The Commensal *Staphylococcus warneri* Makes Peptide Inhibitors of MRSA Quorum Sensing that Protect Skin from Atopic or Necrotic Damage

Morgan M. Severn[1,4], **Young-Saeng K. Cho**[1,4], **Haider S. Manzer**[1], **Zoie L. Bunch**[2], **Ali Shahbandi**[2], **Daniel A. Todd**[2], **Nadja B. Cech**[2], **Alexander R. Horswill**[1,3,*]

[1]Department of Immunology and Microbiology, University of Colorado School of Medicine, Aurora, Colorado, USA

[2]Department of Chemistry and Biochemistry, University of North Carolina at Greensboro, Greensboro, North Carolina, USA

[3]Department of Veterans Affairs, VA Eastern Colorado Healthcare System, Aurora, Colorado, USA

TO THE EDITOR

The opportunistic pathogen *Staphylococcus aureus* accounts for the majority of all skin and soft tissue infections in the United States and infects 30—100% of atopic dermatitis (AD) lesions (Geoghegan et al., 2018; Parlet et al., 2019). Conversely, *S. aureus* only transiently colonizes healthy skin, suggesting that this pathogen may be actively excluded from this environment (Geoghegan et al., 2018). Healthy human skin is home to a diverse community of normal flora, including the coagulase-negative staphylococci (CoNS), and many CoNS directly compete with *S. aureus* through a variety of mechanisms to maintain skin homeostasis and colonization resistance (Parlet et al., 2019).

One mechanism of CoNS colonization resistance is quorum-sensing interference. All staphylococci encode the conserved accessory gene regulator (*agr*) quorum-sensing system, which senses and responds to its cognate autoinducing peptide (AIP) signal in a cell density–dependent manner (Thoendel et al., 2011). *S. aureus agr* is required for skin infection and degradation of the epithelium in AD because most virulence factors, including proteases, lipases, and toxins, are under *agr* control (Nakamura et al., 2020; Thoendel et al., 2011). *S. aureus* encodes four *agr* allelic variants, determined by a hypervariable region

spanning *agrBDC*. Each variant senses and responds to a unique cognate AIP but can be inhibited by noncognate AIPs through intraspecies or interspecies cross-talk (Thoendel et al., 2011). The ubiquitous CoNS skin commensals *S. hominis* and *S. epidermidis* make AIPs that inhibit *S. aureus agr* and mitigate infection (Otto et al., 2001; Williams et al., 2019). However, many other healthy skin CoNS remain understudied. We hypothesize that these common skin colonizers may also play a role in maintaining colonization resistance through quorum sensing interference.

Staphylococcus warneri can be isolated from the head, nares, arms, legs, and feet of healthy individuals, but little is understood about its contributions to colonization resistance (Byrd et al., 2018; Kloos and Schleifer, 1975). We found that conditioned media (CM) from healthy skin isolates of *S. warneri*–inhibited *S. aureus agr*-I signaling in a dose-dependent manner, with no effect on growth (Figure 1a and Supplementary Figure S1). Sequence analysis of the AIP-encoding region (*agrD*) of each *S. warneri* strain revealed two putative *agr* types. The *S. warneri* AIP-I structure was previously identified (Gless et al., 2019), but to our knowledge, *S. warneri agr* allelic variation has not been previously reported. Liquid chromatography-mass spectrometry analysis of CM from an *agr*-I strain (AH4548) confirmed the previously published structure of AIP-I to be YSPc[CTNFF] (Gless et al., 2019), with m/z of 960.3907 (Figure 1b and Supplementary Figure S2). Liquid chromatography-mass spectrometry of CM from the *agr*-II strain (AH5628) revealed an eight amino acid peptide (ANPc[CAMFY]) with a measured m/z value for the [M+H]$^+$ ion of 898.3592 (Figure 1c and Supplementary Figure S3).

