2-O-Sulfated Domains in Syndecan-1 Heparan Sulfate Inhibit Neutrophil Cathelicidin and Promote Staphylococcus aureus Corneal Infection*

Received for publication, April 22, 2015 Published, JBC Papers in Press, April 30, 2015, DOI 10.1074/jbc.M115.660852

Atsuko Hayashida1, Shiro Amano1, Richard L. Gallo3, Robert J. Linhardt1, Jian Liu1, and Pyong Woo Park‡∗†1

Background: Syndecan-1 promotes bacterial infections, but how this is accomplished remains unclear.

Results: Syndecan-1 and 2-O-sulfated heparan compounds specifically enhanced S. aureus corneal virulence and inhibited bacterial killing by CRAMP secreted from degranulated neutrophils.

Conclusion: Specific structural motifs in syndecan-1 HS promote S. aureus corneal infection by inhibiting neutrophil CRAMP.

Significance: This study uncovers a new pathogenic role for syndecan-1 in bacterial infection.

Ablation of syndecan-1 in mice is a gain of function mutation that enables mice to significantly resist infection by several bacterial pathogens. Syndecan-1 shedding is induced by bacterial virulence factors, and inhibition of shedding attenuates bacterial virulence, whereas administration of purified syndecan-1 ectodomain enhances virulence, suggesting that bacteria subvert syndecan-1 ectodomains released by shedding for their pathogenesis. However, the pro-pathogenic functions of syndecan-1 ectodomain have yet to be clearly defined. Here, we examined how syndecan-1 ectodomain enhances Staphylococcus aureus virulence in injured mouse corneas. We found that syndecan-1 ectodomain promotes S. aureus corneal infection in an HS-dependent manner. Surprisingly, we found that this pro-pathogenic activity is dependent on 2-O-sulfated domains in HS, indicating that the effects of syndecan-1 ectodomain are structure-based. Our results also showed that purified syndecan-1 ectodomain and heparan compounds containing 2-O-sulfate motifs inhibit S. aureus killing by antimicrobial factors secreted by degranulated neutrophils, but does not affect intracellular phagocytic killing by neutrophils. Immunodepletion of antimicrobial factors with staphylocidal activities demonstrated that CRAMP, a cationic antimicrobial peptide, is primarily responsible for S. aureus killing among other factors secreted by degranulated neutrophils. Furthermore, we found that purified syndecan-1 ectodomain and heparan compounds containing 2-O-sulfate units potently and specifically inhibit S. aureus killing by synthetic CRAMP. These results provide compelling evidence that a specific subclass of sulfate groups, and not the overall charge of HS, permits syndecan-1 ectodomains to promote S. aureus corneal infection by inhibiting a key arm of neutrophil host defense.

Conclusions: Many microbial pathogens, including viruses, bacteria, and parasites, are thought to exploit the heparan sulfate (HS) moiety of HS proteoglycans (HSPGs) to infect host cells and to evade immune mechanisms (8, 9). HSPGs are expressed ubiquitously on the cell surface and in the extracellular matrix. HSPGs are comprised of one or several HS chains attached covalently to specific core proteins (10). HS binds to and regulates many molecules that have been implicated in the host defense against infectious agents, including cytokines, chemokines, and cationic antimicrobial factors (8, 9).

HS chains are unbranched polysaccharides comprised of repeating disaccharide units of hexuronic acid, either glucuronic (GlcA) or iduronic acid (IdoA), alternating with an unsubstituted or N-substituted glucosamine on which the substituents are either acetate or sulfate (11–13). In HSPG biosynthesis, a non-sulfated HS precursor is polymerized on specific structural motifs in HS that enables mice to significantly resist infection by several bacterial pathogens. Syndecan-1 shedding is induced by bacterial virulence factors, and inhibition of shedding attenuates bacterial virulence, whereas administration of purified syndecan-1 ectodomain enhances virulence, suggesting that bacteria subvert syndecan-1 ectodomains released by shedding for their pathogenesis. However, the pro-pathogenic functions of syndecan-1 ectodomain have yet to be clearly defined. Here, we examined how syndecan-1 ectodomain enhances Staphylococcus aureus virulence in injured mouse corneas. We found that syndecan-1 ectodomain promotes S. aureus corneal infection in an HS-dependent manner. Surprisingly, we found that this pro-pathogenic activity is dependent on 2-O-sulfated domains in HS, indicating that the effects of syndecan-1 ectodomain are structure-based. Our results also showed that purified syndecan-1 ectodomain and heparan compounds containing 2-O-sulfate motifs inhibit S. aureus killing by antimicrobial factors secreted by degranulated neutrophils, but does not affect intracellular phagocytic killing by neutrophils. Immunodepletion of antimicrobial factors with staphylocidal activities demonstrated that CRAMP, a cationic antimicrobial peptide, is primarily responsible for S. aureus killing among other factors secreted by degranulated neutrophils. Furthermore, we found that purified syndecan-1 ectodomain and heparan compounds containing 2-O-sulfate units potently and specifically inhibit S. aureus killing by synthetic CRAMP. These results provide compelling evidence that a specific subclass of sulfate groups, and not the overall charge of HS, permits syndecan-1 ectodomains to promote S. aureus corneal infection by inhibiting a key arm of neutrophil host defense.

References:

1 The abbreviations used are: HS, heparan sulfate; HP, heparin; 2ODS-HP, 2-O-desulfated HP; 6ODS-HP, 6-O-desulfated HP; FNL, formyl-norleucine-leucine-phenylalanine; GlcA, glucuronic acid; GlcA2S, 2-O-sulfated glucuronic acid; H, heparosan; HSPG, heparan sulfate proteoglycan; IdoA, iduronic acid; IdoA2S, 2-O-sulfated iduronic acid; NS-H, N-sulfated heparosan; NS2OS-H, N- and 2-O-sulfated heparosan; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; Sdc1−/−, syndecan-1 null; Sdc4−/−, syndecan-4 null; TSB, tryptic soy broth.

2 The abbreviations used are: HS, heparan sulfate; HP, heparin; 2ODS-HP, 2-O-desulfated HP; 6ODS-HP, 6-O-desulfated HP; FNL, formyl-norleucine-leucine-phenylalanine; GlcA, glucuronic acid; GlcA2S, 2-O-sulfated glucuronic acid; H, heparosan; HSPG, heparan sulfate proteoglycan; IdoA, iduronic acid; IdoA2S, 2-O-sulfated iduronic acid; NS-H, N-sulfated heparosan; NS2OS-H, N- and 2-O-sulfated heparosan; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; Sdc1−/−, syndecan-1 null; Sdc4−/−, syndecan-4 null; TSB, tryptic soy broth.

