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Staphylococcus aureus-Derived Staphopain B, a Potent Cysteine Protease Activator of Plasma Chemerin

Paulina Kulig,* Brian A. Zabel,**§§ Grzegorz Dubin, † Samantha J. Allen, ‡ Takao Ohyama, ‡§ Jan Potempa, ‡ Tracy M. Handel, ‡ Eugene C. Butcher, ‡§ and Joanna Cichy§§§

Chemerin is an attractant for cells that express the serpentine receptor CMKLR1, which include immature plasmacytoid dendritic cells (pDC) and macrophages. Chemerin circulates in the blood where it exhibits low biological activity, but upon proteolytic cleavage of its C terminus, it is converted to a potent chemoattractant. Enzymes that contribute to this conversion include host serine proteases of the coagulation, fibrinolytic, and inflammatory cascades, and it has been postulated that recruitment of pDC and macrophages by chemerin may serve to balance local tissue immune and inflammatory responses. In this work, we describe a potent, pathogen-derived proteolytic activity capable of chemerin activation. This activity is mediated by staphopain B (SspB), a cysteine protease secreted by Staphylococcus aureus. Chemerin activation is triggered by growth medium of clinical isolates of SspB-positive S. aureus, but not by that of a SspBnull mutant. C-terminal processing by SspB generates a chemerin isoform identical with the active endogenous attractant isolated from human ascites fluid. Interestingly, SspB is a potent trigger of chemerin even in the presence of plasma inhibitors. SspB may help direct the recruitment of specialized host cells, including immunoregulatory pDC and/or macrophages, contributing to the ability of S. aureus to elicit and maintain a chronic inflammatory state. The Journal of Immunology, 2007, 178: 3713–3720.

C hemoattractants target host-defense effector cells to local sites of infection and guide developing immune cells to microenvironments suitable for their maturation. The presence of a chemoattractant in a tissue, and the presence of its cognate receptor on a leukocyte, help define migration specificities (1). It is clear that transcriptional control is important for regulating the expression of numerous chemoattractants (2). However, recent literature shows that posttranslational proteolytic processing also provides an important mechanism for the regulation of chemoattractant function (3). For example, metalloprotease activity leads to the inactivation of several chemokines, including the monocyte chemotactic proteins CCL2, 7, 8, and 13 (MCP1–4), and the general leukocyte attractant CXCL12 (SDF1) (4). In certain cases, proteolytically truncated chemokines bind to their cognate receptors, but no longer promote chemotaxis, acting instead as chemokine antagonists capable of dampening inflammation (4). Protease activity can also modulate the function of the chemokine CX3CL1 that is tethered to the cell surface through a mucin-like stalk. Although it is attached to the cell surface, CX3CL1 acts as a cell adhesion molecule, but after proteolytic cleavage, it functions as a soluble chemokine (5, 6). Finally, recent advances reveal the potential for proteases to activate chemoattractants (7, 8).

Chemerin, also known as tazarotene-induced gene 2, has been recently characterized as a ligand for the seven transmembrane, G protein-coupled receptor CMKLR1 (also known as ChemR23 in humans and DEZ in mice). Chemerin mRNA is present in most tissues, including liver, pancreas, and skin (7, 8). The liver, however, appears to be a primary source of chemerin, and is likely to be responsible for the high levels of this protein in plasma (8, 9). Chemerin circulates as an inactive precursor (prochemerin) in blood and requires proteolytic processing for activation. Recently, we and others have demonstrated that active chemerin is generated by serine proteases of the inflammatory, coagulation and fibrinolytic cascades (9, 10). These include neutrophil-derived elastase and cathepsin G, mast cell tryptase, as well as factors VIIa, XIIa, tissue plasminogen activator, urokinase-type plasminogen activator, and plasmin. Proteolytically processed chemerin selectively attracts specific subsets of immunoregulatory APCs, such as immature plasmacytoid dendritic cells (pDCs)* and macrophages that express CMKLR1 (7–11) providing a potential mechanism for controlling immune responses at sites of inflammation or tissue injury.

With the growing prevalence of bacteria-mediated chronic inflammatory diseases (12–15), there is a need to define the mechanisms underlying recruitment of Ag presenting and immune regulatory cells

* Abbreviations used in this paper: pDC, plasmacytoid dendritic cell; SspB, staphopain B; Spl, serine protease-like protein; ScpA, staphopain A; Aur, aureolysin; TFA, trifluoroacetic acid; MS, mass spectrometry.

