity is less potent than that of CCL15(25-92). Checkerboard assays indicate that CCL15(25-92) induces endothelial cell migration in a chemotactic, not a chemokinetic manner. The angiogenic effect of CCL15 was clearly evident in both the in vitro Matrigel tube formation assay and the in vivo CAM assay. Angiogenic responses in the chick systems by CCL15 indicate that chick CCR1 and/or CCR3 homologous to human receptors may exist. CCL15 can also induce endothelial cell sprouting from rat aortic rings in the absence of inflammatory infiltrates, indicating its direct effect in promoting angiogenesis. The fact that CCL15 does not act as an endothelial cell mitogen suggests that the chemotactic, not the mitogenic, effect of CCL15 may be responsible for its angiogenic activity. Recently, it has

been reported that NCC-4, also known as CCL16, has in vitro and in vivo angiogenic activity by activating CCR1 [12]. Although we could not completely exclude the involvement of the other receptors, these results suggest that the angiogenic activity of CCL15 may be mediated via CCR1 and/or CCR3 present on endothelial cells.

Several lines of evidence suggest that a large number of CXC and CC chemokines play an important role in the regulation of endothelial cell function and neovascularization, including proliferation, migration, and differentiation during angiogenesis, and re-endothelialization after injury [7–11,18,19]. In recent years, the involvement of leukocyte infiltration in both physiologic and pathologic angiogenesis has attracted much attention [8]. CCL15, a classical inflammatory chemokine, is involved in the immune response via attraction of almost all types of inflammatory cells. It has been reported that CCL15 could be a novel mediator of atherosclerosis, indicating that CCL15 may be involved in vascular disease [20].

Several forms of CCL15, including CCL15(1-92) and CCL15(25-92), have been studied to characterize the biological activity of this chemokine [4,6,21,22]. We showed that truncated CCL15(25-92) had more potent migratory activity with endothelial cells than CCL15(1-92). Our observations are consistent with reports showing that truncation of NH_2-terminal amino acids significantly increases the biochemical and biological activities of CCL15 [6]. Ckβ8(25-99) is also more potent in its agonistic activities than Ckβ8(1-99) [23]. Several forms of circulating HCC-1, including intact HCC-1(1-74) and HCC-1(9-74), were isolated from plasma [22,24]. In vitro experiments [24,25] showed that proteolysis of full-length HCC-1(1-74) by either trypsin or serine proteases present in conditioned media from several tumor cell lines generated the active HCC-1(9-74). The biological activity of HCC-1(9-74) is significantly higher than that of HCC-1(1-74), suggesting that proteolytic processing may transform a virtually inactive propeptide into a biologically active molecule. Amino-terminal truncation or elongation of chemokines frequently results in either significant enhancement or loss of activity, the appearance of antagonistic activity, or a change in chemokine receptor specificity [22]. Several circulating forms of CCL15 exist in the blood and act on hematopoietic progenitor cells [26]. To delineate the biological significance of truncated forms of CCL15, it may be most important to determine whether CCL15 can be cleaved in vivo by proteolytic enzymes such as plasminogen activators of urokinase type or metalloproteinases which are overexpressed in cancer. In addition, isolation of the naturally occurring CCL15 needs to be further elucidated because amino terminus of natural CCL15 has not yet been determined.

Acknowledgements: This work was supported by an Immunomodulation Research Center grant (to J.K.) from KOSEF and a Vascular System Research Center grant (to Y.S.G.). J.H., K.N.S. and K.H.L. were supported by BK21 program of Ministry of Education.