* This work was supported in part by National Institutes of Health Grants R01 EY021765 and R01 HL107472.
† To whom correspondence should be addressed: Children’s Hospital, Harvard Medical School, 320 Longwood Ave., Enders-461, Boston, MA 02115. Tel.: 617-919-4584; Fax: 617-730-0240; E-mail: pyong.park@childrens.harvard.edu.
‡ From the Division of Respiratory Diseases and **Division of Newborn Medicine, Children’s Hospital, Harvard Medical School, Boston, Massachusetts 02115, †Division of Dermatology, University of California San Diego, La Jolla, California 92033, §Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, Troy, New York 12180, and ‡Division of Chemical Biology and Medicinal Chemistry, University of North Carolina, Chapel Hill, North Carolina 27599
serine residues of HSPG core proteins and then extensively modified by N-deacetylase/N-sulfotransferases, C5 epimerase, 2-O-sulfotransferase (2OST), 6OSTs, and 3OSTs in the Golgi. Unique sulfation patterns of HS are thought to dictate how HSPGs bind to molecules and regulate biological processes, including HS interactions with infectious agents. For example, envelope glycoproteins E1 and E2 of hepatitis C virus require N- and 6-O-sulfate groups for efficient interaction with HS (14), whereas OmCB of Chlamydia trachomatis binds to 6-O-sulfated HS domains (15). These observations suggest that microbes target specific HS modifications to promote their pathogenesis, but whether GAG modifications are indeed important in vivo has yet to be determined. In fact, our knowledge of the role of HSPGs in infections is mostly derived from cell-based experiments performed in vitro, and their physiological significance, relevance, and function in infectious diseases remain largely speculative.

A growing body of evidence suggests that bacterial pathogens subvert syndecan-1 shedding to promote their pathogenesis in various tissues. Syndecan-1 is the predominant HSPG of epithelial cells, a cell type that most bacteria first encounter during their pathogenesis (8). *S. aureus* (16), *P. aeruginosa* (17), and *S. pneumoniae* (18) induce the shedding of syndecan-1 ectodomains from the cell surface through specific virulence factors in cultured epithelial cells. Moreover, in mice, syndecan-1 ablation is a gain of function of mutation where the syndecan-1 null (*Sdc1−/−*) mice are significantly protected from *P. aeruginosa* (19) and *S. aureus* (20) lung infection, and *S. aureus* corneal infection (21) compared with wild type (WT) mice, suggesting that syndecan-1 shedding promotes bacterial pathogenesis. Indeed, inhibition of shedding reduces bacterial virulence, whereas administration of purified syndecan-1 ectodomains or HS, but not other glycosaminoglycans or syndecan-1 core protein devoid of HS, enhances bacterial virulence in mouse models of infection (19, 21). These results indicate that syndecan-1 ectodomains promote bacterial pathogenesis in an HS-dependent manner, but precisely how this is accomplished is incompletely understood.

Here, we examined how HS chains of syndecan-1 ectodomains enhance *S. aureus* virulence in injured corneal tissues. Our results surprisingly showed that 2-O-sulfated domains in syndecan-1 HS promote *S. aureus* corneal infection. Our study also showed that the antimicrobial peptide CRAMP secreted by degranulated neutrophils selectively kills *S. aureus*, and 2-O-sulfate motifs in syndecan-1 HS potently and specifically inhibit the staphylococidal activity of CRAMP. These results reveal a new pathogenic role for discrete domains in syndecan-1 HS in infection.

**Experimental Procedures**

**Materials**—281-2 rat anti-mouse syndecan-1 ectodomain and Ky8.2 rat anti-mouse syndecan-4 monoclonal antibodies were purchased from BD Biosciences (San Jose, CA). Rabbit anti-mouse myeloperoxidase (L607) was from Cell Signaling (Danvers, MA) and goat anti-lactoferrin (C-15) and mouse anti-CRAMP (G-1) antibodies were from Santa Cruz Biotechnology (Dallas, TX). Mouse IgG was from Equitech Bio (Kerrville, TX). *S. aureus* BioParticles Opsonizing Reagent, Live/Dead BacLight Bacterial Viability Kit, Alexa Fluor 488 and 594 Antibody Labeling Kits were obtained from Invitrogen (Carlsbad, CA). Chondroitin sulfate A (CS), formyl-norleucine-leucine-phenylalanine peptide (INLP), and cytochalasin D were from Sigma. CRAMP peptide was synthesized at institutional core programs or obtained from Anaspec (Fremont, CA). Percoll was from GE Healthcare Life Sciences (Pittsburgh, PA) and protein A-agarose and protein G-agarose beads were from Pierce. Syndecan-1 ectodomains were purified from the conditioned medium of normal mammary gland epithelial cells as described previously (21), whereas syndecan-4 ectodomains were partially purified from the conditioned medium by DEAE chromatography and sequential absorption to anti-syndecan-2 and -3 and antisyndecan-1 affinity resins to immunodeplete other syndecans. The partially purified syndecan-4 preparation was determined to contain no syndecan-1, -2, and -3 and glypican-1 and -3 by dot immunoblotting. Porcine mucosal HS and heparin, and N-desulfated, 2-O-desulfated and 6-O-desulfated heparin were from Neoparin (Alameda, CA). Heparosan was purified from *E. coli* K5 and chemoenzymatically N-sulfated or N- and 2-O-sulfated as described (22). Briefly, N-sulfated heparosan was synthesized by incubating chemically deacetylated heparosan with *N*-sulfotransferase and the sulfate donor 3′-phosphoadenosine 5′-phosphosulfate (PAPS), whereas N- and 2-O-sulfated heparosan was made by incubating N-sulfated heparosan with recombinant 2-O-sulfotransferase and PAPS. Disaccharide composition analysis revealed that ~95% of the disaccharides in *N*-sulfated heparosan are comprised of GlcA-GlcNS units, whereas N- and 2-O-sulfated heparosan is composed of about 20% of GlcA2S-GlcNS and 80% of GlcA-GlcNS disaccharide units. All other materials were purchased from Sigma, Thermo Fisher Scientific (Waltham, MA), or VWR (Westchester, PA).

**Mice**—Except for experiments comparing the response of *S. aureus* corneal infection (Fig. 1B), mice on the BALB/c background were used in all experiments since BALB/c mice are considered to be more susceptible to *S. aureus* keratitis compared with BL/6 mice (23). Unchallenged *Sdc1−/−* mice on both the BALB/c and C57BL/6J backgrounds and unchallenged *Sdc4−/−* mice on the C57BL/6J background are healthy with normal growth, reproduction, tissue morphology, complete blood cell counts, and serum chemistry parameters (24–26). Both female and male *Sdc1−/−*, *Sdc4−/−*, and WT mice were used at an age of 8–10 weeks. Mice were maintained in microisolator cages under specific pathogen-free conditions in a 12 h light/dark cycle and fed a basal rodent chow ad libitum. All animal experiments were approved by the Institutional Animal Care and Use Committee of Children’s Hospital, and complied with federal guidelines for research with experimental animals.

