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*J Immunol* 2011; 187:1403-1410; Prepublished online 29 June 2011; doi: 10.4049/jimmunol.1002352
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Regulation of Chemerin Chemoattractant and Antibacterial Activity by Human Cysteine Cathepsins

Paulina Kulig,*1 Tomasz Kantyka,*1 Brian A. Zabej,*1 Magdalena Banaś,* Agnieszka Chyra,* Anna Stefanska,* Hua Tu,‡ Samantha J. Allen,§ Tracy M. Handel,§ Andrzej Kozik,‖ Jan Potempa,‡,# Eugene C. Butcher,‡,** and Joanna Cichy*  

Chemerin, a ligand for the G-protein coupled receptor chemokine-like receptor 1, requires C-terminal proteolytic processing to unleash its chemoattractant activity. Proteolytically processed chemerin selectively attracts specific subsets of immunoregulatory APCs, including chemokine-like receptor 1-positive immature plasmacytoid dendritic cells (pDC). Chemerin is predicted to belong to the structural cathelicidin/cystatin family of proteins composed of antibacterial polypeptide cathelicidins and inhibitors of cysteine proteases (cystatins). We therefore hypothesized that chemerin may interact directly with cysteine proteases, and that it might also function as an antibacterial agent. In this article, we show that chemerin does not inhibit human cysteine proteases, but rather is a new substrate for cathepsin (cat) K and L. Cat K- and L-cleaved chemerin triggered robust migration of human blood-derived pDC in vivo. Furthermore, cat K- and L-truncated chemerin also displayed antibacterial activity against Enterobacteriaceae. Cathepsins may therefore contribute to host defense by activating chemerin to directly inhibit bacterial growth and to recruit pDC to sites of infection. The Journal of Immunology, 2011, 187: 1403–1410.

Chemerin is a recently characterized chemoattractant protein that serves as a ligand for the seven-pass G-protein coupled receptor chemokine-like receptor 1 (CMKLR1) (1, 2). Two additional heptahelical receptors, GPR1 and CCRL2, have been reported to bind chemerin, although they do not appear to directly support chemotaxis (3, 4). Chemerin circulates as an inactive precursor (prochemerin) in blood and undergoes proteolytic processing by host cysteine proteases to generate the bioactive chemotactic/antibacterial agent. The inactive precursor (prochemerin) of human chemerin is a 26-kDa protein that is cleaved by cysteine proteases to yield the 18-kDa bioactive chemotactic fragment, the human cationic antimicrobial peptide of 18 kDa (hCAP18). Cathepsins are degradative enzymes of mammalian cells, participating primarily in intracellular proteolytic pathways (such as Ag processing and presentation), but also extracellular protein turnover. Recent studies show that lysosomal cathepsins can exert their proteolytic activity at extracellular sites (14, 15), where they contribute to a variety of pathophysiological processes, including chronic inflammation associated with obesity (16–18).

Cathelicidins consist of two distinct domains: a highly conserved N-terminal cathelin-like domain with homology to the cystatins and a divergent C-terminal antimicrobial region that varies among species. Only one cathelicidin has been described in humans: human cationic antimicrobial peptide of 18 kDa (hCAP18). hCAP18 is cleaved by neutrophil serine proteases such as pro-
teinase 3 to generate a 37-aa antimicrobial peptide LL-37 and a 103-aa cathelin-like domain (19, 20).

In this study, we found that although chemerin does not inhibit the proteolytic activities of cat L or K, these cysteine proteases are potent activators of chemerin. cat L and K initially and efficiently cleave prochemerin to release a 6-aa peptide from the carboxyl terminus, generating chemS157; the enzymes can also cleave chemerin to release a 38-residue C-terminal peptide, generating chemR125. The activated chemerin is a potent attractant for CMKLR1 cells, including human blood pDC. In addition, we demonstrate that although the smaller chemerin fragment generated by both cathepsins (chemerin R125) does not support chemotaxis of CMKLR1 cells, both chemS157 and chemR125 display comparable anti-inflammatory activity against Enterobacteriaceae.

