CXCL17, an orphan chemokine, acts as a novel angiogenic and anti-inflammatory factor

Wei-Yu Lee, Chun-Jen Wang, Ting-Yu Lin, Chih-Lun Hsiao, and Ching-Wei Luo

Department of Life Sciences and Institute of Genome Sciences, National Yang-Ming University, Taipei, Taiwan

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CHEMOKINES ARE A GROUP OF CHEMOTACTIC CYTOKINES THAT GOVERN the recruitment of various leukocytes to inflammation sites. In addition to promoting the trafficking of leukocytes, recent studies indicate that chemokines also play important roles in many aspects, including tumor growth, angiogenesis, organ sclerosis, and autoimmunity (21). Based on sequence homology and the numbers of amino acids between the first two cysteines, chemokines can be subdivided into C, CC, CXC, and CX3C subgroups (15). Chemokines bind to G protein-coupled receptors (GPCRs) on different target cells to initiate intracellular signaling cascades. Although the physiological roles and cognate receptors of most chemokines have been well characterized (34), the endogenous function as well as the receptor nature of CXCL17, the most recent chemokine ligand to be reported (17), are still unclear.

CXCL17 was originally identified in 2006 by threading methods as having a protein structure similar to the CXC chemokine family (17). Although its physiological functions have not been clearly explored, two studies have proposed that CXCL17 might act as a chemokine that accelerates tumor progression (13, 30). Overexpression of CXCL17 in NIH3T3 or hepatocellular carcinoma cells significantly promotes their tumor formation capability in nude mice. Microarray analyses have indicated that the CXCL17 transcript is elevated dramatically in breast and colon tumors, where its level is tightly coregulated in concert with vascular endothelial growth factor (VEGF) expression. Because of the central role of VEGF in the growth and differentiation of vasculature, CXCL17 has also been suggested as a critical player in tumor angiogenesis (30). Interestingly, contrary to the previous results, CXCL17 is found to act as an antitumor factor during the pancreatic carcinogenesis. CXCL17 in the intraductal papillary mucinous neoplasm induces the migration and accumulation of dendritic cells at the tumor site; this promotes the susceptibility of the tumor cells to cytotoxic T cell-mediated cytolysis (8).

Despite its regulatory roles during tumorigenesis, CXCL17 has been found to be expressed constitutively in the lung and trachea and has been speculated to play a potential role in recruiting blood monocytes or dendritic cells to these tissues (17). A recent study even shows that the CXCL17 peptide exhibits an antimicrobial activity potentially through disrupting bacterial membrane (2). Thus, these studies strongly suggest that CXCL17 is a homeostatic, mucosa-associated chemokine involved in innate immunity as well as sterility of the respiratory tracts. Although its expression has been detected in the adult stomach (17), no further distribution as well as physiological function for CXCL17 in this organ has been elucidated. In addition, distinct from other CXC chemokines with four conserved cysteines (1, 34), CXCL17 contains six cysteine residues in its primary sequence. In this study, we first found that CXCL17 is highly abundant in the gastric mucosa, where its propeptide undergoes endoproteolytic processes during maturation. We further demonstrated that the mature CXCL17 is critical to leukocyte recruitment, angiogenesis, and anti-inflammation.

MATERIALS AND METHODS

Animals and ethics. Sprague-Dawley rats (5–8 wk old) were obtained from the laboratory animal center (National Yang-Ming University, Taipei, Taiwan). For the alcohol-drinking experiments, male rats were raised with standard rat chow and 15% alcohol (vol/vol) or water for 3 days before their stomachs were harvested. For fasting and refeeding treatments, male rats were fasted for 48 h before being fed standard rat chow. The stomachs were harvested at the indicated time intervals for gene quantification.

Animal care and treatments were approved by the Institutional Animal Care and Use Committee of the National Yang-Ming University (permit no. 981205).

Cell lines and cultures. Human embryonic kidney-293T, human THP-1, and human KATO III cells were purchased from ATCC. The J774 murine macrophage-like cells were purchased from the Bioresource Collection and Research Center (Hsinchu, Taiwan). Human HMEC-1 cells were kindly provided by Dr. Hsei-Wei Wang (National Yang-Ming University) (31). For cell cultures, 293T cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen). THP-1 cells were cultured in RPMI medium 1640 (Invitrogen) supplemented...
with 10% fetal bovine serum, 2.5 g/l glucose (Sigma-Aldrich), 1.0 mM sodium pyruvate (Sigma-Aldrich), and 0.05 mM 2-mercaptoethanol (Sigma-Aldrich). KATO III cells were cultured in RPMI medium supplemented with 20% fetal bovine serum. J774 cells were maintained in DMEM-high glucose (Thermo Scientific) supplemented with 10% fetal bovine serum. HMEC-1 cells were cultured in endothelial cell media MV (PromoCell).