**Mouse Model of S. aureus Corneal Infection**—*S. aureus* strains 8325–4 (16), P1 (27), USA300 (28), and Woods (29) were from our culture collection. *S. aureus* strains were grown to late log growth phase in tryptic soy broth (TSB), and the bacterial concentration was approximated by turbidity measurement at 600 nm. After washing, the concentration was adjusted to ~5 × 10⁸ cfu/5 μl. The exact bacterial concentra-
tion in the inoculum was determined by plating out serial dilutions onto TSB agar plates immediately after preparation. A single vertical scratch was made with a 29-gauge needle in one of the corneas of each anesthetized mouse without penetrating beyond the superficial stroma. The other eye served as an uninjured control. A 5 μl suspension of *S. aureus* was applied topically to injured or uninjured corneas, and test reagents were administered topically at the indicated times. The dose of test compounds was selected on preliminary titration experiments. At 7–10 h post-infection, mice were euthanized and the bacterial burden in isolated corneas was determined. Isolated corneas were homogenized in TSB containing 0.1% (v/v) Triton X-100 and serial dilutions of homogenates were plated onto TSB agar plates.

*S. aureus* Killing by Neutrophils, Degranulated Neutrophil Extracts, and CRAMP—Neutrophils were isolated from bone marrows by Percoll gradient density centrifugation as described previously (21). Briefly, isolated femurs and tibias were cleaned and flushed with Hank’s Balanced Salt Solution (HBSS) containing 10 mM HEPES, pH 7.4 (HBSS/HEPES). Bone marrow cells were centrifuged at 300 × g for 10 min, resuspended in 45% Percoll solution, layered on top of a 62 and 81% Percoll gradient solution, and centrifuged at 1500 × g for 30 min. The neutrophil layer between 62 and 81% Percoll was collected, washed, resuspended in HBSS/HEPES, and counted. Neutrophils were incubated with pre-opsinized *S. aureus* Woods strain in HBSS/HEPES containing 5% mouse serum for 2 h at 37 °C. Bacterial killing was enumerated by incubating test samples with 0.1% Triton X-100 in HBSS/HEPES for 30 min and plating serial dilutions onto TSB agar plates. To prepare degranulated neutrophil extracts, isolated neutrophils were stimulated with 1 μM fNLP for 1 h at 37 °C, and the supernatants were collected by centrifugation at 300 × g for 5 min. *S. aureus* killing by fNLP-stimulated neutrophils supernatants or synthetic CRAMP was determined by incubating *S. aureus* with supernatants or CRAMP peptide at the indicated doses for 2 h at 37 °C and plating serial dilutions of the test samples onto TSB agar plates.

**Histology**—Eyes were enucleated from uninfected Wt or infected Wt and Sdc1−/− mice at 7 h post-infection, fixed in 4% paraformaldehyde/PBS for 4 h at room temperature, embedded in paraffin, and sectioned horizontally. Eye sections (5 μm) were stained with Gram solution or immunostained with monoclonal antibodies directly conjugated to Alexa 594 or 488. Stained tissue sections were visualized with a Zeiss Axiolab 40 CFL microscope and pictures were taken with the AxioCam MRm high resolution camera. Adobe Photoshop CS6 was used to process the acquired images.

**Statistical Analyses**—All data are expressed as mean plus or minus S.E. Differences between experimental and respective control groups were examined by Student’s *t* test, and *p* values less than 0.05 were considered statistically significant.

**Results**

**Corneal Epithelial Syndecan-1 Specifically Promotes *S. aureus* Corneal Infection in an HS-dependent Manner**—We initially examined the expression of syndecans in the cornea. Uninfected Wt corneas showed strong expression of syndecan-1 in the epithelium and weak expression in the endothelium (Fig. 1A). Syndecan-4 was expressed in a similar pattern in the corneal epithelium and endothelium, albeit at a lower level than that of syndecan-1 (Fig. 1A). Syndecan-2 was weakly expressed by keratocytes in the corneal stroma and syndecan-3 expression was not detected (data not shown). Based on these results, we examined whether syndecan-1 functions specifically in bacterial keratitis by comparing the response of Wt, Sdc1−/−, and Sdc4−/− mice to *S. aureus* corneal infection. Mouse corneas were injured with a single vertical scratch using a 29-gauge needle without penetrating beyond the superficial stroma and infected topically with 5 × 10⁶ cfu of *S. aureus* strain 8325–4. The corneal bacterial burden was quantified by plating out serial dilutions of infected corneal homogenates. The infectious burden was significantly reduced by over 10-fold in *Sdc1*−/− corneas compared with those of Wt and Sdc4−/− corneas, but similar between Wt and Sdc4−/− corneas (Fig. 1B), indicating that ablation of syndecan-1 enables mice to specifically resist *S. aureus* corneal infection.

We next examined if the protection from *S. aureus* infection by syndecan-1 ablation is restricted to the 8325–4 strain by comparing the response of Wt and *Sdc1*−/− corneas to infection by 8325–4, P1 (clinical blood isolate), and USA300 (MRSA) strains. Although there were differences in the virulence of each bacterial strain, *Sdc1*−/− corneas were significantly protected from infection by all 3 strains tested compared with Wt corneas (Fig. 1C). Furthermore, reinforcing the prevailing view that injured areas are exclusively infected in the model of scarified corneal infection, Gram staining showed an intense accumulation of Gram-positive *S. aureus* at sites where the epithelium was scratched in Wt corneas, but not in *Sdc1*−/− corneas (Fig. 1D). Thus, despite observations suggesting that ablation of syndecan-1 delays wound healing in the cornea and skin (30), and that impairment of wound healing is a major risk factor for infection, these data suggest that syndecan-1 both specifically and prominently promotes *S. aureus* corneal infection.

Next we examined how syndecan-1 specifically enhances *S. aureus* virulence and if certain structural components of syndecan-1 HS are important by comparing the effects of purified syndecan-1 ectodomains, HS, CS, syndecan-1 core protein devoid of both HS and CS chains, and partially purified syndecan-4 ectodomains devoid of other syndecans including syndecan-1. Because syndecan-1 shedding is maximal at 3 h after *S. aureus* corneal infection (21), we administered purified ectodomains and related compounds at 3 h post-infection and quantified the corneal bacterial burden 7 h later. Both purified ectodomain and HS significantly increased the bacterial burden in *Sdc1*−/− corneas by 4- and 3-fold, respectively, whereas CS and core protein did not (Fig. 2A). Purified syndecan-1 ectodomain and HS also similarly enhanced *S. aureus* virulence in *Sdc1*−/− corneas in a dose-dependent manner (Fig. 2B). Incubation of bacteria with purified ectodomain or HS had no effect on bacterial growth in vitro (not shown). Interestingly, partially purified syndecan-4 ectodomains lacking syndecan-1 also significantly increased the corneal bacterial load (Fig. 2A), indicating that other HSPGs also possess critical HS motifs that...
Syndecan-1 is the predominant HSPG in the mouse corneal epithelium and Sdc1<sup>−/−</sup> mice specifically resist <i>S. aureus</i> corneal infection compared with Wt mice. A, eye sections (5 µm) of uninfected Wt mice were immunostained with 5 µg/ml 281-2 anti-mouse syndecan-1 ectodomain or 5 µg/ml Ky8.2 anti-mouse syndecan-4 ectodomain antibodies directly conjugated to Alexa 594 or 488 (original magnification, x200). B, scarified corneas of Wt, Sdc1<sup>−/−</sup>, and Sdc4<sup>−/−</sup> mice on the BL/6J background were infected topically with <i>S. aureus</i> strain 8325-4. The corneal bacterial burden was quantified 7 h after infection. Data shown are mean ± S.E. (n = 10 in Wt, n = 12 in Sdc1<sup>−/−</sup>, and n = 14 in the Sdc4<sup>−/−</sup> groups, *p < 0.05 relative to Wt, Student’s t test). C, scarified corneas of Wt and Sdc1<sup>−/−</sup> mice on the BALB/c background were infected topically with 5 × 10<sup>6</sup> cfu of <i>S. aureus</i> strain 8325-4, P1 or USA300, and the corneal bacterial burden was quantified at 7 h post-infection. Data shown are mean ± S.E. (8325-4: n = 8 for both Wt and Sdc1<sup>−/−</sup>, P1: n = 14 for both Wt and Sdc1<sup>−/−</sup>, and USA300: n = 14 for Wt and n = 12 for Sdc1<sup>−/−</sup>; *p < 0.05 relative corresponding Wt). D, eye sections (5 µm) of Wt and Sdc1<sup>−/−</sup> mice isolated at 7 h after infection with <i>S. aureus</i> 8325-4 were Gram-stained (original magnification, ×200).