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to sites of bacteria infection. *Staphylococcus aureus* is among the most successful of human pathogens. It is a major cause of skin and soft tissue infections and has been implicated in sepsis as well as in several chronic inflammatory disorders, including atopic dermatitis and psoriasis (15). The success of *S. aureus* as a pathogen may reflect an ability to regulate the recruitment and modulate the function of specialized host cells.

In this work, we demonstrate that the secreted cysteine protease staphopain B (SspB), a potential virulence factor that is likely to contribute to the chronicity of *S. aureus* infections, is a potent activator of chemerin. Processing of chemerin by SspB may therefore contribute to the pathologic inflammatory response to staphylococcal invasion through recruitment of immunomodulatory pDC and/or macrophages to sites of infection.

**Materials and Methods**

**Bacterial strains and growth conditions**

Wild-type *S. aureus* strains 8325-4 and COL (16, 17), and isogenic knockout mutants of individual protease genes in the 8325-4 genetic background were used in this study. Staphopain A (∆scaA), SspB (∆SspB), SspB and V8 protease (∆scaA∆V8), and aureolysin (∆Aur) deficient strains (18) were obtained from Dr. L. Shaw (Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA) while the Ssp proteases (∆Ssp) mutant (19) was a gift from Dr. K. Bayles (Department of Microbiology, Molecular Biology, and Biochemistry, University of Idaho, Moscow, ID). Collection of clinical isolates of *S. aureus* samples from joint, blood, and lesions of skin patients suffering from bone infection, sepsis, or atopic dermatitis were donated by Dr. S. Eck (Institute of Medical Microbiology, University Hospital, Jena, Germany) and Dr. M. Kapinska-Mrowiec (Zeromski General Hospital, Krakow, Poland). The *S. aureus* strains were grown in tryptic soy broth (Sigma-Aldrich) at 37°C with shaking at 200 rpm for 14 h. The bacterial density was measured spectrophotometrically and the cell number was calculated using previously determined standard curves. Typically, the bacterial OD (OD₆₀₀) was within 8–10. No major growth differences were observed among *S. aureus* strains as confirmed by serial dilution and CFU counting on tryptic soy agar. The bacteria were centrifuged at 5000 × g for 15 min and conditioned medium was collected, diluted with tryptic soy broth to OD₆₀₀ = 7, and used in chemotaxis assays and zymography.

**Recombinant prochemerin**

Prochemerin with a C-terminal HIS6 tag was cloned into pACGP67 (BD Biosciences) and transfected into Sf-9 cells. The expressed protein has the sequence NH₂-ADPELTEA. . .LPRSPHHHHHH-COOH, where the underlined residues are nonnative. After viral amplification, prochemerin was purified by reverse-phase HPLC purification (Filtron) with a 3-kDa cutoff filter. After a running the solution over nickel-nitrilotriacetic acid (Amersham Biosciences) into 50 mM HEPES, 0.3 M NaCl (pH 8.0), prochemerin was purified by PAGE (13%) under nonreducing conditions. Bands were then visualized with Coomassie to allow the bands of proteolytic activity to be detected as clear bands of lysis against a stained background.

**Purification of chemerin fragments by reverse-phase HPLC**

Human blood pDC transendothelial migration

The Institutional Review Board (Stanford University) approved all human subject protocols and informed consent was obtained for all donations. Human blood was collected and PBMC were harvested following Histopaque 1077 gradient separation. Total PBMC were preincubated 1 h in chemotaxis medium (RPMI 1640 plus 10% FCS, supplemented with l-glutamine, penicillin/streptomycin, sodium pyruvate, and nonessential amino acids) at 10⁷ cells/ml to allow for recovery of receptor expression. Transwell inserts (5-μm pure; Costar) were coated with 2% gelatin and seeded with 10⁴ HUVECs (passage <5), and incubated for 24–48 h. Confluent HUVEC monolayers were confirmed by DifQuick staining (IMEB). Monolayers were rinsed with chemotaxis medium before use. A total of 10⁶ PBMC (100 μl) were added to the top wells, and various concentrations of chemotactants were added to the bottom wells in a 600-μl volume. Migration was assayed for 2 h at 37°C, then the inserts were removed, and the cells that had migrated through the filter to the lower chamber were harvested, stained (Lin FITC (CD3, CD14, CD16, CD19, CD20, CD56), CD11c PE, CD123 CyChrome, HLA-DR allophycocyanin; mAbs from BD Pharmingen and eBioscience), and analyzed by flow cytometry. An equivalent number of beads were added to each tube to allow the cell count to be normalized.