Plasmid construction and protein expression. For amplification of the full-length cDNA of human CXCL17, primers 5'-ATGAACTTTGACTTTTGTAT CTTTCCACACTTCCTATCCGTCTC and 5'-CAGAGGCCGAGCAAGTCCGTT were used. For amplification of the full-length cDNA of rat CXCL17, primers 5'-ATGAACTTTGACTTTTGTAT CTTTCCACACTTCCTATCCGTCTC and 5'-CAAAGGCAGAGCAAAGCTTC were used. To introduce mutated residues into rat CXCL17, PCR-based mutagenesis was performed, using overlapping primers containing mutated sequences. For mammalian cell expression of rat and human CXCL17, the cDNA was cloned into the pcDNA 3.1 vector (Invitrogen). The constructs were designed by adding a FLAG epitope tag at the COOH terminus of human CXCL17, rat CXCL17, and various CXCL17 mutants to facilitate recombinant protein detection. The constructed plasmids were purified and transfected into 293T cells using LipofectAMINE 2000 (Invitrogen). Conditioned media were concentrated and then subjected to Western blotting.

For generation and purification of recombinant human CXCL17 protein from Escherichia coli, the cDNA encoding human CXCL17 propeptide (6-Cys) from Leu24 to Leu119 or mature peptide (4-Cys) from Phe64 to Leu119 was subcloned into the pET21a vector (Novagen). The expression of the recombinant proteins was induced in Escherichia coli strain BL21 (DE3; Novagen) with 1 mM isobutyl-1–1-methylxanthine, or 10 μM lipopolysaccharide (LPS), 300 nM gastrin, 100 nM insulin, 250 μM 3-isobutyl-1–1-methylxanthine, or 10 μM forskolin for 6 h. For LPS, hormone, or chemical washing followed by resuspension in DMEM culture medium. For primary gastric cell culture, collected mucosal layers were further incubated in DMEM buffer with 0.1% collagenase at 37°C for 70 min. After careful scraping, the glandular stomachs harvested from the male rats were washed 2:1:4. One day after transfection, the cells were resuspended in serum-free conditions for 5 h before being treated mouse IL-6 reverse, ATTCCAGATTTCCGCCAGAG; mouse TNFα forward, GCTTGTCTTACATCCT; mouse TNFα reverse, CACTTGTGGTTGCTTAGCA; mouse inducible nitric oxide synthase (iNOS) forward, AGCCAAAG CCTCACTTAC; mouse iNOS reverse, CAATCCTCTATCCGGTTCTC; human VEGF-A forward, ATGCAGAATCATGTTTGGGA; human cyclooxygenase-2 (COX-2) forward, CCCCCCTGCGACCTAC; human COX-2 reverse, GCAACCTT GCCAACATTT; human β-actin forward, CTCCGTGTTGATCGGCCTC; human β-actin reverse, CTGTTGTGATCCACACTCTG.

Molecular cloning and immunohistochemical analyses. The affinity-purified sheep polyclonal antibody against mouse CXCL17 was purchased from R & D Systems. The mouse anti-FLAG monoclonal antibody was from Sigma-Aldrich. The antibodies specific for phosphorylated ERK1/2, p38, or JNK were obtained from Millipore, whereas the antibodies against total MAPKs were purchased from Santa Cruz Biotechnology. For immunoblotting to assess the CXCL17 expression, the stomachs from male rats were separated into the forestomach and granular stomach before homogenization in PBS, whereas the conditioned media from transfected 293T cells were collected and concentrated. The protein amounts in the supernatant were quantified by Micro BCA protein assay kit (Pierce Biotechnology), and ~25 μg of protein was subjected to electrophoresis. Western blotting was performed using the affinity-purified sheep polyclonal antibody against mouse CXCL17 (R & D Systems) or the mouse monoclonal antibody against the FLAG tag (Sigma-Aldrich). For assessment of the kinase phosphorylation levels, J774 cells were preincubated in serum-free conditions for 5 h before being treated with 30 nM CXCL17 for different time intervals. After treatment, the cell lysates were subjected to Western blotting using antibodies specific for phosphorylated (Millipore) or total ERK1/2, p38, and JNK (Santa Cruz Biotechnology).

