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MAJOR ARTICLE

Staphylokinase Promotes the Establishment of *Staphylococcus aureus* Skin Infections While Decreasing Disease Severity

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Skin infections are frequently caused by *Staphylococcus aureus* and can lead to a fatal sepsis. The microbial mechanisms controlling the initiation and progression from mild skin infection to a severe disseminated infection remain poorly understood. Using a combination of clinical data and in vitro and ex vivo assays, we show that staphylokinase, secreted by *S. aureus*, promoted the establishment of skin infections in humans and increased bacterial penetration through skin barriers by activating plasminogen. However, when infection was established, the interaction between staphylokinase and plasminogen did not promote systemic dissemination but induced the opening and draining of abscesses and decreased disease severity in neutropenic mice. Also, increased staphylokinase production was associated with noninvasive *S. aureus* infections in patients. Our results point out the dual roles of staphylokinase in *S. aureus* skin infections as promoting the establishment of infections while decreasing disease severity.

Keywords. staphylococcus; staphylokinase; plasminogen; skin infection; mouse model; pathogen–host relation.

Staphylococcus aureus is a major cause of severe infections in both developed and developing countries [1, 2]. The most common *S. aureus* diseases are skin and soft-tissue infections, ranging from mild and superficial conditions, such as impetigo and folliculitis, to potentially fatal and deep-seated infections, such as cellulitis, pyomyositis, and fasciitis [3]. Even the superficial skin infections sometimes give rise to dangerous systemic dissemination and sepsis, and skin and soft-tissue infections are the most commonly reported sources of systemic bacteremia [4]. On the host side, the risk of transition from minor infection to severe infection is increased by immunosuppression [5]. However, the microbial factors that control this 2-faced behavior of *S. aureus*—a cause of mild disease and of invasive, fatal infection—are still poorly understood.

Staphylokinase (Sak), a protein secreted by many *S. aureus* strains [6], activates human plasminogen (h-plg) into plasmin [7]. Plasmin in turn digests fibrin clots and many components of extracellular matrix and basal membranes [8] and activates latent matrix metalloproteinases, leading to extensive proteolysis [8]. Therefore, Sak could hypothetically enhance staphylococcal escape from the fibrin clots and facilitate systemic bacterial dissemination from the infection site [8–10]. Other bacterial plg activators, secreted by *Yersinia pestis* and streptococci, have been reported to enhance the invasive capacity of bacteria [11, 12]. However, this hypothesis has not been tested in the case of staphylococci.

In this study, we used Sak transgenic *S. aureus* strains, transgenic mice expressing h-plg, and clinical isolate collections to investigate the role played by Sak in skin infections. The secretion of Sak was very important for the establishment of *S. aureus* skin infection.

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However, contrary to previous assumptions, Sak was not involved in the systemic spread of bacteria; by interacting with plasminogen, Sak even limited the severity of infection.

METHODS

Bacterial Strains and Preparation of Bacterial Batches

Congenic *S. aureus* strains, differing in Sak secretion [13], were used: the LS-1*EP* strain does not secrete Sak, the LS-1*sak* strain secretes moderate amounts of Sak, and the LS-1*spa-sak* strain secretes high amounts of Sak.

Two collections of clinical *S. aureus* isolates were used. The first collection consisted of isolates from both primary (n = 60) and secondary (n = 100) skin and soft-tissue infections [14]. Primary infections were defined as spontaneous skin lesions of previously healthy skin, without preceding trauma or preexisting skin disorders, accompanied by clinical signs of local infection (warmth, swelling, tenderness, and purulent discharge). These included cases of cutaneous abscesses, impetigo, folliculitis, and furunculosis. Secondary infections were defined as cases of skin infections with preceding skin trauma or preexisting skin disorders and included postsurgical wound infections, infections of wounds caused by vascular insufficiency, diabetic wound infections, and infections following skin trauma. The majority of infections were localized in the skin. Only 2 cases of primary infections were soft-tissue infections.

The second collection consisted of *S. aureus* isolates from uncomplicated skin infections (n = 49) and invasive infections (n = 159; 37 were patients receiving immunosuppressive drugs, of whom 2 had leukopenia) collected from patients in Skaraborg Hospital, Sweden [5]. Uncomplicated skin infections were defined as cases of skin wounds in conjunction with clinical signs of local infection but without involvement of other organ, no positive culture results for specimens from otherwise sterile organs, no clinical signs of systemic infection (fever [temperature, >38.5°C], tachycardia, and/or hypotension), and no need for in-hospital treatment. Invasive infections were defined as cases with positive cultures of specimens from otherwise sterile internal sites (eg, blood, joint fluid, cerebrospinal fluid, bone biopsy, and samples from deep tissue abscesses), with clinical signs of systemic infection and with a need for in-hospital treatment.

Bacteria were grown at 37°C in tryptic soy broth, with shaking or on horse blood agar plates and were prepared for infection experiments as described previously [13].

Measurement of Sak Secretion by Clinical Isolates of *S. aureus*

Overnight cultures of *S. aureus* isolates were centrifuged (at 3400 ×*g* for 10 minutes at 4°C), and the supernatants were assayed for Sak activity. Fifty microliters of 0.04 mg/mL human glu-plasminogen (Technoclone, Vienna, Austria) in Tris-buffered saline was mixed with 30 µL of supernatant. After incubation for 15 minutes at 37°C, 20 µL of a plasmin-specific chromogenic substrate S-2251 (H-D-Val-Leu-Lys-paranitroanilide, 3 mmol/L, Chromogenix, Milano, Italy) was added. Color development was continuously measured at 405 nm with a SpectraMax 340PC384 reader over a 1-hour period of incubation at 37°C. Samples with a continuous increase in absorbance of at least 0.005 were considered to be Sak positive [13]. The specificity of this chromogenic assay was confirmed in a preliminary experiment in the uncomplicated skin infection group, in which polymerase chain reaction was performed to detect the Sak gene (data not shown).