References

[1] Zlotnik, A. and Yoshie, O. (2000) Immunity 12, 121–127.
[2] Murdoch, C. and Finn, A. (2000) J. Vasc. Res. 37, 1–7.
[3] Murphy, P.M., Baggiolini, M., Charo, I.F., Hebert, C.A., Horuk, R., Matsushima, K., Miller, L.H., Oppenheim, J.J. and Power, C.A. (2000) Pharmacol. Rev. 52, 145–176.
[4] Youn, B.S., Zhang, S.M., Lee, E.K., Park, D.H., Broxmeyer, H.E., Murphy, P.M., Locati, M., Pease, J.E., Kim, K.K., Antol, K. and Kwon, B.S. (1997) J. Immunol. 159, 5201–5205.
[5] Pardigol, A., Forssmann, U., Zucht, H.D., Loetscher, P., Schulz-Knappe, P., Baggiolini, M., Forssmann, W.G. and Magert, H.J. (1998) Proc. Natl. Acad. Sci. USA 95, 6308–6313.
[6] Lee, J.K., Lee, E.H., Yun, Y.P., Kim, K., Kwack, K., Na, D.S., Kwon, B.S. and Lee, C.K. (2002) J. Biol. Chem. 277, 14757–14763.
[7] Strieter, R.M., Polverini, P.J., Arenberg, D.A. and Kunkel, S.L. (1995) Shock 4, 155–160.
[8] Bernardini, G., Ribatti, D., Spinetti, G., Morbidelli, L., Ziche, M., Santoni, A., Capogrossi, M.C. and Napolitano, M. (2003) J. Immunol. Methods 273, 83–101.
[9] Bernardini, G., Spinetti, G., Ribatti, D., Camarda, G., Morbidelli, L., Ziche, M., Santoni, A., Capogrossi, M.C. and Napolitano, M. (2000) Blood 96, 4039–4045.
[10] Salcedo, R., Ponce, M.L., Young, H.A., Wasserman, K., Ward, J.M., Kleinman, H.K., Oppenheim, J.J. and Murphy, W.J. (2000) Blood 96, 34–40.
[11] Salcedo, R., Young, H.A., Ponce, M.L., Ward, J.M., Kleinman, H.K., Murphy, W.J. and Oppenheim, J.J. (2001) J. Immunol. 166, 7571–7578.
[12] Strasly, M., Doronzo, G., Capello, P., Valdembri, D., Arese, M., Mitola, S., Moore, P., Alessandri, G., Giovarelli, M. and Bussolino, F. (2004) Blood 103, 40–49.
[13] Gho, Y.S., Kim, P.N., Li, H.C., Elkin, M. and Kleinman, H.K. (2001) Cancer Res. 61, 4253–4257.
[14] Kim, Y.M., Hwang, S., Kim, Y.M., Pyun, B.J., Kim, T.Y., Lee, S.T., Gho, Y.S. and Kwon, Y.G. (2002) J. Biol. Chem. 277, 27872–27879.
[15] Ades, E.W., Candal, F.J., Swerlick, R.A., George, V.G., Summers, S., Bosse, D.C. and Lawley, T.J. (1992) J. Investig. Dermatol. 99, 683–690.
[16] Gho, Y.S., Kleinman, H.K. and Sosne, G. (1999) Cancer Res. 59, 5128–5132.
[17] Nicosia, R.F. and Ottinetti, A. (1990) In Vitro Cell Dev. Biol. 26, 119–128.
[18] Salcedo, R., Wasserman, K., Young, H.A., Grimm, M.C., Howard, O.M., Anver, M.R., Kleinman, H.K., Murphy, W.J. and Oppenheim, J.J. (1999) Am. J. Pathol. 154, 1125–1135.
[19] Soto, H., Wang, W., Strieter, R.M., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Hedrick, J. and Zlotnik, A. (1998) Proc. Natl. Acad. Sci. USA 95, 8205–8210.
[20] Lee, W.H., Kim, S.H., Jeong, E.M., Choi, Y.H., Kim, D.I., Lee, B.B., Cho, Y.S., Kwon, B.S. and Park, J.E. (2002) Atherosclerosis 161, 255–260.
[21] Zhang, S., Youn, B.S., Gao, J.L., Murphy, P.M. and Kwon, B.S. (1999) J. Immunol. 162, 4938–4942.
[22] Forssmann, U., Magert, H.J., Adermann, K., Escher, S.E. and Forssmann, W.G. (2001) J. Leukoc. Biol. 70, 357–366.
[23] Macphee, C.H., Appelbaum, E.R., Johanson, K., Moores, K.E., Imburgia, C.S., Fornwald, J., Berkhout, T., Brawner, M., Groot, P.H., O'Donnell, K., O'Shannessy, D., Scott, G. and White, J.R. (1998) J. Immunol. 161, 6273–6279.
[24] Detheux, M., Standker, L., Vakili, J., Munch, J., Forssmann, U., Adermann, K., Pohlmann, S., Vassart, G., Kirchhoff, F., Parmentier, M. and Forssmann, W.G. (2000) J. Exp. Med. 192, 1501–1508.
[25] Vakili, J., Standker, L., Detheux, M., Vassart, G., Forssmann, W.G. and Parmentier, M. (2001) J. Immunol. 167, 3406–3413.
[26] Richter, R., Forssmann, W.G. and Henschler, R., (1999). HCC-2 circulates in blood and acts on hematopoietic progenitor cells. In Cambridge Healthtech Institute's Congress on Chemokine and Chemokine Receptors: Disease Targets for Therapeutic Development. McLean, VA.