For immunohistochemical staining, the stomachs from male rats were fixed using Bouin’s fixative for 8 h before paraffin embedding. Blocks were sectioned at 8-μm thickness. Immunohistochemical analyses were performed using the affinity-purified sheep polyclonal antibody against mouse CXCL17 (R & D Systems) or the normal sheep IgG as the primary antibody and the horseradish peroxidase-conjugated rabbit anti-sheep antibody as the secondary antibody. Staining was visualized using the Nova Red kit (DakoCytomation), following the manufacturer’s instructions.

Protein purification for Edman degradation. The lungs from ~15 rats were harvested and homogenized in PBS supplemented with protease inhibitor cocktails (Roche). After filtration, the homogenate was passed through protein A-Sepharose to preabsorb endogenous immunoglobulins before immunoprecipitation was performed. For enriching the CXCL17 protein fragment, the tissue homogenate was supplemented with 10% FBS and 50 ng/ml GM-CSF for 4 days. on day 4, nonadherent cells were collected and cultured for an additional 3 days in the presence of fresh GM-CSF. The marrow-derived macrophages were then collected and used in subsequent experiments.

cDNA isolation and quantification. For gene quantification, the treated cells or target tissues were harvested, and their total RNAs were extracted and reverse transcribed as described previously (22). For quantitative TaqMan real-time PCR, a QuantiTect Probe PCR kit (Qiagen Sciences) was used. The primer pairs and fluorescent probe for rat CXCL17 are as follows: rat CXCL17 forward, AAGCAAGCATGACCAACCAA; rat CXCL17 reverse, GTGAAGCTTTGATGGTACAGT; rat CXCL17 probe, CCTCCGACACTGTCGAG; rat β-actin forward, CTGTTGTGATGATTGCTC; rat β-actin reverse, CTGTTGTGATCCACACTCTG.

For the quantitative SYBR Green real-time PCR, a Maxima SYBR Green quantitative PCR Master Mix kit (Fermentas) was used, and the primer pairs for each gene were listed as follows: mouse c-fos forward, TGGCGAAAATCCTTGTTGT; mouse c-fos reverse, GOGGAGC- CTTCTTACTTACCAT; mouse IL-6 forward, TTCCCATCCTGGTGC; mouse IL-6 reverse, ATTCCACGATTTCCGCCAGAG; mouse c-fos forward, CAGAGCTGCTATCCGTCTC; mouse TNFα forward, GCTTGTCTTACATCCT; mouse TNFα reverse, CACTTGTGGTTGCTTAGCA; mouse inducible nitric oxide synthase (iNOS) forward, AGCCAAAG CCTCACTTAC; mouse iNOS reverse, CAATCCTCTATCCGGTTCTC; human VEGF-A forward, AAGGAGGAGGCGAAATCTC; human VEGF-A reverse, ATGCAGAATCATGTTTGGGA; human cyclooxygenase-2 (COX-2) forward, CCCCCCTGCGACCTAC; human COX-2 reverse, GCAACCTT GCCAACATTT; human β-actin forward, CTCCGTGTTGATCGGCCTC; human β-actin reverse, CTGTTGTGATCCACACTCTG.

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incubated with the anti-CXCL17 antibody and Protein G Mag Sepharose Xtra (GE Healthcare) at 4°C overnight. The immunoprecipitated samples were analyzed by running 15% SDS-PAGE, followed by blotting to a 0.45-μm polyvinylidene difluoride membrane (Millipore). After being stained with freshly prepared Coomassie brilliant blue R-250, the band of interest was excised and subjected to Edman degradation (Misson Biotech).

Transwell assay. The chemoattractant ability of CXCL17 was assessed using an 8-μm-pore size Transwell (Costar). Briefly, 3 × 10⁶ THP-1 cells or 1 × 10⁶ J774 cells were loaded into the upper chamber of Transwell and allowed to transmigrate for 4 h at 37°C in the presence or absence of CXCL17. Transmigrated cells on the bottom side of the filter were fixed by 4% paraformaldehyde and stained with Giemsa solution (Gibco).