**FIGURE 2.** Syndecan-1 ectodomain HS specifically promotes <i>S. aureus</i> corneal infection in a dose-dependent manner. A, scarified corneas of Sdc1<sup>−/−</sup> mice were infected topically with 5–6 × 10<sup>8</sup> cfu of <i>S. aureus</i> 8325-4, administered vehicle (Sdc1<sup>−/−</sup>, control) or 200 ng of purified syndecan-1 ectodomain, HS, CS, core protein (CP), or partially purified syndecan-4 ectodomain at 3 h after infection, and the corneal bacterial burden was quantified at 10 h after infection (n = 16 for the control group, n = 10 for the syndecan-1 ectodomain, HS and syndecan-4 ectodomain groups, n = 4 for the CS and CP groups; *p < 0.05 relative to control). B, scarified Sdc1<sup>−/−</sup> corneas were infected topically with 5 × 10<sup>6</sup> cfu of <i>S. aureus</i>, administered increasing doses of purified syndecan-1 ectodomain or HS at 3 h post-infection, and the corneal bacterial burden was measured at 10 h post-infection (n = 4 for 20 ng, n = 6 for 85 ng, and n = 10 for 200 ng groups for both ectodomain and HS).

2-O-Sulfated Domains in Syndecan-1 HS Promote <i>S. aureus</i> Corneal Infection—We next examined if a specific sulfate modification is important for HS’s ability to enhance <i>S. aureus</i> virulence in the cornea. Structures of disaccharide repeating units enhance <i>S. aureus</i> corneal virulence. More importantly, these data suggest that syndecan-1 functions specifically in <i>S. aureus</i> corneal infection because it is shed specifically by <i>S. aureus</i> infection.
of HS are shown in Fig. 3A. HS primarily exists in nature as an extended helical structure and does not fold into tertiary structures (31). HS functions are largely specified by how it displays its carboxyl motifs. For example, 2-O-sulfate groups of HS are shown in Fig. 3A. Therefore, removal of 2-O-sulfate groups significantly increased the corneal bacterial burden since bacterial interaction with HS is necessary to efficiently sulfate heparan and heparosan using a chemoenzymatic approach (22). We first tested the effects of chemically desulfated heparin compounds on the corneal virulence of S. aureus in mice. We used N-sulfated heparin as a substrate for 2-O-sulfation because N-sulfation is necessary to efficiently sulfate heparan at the 2-O-position. Topical administration of unmodified heparan or N-sulfated heparan had no significant effect on the corneal bacterial burden in Sdc1−/− mice, whereas administration of N- and 2-O-sulfated heparosan significantly increased the bacterial burden by over 5-fold compared with mice that were infected with bacteria only (Fig. 3D). Heparin or heparosan compounds had no effect on bacterial growth in vitro. Altogether, these data indicate that 2-O-sulfated domains in syndecan-1 HS promote S. aureus corneal infection and suggest that they do so by modulating the host response to infection.

Because chemical desulfation is selective, but not specific, we next tested the effects of heparan and heparosan compounds that were sulfated in vitro (Fig. 3B). Heparan is a capsular polysaccharide from bacteria that has a repeating disaccharide unit of -GlcA-GlcNAc-, and is essentially identical in structure to unmodified HS. To confirm that 2-O-sulfate groups are important, we synthesized N-sulfated and N- and 2-O-sulfated heparan using a chemoenzymatic approach (22), and tested their effects on S. aureus virulence in the cornea. We used N-sulfated heparan as a substrate for 2-O-sulfation because N-sulfation is necessary to efficiently sulfate heparan at the 2-O-position. Topical administration of unmodified heparan or N-sulfated heparan had no significant effect on the corneal bacterial burden in Sdc1−/− mice, whereas administration of N- and 2-O-sulfated heparan significantly increased the corneal bacterial burden by over 5-fold compared with mice that were infected with bacteria only (Fig. 3D). Heparin or heparosan compounds had no effect on bacterial growth in vitro. Altogether, these data indicate that 2-O-sulfated domains in syndecan-1 HS promote S. aureus corneal infection, and suggest that they do so by modulating the host response to infection.
S. aureus Subverts 2-O-Sulfated Domains in Syndecan-1 HS

2-O-Sulfated Domains in Syndecan-1 HS Inhibit the Extracellular Killing of S. aureus by Neutrophils—Neutrophils are rapidly deployed to sites of infection and injury, and are indispensable in the innate host defense against the majority of acute bacterial infections, including bacterial keratitis (35, 36). Consistent with these observations, immunodepletion of neutrophils significantly increased S. aureus virulence in Sdc1−/− corneas (21), indicating that neutrophils are essential to rapidly clear S. aureus and enable Sdc1+/− mice to resist S. aureus corneal infection. However, neutrophils do not express syndecan-1 and do not bind to syndecan-1. Syndecan-1 also does not regulate neutrophil influx into corneas infected with S. aureus, and isolated neutrophils of both Sdc1−/− and Wt mice similarly kill S. aureus (21). Moreover, Wt and Sdc1−/− neutrophils are similar in size, granularity, and pattern of GR1 staining, Sdc1−/− neutrophils do not have an inherent defect in their ability to migrate, and Sdc1−/− mice contain normal numbers of circulating neutrophils (25, 37). Instead, syndecan-1 ectodomains have been shown to promote S. aureus corneal infection by inhibiting neutrophil-mediated S. aureus killing in an HS-dependent manner (21).

