Title
Hyaluronan Degradation by Cemip Regulates Host Defense against Staphylococcus aureus Skin Infection.

Permalink
https://escholarship.org/uc/item/3d52h4z7

Journal
Cell reports, 30(1)

ISSN
2211-1247

Authors
Dokoshi, Tatsuya
Zhang, Ling-Juan
Li, Fengwu
et al.

Publication Date
2020

DOI
10.1016/j.celrep.2019.12.001

Peer reviewed
Hyaluronan Degradation by Cemip Regulates Host Defense against Staphylococcus aureus Skin Infection

Tatsuya Dokoshi1, Ling-juan Zhang1, Fengwu Li1, Teruaki Nakatsuji1, Anna Butcher1, Hiroyuki Yoshida2, Masayuki Shimoda3, Yasunori Okada4, Richard L. Gallo1,5,*

1Department of Dermatology, University of California, San Diego, La Jolla, CA 92037, USA
2Biological Science Research, Kao Corporation, Odawara-shi, Kanagawa, Japan
3Department of Pathology, Keio University School of Medicine, Tokyo, Japan
4Department of Pathophysiology for Locomotive and Neoplastic Diseases, Juntendo University Graduate School of Medicine, Tokyo, Japan
5Lead Contact

SUMMARY

Staphylococcus aureus is a major human bacterial pathogen responsible for deep tissue skin infections. Recent observations have suggested that rapid, localized digestion of hyaluronic acid in the extracellular matrix (ECM) of the dermis may influence bacterial invasion and tissue inflammation. In this study we find that cell migration-inducing protein (Cemip) is the major inducible gene responsible for hyaluronan catabolism in mice. Cemip−/− mice failed to digest hyaluronan and had significantly less evidence of infection after intradermal bacterial challenge by S. aureus. Stabilization of large-molecular-weight hyaluronan enabled increased expression of cathelicidin antimicrobial peptide (Camp) that was due in part to enhanced differentiation of preadipocytes to adipocytes, as seen histologically and by increased expression of Pref1, PPARγ, and Adipoq. Cemip−/− mice challenged with S. aureus also had greater IL-6 expression and neutrophil infiltration. These observations describe a mechanism for hyaluronan in the dermal ECM to regulate tissue inflammation and host antimicrobial defense.

In Brief

In this paper, Dokoshi et al. describe how the mammalian hyaluronidase Cemip is induced in the dermis during S. aureus infection. Cemip digests hyaluronan in the skin to regulate reactive adipogenesis and subsequent antimicrobial activity and skin inflammation.

This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Correspondence: rgallo@ucsd.edu.

AUTHOR CONTRIBUTIONS

T.D. and R.L.G. designed and performed experiments, analyzed data, and wrote the paper. F.L. designed and performed experiments and analyzed data. H.Y. designed experiments and analyzed data. M.S. and Y.O. provided Cemip−/− mice.

DECLARATION OF INTERESTS

H.Y. is an employee of Kao Corporation (Odawara-shi, Kanagawa, Japan). R.L.G. is a co-founder, a scientific adviser, a consultant, and an equity holder in MatriSys Biosciences and is a consultant for, receives income from, and has equity in Sente.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.celrep.2019.12.001.
INTRODUCTION

Staphylococcus aureus (S. aureus) and group A Streptococcus (GAS) are the major bacterial pathogens responsible for invasive infections of human skin. The host immune response to these pathogens remains incompletely defined. The majority of research has focused on mechanisms to limit invasion of these bacteria by the actions of resident and recruited immunocytes as well as the innate antimicrobial functions of the epidermis. However, upon disruption of the epidermal barrier, S. aureus or GAS encounters a very different physical environment in the dermal extracellular matrix (ECM). As a consequence, the virulence of these pathogens includes exploitation of ECM components. For example, GAS evades resident leukocyte killing by expressing long chains of hyaluronan (HA) on its surface to mimic the HA-rich ECM in the surrounding environment of the dermis (Cole et al., 2011; Wessels et al., 1991). S. aureus also has adapted to HA and uses its hyaluronidases to facilitate virulence (Ibberson et al., 2014, 2016). Currently, the interplay between bacterial and host HA catabolic systems has left unanswered the central question of how mammalian HA turnover during injury influences microbial resistance. In this study we sought to better understand this host response to infection.

HA is a linear polysaccharide found in the ECM of all vertebrates (Hascall et al., 2004; Toole, 1991). The functions of HA are diverse, as it is necessary for mammalian
development and migration and also serves important functions in cancer and other diseases (Toole et al., 2002). Consistent with the important function of HA, the synthesis and degradation of this polysaccharide is strictly regulated and in constant dynamic equilibrium (Laurent and Fraser, 1992). A family of mammalian HA synthases and hyaluronidases are used in a cell- and tissue-specific manner to regulate tissue HA content (Erickson and Stern, 2012). Importantly, upon injury, HA is rapidly degraded, and this catabolic reaction results in important changes in the local immune response (Noble, 2002; Taylor et al., 2007a). HA fragments interact with Toll-like receptor 4 to activate cell responses during injury and have been proposed to act as a way to complement pathogen detection mechanisms (Taylor et al., 2004). Bacterial hyaluronidases such as HysA expressed by S. aureus degrade HA differently than the mammalian hyaluronidases and thus generate alternative products with distinct functions (Ibberson et al., 2016). Importantly, upon injury, HA is rapidly degraded, and this catabolic reaction results in important changes in the local immune response (Noble, 2002; Taylor et al., 2007a). HA fragments interact with Toll-like receptor 4 to activate cell responses during injury and have been proposed to act as a way to complement pathogen detection mechanisms (Taylor et al., 2004). Bacterial hyaluronidases such as HysA expressed by S. aureus degrade HA differently than the mammalian hyaluronidases and thus generate alternative products with distinct functions (Ibberson et al., 2016). However, despite the important role of HA during injury, the mechanism responsible for local regulation of HA turnover and its contribution to host defense against infection has been unknown.