Materials and Methods

Materials

Recombinant cat B, L, and K, and chemS157 were purchased from R&D Systems. Anti-CD3, -CD14, -CD16, -CD19, -CD20, -CD56 biotin-linked mAbs, as well as FITC-labeled CD123 and allophycocyanin-labeled BDCA-2 were obtained from BD Pharmingen, Miltenyi Biotec, eBioScience, and BioLegend. Recombinant chemerin isoforms, full-length prochemerin, chemerin serum form (chemA155), and SspB-truncated chemerin (chem/SspB; chemS157) were produced as previously described (2, 13, 21). Recombinant Fc–chemerin proteins were produced and purified from Chinese hamster ovary cells via transient transfection and protein A purification. DNA fragments corresponding to the desired chemerin proteolyte were amplified by PCR and cloned in-frame downstream of human IgG1 Fc domain, which is downstream of a secretion signal peptide in mammalian expression vector pLEV113 (LakePharma). There is a 9-aa glycine-rich linker between the Fc and chemerin domains. Plasmid DNA was transfected into Chinese hamster ovary cells using Lefactine transfection reagent (LakePharma), and cell culture supernatant was collected 3 d posttransfection. Fc fusion proteins were purified with Protein A resins (Mab Select SuRe GE Healthcare), and final proteins were formulated in 100 mTris, 150 mM NaCl, and 0.45% NaOAc.

Inhibition assays

Inhibitory activity against cat B, K, and L was assayed fluorometrically using a Molecular Devices Gemini XS system, with an excitation of 350 nm and emission wavelength of 460 nm. Cathepsins were preactivated in a buffer containing 100 mM sodium acetate, pH 5.5, 100 mM NaCl, 10 mM DTT, 1 mM EDTA, and 0.01% Tween 20 for 10 min at 25°C. Cathepsins (0.2 nM) were then incubated with or without chemerin for 30 min at 37°C in the same buffer containing the fluorescent substrates Z-Phe-Arg-AMC (10 μM; Sigma) for cat B and L or Boc-VLK-AMC (10 μM; Peptides International) for cat K, in a total volume of 100 μL. The progress of the reaction was monitored by fluorescence spectrophotometry, and the data were plotted versus time. Maximum velocity values were calculated by linear fits to the time-dependent curve. The residual chemerin activity present in each treatment group is presented as a percentage of the maximum velocity determined in the absence of inhibitor (chemerin + substrate only).

Mass spectrometry

Matrix-assisted laser desorption ionization time-of-flight spectrometry (MS) was performed by the Stanford Protein and Nucleic Acid Bio-technology Facility (Stanford University, Stanford, CA). After trypsin digest of the cat K and L truncated-chemerin band, MS was performed, and the mass values were used in a Mascot search (http://www.matrixscience.com) of public peptide databases. PeptideCutter was used to predict the mass values of tryptic chemerin fragments (http://www.expasy.org). Mass values obtained after collision-induced dissociation (CID) of the predicted C-terminal peptide (f) were compared with the predicted CID mass values for the peptide corresponding to residues 141–157 of chemerin (sequence: AGEDPHSFYFPGQFAFS), using Mascot software (Matrix Science).

Purification of pDC

The Institutional Review Board at Jagiellonian University approved all human subject protocols. Human blood was collected and PBMCs were harvested after LSM1077 (PAA Laboratories) gradient separation, as described by the manufacturer. pDC were enriched from PBMC using negative selection with biotinylated mAbs directed against CD3, CD14, CD16, CD19, CD20, CD56, and anti-biotin MACS Microbeads (Miltenyi Biotec), according to the manufacturer’s recommendations. Cells were blocked with 50–80% autologous plasma and then stained for flow cytometry analysis using mAbs against CD123 and BDCA-2 to identify pDC. Stained cells were analyzed on an LSRII flow cytometer (Becton Dickinson).