Thus, we next explored whether specific structural features of syndecan-1 HS inhibit the host defense activities of neutrophils against S. aureus. We first examined the effects of chemically desulfated heparin and chemoenzymatically sulfated heparan on the killing of pre-opsonized S. aureus by Wt neutrophils isolated from the bone marrow. Heparin significantly inhibited S. aureus killing by neutrophils, and removal of sulfate groups at the N- or 6-O-position had no effect on this inhibitory activity (Fig. 4A). However, 2-O-desulfated heparin lost its capacity to inhibit S. aureus killing by neutrophils, suggesting that 2-O-sulfate groups are consistent. Important with these observations, heparan and N-sulfated heparan only slightly inhibited S. aureus killing by neutrophils, but the marginal difference (<4%) did not reach significance (Fig. 4B). In contrast, N- and 2-O-sulfated heparan significantly inhibited the killing of S. aureus by neutrophils (Fig. 4B). At the doses tested, the heparin and heparan compounds did not affect the viability of isolated neutrophils. These findings suggest that 2-O-sulfated domains in syndecan-1 HS promote S. aureus corneal infection by inhibiting bacterial killing by neutrophils.

Neutrophils can kill bacteria by extracellular or intracellular phagocytic mechanisms. Most of the antimicrobial activity of neutrophils are thought to occur within intracellular phagosomes, but extracellular killing mechanisms involving anti-microbial factors secreted by neutrophil degranulation or released and embedded in neutrophil extracellular traps also comprise an important arm of host defense against infections (36). To determine how 2-O-sulfated domains in syndecan-1 HS inhibit S. aureus killing by neutrophils, we first examined the effects of HS on the rate of phagocytic killing. Wt neutrophils were incubated with pre-opsonized S. aureus in the absence or presence of HS for 15, 30, 60, or 90 min, incubated with gentamycin to kill extracellular bacteria, and the intracellular bacterial load was determined. The number of live intracellular bacteria was similar between the neutrophils incubated with bacteria only and with bacteria and HS at all times examined (Fig. 5A), indicating that HS does not inhibit phagocytic killing mechanisms of neutrophils. In fact, intracellular killing was slightly inhibited by HS at 90 min postincubation, although the difference did not reach significance (Fig. 5A).

Next, we examined the effects of purified syndecan-1 ectodomain and related compounds on the extracellular killing of S. aureus by neutrophils. Neutrophils were pre-treated with cytochalasin D to inhibit phagocytosis and incubated with S. aureus in the absence or presence of purified syndecan-1 ectodomain, HS, CS, heparan, or N- and 2-O-sulfated heparan. Purified ectodomain, HS, and N- and 2-O-sulfated heparan significantly inhibited the extracellular killing of S. aureus by neutrophils, whereas CS and heparan did not (Fig. 5B). Together with the data from the phagocytic killing assay, these findings suggest that 2-O-sulfated domains in syndecan-1 HS promote S. aureus corneal infection by inhibiting antibacterial factors secreted from neutrophils.

We further explored this hypothesis by examining the effects of purified syndecan-1 ectodomain and heparan compounds on the antibacterial activity of degranulated contents collected from neutrophils stimulated with the formylated tripeptide, formyl-norleucine-leucine-phenylalanine (fNLP). Neutrophils express heterogeneous granules and vesicles that contain various peptides and proteins with antimicrobial activity (38). These granules and vesicles are differentially released upon
neutrophil stimulation by both biological and chemical reagents, including microbial products (e.g. formylated peptides, glycolipids, cytokines, and chemokines, and phorbol esters. In general, granules formed during the late stages of neutrophil differentiation are more readily mobilized than granules formed during the early stages of differentiation (39). Stimulation by fNLP released both specific (lactoferrin, CRAMP) and azurophilic (myeloperoxidase) granule contents (not shown). Moreover, we found that fNLP-stimulated neutrophil extracts possess potent staphylocidal activity, which was significantly inhibited by purified ectodomain and 2-O-sulfated heparan compounds (HS, and N- and 2-O-sulfated heparan), but not by those lacking 2-O-sulfate motifs (CS or unmodified heparan) (Fig. 5C). Together, these data indicate that 2-O-sulfated domains in syndecan-1 HS inhibit extracellular killing mechanisms of neutrophils.

**FIGURE 5. 2-O-sulfated domains in syndecan-1 HS inhibit extracellular killing mechanisms of neutrophils.** A, Wt neutrophils (5 x 10^5) were incubated with pre-opsonized S. aureus for 15, 30, 60, or 90 min in the absence or presence of 3 μg/ml HS, washed, incubated with 100 μg/ml gentamycin for 30 min to kill extracellular bacteria, washed, treated with TSB containing 0.1% (v/v) Triton X-100, and detergent lysates were plated out to determine the rate of phagocytic killing (mean ± S.E., n = 5). B, Wt neutrophils (5 x 10^5) were pre-treated with 10 μg/ml cytochalasin D, incubated with S. aureus (2 x 10^7 cfu) in the presence of cytochalasin D without or with 1 μg/ml purified ectodomain, 3 μg/ml HS or CS, or 10 μg/ml H or NS2OS-H for 2 h at 37°C, and bacterial killing was determined (n = 22 for the cytochalasin D group, n = 11 for the +Ecto group, n = 16 for the + HS group, n = 4 for +CS and +H groups, and n = 5 for the +NS2OS-H group; *, p < 0.05 relative to the cytochalasin D group). C, supernatants from neutrophils stimulated with 1 μM fNLP for 1 h were incubated with S. aureus (1.5 x 10^7 cfu) in the absence (fNLP sup) or presence of 1 μg/ml ectodomain, 3 μg/ml HS or CS, or 10 μg/ml H or NS2OS-H for 2 h at 37°C, and bacterial killing was determined (n = 5 in all groups; *, p < 0.05 relative to the fNLP sup group).

Immunoprecipitation with nonspecific mouse IgG or immunodepletion of lactoferrin or myeloperoxidase did not inhibit S. aureus killing by degranulated neutrophil supernatants (Fig. 6A). On the other hand, immunodepletion of the cationic antimicrobial peptide CRAMP significantly inhibited the staphylocidal activity of degranulated neutrophil supernatants (Fig. 6A), suggesting that syndecan-1 HS inhibits CRAMP to inhibit S. aureus killing by neutrophils.

CRAMP kills S. aureus (40) and S. aureus killing by LL-37, the human homologue of CRAMP, is inhibited by HS (41, 42). Consistent with these reports, we found that incubation with synthetic CRAMP for 2 h kills over 90% of S. aureus (Fig. 6B). Importantly, purified syndecan-1 ectodomain, HS, and N- and 2-O-sulfated heparan, but not unmodified heparan without 2-O-sulfate groups, abolished or significantly inhibited S. aureus killing by CRAMP (Fig. 6B). These findings were also confirmed by a live/dead staining assay where most S. aureus cells incubated with CRAMP only or CRAMP and heparan were dead (red), whereas the majority of those incubated with CRAMP and ectodomain, HS, or N- and 2-O-sulfated heparan were alive (green) (Fig. 6C).