Prior attempts to evaluate the function of previously defined mammalian hyaluronidases had not identified the gene responsible for mediating this critical event following infection of the skin. Cell migration-inducing protein (Cemip), alias HA-binding protein involved in HA depolymerization (HYBID) and KIAA1199, has been recently observed to have functional consequences in functions including deafness, bone growth, fibrosis, and tumor invasion (Shimoda et al., 2017; Tang et al., 2019; Yoshida et al., 2013; Yoshino et al., 2017, 2018). In this study we hypothesized that Cemip may initiate HA breakdown during deep tissue infection by S. aureus and could be used to address the role of HA turnover in host defense. Our observations show that Cemip is a critical mammalian hyaluronidase and further show how regulation of this ECM component is a key regulator of innate antimicrobial defense by the dermis.

RESULTS
Cemip Digests Dermal HA during S. aureus Skin Infection

We examined the expression of Cemip in mice following inoculation of S. aureus into the dermis to test if this enzyme may be the hyaluronidase responsible for HA degradation during skin injury. S. aureus was chosen as the model skin pathogen over GAS because it does not synthesize HA itself but does produce a secreted hyaluronidase that confers virulence (Ibberson et al., 2014). S. aureus infection significantly increased Cemip mRNA in whole skin, but the expression of other murine hyaluronidases (Hyal1, Hyal2, Hyal3, and transmembrane protein 2 [TMEM2]) were unchanged (Figure 1A). Immunohistochemical analysis of locally infected tissue showed that Cemip was increased in regions where HA staining was decreased (Figure 1B). Cemip−/− mice failed to show an increase of Cemip mRNA and had a greater amount of HA in the dermis following skin infection (Figures 1B–1D). Furthermore, the decrease in the size of HA that occurs following infection was abolished in Cemip−/− mice (Figure 1E, lanes c and d). Mast cell-deficient mice had less Cemip expression, a finding consistent with a role of histamine in the induction of Cemip (Figure S1). These observations demonstrate that Cemip promotes digestion of HA in the skin during infection by S. aureus.
Loss of Cemip Increases Resistance against *S. aureus*

To evaluate the functional significance of HA digestion by Cemip, tissue injury and *S. aureus* survival were measured in the skin of Cemip−/− mice. Three days after *S. aureus* injection, necrotic lesions on Cemip−/− mice were significantly smaller (Figures 2A and 2B), and fewer live bacteria were evident in the skin (Figures 2C and 2D). Fewer bacteria were also detected in spleens from Cemip−/− mice (Figure 2E). Because the expression of the cathelicidin antimicrobial peptide Camp is strongly associated with resistance to bacterial skin infections (Nizet et al., 2001), we next assessed the relative expression of Camp in the skin of these mice. mRNA for Camp was significantly increased in tissue biopsies from the infected site of Cemip−/− mice (Figure 2F), and more cathelicidin protein was observed in tissue surrounding the infected area of the dermis (Figures 2G–2I). These observations suggest that loss of Cemip function enabled increased Camp expression.

Loss of Cemip Enhances Reactive Adipogenesis

We have recently shown that a major source of cathelicidin expression in the skin comes from the local differentiation of preadipocyte fibroblasts into adipocytes, a process we refer to as reactive adipogenesis (Zhang et al., 2015). Degradation of HA inhibits the capacity of preadipocytes to differentiate into mature adipocytes (Dokoshi et al., 2018; Ji et al., 2014). Therefore, we hypothesized that the digestion of HA by Cemip may inhibit the local adipogenic response and thus suppress the expression of the antimicrobial peptide by these cells. Histological evaluation of the deep dermis showed a greater expansion of subcutaneous white adipose tissue (DWAT) after infection in Cemip−/− mice (Figures 3A and 3B). Consistent with the observation of enhanced reactive adipogenesis by decreasing hyaluronidase activity, there was also significantly increased expression of genes associated with adipogenesis in Cemip−/− mice (preadipocyte factor 1 [Pref-1], peroxisome proliferator-activated receptor gamma [PPARg], and adiponectin) (Figures 3C–3E). Fluorescence-activated cell sorting (FACS) analysis of skin before and after *S. aureus* infection also showed an increase in the population of preadipocytes in the dermis of Cemip−/− mice as defined by CD31-negative, CD45-negative, platelet-derived growth factor receptor-α (PDGFRα)-positive, and spinocerebellar ataxia type 1 (SCA1)-positive cells (Figures 3F and 3G). Taken together, these data show that loss of Cemip results in an increase in dermal reactive adipogenesis.

Loss of Cemip Enhances the Inflammatory Response to Infection

The expression of Camp and other products of reactive adipogenesis can influence inflammation that may amplify the host defense against *S. aureus* (Hancock et al., 2016; Zhang et al., 2016). Therefore, we also investigated the influence of Cemip on resident and circulating lymphoid populations. FACS analysis of resident skin lymphoid cells revealed that Cemip−/− mice had differences in the relative abundance of CD11c dendritic cells, LY6-G neutrophils, and F4/80/Ly6-C monocytes (Figures 4A–4F). Interestingly, under baseline conditions, dendritic cells and neutrophils were both slightly elevated in Cemip−/− mice. Following infection, Cemip−/− mice had relatively fewer dendritic cells and higher numbers of the CD11b, LY6-G, and F4/80/Ly6C positive populations. Neutrophils are a critical cell type for resistance to *S. aureus* infection, and increased numbers of LY6G-positive
neutrophils were evident by immunohistochemistry (Figure 4G), and an increase in IL-6 mRNA as measured by qPCR (Figure 4H) was detected in the skin of S. aureus-infected Cemip−/− mice. We also evaluated systemic responses in Cemip−/− mice infected by S. aureus. Cemip−/− mice showed significantly lower fractions of T-box transcription factor (Tbet)+, retinoid-related orphan receptor gamma T (RORgt)+, interferon gamma (IFNg)+, and IL-17+ T cells in the spleen (Figures S2A–S2F). Overall, the loss of Cemip expression resulted in an enhanced local inflammatory response and decreased systemic inflammatory response after S. aureus infection.