Quantification of nitric oxide production. Nitric oxide levels were determined by measuring the amount of nitrite in cell culture supernatants stained with Griess reagent (Promega) according to the manufacturer’s instructions. Optical density was converted into micromolarity using a curve generated from nitrite standards.

Data analysis. All experimental data reported in the figures are presented as the mean ± SD of at least three independent experiments. Statistical significance was determined by ANOVA. Significance was accepted at P < 0.05 unless otherwise noted.

RESULTS

CXCL17 is highly expressed in the stomach in both a constitutive and inducible manner. CXCL17 is a newly discovered and orphan chemokine of which the physiological function has not yet been characterized. Using real-time PCR to quantify its mRNA levels in 22 different rat tissues, the CXCL17 message was found mainly in the stomach, duodenum, lung, and salivary gland, with the stomach showing the highest expression level (Fig. 1A). Since rodent stomachs consist of a forestomach, which histologically resembles the esophagus, and a glandular stomach, which corresponds to the ordinary human stomach, the CXCL17 mRNA level in these two portions was investigated separately. We found that the CXCL17 level in the glandular stomach is 14.5-fold higher than that in the forestomach (Fig. 1A, right). The stomach is the digestive organ that is constantly in contact with stimuli such as microbiota, drugs, and food antigens. Therefore, such stimulatory effects on the gastric CXCL17 expression were examined further. We found that treatment of primary gastric cells isolated from rat glandular stomachs with bacteria-derived LPS (Fig. 1B) significantly increased CXCL17 expression in a dose-dependent manner. In addition, we also found that CXCL17 transcript was increased when the gastric cells were treated with alcohol (Fig. 1C). Similar results were observed in rats that freely drank 15% alcohol (Fig. 1D). The glandular stomach is responsible for hormone secretion as well as induction. Therefore, we tested such effects on the CXCL17 expression using primary rat gastric cells. In contrast to the gastrin or insulin stimulation, which had a negligible effect, forskolin treatment effectively elevated the CXCL17 transcript level. This elevation was further increased by adding the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine, indicating that CXCL17 transcription is under the control of cAMP signaling (Fig. 1E). In addition, we also found that the CXCL17 level in the stomach does not change during the fasting and refeeding processes (data not shown).

CXCL17 protein secreted from the gastric mucosa is likely to undergo endoproteolytic cleavage. To further characterize the CXCL17 distribution in the stomach and its protein nature, immunohistochemical staining and Western blotting were performed. Our staining data indicated that strong CXCL17 immunoreactivity was observed in the mucosal layer of rat glandular stomach and in the squamous epithelium of mucosal layer of forestomach (Fig. 2A). Western analysis using the anti-CXCL17 antibody also showed a specific band at ~8 kDa in the supernatant of tissue homogenates extracted from both rat stomach portions. The immunointensity indicated that the CXCL17 protein level in the glandular stomach is much higher...
than that in the forestomach (Fig. 2B, left), consistent with the transcript level (Fig. 1A, right). In addition, a single band with similar molecular weight was also detected in the conditioned media secreted from human KATO III gastric carcinoma cells transfected with CXCL17 (Fig. 2B, middle). However, in addition to a band with smaller molecular weight comparable with that in the tissue homogenates, an additional band at ~20 kDa was also detected in conditioned media collected from 293T cells overexpressing either the COOH-terminal FLAG-tagged rat or human CXCL17 (Fig. 2B, right). This strongly suggests the endogenous CXCL17 protein is likely to undergo posttranslational cleavage by endoproteolytic enzymes such as proprotein convertases to allow peptide maturation. Of interest, on the basis of a sequence search, two convertase cleavage sites containing paired basic residues (3) between Cys2 and Cys3 were found in human CXCL17 propeptide, and these are highly conserved in other mammals due to the high-sequence homology (Fig. 2C). Therefore, to verify the correct cleavage site, we designed multiple alanine replacements at these sites of CXCL17 and found that the A61A62A63 mutant completely eliminated the secretion of mature CXCL17 with a smaller molecular weight in the conditioned medium, whereas the protein expression pattern of A72A73 mutant is similar to that of wild-type CXCL17 (Fig. 2D). Alternatively, the immunoprecipitated sample from rat lung homogenates was resolved by SDS-PAGE, and the band corresponding to the endogenous CXCL17 fragment was excised and subjected to NH2-terminal sequencing (Fig. 2E). The obtained sequence, XAVL (X represents undetermined residue), matched the three residues from position 66 to 68 after the first predicted cleavage site of rat CXCL17. Taken together, these results showed that the CXCL17 propeptide, which contains six cysteines, is likely to be cleaved posttranslationally to produce the mature CXCL17 peptide with only four cysteines, which fits the canonical CXC chemokine scaffold. The bioactivities of the six cysteine-containing human CXCL17 propeptides, from Leu24 to Leu119 and designated 6-Cys CXCL17, and the four cysteine-containing mature human CXCL17 propeptides, from Phe64 to Leu119 and designated 4-Cys CXCL17, were then generated for comparison in the following chemoattractant experiments.