Mice

Sak activates h-plg but has little effect on mouse plg [7]. Therefore human plasminogen transgenic mice (h-plg mice) of both sexes, backcrossed with C57BL6/J mice (Charles River, Sulzfeld, Germany), were used [12]. The mice were housed under standard conditions and were used for experiments at the age of 12–25 weeks. For each experiment, the age and sex were matched. The Animal Research Ethical Committee of Gothenburg approved the study.

Measurement of *S. aureus* Invasiveness in a Transwell System

To measure bacterial capacity for penetrating skin barriers, we used a Transwell system (Corning, Lowell, MA) with a 3-µm pore size polyester membrane and a 6.5-mm diameter insert. Overnight cultures of *S. aureus* were washed with phosphate-buffered saline (PBS) and resuspended in fresh tryptic soy broth to an OD_{600} of 0.5 (approximately $1–2 \times 10^9$ colony-forming units [CFU]/mL). One part of the bacterial suspension was mixed with 2 parts of tested material, and 100 µL of the mixture was pipetted into a transwell insert and solidified for 15 minutes at 37°C. Next, inserts were placed in the lower well of the transwell filled with 0.6 mL PBS, and the entire transwell was incubated at 37°C. At regular intervals, the CFU in the lower wells were counted. Details of preparation of test materials are available in the Supplementary Materials.

Measurement of *S. aureus* Invasiveness Ex Vivo

Skin biopsy specimens (diameter, 8 mm) were taken from the shaved backs of h-plg mice, placed floating on human heat-inactivated plasma in cell culture dishes, and incubated for 4 hours at 37°C. Next, 5 µL of fluid from overnight culture of the *S. aureus* strains was placed on the surface of the biopsy specimen. After 24 hours of incubation at 37°C, biopsy specimens were embedded in Tissue-Tek OCT compound (Sakura-Finetek, Gothenburg, Sweden), frozen at −70°C, and processed for immunohistochemistry analysis.

Induction of Neutropenia in Mice

Mice were immunosuppressed by intraperitoneal injections of 300 mg/kg body weight (100 mg/kg for tranexamic acid experiments) of cyclophosphamide (Baxter Medical, Kista, Sweden) 1 and 4 days before infection. For experiments with a duration >5

days, an additional 200 mg/kg of cyclophosphamide was injected on day 4 after infection. Peripheral blood leukocytes during the experiments remained <5% of the normal count.

Experimental Skin Infection

A skin infection model modified from Mölne et al was used [15]. Neutropenic mice had their backs shaven and were injected subcutaneously on each flank with 25 μL of bacterial suspension in PBS containing $1–2 \times 10^6$ CFU/mL of one of the *S. aureus* strains. In some experiments, mice were injected with the same strain on both flanks, while in other experiments, one flank was injected with the LS-1*EP* strain, while the other flank was injected with LS-1*sak* or LS-1*spa-sak*. Seven separate experiments were performed, and the results were pooled.

Evaluation of Skin Infection Severity

On days 3, 5, 7, and 9 after injection, the maximal diameters of the skin lesions were measured with a caliper. The injection sites were inspected daily for signs of spontaneous abscess draining. In some of the experiments, skin biopsy specimens were obtained on days 3 and 9 for histopathologic analysis.

In experiments in which the mice were injected on both flanks with the same *S. aureus* strain, additional tests were performed on days 1, 3, and 9. To examine whether Sak enhances the invasiveness of *S. aureus* in vivo, kidneys, spleens, and livers were removed (as well as skin, on day 9) and homogenized, and their CFU content was counted. To study the impact of Sak on systemic inflammation, blood was collected into ethylenediaminetetraacetic acid tubes and centrifuged at 1100 ×*g* for 20 minutes, and plasma was stored at −20°C.

Histopathologic Analysis

Skin biopsy specimens were prepared as previously described [15] and examined by a pathologist (W. W.) blinded to the experimental groups. The widths and depths of lesions were measured, and the degrees of acute inflammation were scored on a scale from 0 to 3.

Measurement of Inflammatory Markers in Mouse Plasma

Plasma levels of plasminogen activator inhibitor-1 (PAI-1) were measured with Murine PAI-1 Total Antigen Assay enzyme-linked immunosorbent assay (ELISA; Innovative Research, Novi, MI), levels of interleukin 10 (IL-10) were measured with the mouse IL-10 DuoSet ELISA (R&D Systems, Minneapolis, MN), and levels of interleukin 6 (IL-6) were measured with the mouse IL-6 DuoSet ELISA (R&D Systems) with streptavidin-poly-HRP (Sanquin, Amsterdam, Netherlands).

Statistical Analysis

Differences in the frequencies of Sak secretion by clinical strains were analyzed by the χ^2 test. Correlations of Sak secretion and Panton-Valentine leukocidin gene in isolates from primary skin infections were analyzed by the χ^2 test. Differences in amounts of secreted Sak were analyzed with an unpaired *t* test. Differences in the severity of skin infection between the groups were analyzed with a Mann–Whitney test or Wilcoxon signed rank test. Differences in lesion opening were analyzed with a log-rank test. Prism 5.04 software (GraphPad Software, La Jolla, CA) was used, and *P* values of <.05 were considered significant.