S. aureus agr function is critical for skin infection and exacerbation of AD lesions (Geoghegan et al., 2018; Nakamura et al., 2020). To determine whether *S. warneri* AIP-I (Figure 1d) or AIP-II (Figure 1e) could inhibit all of the four *S. aureus agr* classes, we treated fluorescent methicillin–resistant *S. aureus* (MRSA) *agr* reporters (P3::YFP) with increasing doses of each synthetic peptide. Both AIPs had half-maximal inhibitory concentration values in the low nanomolar range against most MRSA *agr* types, except for AIP-I, which weakly inhibited MRSA *agr*-IV (Figure 1f). Notably, AIP-II had potent activity (half-maximal inhibitory concentration, 4 nM) against MRSA *agr*-IV (Figure 1f).

S. epidermidis can also expand in AD lesions, and the *S. epidermidis agr*–regulated protease EcpA was shown to degrade the AD barrier (Byrd et al., 2017; Cau et al., 2021). However, only one study has identified inter-CoNS AIP-mediated cross-talk (Cau et al., 2021). Using fluorescent *S. epidermidis* reporters (P3::sGFP), we found that synthetic AIP-I (Figure 1g) and AIP-II (Figure 1h) were potent inhibitors of *S. epidermidis agr*-I, which is the most common *S. epidermidis agr* type on healthy and AD skin (Olson et al., 2014; Williams et al., 2019). Both AIPs inhibited *agr*-II with low nanomolar half-maximal inhibitory concentration but neither inhibited *agr*-III (Figure 1i). Together, our in vitro data suggest that competition between *S. warneri* and *S. epidermidis* or *S. aureus* may dampen the production of *agr*-regulated virulence factors.

Given the potent activity of both *S. warneri* AIPs in vitro, we determined whether they might also be protective against MRSA-mediated skin damage in vivo. A total of 50 μg of synthetic *S. warneri* AIP-I dampened MRSA *agr* signaling and maintained barrier

integrity as assessed by transepithelial water loss (Figure 2a), with no change in recovered bacteria (Figure 2b) in a mouse model of epicutaneous infection. This finding is similar to our in vitro findings that AIPs inhibited MRSA *agr* activation with no effect on growth. AIP-I application also inhibited erythema, redness, and scaling 72 hours after infection (Figure 2c). We then focused on the characterization of the novel *S. warneri* AIP-II, which had even greater inhibitory activity in vitro than AIP-I. In the epicutaneous model, coinoculation with equivalent numbers of an *S. warneri* AIP-II–producing strain and MRSA significantly decreased skin transepithelial water loss compared to MRSA alone (Figure 2d). No significant differences in MRSA numbers were recovered between the single and coinfection groups (Figure 2e). Coinfection with *S. warneri* also prevented barrier erythema and scaling (Figure 2f). Thus, *S. warneri* and its AIPs may contribute to the repression of *S. aureus* virulence factor production on the skin.

To our knowledge, no study has yet to identify a naturally occurring CoNS AIP inhibitor of MRSA *agr*-IV. MRSA *agr*-IV skin infections are rare but serious. They are most often associated with staphylococcal scalded skin syndrome and the *agr*-regulated production of exfoliative toxins (Jarraud et al., 2002; Thoendel et al., 2011). Given our in vitro half-maximal inhibitory concentration findings, we determined whether *S. warneri* AIP-II could be an effective MRSA *agr*-IV inhibitor in vivo. We found that injection of 50 μg synthetic AIP-II decreased MRSA *agr*-IV dermonecrotic lesion size (Figure 2g) throughout the course of infection and protected animals from systemic weight loss (Figure 2h) and skin injury (Figure 2i).

Together, our results suggest an important protective role for *S. warneri* quorum-sensing crosstalk on the skin. More work is needed to determine the distribution of these *agr* types on healthy skin and whether the absence of *S. warneri* may be correlated with disease. Further studies may reveal other mechanisms of *S. warneri* colonization resistance, such as the production of antimicrobial peptides (Nakatsuji et al., 2017). In future translational applications, *S. warneri* or its molecular products could potentially be harnessed to treat AD or other skin diseases in a biotherapeutic approach (Nakatsuji et al., 2021).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

Data availability statement

All data needed to evaluate the conclusions in the letter are present in the letter and/or the Supplementary Materials.

