Supplementary Methods

Generation and characterization of ΔtagO

In order to inactivate the *S. aureus* homologue of *tagO*, which is 62% similar to the corresponding *B. subtilis* gene, DNA fragments consisting of 1,001 bp upstream and 1,002 bp downstream of *tagO* were amplified by PCR from *S. aureus* SA113 (ATCC 35556) DNA and cloned together with the *ermB* gene into the temperature-sensitive shuttle plasmid pBT2 using suitable restriction enzymes. The resulting plasmid was transferred into *S. aureus* SA113 and *tagO* mutants were enriched by cultivation at 42 °C. The procedures for cloning, homologous recombination, and verification of the mutant were performed essentially as described recently[1-3]. Plasmid pRBtagO was constructed by cloning a 1,720-bp PCR fragment bearing the *tagO* gene together with the putative promoter region (460-bp non-coding upstream DNA) into the shuttle vector pRB473. *E. coli*- and *Staphylococcus*-specific plasmid vectors and molecular methods have been described previously[1,4,5].

Staphylococcal teichoic acids were isolated by extracting crude cell lysates with 2% SDS and releasing WTA from the purified cell walls by treatment with 5% trichloroacetic acid as described recently[2]. For some assays WTA was further purified by ethanol precipitation with 1/10 volume of 3 M sodium acetate (pH 5.1) and 3 volumes of 95% ice-cold ethanol. WTA was separated on polyacrylamide gels containing 18% acrylamide but lacking SDS and visualized by a combined alcian blue and silver stain, essentially as described elsewhere[6]. The amounts of phosphorus and hexosamines in teichoic acid samples were determined by colorimetric assays as described previously[2,7]. The ribitol content was determined by gas chromatography. 100 µl of WTA preparations were heated with 100 µl 6 N HCl at 110 °C for 23 h to convert ribitol to anhydroribitol. 100 µl methanol and 10 µl tert-butanol were added, samples were dried and then derivatized with 50 µl bis(trimethylsilyl)trifluoroacetamide:acetonitrile (1:1) at 110 °C for 2 h. The samples were diluted with 100 µl methylene chloride containing 20 µg n-tetracosane (internal standard) and analyzed on a gas chromatograph using a DB 5 fused silica capillary.

Phages 3A52 and Ø11 were propagated in *S. aureus* SA113 according to standard procedures[5] and their lytic activity was studied as described recently[7]. Bacterial growth was studied in BM broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.1% K_2HPO_4, and 0.1% glucose) or IMDM (Gibco-BRL, Carlsbad, CA) under vigorous aeration after inoculation of the medium with 1/100 of an overnight culture. Survival rates in the stationary phase were investigated in the same way using BM broth. In order to analyze the stability of plasmid pRBtagO, bacteria were cultivated in consecutive cultures lacking antibiotic, which were inoculated 1:100 every 24 h. Viable bacteria were counted by plating diluted bacterial suspensions on agar with or without chloramphenicol

Cotton rat model of nasal colonization

The cotton rat nasal colonization model has recently been described in detail[8]. Briefly, *S. aureus* was grown overnight on Columbia agar supplemented with 2% NaCl. Bacteria were washed in PBS and resuspended in 10 µl PBS per animal to be instilled containing no antibiotics or, in one experiment, 2.5 µg/ml erythromycin, 300 µg/ml spectinomycin, or 10 µg/ml chloramphenicol for Δ*tagO*, Δ*dltA,* or complemented Δ*tagO*, respectively. Six week-old female cotton rats (*Sigmodon hispidus*, bred at the Biosynexus breeding facility) were anesthetized with a combination of Rompun, acepromazine maleate, and Ketamine (2.5 mg/kg, 2.5 mg/kg, and 25 mg/kg, respectively). A 10-µl aliquot of

resuspended *S. aureus* (10^9 CFUs) was intranasally instilled in a drop-wise fashion and distributed equally in each nostril of the anesthetized animal. 7 d after nasal instillation, animals were sacrificed. The nose area was cleansed thoroughly with 70% ethanol, the noses were surgically removed and the nostrils were bisected with scissors. The noses were placed in 500 µl of PBS containing 0.5% Tween-20, vortexed vigorously to release colonizing bacteria, and appropriate amounts of supernatant were plated on tryptic soy agar (TSA) supplemented with 7.5% NaCl. Antibiotics (spectinomycin 300 µg/ml, erythromycin 2.5 µg/ml, or chloramphenicol 10 µg/ml) or lysostaphin (1 µg/ml) were added to the TSA in some experiments to aid in isolation of the bacterial strain used in the particular study. To differentiate nasal colonization by instilled *S. aureus* wild-type that is not antibiotic resistant from indigenous coagulase-negative staphylococci (CoNS), supernatants from dissected noses instilled with wild-type SA113 were plated on plates with and without 1 µg/ml lysostaphin that inhibited growth of *S. aureus* but not CoNS from cotton rat noses[8]. The CFUs on TSA with lysostaphin were subtracted from CFUs on TSA without lysostaphin.

Preparatory experiments demonstrated that elimination of the natural nasal flora with streptomycin or nafcillin prior to instillation did not considerably affect subsequent nasal colonization by *S. aureus*. Moreover, *S. aureus* nasal colonization had no apparent influence on the natural flora. All guidelines of both the U.S. Department of Agriculture and the Biosynexus Inc. Institutional Animal Care and Use Committee were followed.