Additional Methods

Details of measurement of Sak expression by *S. aureus* after stimulation with plg, of immunohistochemistry analysis, of tranexamic acid treatment, and of the immunocompetent skin infection model are available in the Supplementary Materials.

RESULTS

Sak Promotes the Establishment of *S. aureus* Primary Skin Infections

We examined Sak secretion by *S. aureus* clinical isolates from both primary and secondary skin and soft-tissue infections. The majority of the isolates from primary infections (70%) secreted detectable quantities of Sak, whereas only a minority of isolates from secondary infections secreted Sak (41%; $P = .0004$; Figure 1*A*). Isolates from primary skin infections also secreted higher amounts of Sak ($P = .02$; Figure 1*B*), suggesting that Sak might play an important role in the establishment of primary skin infections. There was no correlation between Sak secretion and the presence of the gene of Panton-Valentine leukocidin, another factor known to promote primary skin infections.

Activation of Plasminogen by Sak Helps *S. aureus* Penetrate Skin Physiological Barriers

We used an in vitro system to determine whether Sak secretion helps *S. aureus* spread within tissues in the presence of plasminogen. The same pattern of invasiveness enhanced by Sak was observed in all experiments. The LS-1*spa-sak* strain, which had the highest Sak secretion level, efficiently penetrated keratinocyte monolayers, clots of fibrin, clots of coagulated plasma, and reconstituted basal membranes (Figure 2*A*). The LS-1*sak* strain, which had moderate levels of Sak secretion, penetrated fibrin clots, basal membranes, and keratinocyte monolayers to a lesser degree than LS-1*spa-sak* but still had more invasive capability than strain LS-1*EP*, which secretes no Sak (Figure 2*A*).

Penetration by LS-1*spa-sak* was completely blocked when no plg was provided (Figure 2*B*). Also, the addition of aprotinin, a plasmin inhibitor, dose-dependently suppressed penetration by LS-1*spa-sak* (Figure 2*B*).

Plasminogen Upregulates Sak Expression

To elucidate the potential impact of plg on the expression of Sak by *S. aureus*, we exposed LS-1 congenic strains to plg (0–0.2 mg/mL). This resulted in upregulated Sak messenger RNA expression in both LS-1*sak* and LS-1*spa-sak* as compared to controls in a dose-dependent manner (data not shown).

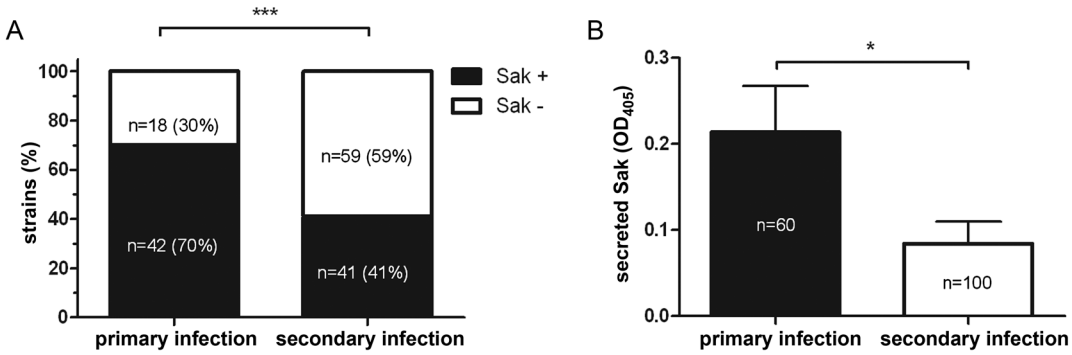

Figure 1. Secretion of staphylokinase in clinical isolates from *Staphylococcus aureus* skin infections is associated with primary infections. *A*, Frequency of staphylokinase secretion among *S. aureus* strains from a collection of primary and secondary skin infections. *B*, Amount of staphylokinase secreted by the strains, shown as means ± standard error of the mean. Frequencies are compared by the χ^2 test; the amounts of secreted staphylokinase are compared by unpaired *t* tests. **P* < .05; ****P* < .001.

Sak Promotes *S. aureus* Penetration Through the Skin

We further examined the capacity of *S. aureus* to penetrate skin ex vivo. When cultures of LS-1*spa-sak* or LS-1*sak* strains were placed on a skin biopsy specimen, they readily penetrated through the epidermis and invaded the dermis, whereas LS-1*EP* remained on the surface of the skin, as determined by fluorescence microscopy (Figure 2*C*).

Activation of Plasminogen by Sak Does Not Promote Systemic Spread From the Infection Site But Reduces Lesion Size in a Model for *S. aureus* Skin Infection

To assess the impact of the Sak–plasminogen interaction on established *S. aureus* skin infections, we injected bacteria subcutaneously into neutropenic wild-type and h-plg mice. Because Sak activates plg only in h-plg mice [13], this allowed assessment of the effect of plg activation by Sak, while immunosuppression enabled the systemic spread of bacteria from the skin.

After subcutaneous injection, bacteria were found in the kidneys, spleens, and livers of some of the animals on days 3 and 9 following infection. No differences were observed in the organs of wild-type and h-plg mice, nor between animals injected with *S. aureus* strains LS-1*EP* and LS-1*spa-sak*, suggesting that the activation of plg by Sak had no effect on bacterial systemic spread (Supplementary Figure 1). Mortality during the experiment reached approximately 40% and did not differ between the experimental groups (data not shown). There were no differences in bacterial counts in infected skin at the end of the experiment (data not shown). However, there were clear differences in local infection severity.