Abbreviations:

AD	atopic dermatitis
agr	accessory gene regulator
AIP	autoinducing peptide
CM	conditioned media
CoNS	coagulase-negative staphylococci
MRSA	methicillin–resistant Staphyloccocus aureus

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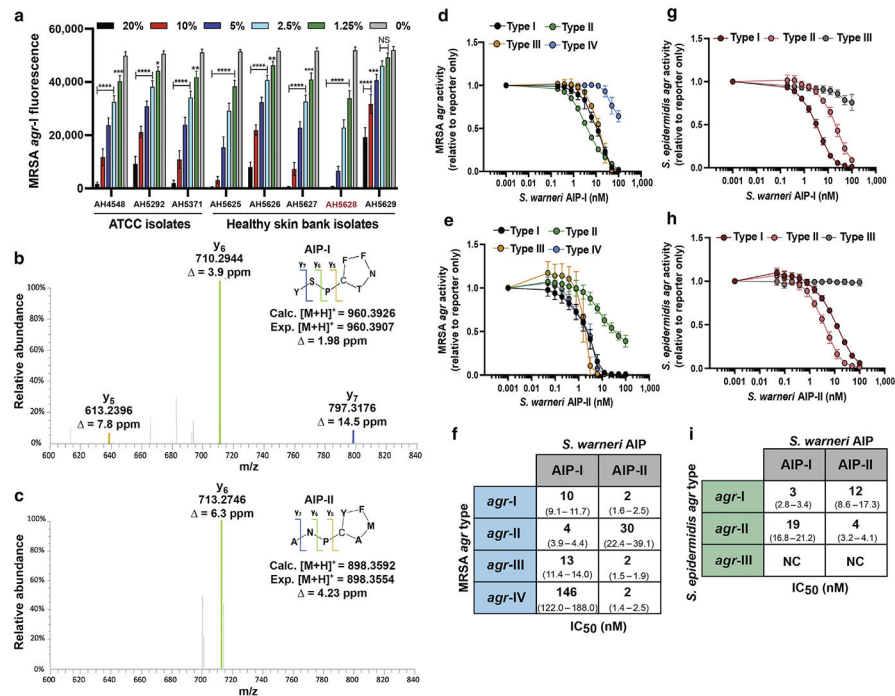
Figure 1. *S. warneri* AIPs inhibit *agr* quorum sensing in opportunistic staphylococcal pathogens.
(**a**) The MRSA *agr*-I P3::YFP reporter strain was incubated with increasing doses of *S. warneri* cell-free CM from healthy skin or ATCC (Manassas, VA) isolates. The representative *S. warneri agr*-II strain is highlighted in red. Significant differences to 0% CM were calculated by ordinary one-way ANOVA followed by Dunnett's MCT. $*P < 0.05$, $**P < 0.01$, $***P < 0.005$, and $****P < 0.0001$. (**b**) MS confirmation of *S. warneri* AIP-I from CM. (**c**) MS identification of *S. warneri* AIP-II from CM. (**d**) MRSA agr-I-IV P3::YFP reporter strains incubated with increasing concentrations of synthetic *S. warneri* AIP-I. (**e**) MRSA *agr*-I-IV P3::YFP reporter strains incubated with increasing concentrations of synthetic *S. warneri* AIP-II. (**f**) Calculated IC_{50} values for synthetic AIP inhibition of MRSA quorum sensing. The 95% confidence intervals are reported in parenthesis. (**g**) *S. epidermidis agr*-I-III P3::sGFP reporters incubated with increasing concentrations of synthetic *S. warneri* AIP-I. (**h**) *S. epidermidis agr*-I-III P3::sGFP reporter strains incubated with increasing concentrations of synthetic *S. warneri* AIP-II. (**i**) Calculated IC_{50} values for synthetic AIP inhibition of *S. epidermidis* quorum sensing. The 95% confidence intervals are reported in parenthesis. The 24-hour fluorescence point is shown for **a**, **d**, **e**, **g**, and **h**. For all experiments, error bars are mean ± SD, and results are pooled from three independent experiments. IC_{50} was calculated with a four-parameter logistic regression curve. *agr*, accessory gene regulator; AIP, autoinducing peptide; Calc., calculated; CM, conditioned media; Exp., expected; IC_{50}, half-maximal inhibitory concentration; MCT, multiple comparisons test; MRSA, methicillin-resistant *Staphylococcus aureus*; MS, mass-spectrometry; NS, not significant.
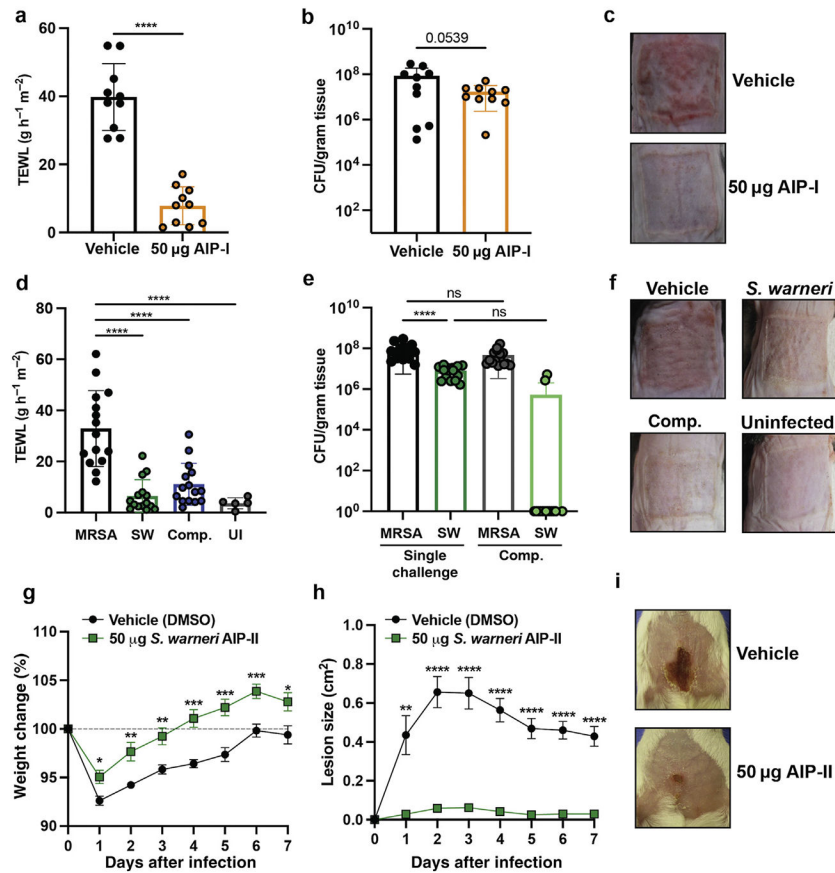
Figure 2. SW protects murine skin from MRSA damage.