In order to study the inhibition of nasal colonization by WTA, cotton rat noses were preinstilled with purified WTA 5 min before instillation with bacteria in three different experiments. Comparatively low numbers of bacteria were used to permit an efficient competition by the preinstilled WTA. Either *S. aureus* SA113 wild-type (3×10^4 CFU) or the clinical isolate MBT 5040 (5×10^5 CFU), which was easy to identify on agar plates because of its streptomycin resistance[8], were used in experiments one or two and three, respectively. WTA from SA113 wild-type in 10 µl PBS (50 µg in experiment one and three or 200 µg in experiments two) were applied. In experiment one, treatment with 50 µg WTA was repeated on day one and two. Ten, six, and twenty animals were used in experiments one, two, and three, respectively. In each experiment, 50% of the animals were pretreated with WTA in PBS or with PBS alone as a control.

Interactions with epithelial cells

Primary human nasal epithelial cells (HNEC) and the required medium components were purchased from Oligene (Berlin, Germany). They were cultured according to the manufacturer's instructions and used up to passage number four. The human alveolar epithelial cell line A549 (ref. 9) was cultured in DMEM-F12 medium (Gibco-BRL), supplemented with 10% heat-inactivated fetal bovine serum and 2 mM glutamine. All types of cells were seeded to 24-well culture plates at 1×10^4/well (HNEC) or 5×10^4/well (A549) and incubated at 37 °C under 5% CO_2.

Bacteria grown to mid-logarithmic phase in Mueller Hinton Broth were washed three times and resuspended in PBS. They were labeled with 0.1 mg/ml of fluorescein isothiocyanate (FITC) at 37 °C for 1 h, washed three times with PBS, and resuspended in RPMI (Sigma, St. Louis, MO). Cell numbers were adjusted using a Neubauer chamber. Confluent epithelial cell monolayers grown in 24-well plates (approximately 4×10^4 HNEC/well or 7×10^5 A549/well) were washed twice with RPMI and inoculated with FITC-labeled bacteria. Dose-dependency of bacterial adherence was confirmed by using increasing multiplicities of infection (MOI), ranging from 5 to 100 (data not shown) and a MOI of 50 was used. After incubation for 1 h at 37 °C under 5% CO_2, the wells were

washed three times with RPMI and fixed with 3.5% paraformaldehyde in PBS. No morphological changes in the cells were observed after this procedure in control wells. Adherent bacteria/mm^2 were counted using a fluorescence microscope. Experiments were run in triplicate and up to ten random fields were counted in each well. Under these conditions, 10.0 ± 1.0% of the applied *S. aureus* wild-type cells adhered to HNEC (mean and SD of five experiments).

To evaluate the effect of purified WTA on adhesion of *S. aureus* to epithelial cells, confluently grown HNEC or A549 were preincubated with different concentrations of purified WTA (125 µg/ml, 250 µg/ml, or 500 µg/ml) isolated from *S. aureus* wild-type or Δ*dltA* dissolved in RPMI. In the case of Δ*tagO,* equal volumes of samples prepared by the same method but lacking WTA were applied. After 1 h of incubation the cells were washed and inoculated with FITC-labeled bacteria as described above.

IL-8 induction was studied by incubating HNEC with *S. aureus* strains under conditions described for adherence studies except that the bacteria were not labeled and were inactivated after 1 h by addition of gentamycin (100 µg/ml) followed by incubation for further 8 h. IL-8 was quantified by ELISA (R&D Systems, Mineapolis, MN).

Adherence of WTA-coated microspheres

Amine-modified fluorescent microspheres (FluoSpheres, 1.0 µm diameter, Molecular Probes, Eugene, OR), washed with PPB (10 mM potassium phosphate buffer, pH 7.5), were incubated with 500 µg/ml WTA for 30 min. WTA-coated beads were washed twice and resuspended in PPB containing 1% BSA to block hydrophobic areas. The amount of adsorbed WTA was determined and found to be the same for wild-type and Δ*dltA* by measuring the amount of GlcNAc released by boiling 100 µl of WTA-coated beads without BSA at 100 °C for 10 min. The WTA-coated beads were diluted in RPMI and used in adhesion assays on confluently grown cells with MOIs of 60, 30, and 15 (HNEC) or 50, 25 and 12.5 (A549) as described for FITC-labeled bacteria. The relative fluorescence at 505/515 nm per well was quantified with a fluororeader. In some experiments the numbers of beads/mm^2 epithelial cells were also counted microscopically.

Adherence to fibronectin

96-well microtiter plates were coated with 20 µg fibronectin/well (Sigma) in 50 mM sodium carbonate buffer (pH 9.6) for 15 h at 4 °C. Subsequently, wells were blocked with 3% BSA in TBS (25 mM Tris-HCl, 100 mM NaCl, pH 7.5) for 2 h and washed twice with TBS. Bacteria were grown in IMDM to mid-logarithmic phase, washed twice with TBS, and adjusted to 1 x 10^9 cells/ml using a Neubauer chamber. 200 µl of bacterial suspensions were added to each well. After 1 h incubation at 37 °C the wells were washed three times with TBS, stained with safranin for 1 min, and A_{492} was determined.

Antimicrobial peptides

Inactivation of bacteria by antimicrobial peptides was analyzed and hNP1-3 was purified from human granulocytes as described recently[18,23], except that 1x10^8 CFU/ml were incubated with 100 µg/ml hNP1-3, 10 µg/ml LL-37, or 500 µg/ml lactoferrin in the presence of 0.05% human serum albumin. LL-37 was synthesized by solid-phase peptide synthesis and purified by reversed-phase preparative HPLC. The purity and identity of the peptides were confirmed by HPLC and mass spectrometry. Lactoferrin was purchased from Sigma.

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