Chemotaxis assay

Cathepsins were incubated with recombinant human prochemerin for 10 min at 37°C and then tested in an in vitro chemotaxis assay using murine pre-B lymphoma L1.2 cells stably transfected with human recombinant CMKLR1 (CMKLR1/L1.2) or purified human blood pDC. In each case, enzymatic digestion was stopped by placing samples on ice and diluting with chemotaxis medium (RPMI 1640 containing 10% FBS). Where indicated, recombinant chemerin isoforms, Fc–chemerin fusion proteins, or Fc alone were used. A total of 100 μL cells (2.5 × 10^5 cells/well) was added to the top well of 5-μm pore transwell inserts (Costar), and test samples were added to the bottom well in a 600-μL volume. Migration was assayed for 2 h at 37°C. The inserts were then removed, and cells that had migrated through the filter to the lower chamber were collected and counted by flow cytometry (FACSCalibur; BD Biosciences). The results are presented as percentage input migration. CXCL12 served as a positive control.

Antimicrobial assay

Escherichia coli (HB101, a conventional laboratory strain) and Klebsiella pneumoniae (a clinical isolate from human bronchoalveolar lavage fluid) were used in this study. The antimicrobial activity of the indicated chemerin forms was estimated using a microtiter broth dilution assay (22). A single colony of bacteria was inoculated into 20 mL Mueller–Hinton broth (MHB; Difco) and incubated overnight at 37°C; subcultured once at 1:100 dilution in MHB, and then grown for 2–3 h to midlogarithmic phase. Cell numbers were calculated using previously determined standard curves, and for subsequent experiments, bacteria were used at 2–7 × 10^7 CFU/mL. Bacterial suspensions (90 μL) in MHB were mixed with 10 μL diluent (10 mM HEPES, 100 mM Tris, 150 mM NaCl, and 0.45% NaOAc, or MHB) control or 10 μL different concentrations of chemerin, Fc–chemerin fusion proteins, Fc alone, or LTB4 (Sigma) to a final volume of 1 mL (Microbiology Facility) and incubated at 37°C for the indicated times. After serial dilutions with MHB, the diluted mixture was plated on MHB agar plates and incubated at 37°C overnight for enumeration of CFU. In selected experiments, samples of the bacteria/peptide mixtures were also analyzed by flow cytometry and by spectrophotometry. These methods produced comparable results to the colony-forming assay (data not shown).

Results

cat L and K activate chemerin chemoattractant activity

We initially tested chemerin for cystatin-like activity. Substrate hydrolysis by cat B, L, or K was not significantly inhibited by prochemerin, serum-form bioactive chemerin (chemA155), or SspB-activated chemerin (chemS157), even at 10:1 and 100:1 molar ratios of chemerin-to-cathepsin (Fig. 1, Table I). The general cysteine proteinase inhibitor E64 efficiently abolished the activity of all cathepsins examined (Fig. 1). Thus, chemerin does not appear to function as a cystatin.

Prochemerin was incubated with purified cat B, L, or K and tested for attractant activity to test whether prochemerin is a substrate for human cysteine proteases. A controlled digest of prochemerin, using low concentrations of either cat L or K (1000-fold less than prochemerin) generated a single primary proteolytic product (Fig. 2A). Two apparent cleavage products were generated when cat L and K concentrations were increased 10–50 times, suggesting further digestion of prochemerin. Under similar conditions, cat B did not cleave prochemerin (data not shown). Interestingly, the two specific >10-kDa chemerin cleavage products generated by cat L and K can be distinguished by PAGE only under reducing conditions (Fig. 2A). Because prochemerin contains three disulfide bonds, these data suggest that under non-reducing conditions, the dual-cleaved chemerin products remain associated with the holo-molecule through S–S bonds (Fig. 2A).
CMKLR1-transfected L1.2 cells migrated significantly to cat L- or K-treated prochemerin (Fig. 2B). The chemotactic response of CMKLR1/L1.2 transfectants was dependent on cathepsin concentration, with cat L and K eliciting maximal effects on chemerin-mediated migration at a 1:100 prochemerin/cathepsin ratio (Fig. 2B). Although chemerin treated with cat L appeared to elicit slightly higher chemotactic response compared with cat K-treated chemerin (Fig. 2B), the difference was not statistically significant. Freshly isolated CMKLR1+ human blood pDC also migrated in response to cathepsin-cleaved chemerin, suggesting that these enzymes may be involved in pDC recruitment (Fig. 2C). No cell migration was detected in the absence of chemerin, or when prochemerin or the cathepsins were tested alone (Fig. 2). Compared with cat L and K, cat B had negligible effects on chemerin chemoattractant activity (data not shown). Taken together, these data suggest that incubation of cat L and K with prochemerin results in generation of bioactive chemerin chemoattractant.