When the LS-1*EP* strain, which does not secrete Sak, was injected, the size of the lesion was similar, regardless of whether wild-type or h-plg mice were used (Figure 3*A*). In contrast, the *S. aureus* strain LS-1*spa-sak* resulted in significantly decreased lesion sizes in h-plg animals as compared to wild-type mice (Figure 3*A*). Lesion size was also decreased in h-plg mice injected with LS-1*sak* (Supplementary Figure 2), and the effect was smaller than in the case of LS-1*spa-sak*, suggesting a dose dependence of the effect. The capacity of the Sak-plg interaction to decrease the lesion size was also observed when lesion sizes were compared directly between LS-1*spa-sak* and LS-1*EP* strains in h-plg mice (Figure 3*B*).

When h-plg mice infected with the LS-1*spa-sak* strain were treated with tranexamic acid, which inhibits Sak-induced plg activation [16], the sizes of skin lesions increased ($P = .02$; Figure 3C). This further demonstrated that plg activation was responsible for the diminished severity of skin infections.

Activation of Plasminogen by Sak Promotes the Opening and Drainage of Skin Lesions

During the experimental *S. aureus* skin infection, some of the lesions spontaneously opened and drained. In wild-type mice, this was rare and irrespective of the injected bacterial strain (Figure 3*D*). However, in h-plg animals, there were significantly more open lesions when the LS-1*spa-sak* strain was injected ($P = .0009$; Figure 3*D*).

Activation of Plasminogen by Sak Reduces Tissue Damage in *S. aureus* Skin Infections

Skin lesions caused by LS-1*spa-sak* were further investigated histopathologically. Both the width and depth of lesions were significantly smaller on day 9 in h-plg animals than in wild-type mice (Figure 4*A*). There was also a trend toward decreased signs of acute inflammation in lesions from h-plg mice (Figure 4*B*).

The differences in histopathologic findings were especially striking between open lesions in h-plg animals and lesions in wild-type mice. Lesions in wild-type mice were still inflamed and necrotic, lacked granulation tissue or reepithelialization, and had large numbers of bacterial clusters in the dermis and