**Intracellular calcium mobilization**

Chemotactant-stimulated Ca²⁺ mobilization was performed following Alliance for Cell Signaling protocol ID P000000210. CMKLRL1/L1.2 transfectants (5 × 10⁶/ml) were loaded with 4 μM Fluo4-AM, 0.16% pluronic acid F-127 (Invitrogen Life Technologies) in modified Iscove’s medium (Invitrogen Life Technologies) plus 1% FBS for 30 min at 37°C. The samples were mixed every 10 min during loading, washed once, resuspended at 2 × 10⁶/ml in the same buffer, and allowed to rest at room temperature in the dark for 20 min. Changes in Fluo4 fluorescence in the labeled cells following treatment with chemotactants were measured over real-time with a FACSscan flow cytometer and CellQuest software (BD Biosciences) at room temperature under stirring conditions (500 rpm). Fluorescent data were acquired continuously up to 512 s at 1-s intervals. The samples were analyzed for 45 s to establish their basal state, then removed from the nozzle for addition of stimuli, then returned to the nozzle for continued data acquisition. Mean channel fluorescence over time was analyzed with FlowJo software (Tree Star).

**SDS-PAGE**

Recombinant prochemerin was incubated with pure SspB at 2:1 molar ratio at 37°C for 10 min in a buffer containing 50 mM Tris-HCl (pH 7.6), 2.5 mM EDTA, and 1 mM DTT. The enzymatic digestion was stopped by addition of the SDS gel loading buffer. Samples were resolved by SDS-PAGE (13%) under nonreducing conditions. Bands were then visualized using the Coomassie G250 stain (Bio-Rad).

**Gelatin zymography**

Bacteria growth medium were resolved by SDS-PAGE (8%) containing 0.1% gelatin (type I from porcine skin; Sigma-Aldrich). After electrophoresis, the gel was washed in 2.5% Triton X-100 for 30 min at room temperature, followed by 40-h incubation at 37°C in buffer containing 50 mM Tris (pH 7.7), 5 mM CaCl₂, and 0.02% Na₂SO₄. The gel was then stained with Coomassie to allow the bands of proteolytic activity to be detected as clear bands of lysis against a stained background.

**Purification of chemerin fragments by reverse-phase HPLC**

For analytical applications, recombinant prochemerin was incubated with pure SspB at 2:1 molar ratio at 37°C for 10 min in a buffer containing 50 mM Tris-HCl (pH 7.6), 2.5 mM EDTA, and 1 mM DTT. The reaction was stopped by addition of trifluoroacetic acid (TFA) to a final concentration of 0.5%. Chemerin cleavage products were isolated using a Waters 501 HPLC System on a μBondapak C18 column (3.9 × 300 mm) also obtained from...
Activation of recombinant prochemerin by *S. aureus*-conditioned medium. *A*, A total of 160 nM recombinant prochemerin was incubated for 10 min at 37°C with *S. aureus* growth medium (*S. aureus* GM). Samples were diluted 15 times and CMKLRI/L1.2 migration was assessed by in vitro transwell chemotaxis. Cell migration to growth medium only was used as a negative control. *B*, A total of 160 nM recombinant prochemerin was incubated for 10 min at 37°C with *S. aureus* growth medium from either wild-type *S. aureus* strain 8325-4 (WT) or various protease-deficient (Δ) strains: ScpA; SspB; V8; Aur; Spl. After the samples were diluted 15 times, CMKLRI/L1.2 migration was assessed by in vitro transwell chemotaxis. Cell migration to prochemerin alone was used as a control. The mean from duplicate wells of three or four experiments ± SD is shown. *, p < 0.05; **, p < 0.001 by Student’s *t* test comparing (A) prochemerin alone vs prochemerin + *S. aureus* GM or (B) comparing various protease-deficient mutants with WT.