Identification of the cathepsin chemerin cleavage sites
We used MS to determine the cathepsin chemerin cleavage sites. The mass value for the larger chemerin cleavage product (calculated mass [M+H]+, 16,155 Da; mass difference 1,3 Da; mass difference 1,3 Da) from cat L and K (indicated by arrowheads in Fig. 2A) was isolated, digested with trypsin, and analyzed by mass spectrometry to confirm the processing site. A peptide fragment with a mass value of 1903.8 Da was identified. This fragment includes chemerin residues 18–157 (ADPELT…GQFAFS, chem157S). The larger protein band (indicated by arrowheads in Fig. 2A) was isolated, digested with trypsin, and analyzed by mass spectrometry to confirm the processing site. A peptide fragment with a mass value of 1903.8 Da was identified. This fragment includes chemerin residues 18–157 (ADPELT…GQFAFS, chem157S).

Table I. Comparison of chemerin isoforms used in this study

<table>
<thead>
<tr>
<th>Chemerin Form</th>
<th>Abbreviations</th>
<th>C-Terminal Amino Acid Sequence</th>
<th>References</th>
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<tr>
<td>Prochemerin (full-length chemerin)</td>
<td>chem</td>
<td>I1 VLGRLVHCPEHQTQLRLEAEHEQETQCLRVRQAGEDPHSHFYFPQGQFAE5KLPR 163</td>
<td>7</td>
</tr>
<tr>
<td>Serum form</td>
<td>chemA155</td>
<td>I1 VLGRLVHCPEHQTQLRLEAEHEQETQCLRVRQAGEDPHSHFYFPQGQFAE5KLPR 155</td>
<td>7, 21</td>
</tr>
<tr>
<td>SspB-treated chemerin</td>
<td>chem/SspB; chemS157</td>
<td>I1 VLGRLVHCPEHQTQLRLEAEHEQETQCLRVRQAGEDPHSHFYFPQGQFAE5KLPR 157</td>
<td>13</td>
</tr>
<tr>
<td>Cat L-treated chemerin, Cat K-treated chemerin</td>
<td>chemS157</td>
<td>I1 VLGRLVHCPEHQTQLRLEAEHEQETQCLRVRQAGEDPHSHFYFPQGQFAE5KLPR 148</td>
<td>13</td>
</tr>
<tr>
<td>Cat L-treated chemerin, Cat K-treated chemerin</td>
<td>chemR125</td>
<td>I1 VLGRLVHCPEHQTQLRLEAEHEQETQCLRVRQAGEDPHSHFYFPQGQFAE5KLPR 125</td>
<td>This study</td>
</tr>
</tbody>
</table>

*Residue position is italic. Nonnative amino acids are shown in bold.
corresponding to a nontryptic peptide comprising amino acids 141–157 from the C terminus of chemerin (Fig. 3A, B). This peptide confirmed the initial and predominant cat K- and L-mediated chemerin cleavage site as NH2...AFS↓KAL...COOH. Further microsequencing of this peptide by CID (tandem MS/MS) confirmed the sequence (Fig. 3C). Interestingly, this cleavage site is identical to a previously identified endogenous active human chemerin isoform isolated from ascites fluid (1), and to a main chemerin isoform generated by the S. aureus-secreted cysteine protease SspB (chem/SspB; Table I).

The mass value for the smaller chemerin cleavage product generated by cat L and K (experimental mass [M+H]+, 12,442 Da; calculated mass [M+H]+, 12,437 Da; Δ mass, 5 Da) corresponds to chemerin residues 18–125 (ADPELT……ETQVLR, chemR125). MS and CID analysis of the tryptic digests confirmed that the most distal tryptic or nontryptic fragment was “LVHCPIETQVLR” (fragment d in Fig. 3B, data not shown). Thus, prolonged incubation (data not shown) or incubation with 10–50 times higher concentrations of cat K or L (Fig. 2A) cleaves chemerin at position NH2...QVLR↓EAEE...COOH, and the released C-terminal peptide likely remains linked to the holo-molecule through a disulfide bond, as indicated by Fig. 2A.