CXCL17 induced the expression of proangiogenic factors in treated THP-1 monocytes. Although the full-length CXCL17 propeptide has been proven previously to induce migration of isolated human peripheral blood monocytes (17), its physiological effect on monocytes has not yet been well explored. For convenient in vitro tests, human THP-1, a widely used mono-

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cytoid cell line derived from a patient with monocytic leukemia (23), was used for the following assays. Using Transwell migration assay, we showed that both purified CXCL17 proteins exhibit the ability to induce migration of THP-1 monocytes, with the 4-Cys mature peptide showing the chemoattractant potency to be approximately twofold higher than that of the 6-Cys propeptide (Fig. 3A). In addition to having higher chemoattractant potency, the ability of 4-Cys CXCL17 in the induction of proangiogenic factors in treated THP-1 monocytes was also evaluated. Our results indicated that treatment with 4-Cys mature CXCL17 increased the VEGF-A mRNA level of THP-1 cells dose dependently (Fig. 3B), whereas this phenomenon was completely abolished by heat inactivation of CXCL17 (data not shown). This further supported the hypothesis that the observed cytokine induction was not due to nonspecific heat-stable endotoxins coisolated during CXCL17 purification.

An increase in VEGF-A protein production was also observed in CXCL17-treated THP-1 conditioned medium (Fig. 3C). Conditioned media collected from THP-1 treated with or without 4-Cys CXCL17 were then assessed to determine their potencies with respect to augmenting the endothelial level of COX-2, which is known to be a key mediator involved in the protection and healing/repairing processes of diverse tissues, including the stomach (16, 28). When HMEC-1 microvascular endothelial cells were treated with 4-Cys CXCL17 alone, there was no direct effect on the level of COX-2 mRNA (Fig. 3D). However, compared with the control THP-1 conditioned medium without CXCL17 treatment, treatment with the medium collected from 4-Cys CXCL17-treated THP-1 cells increased the transcript level of COX-2 in HMEC-1 cells significantly, suggesting that CXCL17 is capable of accelerating the protein secretion of proangiogenic factors from activated THP-1 monocytes.

**CXCL17 induced MAPK activation in treated macrophages.** The protein scaffold and sequence of CXCL17 across mammalian species are highly conserved (Fig. 2C). In addition to attracting monocytes (Fig. 3) (17), we further found that recombinant CXCL17 can induce the migration of mouse macrophages. Transwell migration assays of J774 macrophages indicated that the chemoattractant potency of the 4-Cys mature CXCL17 was twofold higher than that of the 6-Cys CXCL17 propeptide (Fig. 4A). It has been suggested that the receptor for CXCL17 is likely to be a GPCR (17). Therefore, the potential downstream early responding gene and MAPK signaling underlying GPCR activation were characterized. As shown in Fig. 4B, the 4-Cys mature CXCL17 dose-dependently induced the nuclear c-fos expression in J774 macrophages. Western blotting against MAPKs indicated that treatment with the mature 4-Cys CXCL17 led to the time-dependent stimulation of ERK1/2 and P38 phosphorylation, but not JNK phosphorylation, in J774 macrophages (Fig. 4C). These results suggest that macrophages contain the endogenous receptor(s), which is likely to be GPCR(s) (17), for CXCL17.