**FIGURE 1.** Activation of recombinant prochemerin by *S. aureus*-conditioned medium. *A*, A total of 160 nM recombinant prochemerin was incubated for 10 min at 37°C with *S. aureus* growth medium (*S. aureus* GM). Samples were diluted 15 times and CMKLRI/L1.2 migration was assessed by in vitro transwell chemotaxis. Cell migration to growth medium only was used as a negative control. *B*, A total of 160 nM recombinant prochemerin was incubated for 10 min at 37°C with *S. aureus* growth medium from either wild-type *S. aureus* strain 8325-4 (WT) or various protease-deficient (Δ) strains: ScpA; SspB; V8; Aur; Spl. After the samples were diluted 15 times, CMKLRI/L1.2 migration was assessed by in vitro transwell chemotaxis. Cell migration to prochemerin alone was used as a control. The mean from duplicate wells of three or four experiments ± SD is shown. *, p < 0.05; **, p < 0.001 by Student’s *t* test comparing (A) prochemerin alone vs prochemerin + *S. aureus* GM or (B) comparing various protease-deficient mutants with WT.

Recombinant prochemerin is activated by a cysteine protease secreted by *S. aureus*

To determine whether proteolytic enzymes produced by the human pathogen *S. aureus* generate active chemerin, bacteria growth medium was incubated with recombinant full-length (proform) chemerin. As demonstrated in Fig. 1A, CMKLRI/L1.2 cells migrated significantly in response to prochemerin treated with *S. aureus* growth medium. No cell migration was detected when the growth medium was tested in the absence of prochemerin, or when prochemerin was tested alone, indicating that prochemerin is activated by factors secreted by *S. aureus*.

A number of different proteases are secreted by *S. aureus*. These include a metalloprotease (Aur), a serine glutamyl endopeptidase (V8 protease), and serine protease-like proteins (Spl), as well as two cysteine proteases known as ScpA and SspB. The involvement of each protease in *S. aureus*-mediated prochemerin processing was examined using growth medium of isogenic mutants deficient in selected proteases. As shown in Fig. 1B, prochemerin activation was completely abrogated in the SspB mutant (ΔSspB), in the double-deficient ΔSspBΔV8 mutant. Generation of active chemerin was also significantly diminished in the absence of Aur (ΔAur). In contrast, deficiency of the Spl proteases (ΔSpl), or...
ScpA (ΔScpA), did not have a significant effect on chemerin activation. These data indicate that SspB and to a lesser extent Aur are responsible for S. aureus-mediated chemerin activation.

Because Aur contributes to the activation of the V8 protease, which in turn activates SspB (26–28), the lack of Aur or V8 protease may indirectly affect chemerin activation by reducing the levels of functional SspB produced by these mutants. To determine whether Aur and the V8 protease are capable of activating chemerin directly, we incubated recombinant prochemerin with purified Aur, the V8 protease, and SspB. As demonstrated in Fig. 2A, while SspB was able to activate prochemerin, neither Aur nor the V8 protease were able to activate the chemoattractant. Thus, among enzymes secreted by S. aureus, SspB is an exclusive prochemerin-activating agent. Furthermore, purified SspB restored the ability of SspB-deficient medium to trigger the chemerin activity (Fig. 2B). This finding supports the notion that prochemerin activation by S. aureus growth medium results from a direct effect mediated by SspB and not from clonal or growth differences among staphylococcal strains. Moreover, purified, enzymatically inactive SspB (inSspB) protein, (the catalytic Cys to Ala mutant) (23) was unable to trigger prochemerin activation, confirming that proteolytic activity of SspB is required for S. aureus-mediated chemoattractant activation (Fig. 2A).