**CHEMERIN S157, but not chemerin R125, is chemotactically active**

Because separation of the smaller cleavage product by HPLC required reducing the S-S bonds, which would likely alter its secondary structure and possibly bioactivity, to determine the relative biological activities of the two cathepsin-generated chemerin products, we generated recombinant Fc-chemerin, abbreviated further as Fc-chemS157 (the larger cleavage product) and Fc-chemR125 (the smaller cleavage product). The Fc-chemerins have a glycine linker on the N terminus that is connected to the Fc domain of human IgG1; thus, the C terminus of the Fc-chemerin fusion proteins remained native. Fc alone or Fc-chemR125 failed to trigger CMKLR1+ cell migration at every concentration tested (up to 50 nM) (Fig. 4). However, chemS157 was the most potent attractant for CMKLR1+ cells, with 1 nM eliciting a maximum 42% cell migration. Recombinant, commercially available chemerin S157 and HPLC-purified chemerin cleavage product of SspB

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**FIGURE 2.** cat L- and K-activated chemerin triggers CMKLR1/L1.2 cell and human blood pDC chemotaxis. A, Various concentrations of cat L or K were incubated with 2.9 μM chemerin, and the resulting products were separated by SDS-PAGE under either reducing (left panel) or nonreducing (right panel) conditions. Arrowheads indicate the two predominant chemerin cleavage products (two-pointed arrowheads signify the larger, three-pointed arrowheads signify the smaller cleavage products). Representative of n = 3 performed with similar results. B and C, cat K- or L-activated chemerin was tested in in vitro transwell chemotaxis using CMKLR1/L1.2 transfectants (B) or human blood pDC (C). The chemerin samples described in A were diluted to a final concentration of 1 nM and tested in chemotaxis assays. CXCL12 (10 nM) was used as a positive control for pDC chemotaxis (C). The mean ± SD from duplicate wells of three or four experiments is shown. *p < 0.05 by Student t test comparing cathepsin-treated chemerin versus prochemerin, or CXCL12 versus (−) control.
were used interchangeably as chemS157, because both gave similar results in chemotaxis assays (data not shown). In previous work, we and others have shown that chemS157 is a chemotactant for pDC, NK cells, and monocyte-derived dendritic cells and macrophages (1, 11, 13).

The Fc-chemS157 fusion protein also triggered statistically significant CMKLR1/L1.2 cell migration at concentrations of 5, 10, and 50 nM, although the response was not as robust as the unmodified form. ChemA155 also triggered CMKLR1+ cell migration, although it should be noted that this recombinant protein has a nonnative "PH" C-terminal dipeptide following the native terminal alanine (residue 155; Table I), which may alter its activity.

Chemerin displays antibacterial activity

The predicted homology between chemerin and the antimicrobial cathelicidins, coupled with the expression of chemerin in the skin and other epithelial cell surfaces continually exposed to bacterial challenge (9), led us to evaluate possible antibacterial activities of chemerin. Different chemerin isoforms were first tested for antibacterial activity against a laboratory strain of E. coli. Human cathelicidin LL-37 (3 μM) was used as a positive control peptide inhibitor of bacterial growth (20). As demonstrated in Fig. 5A, full-length chemerin (prochemerin, 2 μM) significantly inhibited the growth of E. coli when incubated for 24 h, leading to survival of 59 ± 13% of bacteria compared with vehicle-treated E. coli set as 100%. Notably, truncated chemerin was an even more effective inhibitor of bacterial growth. The primary chemerin cleavage product generated by cat K and L (chemS157; Table I) was significantly more effective than prochemerin in inhibiting bacterial growth.
growth, resulting in $33 \pm 15\% \text{ E. coli survival (Fig. 5A)}$. Truncated chemA155 also significantly reduced $E. coli$ survival ($39 \pm 19\%$) versus prochemerin (Fig. 5A). We also tested chemerin (2 $\mu$M) for antibacterial activity against a clinical isolate of $K. pneumoniae$. As demonstrated in Fig. 5A, incubation of $K. pneumoniae$ with prochemerin resulted in $58 \pm 14\%$ viable bacteria compared with control, whereas chemA155 and chemS157 reduced $K. pneumoniae$ survival to $32 \pm 11$ and $46 \pm 13\%$, respectively. Thus, similar to $E. coli$, the truncated form of chemerin was more effective at inhibiting $K. pneumoniae$ growth than prochemerin. However, it should be noted that 24-h incubation of prochemerin with either $E. coli$ or $K. pneumoniae$ also resulted in some truncation of prochemerin (data not shown), suggesting that native protein requires removal of inhibitory C-terminal sequence to display full antibacterial activity. Taken together, these data suggest that proteolytic cleavage increases the antimicrobial activity of chemerin.