Together with data from the immunodepletion experiments, these data suggest that syndecan-1 HS specifically inhibits CRAMP in degranulated neutrophil extracts to promote...
S. aureus corneal infection. However, why syndecan-1 HS targets CRAMP and not other cationic antimicrobial peptides with anti-staphylococcal activity is not clear. Because most antimicrobial peptides and proteins kill S. aureus at relatively high doses, perhaps the concentration of other staphylocidal factors released from degranulated neutrophils by fNLP stimulation did not reach the effective dose. Also, since mouse neutrophils do not express defensins (43), which are inhibited by syndecan-1 HS (19), it is quite possible that syndecan-1 ectodomain may inhibit multiple cationic antimicrobial peptides in clinical S. aureus keratitis. Regardless, our data clearly show that 2-O-sulfated domains in syndecan-1 HS specifically and potently inhibit S. aureus killing by CRAMP, degranulated neutrophil extracts and intact neutrophils, and promote S. aureus corneal infection in vivo.

Discussion

We report here that syndecan-1 is abundantly and selectively expressed in the corneal epithelium, and that HS chains of syndecan-1 promote S. aureus corneal infection in mice. While cell surface HSPGs are widely thought to promote infection by serving as attachment sites for pathogens, syndecan-1 does not bind directly to S. aureus and does not facilitate S. aureus adhesion to cultured corneal epithelial cells (21). Instead, syndecan-1 is shed from the cell surface during S. aureus corneal infection and the shed ectodomain enhances S. aureus virulence in corneal tissues. Our results here indicate that HS chains of syndecan-1 ectodomain promote pathogenesis by impeding bacterial clearance by neutrophils. We found that syndecan-1 ectodomain does not inhibit phagocytic killing of S. aureus by neutrophils, but that it significantly inhibits S. aureus killing by antimicrobial factors secreted by degranulated neutrophils. Our studies also showed that the antimicrobial peptide CRAMP is the primary target of syndecan-1 ectodomains in degranulated neutrophil extracts. Furthermore, our study showed that 2-O-sulfate groups in HS are essential for all of these pro-pathogenic activities of syndecan-1 ectodomain. Together, these observations suggest a new function of syndecan-1 in microbial pathogenesis where 2-O-sulfate motifs in its HS chains promote S. aureus corneal infection by inhibiting S. aureus killing by neutrophil CRAMP.

Our results showing that Sdc1−/− mice, but not Sdc4−/− mice, are significantly protected against S. aureus corneal infection suggest that syndecan-1 functions specifically in this infectious disease, despite similar cellular distribution patterns of syndecan-1 and -4 in the corneal epithelium. How this is accomplished is incompletely understood. Our data indicate...
S. aureus Subverts 2-O-Sulfated Domains in Syndecan-1 HS

that both purified syndecan-1 ectodomains and partially purified syndecan-4 ectodomains devoid of other syndecans are capable of enhancing S. aureus corneal virulence. However, syndecan-1 is expressed at a higher level than that of syndecan-4 in the corneal epithelium, so the abundance of syndecan-1 may overwhelm the potential effects of syndecan-4 in S. aureus corneal infection. Furthermore, α-toxin is a major virulence factor for S. aureus corneal infection (44, 45), and we previously found that α-toxin stimulates the shedding of syndecan-1, but not syndecan-4 ectodomains in cell culture-based assays (16), suggesting that the specific functions of syndecan-1 in corneal tissues are also controlled by its susceptibility to α-toxin-induced ectodomain shedding.

Alternatively, because our results suggest that discrete HS domains enhance S. aureus virulence in the cornea, syndecan-1 HS may contain unique structural features that enable it to function specifically in S. aureus corneal infection. However, opposing this idea are the findings that only minor structural and functional differences are found in HS chains of syndecan-1 and -4 in mouse mammary gland epithelial cells (46) and of syndecan-4 and glypicans in rat embryonic fibroblasts (47), suggesting that structural features of HS chains are cell type specific and not core protein specific. Taken together, these observations suggest that the prominent functions of syndecan-1 in S. aureus corneal infection are considered to be a reflection of its abundant expression and selective shedding by S. aureus α-toxin.

Our results also showed that syndecan-1 HS does not affect intracellular phagocytic killing of S. aureus by neutrophils, but instead inhibits an extracellular killing mechanism of S. aureus by CRAMP secreted upon neutrophil degranulation. Because S. aureus possesses multiple mechanisms to evade intracellular phagosomal killing (48–50), perhaps S. aureus subverts syndecan-1 only to protect itself from extracellular killing by degranulated antimicrobial factors. CRAMP belongs to the cathelicidin family of antimicrobial peptides, which includes human LL-37, porcine PR-39, and bovine bicatrin, among others (51). The importance of the cathelicidins in host defense is quite clear from both animal and cell-based infection studies. For example, LL-37 kills a wide variety of microbial pathogens, including viruses, bacteria and fungi, and is chemotactic for leukocytes (51). Knock-out mice lacking the CRAMP gene (Cnlp-/-) show increased morbidity or mortality in group A Streptococcus skin infection (52), Klebsiella pneumoniae lung infection (53), P. aeruginosa corneal infection (54), E. coli urinary tract infection (55), and Citrobacter rodentium intestinal infection (56). These observations suggest that despite the fact that antimicrobial peptide functions are considered largely redundant, the cathelicidins appear to be essential host defense factors in certain infectious diseases. Based on these observations, we propose that subversion of syndecan-1 HS to inhibit neutrophil CRAMP may be a critical virulence activity of S. aureus in the cornea.

It is important to note that CRAMP is not only expressed by neutrophils, but also by macrophages and epithelial cells (51), suggesting that syndecan-1 HS may also inhibit CRAMP produced by other cell types in S. aureus corneal infection. However, the observation that CRAMP is not expressed in injured but uninfected mouse corneas (57) suggest that epithelial cell-derived CRAMP is not targeted for inhibition by syndecan-1, although epithelial cells are thought to make antimicrobial peptides on request. More importantly, we previously found that neutrophil depletion significantly enhances S. aureus virulence in the Sdc1−/− cornea (21), which provides additional evidence that neutrophil-derived, and not epithelial cell-derived CRAMP is important.

One of the major findings of our study was that 2-O-sulfated groups are essential for the ability of syndecan-1 HS to inhibit CRAMP- and neutrophil-mediated S. aureus killing, and to promote S. aureus corneal infection in mice. These observations were surprising because many HS activities are thought to depend more on the overall organization of HS domains and on the overall net charge of the glycosaminoglycan than on specific modifications (13). However, the structural basis of how HS regulates biological molecules and their processes is still being worked out. What is known for 2-O-sulfation is that it is essential for normal development since mice deficient in 2-O-sulfotransferase die few days after birth due to renal agenesis and CNS and skeletal abnormalities (58). Regulation of several growth factor activities by HS, such as FGF signaling and Wnt signaling (59, 60), and uptake of plasma lipoproteins by hepatocytes (33) have also been shown to be dependent on the presence of 2-O-sulfated uronic acids. However, how 2-O-sulfate groups in HS mediate the inhibition of neutrophil CRAMP-mediated host defense and enhance S. aureus virulence in the cornea have yet to be elucidated. Because HS binds to antimicrobial peptides and inhibit their antibacterial activity by interfering with peptide binding to target bacterial cells (19), syndecan-1 HS is also expected to inhibit CRAMP in a similar manner. A particular binding site in CRAMP may directly bind to 2-O-sulfated uronic acids in syndecan-1 HS or bind to a conformation of syndecan-1 HS that is dictated by 2-O-sulfated motifs. In fact, spacing of cationic amino acids is important for the anti-bacterial activity of LL-37 (61), suggesting that similar spacing of cationic residues in CRAMP may allow this peptide to avidly bind to 2-O-sulfate motifs in syndecan-1 HS.