**DISCUSSION**

The results of this study highlight the recently appreciated role of the ECM and resident, non-lymphoid cells in the dermis to play an important role in host defense against bacterial infection. Large-molecular weight HA is highly abundant and is the major component of the ECM (Tammi et al., 1994). HA digestion into small fragments after injury has been shown to have important implications for inflammatory responses in vivo (Jiang et al., 2005; Muto et al., 2014; Noble et al., 1996; Taylor et al., 2004) and can modify infection by GAS through digestion of the HA-rich bacterial capsule of this organism (Schommer et al., 2014). However, a clear understanding of the mechanism responsible for HA catabolism or its connection to host antimicrobial defense has not been previously defined. In this study, we show that Cemip is responsible for the increase in endogenous hyaluronidase activity seen during deep tissue infection. These observations provide important new insight into the mechanisms that function in the dermis to resist invasive S. aureus infection.

Several complementary experimental observations made here support the conclusion that Cemip digests HA during skin infection. These include observations of increased transcript abundance, increased protein abundance, and decreased large–molecular weight HA corresponding to the timing, localization, and hyaluronidase activity of Cemip. There was no evidence of an increase in the expression of other hyaluronidases such as Hyal1, Hyal2, Hyal3, and TMEM2. HYAL4 has chondroitinase, not hyaluronidase activity (Kaneiwa et al., 2012), and expression of PH-20 is restricted to testes (Cherr et al., 1996), and these were therefore not examined. A loss of hyaluronidase activity was apparent in Cemip−/− mice, as they did not show the decrease in the size of HA after infection that was observed in controls, and they had less loss of total large–molecular weight HA as measured by staining with HA-binding protein or ELISA. It has also been suggested that reactive oxygen species are also involved in HA degradation after tissue injury (Bates et al., 1984). Our observations do not exclude this as an additional mechanism, or that other enzymes contributed by the host or the bacteria themselves could be also contributing to the turnover in HA. On the contrary, as it has been estimated that an adult human contains 15 g of HA and that about one-third turns over daily (Tammi et al., 1991), it is very likely that other hyaluronidases are participating in the steady-state turnover of HA. Furthermore, S. aureus itself can contribute hyaluronidase activity to the site of infection through expression of HysA, an enzyme secreted by the pathogen and associated with virulence (Ibberson et al., 2014). The findings of this report to not exclude contribution of other enzymes to balance of HA but do clearly show that Cemip is responsible for a major fraction of the local increase in HA breakdown that occurs during S. aureus infection.
Further studies are required to better define the cell of origin of Cemip, which originally was discovered in dermal fibroblasts. In preliminary experiments, we have performed single-cell RNA sequencing (RNA-seq) of whole tissue and in vitro analysis of potential candidate cell types that may express Cemip. These studies have not yet convincingly defined a primary cell type responsible for its synthesis but suggest that a fibroblast cell type may be the origin. Cemip has also been shown to be induced by histamine (Yoshino et al., 2018), and as histamine in skin is primarily released by mast cells, we examined if these cells could contribute to the response we observed. Mast cell-deficient mice had somewhat less Cemip but were still able to show increased expression after infection (Figure S2).

The most direct explanation for increased resistance to S. aureus in Cemip−/− mice is the increase in Camp produced by the rapid, local differentiation of preadipocyte fibroblasts to mature fat (Zhang et al., 2015). A persistence of high–molecular weight HA enables this adipogenic response and results in much greater expression of Camp in the dermis at the site of infection. We have previously shown that increased hyaluronidase-1 activity (in contrast with the loss of hyaluronidase activity seen here in Cemip−/− mice) will inhibit reactive adipogenesis and Camp expression (Dokoshi et al., 2018). Our findings described in Figures 2 and 3 show that loss of Cemip enables the skin to respond to S. aureus infection by increasing expression of Camp, expanding DWAT and enhancing gene expression associated with adipogenesis.

The present observations suggest that local induction of hyaluronidase activity has a negative consequence to the host, as it enables greater bacterial proliferation and infection than when Cemip is deleted. Why then has this activity been maintained? We speculate that the presence of inducible hyaluronidase activity serves two complementary purposes. First, prior work has shown that low–molecular weight fragments of HA generated by hyaluronidases are potent danger-associated molecular patterns (DAMPs) and serve to alert the host of injury even under aseptic conditions (Mummert, 2005; Taylor et al., 2007b; Yamasaki et al., 2009). Consistent with the systemic alarmin function of HA fragments as a DAMP, we observed a greatly decreased induction of CD4+ cells expressing Tbet, GATA3, RORγt, INFγ, and 1 IL-17 in Cemip−/− mice following infection. Thus, Cemip may provide one of many systemic signals of injury. A second beneficial consequence of transient, inducible hyaluronidase activity is that the digestion of HA inhibits excess local inflammatory responses. Hyaluronidase expression can inhibit antigen presentation (Muto et al., 2014), thus potentially preventing unwanted allergic sensitization to common antigens that become accessible during injury. Cemip−/− mice studied here had greater IL-6 and neutrophil infiltration, a reaction that may have helped fight infection. However, the immunological consequences to infection in Cemip−/− mice are complicated, as a lower bacterial burden and higher Camp expression can indirectly alter local innate and adaptive immune responses. Furthermore, HA itself influences many different aspects of cell differentiation and migration (West et al., 1985). Major clinical developmental phenotypes result from mutations in hyaluronidases such as mucopolysaccharidosis type IX from Hyal1 (Natowicz et al., 1996), bone defects, and cardiopulmonary dysfunction from loss of Hyal2 (Jadin et al., 2008; Chowdhury et al., 2013). Deafness and other abnormalities are associated with defects in HYBID (Shimoda et al., 2017; Tang et al., 2019; Yoshida et al., 2013; Yoshino et al., 2017, 2018). Thus, as is frequently the case, this gene has several essential
functions that extend beyond the immune defense role we have defined here. Past and current findings suggest that the influence of HA turnover on host defense functions is complex and likely acts in multiple ways beyond direct antimicrobial activity.

In summary, this work has solved the long unanswered question of what induces digestion of HA in the dermis after infection. This further clarifies the molecular steps necessary for the dermis to resist deep tissue infection by *S. aureus*. HA is an essential component of ECM of many organs and often exploited by microbes through molecular mimicry. The fundamental roles of elements of the ECM in immune defense are specific to context and may vary by organ and microorganism. Understanding how the skin initiates the digestion of HA can have important diagnostic and therapeutic implications for many infectious and inflammatory diseases.