The inhibition of $E. coli$ growth by chemerin was detectable after just 8 h (Fig. 5B). Treatment with prochemerin, chemA155, or chemS157 diminished the survival of $E. coli$ to $65 \pm 7, 53 \pm 10$, and $60 \pm 5\%$ of control, respectively (Fig. 5B). The chemerin-mediated decrease in bacterial viability grew even more pronounced at prolonged incubation times; after 24 h, for example, the percentage of viable bacteria decreased to $53 \pm 9, 35 \pm 9$, and $35 \pm 4\%$, respectively (Fig. 5B). With the exception of 4 h, when prochemerin seemed to be more effective compared with chemA155 and chemS157 ($87 \pm 5, 95 \pm 3, 94 \pm 4\%$, respectively), the truncated chemerin forms demonstrated stronger antibacterial activity against $E. coli$ (Fig. 5B). Interestingly, chemerin-mediated inhibition of $E. coli$ survival was detectable over a relatively wide range of concentrations, from 2 $\mu$M (the highest concentration tested leading to statistically significant growth inhibition) to as little as 0.125 to 0.0625 $\mu$M for chemA155 and chemS157 (Fig. 5C). This was in contrast with LL-37, which was highly effective in inhibiting $E. coli$ growth when used at 3 $\mu$M (Fig. 5A) but had almost no effect when tested at 1.5 $\mu$M (data not shown). The enhanced antibacterial activity of the truncated chemerin isoforms compared with prochemerin suggests an inhibitory role for the prochemerin C-terminal peptide. Addition of chemically synthesized C-terminal peptide KALPRS that is released from prochemerin by SspB, cat K, and cat L, however, did not reduce the antibacterial activity of the larger truncated chemerin forms (data not shown). These data suggest that after release from the core protein, the C-terminal peptide no longer plays an inhibitory role. Interestingly, both chemerin Fc fusion proteins, Fc-chemS157 and Fc-chemR125, displayed comparable antibacterial activity against $E. coli$ (Fig. 5D). These data suggest that in contrast with chemotactic activity, the antibacterial properties of chemerin appear to be localized closer to the N terminus, because removal of 38 residues from the C terminus did not abrogate its antibacterial activity.

Discussion

In this article, we identify a novel antimicrobial activity associated with chemerin, and show that host-derived cat L and K can cleave and activate the leukocyte attractant activity of chemerin, as well as enhance its antibacterial effects.