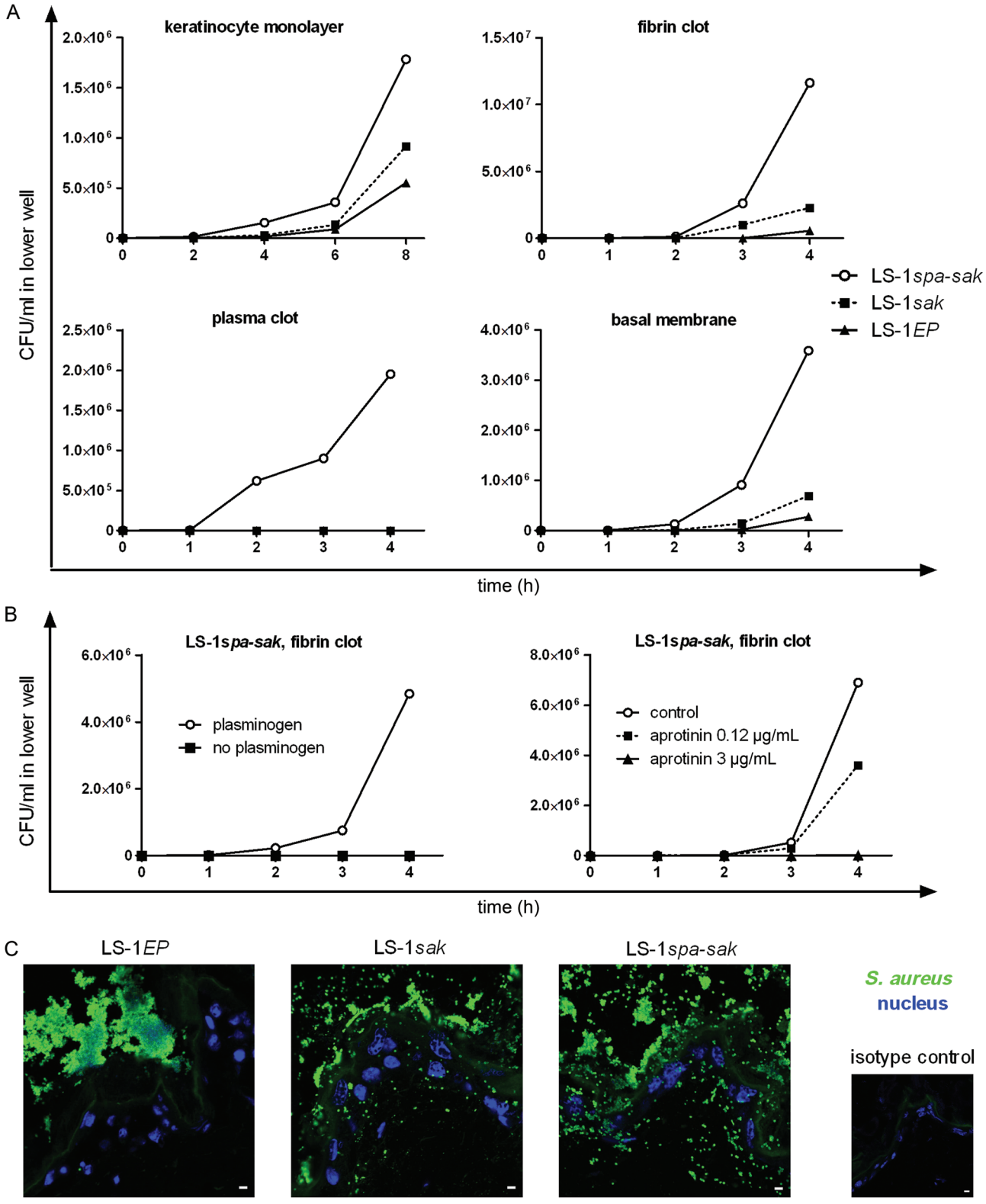

Figure 2. Activation of plasminogen by staphylokinase promotes penetration of *Staphylococcus aureus* through physiological barriers. *A*, Penetration of *S. aureus* strains through a keratinocyte cell monolayer, fibrin clot, plasma clot, and reconstituted basal membrane. Data from a representative experiment are shown. *B*, Penetration of *S. aureus* LS-1*spa-sak* through a fibrin clot in the presence/absence of plasminogen or aprotinin (plasmin inhibitor). Data from a representative experiment are shown. *C*, Fluorescence microscopy images of *S. aureus* strains penetrating into skin biopsy specimens from h-plg mice. Cell nuclei are stained blue with DAPI. *S. aureus* are stained green with anti–*S. aureus* antibodies conjugated to Alexa Fluor 488. Slight nonspecific green staining of the stratum corneum marks the border of the skin, with the dermis in the lower part of the image and the area outside of the skin in the upper part of the image. Representative images are shown. Scale bar, 10 µm.