**Identification of the SspB cleavage site**

To determine the SspB-mediated cleavage site(s), recombinant prochemerin was incubated with purified SspB and then subjected to a separation by HPLC (Fig. 3A) or SDS-PAGE (Fig. 3B). As expected, coincubation of prochemerin with SspB resulted in limited proteolysis of the chemoattractant (Fig. 3B). HPLC fractions (labeled I-III) were analyzed by MALDI-TOF mass spectrometry (Fig. 3A, inset). Fraction I (1590.8 Da) corresponds to the C-terminal fragment of chemerin encompassing residues from 141 to 146, plus the proline linker and C-terminal HIS6 tag (Fig. 3, C and D). The larger portion of chemerin (16,155.4 Da) corresponding to residues 1–140, was collected in fractions II and III. Identification of two matching chemerin fragments corresponding to the C- and N-terminal portion of the molecule (1,590.8 and 16,155.4 Da, respectively) revealed the SspB-mediated chemerin cleavage site to be NH₂..AFS₂KAL..COOH (Fig. 3, C and D). Fraction II also contained an additional peptide of molecular mass 14,963.1 Da, corresponding to residues 1–130, indicating an additional cleavage event. In this case, however, the matching C-terminal peptide or its further degradation products could not be recovered. The 16,155.4 Da cleavage product of SspB, further referred to as “chem16”, was the primary product observed under conditions when prochemerin...
was treated with SspB at 100:1 molar ratio for 150 min, in a buffer containing 50 mM HEPES, 100 mM NaCl (pH 7.2), or at 1000:1 molar ratio for 90 min. in a buffer containing 50 mM Tris- HCl (pH 7.6), 2.5 mM EDTA, and 1 mM DTT (data not shown). Longer incubation resulted in further truncation of prochemerin and the generation of the 14,963.1 Da product, as described above and referred to as “chem15”. Similar SspB cleavage fragments were detected when recombinant prochemerin lacking five of the six histidines in the C-terminal HIS6 tag was used, indicating that SspB-mediated prochemerin cleavage is not affected by the presence of the HIS6 tag (data not shown).

To confirm the C-term processing site for the primary chemerin product generated by SspB, we performed MALDI-TOF MS (mass spectrometry) of chem16 and identified a mass value corresponding to the nontryptic C-terminal peptide predicted by the MS analysis presented in Fig. 3. Microsequencing of this peptide by collision induced dissociation (tandem MS/MS) confirmed the sequence, thus identifying the primary SspB processing site (data not shown).

SspB-activated chemerin triggers a functional response in CMKLR1-positive cells

We characterized the bioactivity of the two SspB-generated chemerin isoforms using in vitro chemotaxis and calcium mobilization assays. Chem16 is a potent chemoattractant, eliciting a robust, dose-dependent chemotactic response in CMKLR1/L1.2 transfectants. Just 4 pM of chem16 was sufficient to induce a detectable migratory response, with 1 nM eliciting the maximal amplitude of migration (Fig. 4A). Chem16 also triggers intracellular calcium mobilization (Fig. 4B) in a dose-dependent fashion. In contrast, the bioactivity profile of chem15 was similar to the relatively inactive prochemerin in both chemotaxis and calcium flux assays (Fig. 4). Chem15 is a poor chemoattractant, requiring concentrations over 6000 times higher than chem16 to elicit the same magnitude of response (Fig. 4A). Taken together, these data suggest that SspB-mediated prochemerin cleavage results in generation of the biologically active chem16 form, which is then further truncated to an inactive chem15 form. To characterize any inhibitory effect chem15 may have on chem16, CMKLR1/L1.2 transfectants were simultaneously exposed to both isoforms. Chem15 did not inhibit the chemotactic response to chem16, even at a molar ratio of 500:1, respectively (Fig. 4C). We conclude that chem15 exerts biological effects on CMKLR1-expressing cells despite the presence of the truncated 15 kDa form.

We next tested to see whether SspB-activated chemerin could act as a chemoattractant for human blood plasmacytoid dendritic cells, a circulating leukocyte population that expresses CMKLR1 and is chemerin responsive (8). In in vitro transendothelial migration assays, pDC migrated significantly to picomolar concentrations of SspB-activated chemerin (mean 9.5 ± 3.5% SEM input migration to 16–64 pM chem16 vs 0.2 ± 0.1% background migration) (Fig. 5). pDC also migrated well to nanomolar concentrations of the general leukocyte attractant CXCL12 (SDF1) (19.6 ± 7.2%). SspB-activated chemerin may therefore contribute to pDC influx to sites of S. aureus infection.

S. aureus strains isolated from clinical patients activate chemerin

To determine whether SspB can serve as the pathophysiologic trigger of chemerin activity, we evaluated sets of clinical S. aureus isolates for their ability to activate prochemerin. These included isolates from patients suffering from sepsis, septic arthritis and atopic dermatitis. The selected bacterial strains displayed proteolytic activity as evidenced by zymographic analysis of the growth medium (Fig. 6A). The effect of clinical isolates on the generation of active chemerin was compared with the effects of the widely used laboratory strains 8325-4 and COL. As demonstrated in Fig. 6B, chemerin activity was triggered by all three clinical isolates tested, as well as by strain 8325-4, but not by the laboratory strain COL. Strain 8325-4 was the most potent in inducing prochemerin activation, which is consistent with its high-level of proteolytic activity (18). Likewise, the lack of prochemerin activation by the COL strain can be explained by the negligible SspB proteolytic activity (18).
activity (Fig. 6A). These data indicate that SspB may be involved in generating a chemotactic gradient of chemerin at sites of S. aureus infection in humans.