Various serine proteases have been reported to effectively convert chemerin to a potent chemottractant in vitro. There is also a single example of a cysteine protease, $S. aureus$-derived SspB that can efficiently activate human chemerin. In addition, host-originating cathepsin S and calpains have been reported to process mouse chemerin, although in this case, the proteolysis of the C terminus generates chemerin variants equipped primarily with anti-inflammatory properties (13, 23). Cysteine cathepsins of the papain-like family are normally confined to the endosomal/lysosomal network. However, there is evidence that certain cathepsins are also active extracellularly, either in association with the cell surface or in soluble form (14). Some cells such as macrophages and fibroblasts constitutively secrete cysteine cathepsins aszymogens (14). Moreover, macrophages have been reported to deploy enzymatically active cat B, L, and S, and exhibit an elastin-degrading phenotype, indicating that macrophages can mobilize
cysteine cathepsins to participate in the pathophysiologic remodeling of the extracellular matrix (17). Massive amounts of extracellular cathepsins, probably released from macrophages, are found in the bronchial tree of patients suffering from acute pulmonary inflammation (24). In addition, cat K is strongly implicated in maintaining the homeostasis of the extracellular matrix in the lung (25). Because chemerin mRNA is abundantly produced in lung (1, 2), collectively, these data suggest that either cat L or K may be involved in chemerin processing in this organ. Alternatively, significant expression and/or activity of cat K and L in the joints of patients with rheumatoid arthritis and skin dermatoses, respectively (26, 27), together with reported chemerin immuno-reactivity or bioactivity, or both, in psoriasis skin and inflamed synovial fluid (1, 9, 28), suggest that these cathepsins may play a role in chemerin cleavage in joints and skin. Because cathepsin-mediated processing releases chemerin attractant activity, these enzymes may have an important regulatory role in immune cell migration. Notably, the presence of pDC in lung, as well as the inflamed joints and psoriatic skin (1, 9, 28, 29), supports the notion that cat K and L, through the generation of active chemerin, may contribute to pDC recruitment to these sites.

Our data also uncover a novel role for chemerin as a host-expressed antibacterial agent in host defense. Despite low primary sequence homology between chemerin and antibacterial cathelicidins, the conserved positioning of key cysteine residues leads to a predicted shared similar tertiary structure, although recent NMR assignment of human chemerin does not exclude a different fold (30). LL-37, the 37-aa C-terminal derivative of human cathelicidin hCAP18, is well known for its potent and broad-spectrum bacterial killing activity. However, chemerin is structurally similar to the cathelin-like N-terminal region. Interestingly, the cathelin-like domain of hCAP18 has been reported to possess antimicrobial activity, although the mechanism by which it inhibits bacterial growth is not known (20).

Chemerin may exert antimicrobial activity on the surface of skin and/or lung where it is locally expressed (1, 9). For example, the respiratory surface is continually exposed to pathogenic organisms, such as K. pneumoniae, which, as shown in this report, might be a direct chemerin target. Although either prochemerin or the C-terminal truncated chemerin forms displayed antibacterial activity, C-terminal processing augmented the inhibitory effect of chemerin on the growth of Enterobacteriaceae. However, our data suggest that prochemerin is also processed by bacterial proteases during incubation, although the protease(s) responsible remain to be identified. It will be interesting to map the specific chemerin domains/regions responsible for its antimicrobial activity. Our preliminary data suggest that most of the antibacterial activity is associated with the chemerin region(s) located within 65–115 aa (data not shown). This is consistent with our data showing that Fc-chemS157 and Fc-chemR125 have similar antibacterial activity, although the inhibitory C-terminal peptide must be removed for full antibacterial effects.

Although the antimicrobial effects of chemerin on E. coli and K. pneumoniae were less potent compared with the classical antibacterial peptide LL-37, chemerin showed bactericidal properties at much lower concentrations. In general, pore-forming antimicrobial peptides, such as LL-37, require micromolar concentrations for activity. However, some antibacterial peptides, such as Lactococcus-derived nisin, operate in the nanomolar range (31). This ability is attributed to docking to a specific component on the bacteria cell wall for subsequent pore formation, or to the dual-killing mechanisms of the peptide, which in addition inhibits bacterial cell wall biosynthesis (32). Chemerin might use a similar strategy to exert its antimicrobial activity in the nanomolar range. However, because antimicrobial properties can be sensitive to pH and ionic composition of the peptide environment (31), it will be important to determine whether chemerin operates in conditions similar to those found in the skin, bronchial tree, or both.

Thus, our work uncovers a novel antibacterial property of chemerin and characterizes the activation of chemerin by host-derived cysteine proteases of the cathepsin family, and adds a new dimension to the ways chemerin may modulate and augment immunity.

Acknowledgments

We are grateful to Dr. M. Bulanda and K. Palaga for help with bacteria collection.

Disclosures

The authors have no financial conflicts of interest.

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