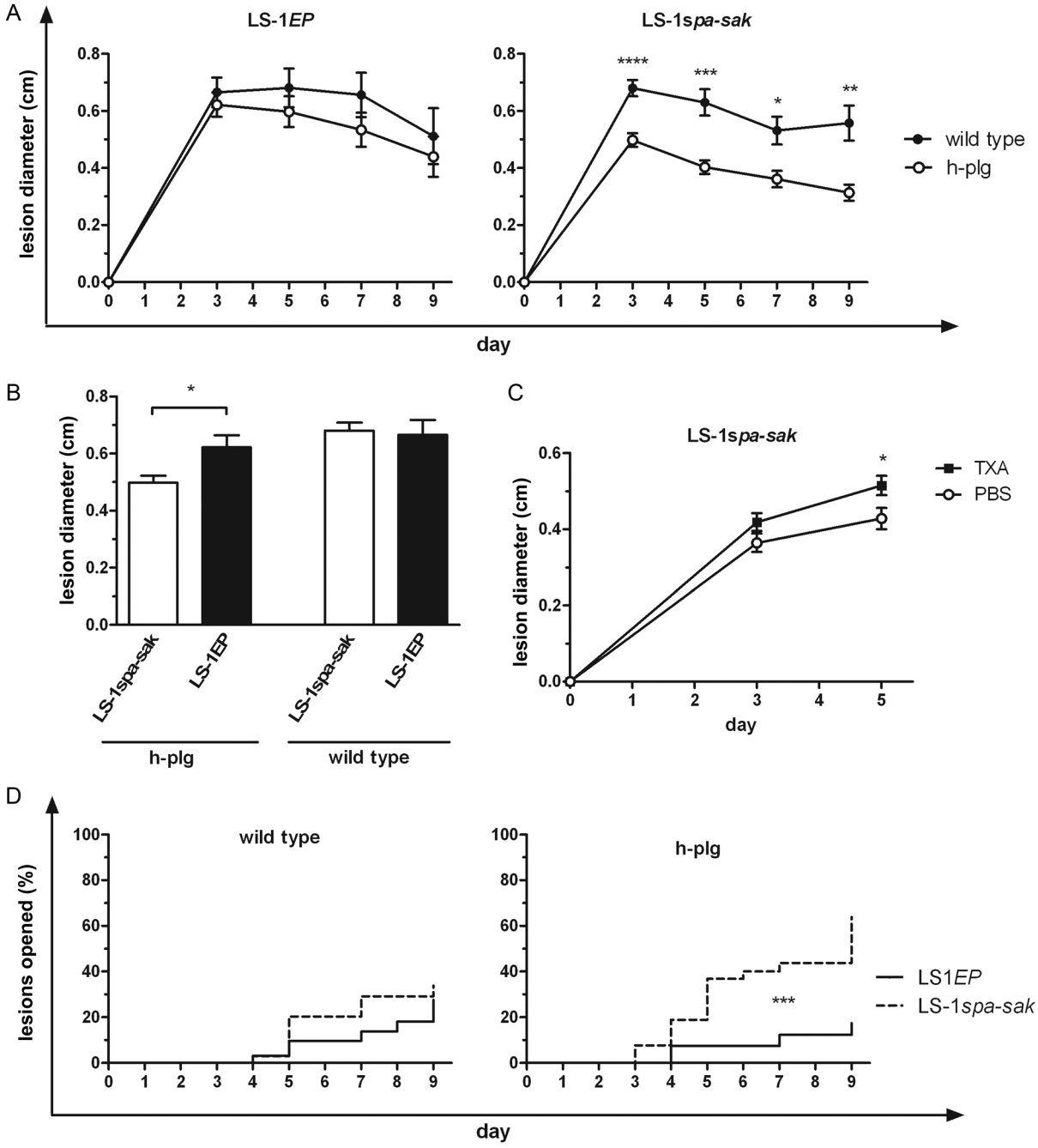

Figure 3. Activation of plasminogen by staphylokinase reduces the severity of *Staphylococcus aureus* skin infection. *A*, Sizes of skin lesions caused by the injection of the *S. aureus* LS-1*EP* strain (wild type, n = 35; h-plg, n = 41) or the LS-1*spa-sak* strain (wild type, n = 64; h-plg, n = 65) into wild-type and h-plg mice. *B*, Sizes of skin lesions on day 3 after injection of *S. aureus* strains LS-1*EP* (wild type, n = 35; h-plg, n = 41) and LS-1*spa-sak* (wild type, n = 64; h-plg, n = 65) into h-plg and wild-type mice. *C*, Sizes of skin lesions in h-plg mice treated with tranexamic acid (TXA), an inhibitor of plasminogen activation (n = 19), or with phosphate-buffered saline (n = 18) after injection of *S. aureus* LS-1*spa-sak*. *D*, Opening of skin lesions caused by the injection of *S. aureus* strains LS-1*EP* (wild type, n = 36; h-plg, n = 27) and LS-1*spa-sak* (wild type, n = 38; h-plg, n = 39) into wild-type and h-plg mice In panels *A–C*, data are shown as means ± standard error of the mean, and the groups were compared by the Mann–Whitney test. For panel *D*, the opening between the groups is compared by the log-rank test. **P* < .05; ***P* < .01; ****P* < .001; *****P* < .0001.

subcutaneous tissues (Figure 4*C*), while the open lesions in h-plg animals exhibited localized ulceration and evidence of the healing process, with reepithelialization and formation of granulation tissue under opened ulcers (Figure 4*D*). No significant differences in skin histopathologic findings were visible on day 3 after infection(data not shown).

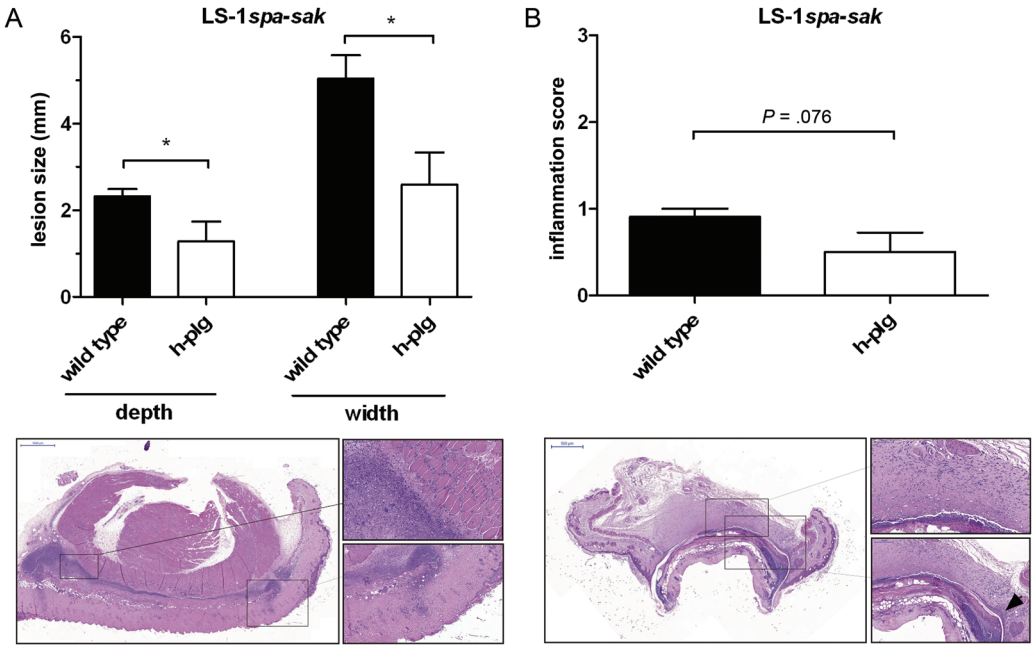

Figure 4. Activation of plasminogen by staphylokinase reduces tissue damage in *Staphylococcus aureus* skin infection. *A*, Skin lesion size measured in histopathologic sections on day 9 after injection of the *S. aureus* LS-1 *spa-sak* strain into wild-type (n = 11) and h-plg (n = 6) mice. *B*, Scores of acute inflammation in histopathologic sections on day 9 after injection of the *S. aureus* LS-1 *spa-sak* strain into wild-type (n = 11) and hpl-g (n = 6) mice. In panels *A* and *B*, data are shown as means ± standard error of the mean, and the groups are compared by the Mann–Whitney test. **P* < .05. *C* and *D*, Pathological changes on day 9 in the skin of mice infected with *S. aureus* LS-1 *spa-sak*. Representative images of "open" and "unopened" lesions are shown (*C*). In wild-type mice, diffuse acute inflammation and necrosis are present in subcutaneous tissues, along with the presence of bacteria in the dermis, subcutis, and muscles, but ulceration and granulation formation are not present. *D*, In h-plg mice, after the "opening" of the lesion, localized ulceration is visible, accompanied by evidence of the healing process, with granulation formation and reepithelialization (arrow).

Activation of Plasminogen by Sak Reduces Systemic Inflammation in *S. aureus* Skin Infection

The levels of systemic inflammatory markers were already elevated on day 1 after infection, but no differences were observed between the groups (Figure 5). On day 3, the levels of IL-6, PAI-1, and IL-10 were significantly lower in h-plg mice than in wild-type mice (Figure 5). On day 9, the levels of inflammatory markers dropped to very low values, and no