**STAR METHODS**

**LEAD CONTACT AND MATERIALS AVAILABILITY**

This study did not generate new unique reagents. Further information and requests for plasmids, resources, and reagents should be directed to and will be fulfilled by the Lead Contact, Richard L. Gallo (rgallo@ucsd.edu).

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

**Animals and animal care**—Cemip (KIAA1199) KO mice were generated using a gene targeting Cre-loxP system as described in the other report (Shimoda et al., 2017; Yoshino et al., 2017). Wild-type mice (C57BL/6 mice) and K14-cre transgenic mice were obtained from The Jackson Laboratory. Scf.ifie mice (a gift from Dr. Dinald at University of California San Diego). K14-cre transgenic mice were bred with Scf.ifie mice for the generation of K14-cre Scf.ifie mice. K14-cre littermate controls were used in all experiments. All animal experiments were approved by the University of California, San Diego, Institutional Animal Care and Use committee. For all animal studies, animals were randomly selected without formal pre-randomization and quantitative measurements were done without the opportunity for bias.

**Bacterial strains**—*S. aureus* strain USA300 is a predominant community-associated Methicillin-resistant *S. aureus* (MRSA) strain and AH4807, a USA300 MRSA strain was tested in a manner that was similar to previously described (Muhs et al., 2017; Paharik et al., 2017), was kindly provided by Alexander Horswill (Department of Immunology & Microbiology at the University of Colorado).

**Mouse model of *S. aureus* skin infection**—Skin infection experiments were done as described before (Nizet et al., 2001). *S. aureus* strain USA300 was used for infection. In brief, the backs of sex-matched and age-matched (8 week to 12 week) adult wild-type or Ella/Hyal1 mice were shaved and hair removed by chemical depilation (Nair) then injected subcutaneously with 100 μL of a mid-logarithmic growth phase of *S. aureus* (2×10^6 CFU of bacteria) in PBS. Mice were sacrificed after day 3 and 8 mm skin punch biopsy comprising the center of the injection site was harvested. Infected skin surrounding the infection center.
(6–8 mm) void of center abscess was carefully dissected out for RNA extraction or CFU determination. Skin biopsies were homogenized in 1 mL Trizol (for RNA) or PBS (for CFU counting) with 2 mm zirconia beads in a mini-bead beater 16 (Biospect, Bartlesville, OK). To count CFU, homogenized skin samples were serially diluted, plated onto Tryptic Soy Agar, and enumerated after 18 hours to quantify the CFU per gram of tissue. For in vivo live bacterial imaging, mice were imaged under isoflurane inhalation anesthesia (2%). Photons emitted from luminescent bacteria were collected during a 1 min exposure using the Xenogen IVIS Imaging System and living image software (Xenogen, Alameda, CA). Bioluminescent image data are presented on a pseudocolor scale (blue representing least intense and red representing the most intense signal) overlaid onto a gray-scale photographic image. Using the image analysis tools in living image software, circular analysis windows (of uniform area) were overlaid onto regions of interest and the corresponding bioluminescence values (total flux) were measured.

**Study approval**—All animal experiments were approved by the University of California, San Diego, Institutional Animal Care and Use committee. For all animal studies, animals were randomly selected without formal pre-randomization and quantitative measurements were done without the opportunity for bias.

**METHOD DETAILS**

**Chemicals and reagents**

*Rat anti-Cemip antibodies were provided by KAO company*: Rabbit anti-CAMP antibodies were made from our lab as described previously (Dorschner et al., 2001); rabbit anti-PREF1/DLK antibodies are from Abcam (Cambridge, MA); BODIPY® FL dye was purchased from Thermo Fisher (Houston, TX). HA binding protein was purchased from Millipore., mouse-Hyal1, Hyal2, KIAA1199, TMEM2, HAS1, HAS2, HAS3, ZFP423, Pref1, PPARg, Adipoq, CEBPA, CAMP, IL6, TNF Taqman gene expression assay were purchased from Life Technologies Corporation (Grand Island, NY).

**Reverse transcription-quantitative PCR (RTqPCR) analyses**—RTqPCR was used to determine the mRNA abundance as described previously (Morioka et al., 2008). Total cellular RNA was extracted using the PureLink RNA Mini Kit (Life Technologies Corporation, Grand Island, NY) and mRNA were purified by using Dynabeads mRNA Purification Kit(Life technologies). 100 ng of mRNA was reverse transcribed to cDNA using iScript cDNA synthesis kit (Bio-Rad Laboratories, Inc. Hercules, CA). Quantitative, real-time PCR was performed on the CFX96 real time system (Biorad) using predeveloped Taqman gene expression assay (Applied Biosystems). The expression of β-Actin gene was used as a house keeping gene to normalize data.

**Histology and immunohistochemistry (IHC)**—Tissue biopsies were directly embedded in OCT compound or paraffin. Paraffin embedded tissues are used for Hematoxylin/Eosin (H&E) staining, and frozen sections were fixed in 4% PFA for 20 mins or 100% acetone prior to immunofluorescence staining. For IHC, fixed and permeabilized frozen tissue sections were blocked with Image-iT FX reagent (Invitrogen) before incubating with primary antibodies followed by appropriate 488- or 568-coupled secondary antibodies.
Nuclei were counterstained with DAPI. All images were taken with an Olympus BX41 microscope (widefield) or Zeiss LSM510 confocal microscope as indicated.