**CXCL17 suppressed LPS-induced proinflammatory cytokine and nitric oxide production in either J774 or primary macrophages.** Mature 4-Cys CXCL17 contains a canonical CXC chemokine scaffold and is capable of activating the MAPK signaling in treated J774 macrophages. Surprisingly, we did not observe any positive effect on the proinflammatory cytokine augmentation when J774 macrophages were treated...
with the mature 4-Cys CXCL17 peptide alone or any attenuation or additive effect on LPS induction of proinflammatory cytokine production when macrophages were cotreated with LPS and the mature 4-Cys CXCL17 peptide for 6 h (Fig. 5, A–C). Since CXCL17 is constitutively expressed at a high level in the stomach (Figs. 1 and 2), we speculated that CXCL17 would act as an anti-inflammatory rather than an inflammatory factor. Indeed, following the pretreatment with 4-Cys CXCL17 overnight, CXCL17 priming did significantly attenuate the LPS-induced expression of proinflammatory genes such as IL-6 (Fig. 5D), TNFα (Fig. 5E), and iNOS (Fig. 5F) as well as nitric oxide production (Fig. 5G). These results suggest that CXCL17 acts as a novel anti-inflammatory chemokine that causes the hyporesponse of macrophages when these cells are stimulated by, for example, LPS.

To confirm such anti-inflammatory effects of CXCL17, primary macrophage cells derived from mouse bone marrow were used for similar treatments (6). In marrow-derived macrophages, when primed with CXCL17, the LPS-induced expression levels of IL-6 (Fig. 6A) and TNFα (Fig. 6B) were significantly dampened, whereas the iNOS level also showed a tendency to decrease (Fig. 6C).

**DISCUSSION**

To our knowledge, this is the first report that characterizes the protein nature, gastric expression, and novel angiogenic and anti-inflammatory functions of CXCL17. Unlike other CXC chemokines with four conserved cysteines, CXCL17 contains six cysteine residues in its primary sequence. Pisabarro et al. (17) had predicted that the disulfide bridges of CXCL17 are potentially formed between C1 and C3 and C2 and C4, which would lead to a nonnative CXC chemokine fold with two appending free cysteines, C5 and C6, at the COOH terminus. Contrary to this prediction, our results in Fig. 2 concluded that CXCL17 is likely to undergo an endoproteolytic process to form a four-cysteine-containing mature peptide with a canonical CXC chemokine scaffold. Such a posttranslational proteolytic process is relatively common in peptide hormone production (19). Thus, the disulfide bond arrangement of mature CXCL17 is predicted as C3–C5 and C4–C6 linkages based on the monomeric structures of other characterized chemokines (4, 5). However, currently, we are not able to perform the disulfide mapping experiment by proteolytic digestion and tandem mass spectrometry because the linkage between C3 and C4 of the Cys-Pro-Cys sequence cannot be cleaved by any protease, and this then results in inseparability of the digested product in mass analysis. Future experiments using NMR or crystallization will help to demonstrate our prediction.

Up to the present, CXCL17 is still an orphan chemokine. Intriguingly, a previous study reports that pertussis toxin effectively inhibits the CXCL17-driven migration of dendritic cells (17), suggesting that the receptor for CXCL17, like other CXC chemokines, is likely to be a G protein-coupled GPCR. There are still six orphan GPCRs potentially sorted as chemokine receptor candidates (9). Among them, we finished testing ADMR, CCRL2, CMKRL2, duffy antigen, and RDC1 using our established universal GPCR reporting system (27). However, no evidence that any of these receptors can be activated by CXCL17 was shown (data not shown). Because several chemokine receptors are known to bind to multiple chemokines and vice versa (34), it might be still worthwhile to screen all known CXC chemokine receptors for unveiling the receptor nature of CXCL17.

Although both the 6-Cys propeptide and 4-Cys mature peptide of CXCL17 can induce the migration of monocytes and macrophages, our data indicated that the 4-Cys mature peptide may exhibit much higher chemoattractant potency (Figs. 3A and 4A). This suggests that the appending NH2-terminal se-
sequence of CXCL17 may interfere with the receptor-binding process and/or affinity. Of interest, the similar maturation as well as regulatory mechanism has also been proposed in other chemokines. For example, a proteolytic processing at the NH2 terminus by serine proteases is needed to convert the propeptide of CCL14a into a high-affinity agonist of CCR1 and CCR5 (7). Likewise, the proform of CXCL7, also named connective tissue-activating peptide III, shows only negligible neutrophil chemotaxis unless the additional residues at the NH2 terminus are cleaved (26). A crystal structural analysis suggests that these NH2-terminal-appending residues may fold back to mask the critical receptor binding motif and thereby stabilize the inactive state of CXCL7 during production (11). In addition, based on the structure of the NH2-terminal extension of CXCL7, it has also been proposed that this appending region may contribute to a helix-helix interaction for stabilizing the oligomerization of CXCL7, which may contribute to other biological functions. Indeed, measurement of the molecular mass by gel permeation chromatography has indicated that the CXCL7 propeptide predominantly forms a tetramer. Although showing no chemotactic activity to neutrophils compared with the mature CXCL7 peptide, the CXCL7 propeptide tetramer exhibits a much higher potency to induce histamine release from the basophilic cells (18). Therefore, like that of CCL14a and CXCL7, more studies will be needed to explore the role of the appending NH2-terminal sequence of CXCL17 in receptor interaction, protein folding, and/or other uncharacterized functions.