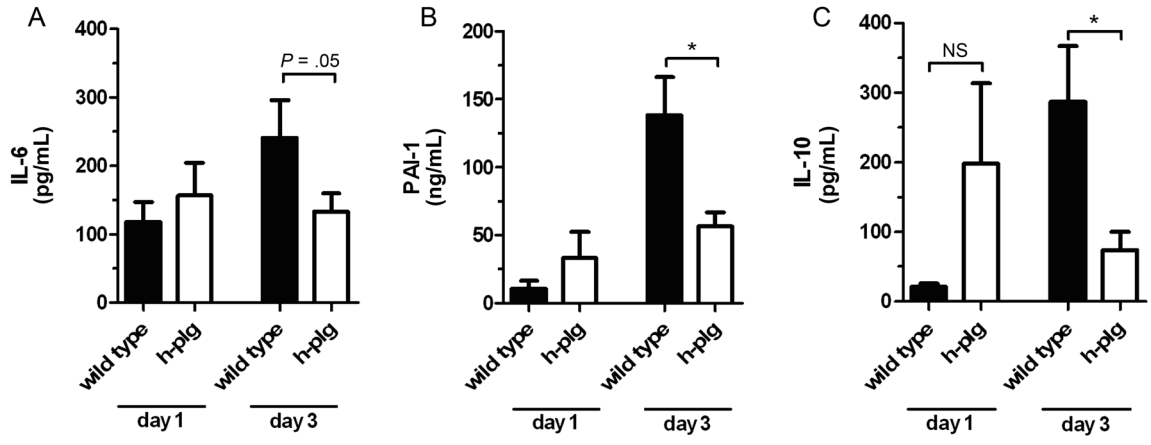

Figure 5. Activation of plasminogen by staphylokinase reduces the levels of inflammation markers in *Staphylococcus aureus* skin infection. *A–C*, Levels of interleukin 6 (IL-6; *A*), plasminogen activator inhibitor 1 (PAI-1; *B*), and interleukin 10 (IL-10) in the plasma of mice on day 1 (wild type, n = 4–7; h-plg, n = 6–8) and day 3 (wild type, n = 10; h-plg, n = 9) of skin infection with the *S. aureus* LS-1 *spa-sak* strain. Data are shown as means ± standard error of the mean. Groups were compared by the Mann–Whitney test. Abbreviation: NS, not significant. **P* < .05.

differences were observed between the groups (data not shown).

Sak Has No Effect on Infection Severity in Immunocompetent Mice

To study the impact of Sak on disease severity in immunocompetent mice, LS1*spa-sak* and LS1*EP* strains were injected subcutaneously into 2 flanks of healthy mice. LS1*spa-sak* tended to induce smaller lesions in h-plg mice, compared with LS1*EP*, on day 3, but no statistically significant differences were identified (Supplementary Figure 3).

Sak Is Associated With Uncomplicated *S. aureus* Infections in Humans

We examined Sak secretion by *S. aureus* clinical isolates from both uncomplicated skin infections (n = 49) and invasive infections (n = 159). There was no difference in the frequency of isolates secreting detectable quantities of Sak between the 2 groups (Figure 6*A*). However, isolates from invasive infections secreted lower amounts of Sak than isolates from uncomplicated infections (Figure 6*B*), suggesting that high Sak secretion might predict a less invasive course of *S. aureus* infection in humans. There were no differences in Sak secretion between the isolates from patients receiving immunosuppressive drugs and the isolates from immunocompetent patients.

DISCUSSION

The skin is a major barrier for infection in the body [3], so we hypothesized that Sak would aid in the initiation of skin infection. Activation of plg by bacteria has been speculated to be an important virulence mechanism enhancing bacterial invasiveness [8–10]. Indeed, we observed that the activation of plg by Sak stimulates the penetration of *S. aureus* through fibrin clots, coagulated plasma, reconstituted basal membranes, and keratinocyte monolayers. Importantly, cathelicidins secreted by skin keratinocytes upon contact with *S. aureus* [17] could additionally potentate local Sak activity [18].With data from ex vivo skin biopsy specimens and clinical *S. aureus* isolates, we confirmed that Sak facilitates *S. aureus* penetration from the epidermis into the dermis and plays an important role in the establishment of primary skin and soft-tissue infections. Panton-Valentine leukocidin, a cytotoxin secreted by *S. aureus*, is also known to be associated with primary skin infections [14, 19]. Yet, the lack of a correlation between Sak secretion and the presence of Panton-Valentine leukocidin suggests that they are 2 independent factors promoting skin infections.

In the skin, plg is present mainly in the basal layer of the epidermis and in the walls of hair follicles [20]. *S. aureus* colonizing skin is probably able to activate this plg with Sak, especially when skin microdamage is present, consequently leading to local breakdown of the basal membrane, lysis of the extracellular matrix, and interrupted cell adhesions. This paves the way for bacterial penetration from the epidermis into the dermis and establishment of infection.

Keeping with the original hypothesis of Sak being a virulence factor for bacterial dissemination, we further examined whether the secretion of Sak affects *S. aureus* metastasis from the skin in neutropenic mice. Surprisingly, the activation of plg by Sak had no impact on bacterial dissemination. Intriguingly, activation of plg by Sak even reduced the severity of skin infections. Plg activation caused a decrease in lesion size, visible both clinically and histopathologically. Systemic signs of inflammation were also lower in mice where Sak activated plg.