**Flow cytometry analyses**—Colon collected from control or DSS-treated mice was cut into small pieces then digested with 2.5 mg/mL Collagenase D and 30 ng/mL DNase1 for 2 hours at 37°C then filtered through a 30 μm filter to generate single cell suspension for FACS analyses. Cells were then stained with zombie violet viability dye (BioLegend, 423114), blocked with anti-mouse CD16/32 (eBioscience, 14016185), followed by staining with antibody cocktails for preadipocytes or immune cells. The antibody cocktail for preadipocytes includes AF488-SMA (eBioscience, 53976082), PECy7-CD45 (BioLegend, 147704), PerCy5.5-CD31 (BioLegend, 102522), PE-Thy1 (BioLegend, 105308), APC-PDGFRa (eBioscience, 17140181), BV605-SCA1 (BioLegend, 108133) and AF700-CD24 (BioLegend, 108136). The antibody cocktail for immune cells includes PECy7-CD11b (BioLegend, 101216), FITC-Ly6G (eBioscience, 11593182), PE-F4/80 (eBioscience, 12480182), APC-CD11c (BioLegend, 117310), AF700-MHCII (eBioscience, 5632182), APC-Cy7-CD3 (BioLegend, 100222), Tbet (Fisher Scientific, 562467), GATA3 (BioLegend, 653807), RORgt (eBioscience, 12–6981-80), IFN-gamma (BioLegend, 505809), IL-17 (BioLegend, 506929), Foxp3 (eBioscience, 48–5773-80) and Fixable Viability Dye eFluor 506 (eBioscience, 65–0866-14)FACS analyses for surface expression of preadipocyte or immune cell markers were performed by the BD FACSCanto RUO machine and analyzed by FlowJo V10 software. Dead cells stained positive with zombie violet dye were excluded from the analyses.

**Hyaluronan (HA) analysis**—Glycosaminoglycan (GAGs), including HA were extracted from murine skin as previously described (Muto et al., 2014). Samples were homogenized and treated overnight with protease (0.16 mg/ml; Sigma-Aldrich) to degrade protein, followed by purification by anion exchange chromatography using DEAE Sephacel (Amersham Biosciences). Columns were washed with a low-salt buffer (0.15 M NaCl in 20 mM sodium acetate; pH 6.0) and eluted with 1 M NaCl. Glycans were desalted by PD10 (GE Healthcare). HA concentrations were measured ELISA Duo Set (R&D Systems). The size distribution of HA was analyzed by agarose gel electrophoresis (Lee and Cowman, 1994). The HA sample was mixed with TAE buffer containing 2 M sucrose and electrophoresed at 2 V/cm for 10 hours at room temperature. The gel was stained overnight under light-protective cover at room temperature in a solution containing 0.005% Stains-All in 50% ethanol, and destained in water. Hyalose ladders (Hyalose) were used for standards.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Experiments were repeated at least three times with similar results. Statistical significance was determined using Student’s unpaired two-tailed t test, or one-way ANOVA multiple comparison test as indicated in the legend (*p < 0.05, **p < 0.01, ***p < 0.001).

**DATA AND CODE AVAILABILITY**

The published article includes all datasets generated or analyzed during this study.
Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

T.D. and R.L.G. were funded by NIH grant P01HL107150. L.Z., T.N., and R.L.G. are funded by NIH grants R01AR069653 and R37AI052453.

REFERENCES

Bates EJ, Harper GS, Lowther DA, and Preston BN (1984). Effect of oxygen-derived reactive species on cartilage proteoglycan-hyaluronate aggregates. Biochem. Int 8, 629–637. [PubMed: 6548142]
Cherr GN, Meyers SA, Yudin AI, VandeVoort CA, Myles DG, Primak-off P, and Overstreet JW (1996). The PH-20 protein in cynomolgus macaque spermatozoa: identification of two different forms exhibiting hyaluronidase activity. Dev. Biol 175, 142–153. [PubMed: 8608861]
Chowdhury B, Hemming R, Hombach-Klonisch S, Flamion B, and Triggs- Raine B (2013). Murine hyaluronidase 2 deficiency results in extracellular hyaluronan accumulation and severe cardiopulmonary dysfunction. J. Biol. Chem 288, 520–528. [PubMed: 23172227]
Cole JN, Barnett TC, Nizet V, and Walker MJ (2011). Molecular insight into invasive group A streptococcal disease. Nat. Rev. Microbiol 9, 724–736. [PubMed: 21921933]
Dokoshi T, Zhang LJ, Nakatsuji T, Adase CA, Sanford JA, Paladini RD, Tanaka H, Fujiya M, and Gallo RL (2018). Hyaluronidase inhibits reactive adipogenesis and inflammation of colon and skin. JCI Insight 3, 123072. [PubMed: 30385720]
Dorschner RA, Pestonjamasp VK, Tamakuwala S, Ohtake T, Rudisill J, Nizet V, Agerberth B, Gudmundsson GH, and Gallo RL (2001). Cutaneous injury induces the release of cathelicidin anti-microbial peptides active against group A Streptococcus. J. Invest. Dermatol 117, 91–97. [PubMed: 11442754]
Erickson M, and Stern R (2012). Chain gangs: new aspects of hyaluronan metabolism. Biochem. Res. Int 2012, 893947. [PubMed: 22216413]
Hancock RE, Haney EF, and Gill EE (2016). The immunology of host defence peptides: beyond antimicrobial activity. Nat. Rev. Microbiol 16, 321–334. [PubMed: 27087664]
Hascall VC, Majors AK, De La Motte CA, Evanko SP, Wang A, Drazba JA, Strong SA, and Wight TN (2004). Intracellular hyaluronan: a new frontier for inflammation? Biochim. Biophys. Acta 1673, 3–12. [PubMed: 15238245]
Ibberson CB, Jones CL, Singh S, Wise MC, Hart ME, Zurawski DV, and Horswill AR (2014). Staphylococcus aureus hyaluronidase is a CodY-regulated virulence factor. Infect. Immun 82, 4253–4264. [PubMed: 25069977]
Ibberson CB, Parlet CP, Kwiecinski J, Crosby HA, Meyerholz DK, and Horswill AR (2016). Hyaluronan modulation impacts Staphylococcus aureus biofilm infection. Infect. Immun 84, 1917–1929. [PubMed: 27068096]
Jadin L, Wu X, Ding H, Frost GI, Onclinx C, Triggs-Raine B, and Flamion B (2008). Skeletal and hematological anomalies in HYAL2-deficient mice: a second type of mucopolysaccharidosis IX? FASEB J. 22, 4316–4326. [PubMed: 18772348]
Ji E, Jung MY, Park JH, Kim S, Seo CR, Park KW, Lee EK, Yeom CH, and Lee S (2014). Inhibition of adipogenesis in 3T3-L1 cells and suppression of abdominal fat accumulation in high-fat diet-feeding C57BL/6J mice after downregulation of hyaluronic acid. Int. J. Obes 38, 1035–1043.
Jiang D, Liang J, Fan J, Yu S, Chen S, Luo Y, Prestwich GD, Mascarenhas MM, Garg HG, Quinn DA, et al. (2005). Regulation of lung injury and repair by Toll-like receptors and hyaluronan. Nat. Med 11, 1173–1179. [PubMed: 16244651]
Kaneiwa T, Miyazaki A, Kogawa R, Mizumoto S, Sugahara K, and Yamada S (2012). Identification of amino acid residues required for the substrate specificity of human and mouse chondroitin sulfate hydrolase (conventional hyaluronidase-4). J. Biol. Chem 287, 42119–42128. [PubMed: 23086929]
Laurent TC, and Fraser JR (1992). Hyaluronan. FASEB J. 6, 2397–2404. [PubMed: 1563592]
Lee HG, and Cowman MK (1994). An agarose gel electrophoretic method for analysis of hyaluronan molecular weight distribution. Anal. Biochem 219, 278–287. [PubMed: 8080084]