SspB activates endogenous human plasma chemerin
Plasma represents a significant reservoir for proform chemerin in vivo, where it circulates at low nanomolar concentrations (3). Because plasma contains a high concentration of protease inhibitors (10% of total protein content), to evaluate the pathophysiologic relevance of chemerin activation by SspB it was important to determine whether or not SspB can process prochemerin in the presence of plasma inhibitors. As shown in Fig. 7, incubation of purified SspB with human plasma resulted in prochemerin activation, suggesting that the chemerin-activating potential of SspB is not blocked by plasma protease inhibitors. Moreover, the chemotactic effect of SspB-treated plasma on CMKLR1-expressing cells was comparable to the effect of serum (Fig. 7), which contains high levels of active chemerin that results from processing by serine proteases activated during clotting/fibrinolysis (9).

Discussion
In this work, we show that SspB, an S. aureus-derived cysteine protease, is a potent chemerin activating enzyme. The predicted three-dimensional structure of chemerin suggests that it is related to the cystatin family (3, 7). The physiologic role of cystatins as inhibitors of cysteine proteases is exerted through tight complex formation with target substrates (29). Thus, the homology shared between cystatin and prochemerin is predictive of interactions between prochemerin and cysteine proteases. Initially, we tested to see whether prochemerin could function as a cystatin, but found that it did not display significant inhibitory activity toward human cysteine proteases (cathepsins) (data not shown). We did, however, discover that a specific bacterial-derived cysteine protease interacts directly with prochemerin, as described in this report. This interaction does not result in inhibition of SspB proteolytic activity (data not shown) but, instead, results in activation of chemerin. It will be interesting to see whether host cysteine proteases, or other pathogen-derived cysteine proteases, are similarly able to activate chemerin.

Several lines of evidence from our present work indicate that SspB may have a physiologically relevant role in chemerin activation. First, endogenous SspB released into S. aureus growth medium activates chemerin, suggesting that SspB triggers the chemerin chemotactic activity in the context of other S. aureus secreted enzymes. This is important, because high levels of proteolytic activity often result in protein cleavage at multiple sites, leading to nonspecific protein degradation. Second, SspB generates active chemerin with a six amino acid C-terminal truncation. This particular isoform is identical with an endogenous active human chemerin isoform isolated from ascites fluid (7). Third, the concentration of host proteases known to activate chemerin, including factor XII and VII, as well as plasmin, are in the range of $10^{-7}$–$10^{-5}$ M (9). Therefore, the effect of SspB appears to be pathophysiologically significant because at the concentration of

![FIGURE 5.](image)

**FIGURE 5.** SspB-activated chemerin is a potent chemoattractant for human blood pDC. Transendothelial migration was investigated using Transwell inserts coated with HUVEC monolayers. Total PBMC were tested and the migrated cells were collected and stained. pDC were identified as Lin-CD123+CD11c- cells. A total of 20 nM CXCL12 was used as a positive control. The optimal concentration of chem16 for eliciting pDC chemotaxis was 16–64 pM. The percent input migration of pDC is displayed (mean ± SEM), n = 7 experiments with three different donors; *p < 0.05 in pairwise comparisons with background migration “(-)” no chem by Mann-Whitney rank sum test.

![FIGURE 6.](image)

**FIGURE 6.** Clinical isolates of S. aureus activate chemerin. Growth medium was harvested from cultures of S. aureus derived from the indicated anatomical sites from patients with sepsis, bone infection, or atopic dermatitis, or from the standard laboratory strains 8325 and COL. The growth medium was then tested by gelatin zymography (A) or incubated with 160 nM recombinant chemerin for 10 min at 37°C (B). A. Protease activity of indicated strains is visualized using a gelatin-containing gel. The position of SspB is indicated. Two bands represent different maturation products of SspB (18). B, CMKLR1/L1.2 migration was assessed by in vitro transwell chemotaxis, after diluting the samples 1/15 with chemotaxis medium. The mean from duplicate wells of two experiments ± range is shown.