Deposition of fibrin, normally used by the body to trap bacteria and limit their spread [21], have a different role in staphylococcal infections. *S. aureus* uses deposited fibrin as a protective barrier against immune response and as a scaffold for developing infectious foci [22, 23], which is needed for full virulence [24]. The secretion of Sak and subsequent generation of plasmin and fibrinolysis might damage this protective fibrin shield and diminish virulence. This, however, might not be the case in our model, as appearance of phagocytes was greatly delayed in neutropenic mice. Plasmin is also involved in regulation of inflammation [25, 26],

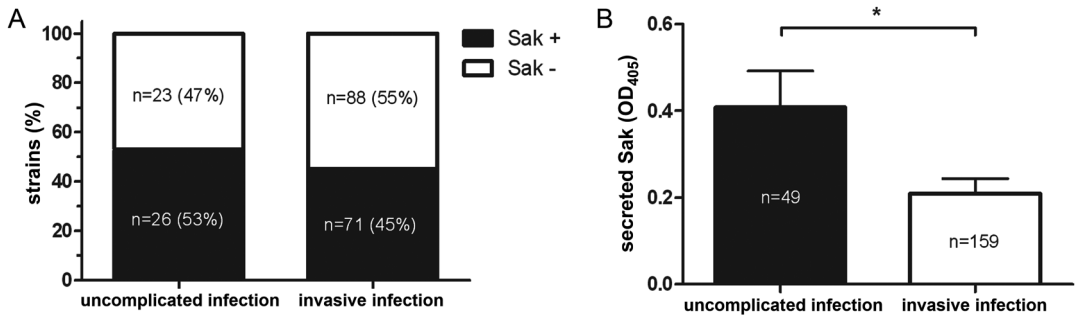

Figure 6. Secretion of staphylokinase in clinical *Staphylococcus aureus* isolates is associated with milder infections. *A*, Frequency of staphylokinase secretion among *S. aureus* strains from a collection of uncomplicated skin infections and severe invasive infections. *B*, Amount of staphylokinase secreted by the strains, shown as means ± standard error of the mean. The χ^2 test identified no significant differences in the frequencies. The amounts of secreted staphylokinase were compared by an unpaired *t* test. $P < .05$.

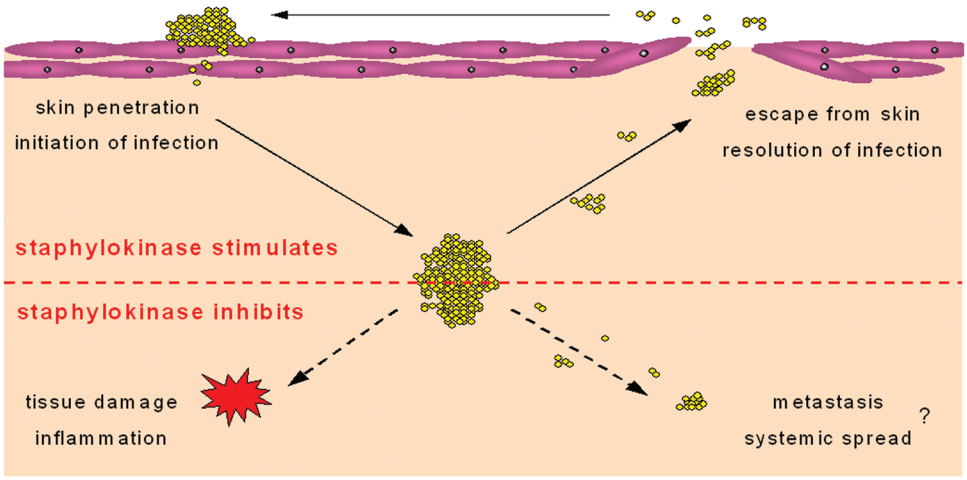

Figure 7. Proposed mechanism of staphylokinase activity in skin infections. The activation of plasminogen by staphylokinase promotes the penetration of *Staphylococcus aureus* into the skin from the surface and the establishment of infection, but it does not support further penetration and systemic spreading. Furthermore, once infection is already established, staphylokinase reduces virulence. Staphylokinase also accelerates healing by draining the infected lesions onto the skin surface; at the same time, this drainage might allow bacteria to spread to new sites on the skin.

stimulates wound healing [27, 28], and could destroy bacterial attachment sites in the extracellular matrix, which could explain why the induction of plasmin by Sak leads to less skin damage. These ideas are supported by the observations that plg and plasmin activity play a protective role in various *S. aureus* infections [26, 29, 30]. It is not obvious how activation of plg by Sak contributed to decreased infection severity in immunocompromised mice, but the possibility of Sak reducing the infection severity was previously suggested in systemic infections [13, 31]. The assumption that plg activation increases the bacterial virulence seems to be oversimplified, and microorganisms can sometimes activate plg as part of a nonvirulent interaction with the host.

In contrast to the antivirulence effect in neutropenic mice, Sak had no impact on the skin infections in immunocompetent mice. This is likely because the strongly activated innate immunity in skin (eg, large release of oxidants and proteolytic enzymes from activated neutrophils) overshadows the effect of Sak on virulence. Nevertheless, in clinical isolates from patients (who were mainly immunocompetent), we noted a significant association between higher Sak secretion and decreased invasiveness. Even if the effect of Sak in decreasing virulence is limited to immunocompromised individuals, our finding has clinical importance because immunosuppression is a major risk factor for systemic spread of staphylococcal infection [5].

Another effect of Sak was the spontaneous drainage of abscesses; instead of enhancing bacterial penetration into deeper sites, Sak promoted migration toward the epidermis. This could be due to the location of skin plg mainly in the epidermis' basal layer [20]; its activation could result in a spatial gradient of plasmin, causing preferential lysis in the direction from the infectious foci toward the surface. Drainage of suppurative lesions may be advantageous to bacteria; after drainage to the skin surface, *S. aureus* can spread to new sites on the skin and to new hosts.

In conclusion, the interaction of Sak with plg has a dual effect in *S. aureus* skin infections (Figure 7). During the initiation phase, Sak promotes bacterial penetration into the skin and the establishment of infection. Once the infection is established, Sak might limit infection severity in immunocompromised individuals.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

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