Morioka Y, Yamasaki K, Leung D, and Gallo RL (2008). Cathelicidin antimicrobial peptides inhibit hyaluronan-induced cytokine release and modulate chronic allergic dermatitis. J. Immunol 181, 3915–3922. [PubMed: 18768846]

Muhs A, Lyles JT, Parlet CP, Nelson K, Kavanaugh JS, Horswill AR, and Quave CL (2017). Virulence inhibitors from Brazilian peppertree block quorum sensing and abate dermonecrosis in skin infection models. Sci. Rep 7, 42275. [PubMed: 28186134]

Mummert ME (2005). Immunologic roles of hyaluronan. Immunol. Res 31, 189–206. [PubMed: 15888911]

Muto J, Morioka Y, Yamasaki K, Kim M, Garcia A, Carlin AF, Varki A, and Gallo RL (2014). Hyaluronan digestion controls DC migration from the skin. J. Clin. Invest 124, 1309–1319. [PubMed: 24487587]

Natowicz MR, Short MP, Wang Y, Dickensin GR, Gebhardt MC, Rosenthal DI, Sims KB, and Rosenberg AE (1996). Clinical and biochemical manifestations of hyaluronidase deficiency. N. Engl. J. Med 335, 1029–1033. [PubMed: 8793927]

Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, Pestonjamasp V, Piraino J, Huttner K, and Gallo RL (2001). Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature 414, 454–457. [PubMed: 11719807]

Noble PW (2002). Hyaluronan and its catabolic products in tissue injury and repair. Matrix Biol. 21, 25–29. [PubMed: 11827789]

Noble PW, McKee CM, Cowman M, and Shin HS (1996). Hyaluronan fragments activate an NF-kappa B/I-kappa B alpha autoregulatory loop in murine macrophages. J. Exp. Med 183, 2373–2378. [PubMed: 8642348]

Paharik AE, Parlet CP, Chung N, Todd DA, Rodriguez EI, Van Dyke MJ, Cech NB, and Horswill AR (2017). Coagulase-negative staphylococcal strain prevents Staphylococcus aureus colonization and skin infection by blocking quorum sensing. Cell Host Microbe 22, 746–756.e5. [PubMed: 29199097]

Schommer NN, Muto J, Nizet V, and Gallo RL (2014). Hyaluronan breakdown contributes to immune defense against group A Streptococcus. J. Biol. Chem 289, 26914–26921. [PubMed: 25122767]

Shimoda M, Yoshida H, Mizuno S, Hirozane T, Horiuichi K, Yoshino Y, Hara H, Kanai Y, Inoue S, Ishijima M, and Okada Y (2017). Hyaluronan-binding protein involved in hyaluronan depolymerization controls endochondral ossification through hyaluronan metabolism. Am. J. Pathol 187, 1162–1176. [PubMed: 28284715]

Tammi R, Säämänen AM, Maibach HI, and Tammi M (1991). Degradation of newly synthesized high molecular mass hyaluronan in the epidermal and dermal compartments of human skin in organ culture. J. Invest. Dermatol 97, 126–130. [PubMed: 2056182]

Tammi R, Agren UM, Tuukkanen AL, and Tammi M (1994). Hyaluronan metabolism in skin. Prog. Histochem. Cytochem 29, 1–81.

Tang Z, Ding Y, Shen Q, Zhang C, Li J, Nazar M, Wang Y, Zhou X, and Huang J (2019). KIAA1199 promotes invasion and migration in non-small-cell lung cancer (NSCLC) via PI3K-Akt mediated EMT. J. Mol. Med. (Berl.) 97, 127–140. [PubMed: 30478628]

Taylor KR, Trowbridge JM, Rudisill JA, Termeer CC, Simon JC, and Gallo RL (2004). Hyaluronan fragments stimulate endothelial recognition of injury through TLR4. J. Biol. Chem 279, 17079–17084. [PubMed: 14764599]

Taylor KR, Yamasaki K, Radek KA, Di Nardo A, Goodarzi H, Golenbock D, Beutler B, and Gallo RL (2007a). Recognition of hyaluronan released in sterile injury involves a unique receptor complex dependent on TLR4, CD44 and MD-2. J. Biol. Chem 282, 18265–18275. [PubMed: 17400552]

Taylor KR, Yamasaki K, Radek KA, Di Nardo A, Goodarzi H, Golenbock D, Beutler B, and Gallo RL (2007b). Recognition of hyaluronan released in sterile injury involves a unique receptor complex dependent on Toll-like receptor 4, CD44, and MD-2. J. Biol. Chem 282, 18265–18275. [PubMed: 17400552]

Toole BP (1991). Proteoglycans and Hyaluronan in Morphogenesis and Differentiation (Plenum).
Toole BP, Wight TN, and Tammi MI (2002). Hyaluronan-cell interactions in cancer and vascular disease. J. Biol. Chem 277, 4593–4596. [PubMed: 11717318]