![FIGURE 7.](image)

**FIGURE 7.** SspB activates human plasma chemerin in the presence of endogenous plasma protease inhibitors. Human plasma was collected in the presence of sodium citrate and incubated (1:1 v/v) with 1 × 10⁻³ M purified SspB at 37°C for 10 min. Matched serum isolated from the same donor was also collected. Plasma treated with SspB, and an equivalent volume of plasma or serum, was diluted 1/15 into chemotaxis medium, and then tested in a chemotaxis assay with CMKLR1/L1.2 and L1.2 cells. The mean from duplicate wells of two experiments ± range is shown.
I × 10^{-7} M, SspB is a very effective trigger of chemerin activation in plasma, yielding chemotactic activity comparable to that generated by all combined host proteases released during blood coagulation and fibrinolysis. Fourth, active chemerin accumulates in SspB-treated plasma despite an abundance of various inhibitors that keep host-derived proteases under the tight control. Importantly, host proteases implicated thus far as chemerin activators, such as neutrophil elastase, cathepsin G, plasmin, urokinase-type plasminogen activator, and tissue plasminogen activator (9, 10) would be expected to be opposed by plasma inhibitors, including α1-protease inhibitor, α1-antichymotrypsin, α2-antiplasmin, and plasminogen activator inhibitor (30–32). At early stages of acute inflammation, the protease-antiprotease balance likely shifts in favor of proteolysis. However, as the inflammatory response continues, the plasma concentration of protease inhibitors increases (30, 31). Therefore, at later stages of the inflammatory response or during ongoing inflammation, host proteases are neutralized by plasma inhibitors. In contrast to host proteases, SspB remains active in plasma, suggesting that it can serve as a chemerin activator at any stage of the inflammatory response. Furthermore, SspB may contribute to chemerin processing during skin and soft tissue infections associated with significant edema, where plasma infiltrate is likely to be a primary source of prochemerin.

Collectively, our findings support the view that S. aureus-derived SspB, through the generation of active chemerin, may play a role in the selective recruitment of immature pDC and/or macrophages to S. aureus-infected inflammatory sites. Although SspB activity at S. aureus-infected sites has yet to be quantified, functional SspB is likely to be present within these lesions. Notably, inactivation of SspB and its processing enzyme, the V8 protease, results in attenuation of S. aureus virulence (18, 33). Thus while chemerin processing by SspB may provide a host distress “SOS signal” to initiate pDC and/or macrophage-mediated immunologic reactions, it is more likely to be an ongoing contributing factor to the pathological outcome of staphylococcal infections. S. aureus infection could lead to the chronic and excessive generation of local levels of active chemerin, resulting in the accumulation of immature pDC and macrophages and establishing a microenvironment permissive for chronic inflammation.

Although several theories have been put forward, it remains unclear why the host defense response is ineffective in clearing chronic S. aureus infections and how the organism contributes to the maintenance of a chronic inflammatory state (34). In this context, it is relevant that the two major cell subtypes that express CMKLR1, immature pDC and macrophages, are both multifunctional cell types that can contribute both to the generation and suppression of pathologic inflammation. Although a comprehensive analysis of pDC function remains incomplete, current data suggest that pDC may occupy a central stage as regulators of immune responses, able to modulate the functions of cells involved in both innate and acquired immunity, including NK, myeloid dendritic cells as well as T and B cells (35). Moreover, immature pDC can differentiate either into proinflammatory APC, capable for example of producing large amounts of type I IFNs in response to viral and microbial stimuli (36), or into potent inducers of T regulatory cells, thus suppressing local immune responses (37–39). Immature pDC circulate normally in blood and lymphoid organs, and are recruited to peripheral tissues during inflammation. Indeed, recent findings point to pDC as important contributors to chronic immune-based disorders such as lupus erythematosus and psoriasis (40–43). Similarly, macrophages are emerging as malleable immune regulatory cells, associated with chronic inflammatory conditions, including atopic dermatitis (44). Thus the cells recruited by active chemerin are capable of complex regulation of immune responses. One possibility therefore is that S. aureus recruits these cells and manipulates their immunoregulatory potential to its own survival advantage. This would be consistent with the behavior of S. aureus to both initiate and maintain a state of chronic pathologic inflammation.

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Disclosures

The authors have no financial conflict of interest.

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