(**a**) TEWL at 72 hpi with vehicle (DMSO) or 50 μg SW synthetic AIP-I (n = 10). Error bars are mean ± SD. (**b**) MRSA CFUs from vehicle or AIP-I treatment 72 hpi. Significant differences from MRSA alone (vehicle) were analyzed by ordinary one-way ANOVA followed by Dunnet's MCT for TEWL or Tukey's MCT for CFUs. ****$P < 0.0001$. (**c**) Representative images of back skin 72 hpi. (**d**) TWEL at 72 hpi for indicated groups (n = 15). (**e**) CFUs recovered from indicated groups 72 hpi (n = 15). Significant differences from MRSA alone (vehicle) were analyzed by ordinary one-way ANOVA followed by Dunnet's MCT for TEWL or Tukey's MCT for CFU data. ****$P < 0.0001$. (**f**) Representative images of back skin 72 hpi with indicated groups. (**g**) A 7-day weight change for groups administered vehicle (DMSO) or 50 μg synthetic SW AIP-II (n = 11 for vehicle, n = 10 for AIP). (**h**) A 7-day lesion-size change for groups administered vehicle (DMSO) or 50 μg synthetic SW AIP-II. Significant differences between groups were analyzed by multiple unpaired Student's *t*-tests with FDR correction. *$P < 0.05$, **$P < 0.005$, and ***$P < 0.0005$. (**i**) Representative images at 3 dpi for indicated groups. AIP, autoinducing peptide; CFU, colony-forming unit; Comp., competition; FDR, false discovery rate; hpi, hour post infection; MCT, multiple comparisons test; MRSA, methicillin-resistant *Staphylococcus aureus*; ns, not significant; TEWL, transepithelial water loss; SW, *Staphyloccocus warneri*; UI, uninfected.