Furthermore, the antibacterial activity of LL-37 is dependent on the extent of amphipathic α-helicity (62). In water, however, LL-37 exhibits a disordered structure consistent with the fact that the energy provided by the hydrogen bonds are not sufficient to overcome the entropic energy associated with folding in short polypeptides, suggesting that the α-helical content of cathelicidins increases when mobilized to kill pathogens. Considering the fact that hydrogen bonds of short peptide α-helices are readily broken by water, perhaps binding of highly anionic molecules like syndecan-1 HS, which would accompany water, may disrupt the α-helicity of CRAMP and hence its anti-staphylococcal activity. Alternatively or concurrently, syndecan-1 HS binding may prevent CRAMP oligomerization, which is considered important for cathelicidins to form toroidal pores in bacterial membranes (62). Additional studies are required to precisely determine how 2-O-sulfated domains in syndecan-1 HS inhibit CRAMP activity.

Another interesting finding of this study was that 2-O-sulfated heparosan inhibits CRAMP- and neutrophil-mediated S. aureus killing, and enhances S. aureus virulence in mouse...
S. aureus Subverts 2-O-Sulfated Domains in Syndecan-1 HS

corneas. HS 2-O-sulfation can occur on both IdoA and GlcA, but is mostly found on IdoA in native HS and heparin due primarily to a higher affinity of 2-O-sulfotransferase for IdoA over GlcA (63). However, heparosan only contains GlcA, suggesting an intriguing possibility where 2-O-sulfated GlcA (GlcA2S)-containing domains in syndecan-1 HS mediate its pro-pathogenic activities in S. aureus corneal infection. IdoA can assume both a \( ^4C_1 \) chair and \( ^3S_2 \) skew boat forms as these have nearly identical energy conformations (64), allowing IdoA to bind to HS-binding ligands in either conformation. This conformational flexibility of IdoA is thought to be important for the ability of heparin and HS to interact with many proteins. In contrast, GlcA prefers a \( ^4C_1 \) conformation, giving a relatively rigid structural unit in HS and heparin and potentially mediate specific interactions with certain HS-binding ligands (64). Thus, syndecan-1 HS binding to CRAMP may be dictated by regions containing GlcA2S and that this interaction mediates the highly specific activities of syndecan-1 HS and 2-O-sulfated heparan compounds in S. aureus corneal infection. However, our results do not completely exclude the possibility that IdoA2S in the \( ^4C_1 \) chair conformation can function similarly.

In addition, although GlcA2S is a rare modification and the content of GlcA2S in corneal syndecan-1 HS is not known, it is worth noting that certain tissues, such as the liver (65) and brain (66), have a higher proportion of GlcA2S, suggesting a possibility where corneal epithelial tissues may also contain higher levels of this sulfate modification. Interestingly, an unusual N-unsubstituted and 3-O-sulfated glucosamine unit has been shown to mediate the specific binding of herpes simplex virus gD protein (67), suggesting that certain pathogens may subvert rare HS modifications in syndecan-1 for their pathogenesis.

In sum, our findings reveal a new pathogenic role for syndecan-1 in S. aureus corneal infection where its shed ectodomain inhibits neutrophil-mediated defense mechanisms crucial to the clearance of S. aureus in the cornea. Although the normal functions of syndecan-1 in the cornea remain to be elucidated, our findings suggest a possible beneficial role of inhibiting syndecan-1 HS and, in particular, the activity of 2-O-sulfated HS domains in treating S. aureus keratitis, for which new interventions are needed. Furthermore, CRAMP is not only important for host defense against S. aureus, but has also been shown to be critical in infections caused by other major pathogens of the ocular surface, such as P. aeruginosa (54) and S. pneumoniae (68). These observations suggest that neutralizing syndecan-1 HS to enhance CRAMP activity may be a viable option for the treatment of infectious keratitis caused by multiple bacterial pathogens of the ocular surface.

References

1. Whitcher, J. P., Srinivasan, M., and Upadhyay, M. P. (2001) Corneal blindness: a global perspective. Bull. World Health Organ. 79, 214–221

2. Wilhelmsen, K. R. (2002) Indecision about corticosteroids for bacterial keratitis: evidence-based update. Ophthalmology 109, 835–842; quiz 843

3. Bourrier, T., Thomas, F., Borderie, V., Chaumeil, C., and Laroche, L. (2003) Bacterial keratitis: predisposing factors, clinical and microbiological review of 300 cases. Br. J. Ophthalmol. 87, 834–838

4. Limberg, M. B. (1991) A review of bacterial keratitis and bacterial conjunctivitis. Am. J. Ophthalmol. 112, 25–95

5. Green, M., Apel, A., and Stapleton, F. (2008) Risk factors and causative organisms in microbial keratitis. Cornea 27, 22–27

6. Schaefer, F., Bruttin, O., Zografos, L., and Guex-Crosier, Y. (2001) Bacterial keratitis: a prospective clinical and microbiological study. Br. J. Ophthalmol. 85, 842–847

7. Ly, C. N., Pham, J. N., Badenoeh, P. R., Bell, S. M., Hawkins, G., Rafferty, D. L., and McClellan, K. A. (2006) Bacteria commonly isolated from keratitis specimens retain antibiotic susceptibility to fluoroquinolones and gentamicin plus cephalothin. Clin. Experiment Ophthalmol. 34, 44–50

8. Teng, Y. H., Aquino, R. S., and Park, P. W. (2012) Molecular functions of syndecan-1 in disease. Matrix Biol. 31, 3–16

9. Bartlett, A. H., and Park, P. W. (2010) Proteoglycans in host-pathogen interactions: molecular mechanisms and therapeutic implications. Expert Rev. Mol. Med. 12, e5

10. Park, P. W., Reizes, O., and Bernfield, M. (2000) Cell surface heparan sulfate proteoglycans: selective regulators of ligand-receptor encounters. J. Biol. Chem. 275, 29923–29926

11. Bernfield, M., Götte, M., Park, P. W., Reizes, O., Fitzgerald, M. L., Linseau, J., and Zako, M. (1999) Functions of cell surface heparan sulfate proteoglycans. Annu. Rev. Biochem. 68, 729–777

12. Esko, J. D., and Selleck, S. B. (2002) Order out of chaos: assembly of ligand binding sites in heparan sulfate. Annu. Rev. Biochem. 71, 435–471

13. Kreuger, J., Spillmann, D., Li, J. P., and Lindahl, U. (2006) Interactions between heparan sulfate and proteins: the concept of specificity. J. Cell Biol. 174, 323–327