Wang Z, Mascarenhas N, Eckmann L, Miyamoto Y, Sun X, Kawakami T, and Di Nardo A (2017). Skin microbiome promotes mast cell maturation by triggering stem cell factor production in keratinocytes. J. Allergy Clin. Immunol 139, 1205–1216.e6. [PubMed: 27746235]

Wessels MR, Moses AE, Goldberg JB, and DiCesare TJ (1991). Hyaluronic acid capsule is a virulence factor for mucoid group A streptococci. Proc. Natl. Acad. Sci. U S A 88, 8317–8321. [PubMed: 1656437]

West DC, Hampson IN, Arnold F, and Kumar S (1985). Angiogenesis induced by degradation products of hyaluronic acid. Science 228,1324–1326. [PubMed: 2408340]

Yamasaki K, Muto J, Taylor KR, Cogen AL, Audish D, Bertin J, Grant EP, Coyle AJ, Misaghi A, Hoffman HM, and Gallo RL (2009). NLRP3/cryopyrin is necessary for interleukin-1beta (IL-1beta) release in response to hyaluronan, an endogenous trigger of inflammation in response to injury. J. Biol. Chem 284, 12762–12771. [PubMed: 19258328]

Yoshida H, Nagaoka A, Kusaka-Kikushima A, Tobiishi M, Kawabata K, Sayo T, Sakai S, Sugiyama Y, Enomoto H, Okada Y, and Inoue S (2013). KIAA1199, a deafness gene of unknown function, is a new hyaluronan binding protein involved in hyaluronan depolymerization. Proc. Natl. Acad. Sci. U S A 110, 5612–5617. [PubMed: 23509262]

Yoshino Y, Ishisaka M, Tsuruma K, Shimazawa M, Yoshida H, Inoue S, Shimoda M, Okada Y, and Hara H (2017). Distribution and function of hyaluronan binding protein involved in hyaluronan depolymerization (HYBID, KIAA1199) in the mouse central nervous system. Neuroscience 347, 1–10. [PubMed: 28189611]

Yoshino Y, Goto M, Hara H, and Inoue S (2018). The role and regulation of TMEM2 (transmembrane protein 2) in HYBID (hyaluronan (HA)-binding protein involved in HA depolymerization/ KIAA1199/CEMIP)-mediated HA depolymerization in human skin fibroblasts. Biochem. Biophys. Res. Commun 505, 74–80. [PubMed: 30241936]

Zhang LJ, Guerrero-Juarez CF, Hata T, Bapat SP, Ramos R, Plikus MV, and Gallo RL (2015). Innate immunity. Dermal adipocytes protect against invasive Staphylococcus aureus skin infection. Science 347, 67–71. [PubMed: 2554785]

Zhang LJ, Sen GL, Ward NL, Johnston A, Chun K, Chen Y, Adase C, Sanford JA, Gao N, Chensue M, et al. (2016). Antimicrobial peptide LL37 and MAVS signaling drive interferon-β production by epidermal keratinocytes during skin injury. Immunity 45, 119–130. [PubMed: 27438769]
Highlights

- *Cemip* is induced following skin infection and digests hyaluronan
- Hyaluronan is required for optimal reactive adipogenesis and antimicrobial activity
- Digestion of hyaluronan by *Cemip* inhibits reactive adipogenesis
- *Cemip* loss increases inflammation and antimicrobial activity following skin infection
Figure 1. Cemip Is Necessary for HA Digestion after Infection

(A) The expression of transcripts for five known mammalian hyaluronidases in murine skin is shown before and 3 days following infection by *S. aureus* (n = 4 control or 6 skin infection mice/group).

(B) Mouse dermis stained for HA (green) or Cemip (red) or DAPI (blue) in representative sections of skin from control and Cemip^{−/−} mice before and 3 days after *S. aureus* infection. Dotted lines outline regions of HA loss. Infection was to the upper right in all fields shown. Scale bar, 20 μm.

(C) mRNA expression from skin measured by qPCR for Cemip (n = 6 mice/group).

(D) HA abundance measured by ELISA in skin extracts (n = 3 mice/group).

(E) Gel electrophoresis and staining for HA. (a) Wild-type skin, (b) Cemip^{−/−} skin, (c) wild-type skin 3 days after *S. aureus*, and (d) Cemip^{−/−} 3 days after *S. aureus*. Arrow indicates accumulation of low-molecular weight HA as seen only in control mice after *S. aureus*. All error bars indicate mean ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001 (t test).
Figure 2. Cemip^{−/−} Mice Resist Infection by *S. aureus*

(A) Skin lesions on control and Cemip^{−/−} mice 3 days after inoculation with 1 × 10^6 colony-forming units (CFU) of *S. aureus*.

(B) Measurements of lesion size on mice after inoculation with *S. aureus* as in (A).

(C) Representative images taken by IVIS.

(D) Quantification of luminescence in region of interest (ROI) of skin from control and Cemip^{−/−} mice 3 days after inoculation with 1 × 10^6 CFU of bioluminescent *S. aureus*.

(E) CFU count of *S. aureus* recovered from the spleen 3 days after skin infection as in (A).

(F) mRNA expression from skin measured by qPCR of *Camp* (n = 6 control and for *S. aureus*+).

(G) Immunohistochemical staining for cathelicidin (red) and DAPI (blue) in representative sections of skin from control and Cemip^{−/−} mice 3 days after *S. aureus* infection. Scale bar, 50 μm.

(H) Tissue extracts were subjected to immunoblotting analyses for *Camp* and beta-actin.

(I) Quantification of the ratio of *Camp* to beta-actin.

All error bars indicate mean ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001 (t test).
Figure 3. Loss of Cemip Enhances Reactive Adipogenesis

(A) Representative histological images of skin from mice at day 0 and 3 days after infection with *S. aureus*. Tissue was stained with H&E. Brackets delineate dermal region occupied by adipocytes. Scale bar, 50 μm.

(B) Quantification of the adipose tissue thickness indicated scale bar (n = 8 for control and Cemip−/− infection mice/group).

(C-E) qRT-PCR of the relative abundance of transcripts for (C) Pref1, (D) PPARγ, and (E) Adipoq as normalized to β-actin (n = 4 for normal condition, n = 8 for infection mice/group).