In addition to attracting monocytes, we found that the mature CXC17 is capable of inducing the secretion of proangiogenic stimulators from treated monocytes, and this further increases COX-2 expression in HMEC-1 endothelial cells (Fig. 5).
3). COX-2 has been known to play a central role in reepithelialization and reconstruction of gastric glands in the mucosal lesion (10, 24). In addition, COX-2 is also strongly expressed in endothelial cells in the ulcer bed (12), where it exhibits healing and cytoprotective activity against ulceration. Although the profile of proangiogenic stimulators secreted by CXCL17-treated THP-1 monocytes has as yet not been well characterized, we have demonstrated that both the mRNA and protein levels of VEGF-A, the most potent stimulator of angiogenesis and tissue remodeling, are increased in these treated cells (Fig. 3, B and C). Of interest, a previous study also found preliminarily that the CXCL17 transcript is tightly co-expressed with the VEGF transcript during breast and colon tumor formation, where CXCL17 is proposed to accelerate tumor angiogenesis (30). Therefore, our result in Fig. 3 may further support the angiogenic function of CXCL17 and clarify the relationship between CXCL17 and VEGF-A in the previous study. In addition to promoting tumor angiogenesis, VEGF-A has also been demonstrated to be an essential factor in the healing process of normal tissues such as the gastrointestinal tracts. For example, administration of platelet suspension can accelerate gastric ulcer healing in rats, whereas this effect is directly reversed by neutralizing the suspension with an anti-VEGF antibody (25). Thus, although more evidence is needed, we speculated that CXCL17 may be involved in the routine repair process of normal tissues such as the gastric mucosa.

Surprisingly, in contrast to other chemokines, we found that CXCL17 significantly suppressed the production of the proinflammatory cytokines and factors from either J774 or primary macrophages (Figs. 5 and 6). Since the endogenous receptor(s) of CXCL17 has not been identified, we are currently not able to explain the possible mechanism in details. However, unlike other inflammatory chemokines, which normally are maintained at negligible levels in tissues and are then dramatically induced only upon stimulation or when inflammation occurs (29, 33), CXCL17 is constitutively expressed in the gastric mucosa at a relatively high level (Figs. 1 and 2). Therefore, it will be physiologically reasonable that CXCL17 acts as an anti-inflammatory rather than an inflammatory factor in the stomach, where it routinely contacts with resident macrophages. It is known that the stomach constantly contacts external inflammatory stimuli such as microbial flora, ingested foodstuffs, and gastric acid; this may further lead to gastric lesion by increasing proinflammatory cytokines such as IL-6 and TNFα within a local gastric area (20, 32). Therefore, we speculated that the anti-inflammatory effects of CXCL17 may protect the stomach by helping the stomach to maintain a noninflamed status while contacting these inflammatory stimuli. Although future experiments involving receptor identification and animal tests will be required for verifying this hypothesis, the proposed anti-inflammatory characteristic of CXCL17 is absolutely novel, and its angiogenic effect has also been strengthened in our study. Thus, this information may provide a potential biological hint for the treatment of gastric diseases such as ulceration and gastritis in the future.

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DISCLOSURES

The authors have no conflict of interests, financial or otherwise, to disclose.
AUTHOR CONTRIBUTIONS

W.-Y.L. and C.-W.L. did the conception and design of the research; W.-Y.L., C.-J.W., T.-Y.L., C.-L.H., and C.-W.L. performed the experiments; W.-Y.L. and C.-W.L. analyzed the data; W.-Y.L. and C.-W.L. prepared the figures; W.-Y.L. and C.-W.L. edited and revised the manuscript; C.-W.L. drafted the manuscript; C.-W.L. approved the final version of the manuscript.

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