14. Kobayashi, F., Yamada, S., Tagawa, S., Kataoka, C., Naito, S., Hama, Y., Tani, H., Matsuura, Y., and Sugahara, K. (2012) Specific interaction of the envelope glycoproteins E1 and E2 with liver heparan sulfate involved in the tissue tropism of infection by hepatitis C virus. Glycocon. J. 29, 211–220

15. Fechner, T., Stallmann, S., Moelken, K., Meyer, K. L., and Hegemann, J. H. (2013) Characterization of the interaction between the chlamydial adhesin OmcB and the human host cell. J. Bacteriol. 195, 5323–5333

16. Park, P. W., Foster, T. J., Nishi, E., Duncan, S. J., Klagsbrun, M., and Chen, Y. (2004) Activation of syndecan-1 ectodomain shedding by Staphylococcus aureus alpha-toxin and beta-toxin. J. Biol. Chem. 279, 251–258

17. Park, P. W., Pier, G. B., Preston, M. J., Goldberger, O., Fitzgerald, M. L., and Bernfield, M. (2000) Syndecan-1 shedding is enhanced by LsaA, a secreted virulence factor of Pseudomonas aeruginosa. J. Biol. Chem. 275, 3057–3064

18. Chen, Y., Hayashida, A., Bennett, A. E., Hollingshead, S. K., and Park, P. W. (2007) Streptococcus pneumoniae sheds syndecan-1 ectodomains through ZmpC, a metalloprotease virulence factor. J. Biol. Chem. 282, 159–167

19. Park, P. W., Pier, G. B., Hinkes, M. T., and Bernfield, M. (2001) Exploitation of syndecan-1 shedding by Pseudomonas aeruginosa enhances virulence. Nature 411, 98–102

20. Hayashida, A., Bartlett, A. H., Foster, T. J., and Park, P. W. (2009) Staphylococcus aureus beta-toxin induces acute lung injury through syndecan-1. Am. J. Pathol. 174, 509–518

21. Hayashida, A., Amano, S., and Park, P. W. (2011) Syndecan-1 promotes Staphylococcus aureus corneal infection by counteracting neutrophil-mediated host defense. J. Biol. Chem. 286, 3288–3297

22. Chen, J., Jones, C. L., and Liu, J. (2007) Using an enzymatic combinatorial approach to identify anticoagulant heparan sulfate structures. Chem. Biol. 14, 986–993

23. Girgis, D. O., Sloop, G. D., Reed, J. M., and O’Callaghan, R. J. (2004) Susceptibility of aged mice to Staphylococcus aureus keratitis. Curr. Eye Res. 29, 269–275

24. Hayashida, K., Chen, Y., Bartlett, A. H., and Park, P. W. (2008) Syndecan-1 is an in vivo suppressor of Gram-positive toxic shock. J. Biol. Chem. 283, 19895–19903

25. Hayashida, K., Parks, W. C., and Park, P. W. (2009) Syndecan-1 shedding facilitates the resolution of neutrophil inflammation by removing sequestered CXC chemokines. Blood 114, 3033–3043

26. Echtermeyer, F., Streit, M., Wilcox-Adelman, S., Saoncella, S., Denhez, F., Detmar, M., and Goetinck, P. (2001) Delayed wound repair and impaired angiogenesis in mice lacking syndecan-4. J. Clin. Invest. 107, R9–R14

27. Sherritz, R. J., Carruth, W. A., Hampton, A. A., Byron, M. P., and Solomon, D. D. (1993) Efficacy of antibiotic-coated catheters in preventing subcu-
28. Miller, L. G., Perdreau-Remington, F., Rieg, G., Mehdi, S., Perlroth, J., Bayer, A. S., Tang, A. W., Phung, T. O., and Spellberg, B. (2005) Necrotizing fasciitis caused by community-associated meticillin-resistant Staphylococcus aureus in Los Angeles. N. Engl. J. Med. 352, 1445–1453

29. Park, P. W., Roberts, D. D., Grosso, L. E., Parks, W. C., Rosenboom, J., Abrams, W. R., and Mecham, R. P. (1991) Binding of elastin to Staphylococcus aureus. J. Biol. Chem. 266, 23399–23406

30. Stepp, M. A., Gibson, H. E., Gala, P. H., Iglesia, D. D., Pajoohesh-Ganji, A., Pal-Ghosh, S., Brown, M., Aquino, C., Schwartz, A. M., Golberger, O., Hinkes, M. T., and Bernfield, M. (2002) Defects in keratinocyte activation during wound healing in the syndecan-1-deficient mouse. J. Cell Sci. 115, 4517–4531

31. Mullloy, B., Forster, M. J., Jones, C., and Davies, D. B. (1993) N.m.r., and molecular-modelling studies of the solution conformation of heparin. Biochem. J. 293 (Pt 3), 849–858

32. Kobayashi, T., Habuchi, H., Tamura, K., Ide, H., and Kimata, K. (2007) Essential role of heparan sulfate 2-O-sulfotransferase in chick limb bud patterning and development. J. Biol. Chem. 282, 19589–19597

33. Stanford, K. L, Wang, L., Castagnola, J., Song, D., Bishop, J. R., Brown, J. R., Lawrence, R., Bai, X., Habuchi, H., Tanaka, M., Cardoso, W. V., Kimata, K., and Esko, J. D. (2010) Heparan sulfate 2-O-sulfotransferase is required for triglyceride-rich lipoprotein clearance. J. Biol. Chem. 285, 286–294

34. Garner, O. B., Bush, K. T., Nigam, K. B., Yamauchi, Y., Xu, D., Esco, J. D., and Nigam, S. K. (2011) Stage-dependent regulation of mammary ductal branching by heparan sulfate and HGF-cMet signaling. Dev. Biol. 355, 394–403

35. Gronert, K. (2010) Resolution, the grail for healthy ocular inflammation. Exp. Eye. Res. 91, 478–485

36. Borregaard, N. (2010) Neutrophils, from marrow to microbes. N. Engl. J. Med. 363, 657–670

37. Li, Q., Park, P. W., Wilson, C. L., and Parks, W. C. (2002) Matrilysin side chains of the major antimicrobial region of human cathelicidin LL-37. J. Biol. Chem. 277, 23511–23517

38. Borregaard, N. (2010) Bactericidal activity of mammalian cathelicidin-derived peptides. J. Immunol. 184, 5473–5478

39. Slominski, A., Tosti, A., and Tomson, B. (2003) Age-related changes of the skin environment. J. Investig. Dermatol. 121, 3S–7S

40. Slominski, A., Tosti, A., Petrczko, A., and Tomson, B. (2003) The skin barrier and aging. J. Investig. Dermatol. 121, 9S–15S

41. Slominski, A. (2003) The role of the skin barrier in the pathogenesis of skin diseases. J. Investig. Dermatol. 121, 16S–22S

42. Slominski, A., Tosti, A., and Tomson, B. (2003) Age-related changes of the skin environment. J. Investig. Dermatol. 121, 3S–7S