(F) Flow cytometry analysis of single-cell suspensions from the skin showing expression of PEGFRα from control, Cemip−/−, control infection, and Cemip−/− infection. Cells were gated on CD31-negative, CD45-negative, and SCA-1-positive.

(G) Statistical comparison of the percentages of the cells in the indicated gates shown in (F). All error bars indicate mean ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001 (t test).
Figure 4. Loss of Cemip Enhances Local Skin Inflammation in Response to S. aureus

(A–F) Flow cytometry analysis of single-cell suspensions from the skin showing expression of CD11c/MHCII, Ly6G/CD11b, and LyG-C cells isolated from control, Cemip−/− control infection, and Cemip−/− infection. Cells were gated on CD3-negative. Numbers represent the percentages of the cells in the indicated gate.

(G) Representative sections of skin from control and Cemip−/− mice at 3 days after S. aureus infection. Tissues are stained with red with Gr-1 antibody and blue with DAPI. Scale bar, 20 μm.

(H) qRT-PCR of the relative abundance of transcripts for IL-6 as normalized to β-actin (n = 4 for normal condition, n = 8 for infection mice/group).

All error bars indicate mean ± SEM. *p < 0.05, **p < 0.01, and ***p < 0.001 (t test).
## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies          |        |            |
| Anti-CAMP           | (Dorschner et al., 2001) | N/A |
| Anti-PREF1/DLK      | Abcam  | Cat# ab21682 |
| BODIPY®             | Thermo Fisher | Cat# A-577 |
| Anti-HA binding protein | Millipore | Cat# 385910 |
| Anti-Cemip          | (Shimoda et al., 2017) | N/A |
| Zombie violet viability dye | BioLegend | Cat# 423114 |
| Anti-mouse CD16/32  | eBioscience | Cat# 14016185 |
| Anti-mouse SMA      | eBioscience | Cat# 53976082 |
| Anti-mouse CD45     | BioLegend | Cat# 147704 |
| Anti-mouse CD31     | BioLegend | Cat# 102522 |
| Anti-mouse Thy1     | BioLegend | Cat# 105308 |
| Anti-mouse PDGFRa   | eBioscience | Cat# 17140181 |
| Anti-mouse SCA1     | BioLegend | Cat# 108133 |
| Anti-mouse CD24     | BioLegend | Cat# 108136 |
| Anti-mouse CD11b    | BioLegend | Cat# 101216 |
| Anti-mouse Ly6G     | eBioscience | Cat# 11593182 |
| Anti-mouse F4/80    | eBioscience | Cat# 12480182 |
| Anti-mouse CD11C    | BioLegend | Cat# 117310 |
| Anti-mouse MHCII    | eBioscience | Cat# 56352182 |
| Anti-mouse CD3      | BioLegend | Cat# 100222 |
| Anti-mouse Tbet     | Fisher Scientific | Cat# 562467 |
| Anti-mouse GATA3    | BioLegend | Cat# 653807 |
| Anti-mouse RORgt    | eBioscience | Cat# 12–6981-80 |
| Anti-mouse IFN-gamma | BioLegend | Cat# 505809 |
| Anti-mouse IL-17    | BioLegend | Cat# 506929 |
| Anti-mouse Foxp3    | eBioscience | Cat# 48–5773-80 |
| Bacterial and Virus Strains |        |            |
| S. aureus strain USA300 | (Muhs et al., 2017) | N/A |
| Chemicals, Peptides, and Recombinant Proteins |        |            |
| protease            | Sigma-Aldrich | Cat# P1236 |
| DEAE Sephacel       | Amersham Biosciences | Cat# 17070901 |
| Prepacked Disposable PD-10 Columns | GE Healthcare | Cat# 17085101 |
| Hyalose ladders HiLadder | Hyalose | Cat# HYA-HILAD-20 |
| Critical Commercial Assays |        |            |
| DuoSet ELISA Ancillary Reagent Kit | R&D Systems | Cat# DY007 |
| iScript cDNA synthesis kit | Bio-Rad | Cat# 1708890 |
| PureLink RNA Mini Kit | Life Technologies | Cat# 12183025 |
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Experimental Models: Organisms/Strains** |
| Mouse:C57BL/6J | Jackson Laboratory | Stock No: 000664 |
| Mouse:Cemip (KIAA1199) KO | KAO (Shimoda et al., 2017) | N/A |
| Mouse:Scf^{fl/fl} | Di Nardo Lab (Wang et al., 2017) | N/A |
| Mouse:B6N.Cg-Tg(KRT14-cre)1Amc/J | Jackson Laboratory | Stock No: 018964 |
| **Oligonucleotides** |
| Actb | ThermoFisher | Mmt02619580_g1 |
| Hyal1 | ThermoFisher | Mmt00476206_m1 |
| Hyal2 | ThermoFisher | Mmt01230688_g1 |
| Hyal3 | ThermoFisher | Mmt00662097_m1 |
| KIAA1199 | ThermoFisher | Mmt00479221_m1 |
| TMEM2 | ThermoFisher | Mmt00459599_m1 |
| HAS1 | ThermoFisher | Mmt03048195_m1 |
| HAS2 | ThermoFisher | Mmt00515089_m1 |
| HAS3 | ThermoFisher | Mmt00515092_m1 |
| ZFP423 | ThermoFisher | Mmt00677660_m1 |
| Pref1 | ThermoFisher | Mmt00494477_m1 |
| PPARg | ThermoFisher | Mmt00440940_m1 |
| Adipoq | ThermoFisher | Mmt00456425_m1 |
| CEBPA | ThermoFisher | Mmt00514283_s1 |
| CAMP | ThermoFisher | Mmt00438285_m1 |
| IL6 | ThermoFisher | Mmt00446190_m1 |
| TNF | ThermoFisher | Mmt00443258_m1 |
| **Software and Algorithms** |
| FlowJo | FlowJo | [https://www.flowjo.com/](https://www.flowjo.com/) |
| IVIS living image software | Xenogen | [https://www.perkinelmer.com/](https://www.perkinelmer.com/) |
| Prism | GraphPad Software | [https://www.graphpad.com/](https://www.graphpad.com/) |