Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections

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Colonization of the anterior nares in ~37% of the population is a major risk factor for severe *Staphylococcus aureus* infections. Here we show that wall teichoic acid (WTA), a surface-exposed staphylococcal polymer, is essential for nasal colonization and mediates interaction with human nasal epithelial cells. WTA-deficient mutants were impaired in their adherence to nasal cells, and were completely unable to colonize cotton rat nares. This study describes the first essential factor for *S. aureus* nasal colonization.

S. aureus is a major human pathogen in hospital- and community-acquired infections, causing wound infections, bacteremia and sepsis with a high mortality rate[1]. Eradication of nasal colonization with the antibiotic mupirocin markedly decreases the risk of postoperative infection[2,3], but the emergence of mupirocin resistance demands new strategies to interfere with colonization. The molecular basis of *S. aureus* nasal colonization has remained unknown.

The Gram-positive cell envelope contains wall teichoic acids (WTA), which are complex surface-exposed polymers[4]. *S. aureus* produces WTA composed of ~40 ribitol phosphate repeating units modified with *N*-acetylglucosamine (GlcNAc) and D-alanine (**Fig. 1a**)[4]. The *Bacillus subtilis* gene *tagO* was recently implicated in WTA biosynthesis and is essential for viability[5]. The similarity of the TagO gene product to UDP-*N*-acetylglucosamine transferases suggests a role in the first step of WTA synthesis, the transfer of GlcNAc to bactoprenol[5]. To evaluate the possible involvement of WTA in nasal colonization, we inactivated the *tagO* homolog in *S. aureus* SA113 and obtained a viable mutant (Δ*tagO*). WTA was absent in Δ*tagO* but reappeared upon complementation with a plasmid-encoded *tagO*, as shown by gel electrophoresis and analysis of phosphate, GlcNAc and ribitol, the hallmark of WTA (see **Supplementary Fig. 1** and **Supplementary Note** online for details). The *S. aureus* phages 3A52 and ∅11, which use WTA as a receptor[6], were completely inactive against Δ*tagO*, further demonstrating that Δ*tagO* is devoid of WTA (data not shown). Generation times in rich and minimal media, under conditions relevant in the anterior nares (30 °C with good aeration), and survival rates in the stationary phases of wild-type and Δ*tagO* bacteria were very similar (**Supplementary Fig. 1 online**). The Δ*tagO* mutant showed only slightly reduced growth at 37 °C in rich medium. Patterns of cell wall–anchored proteins in wild-type and Δ*tagO* bacteria showed no differences, indicating that the lack of WTA has no major impact on other surface molecules.

We investigated the role of WTA in *S. aureus* nasal colonization in a cotton rat (*Sigmodon hispidus*) model[7]. This model reflects the situation in human *S. aureus* carriers well, as cotton rat nares have histological properties similar to those of humans, with squamous, columnar and pseudostratified epithelial areas[8]. Cotton rats are susceptible to many human respiratory pathogens, and follow disease courses similar to those observed in humans[9]. Rats were intranasally instilled with equal numbers of wild-type or Δ*tagO* bacteria, which were enumerated 7 d later. Whereas all rats instilled with wild-type bacteria were colonized, no *S. aureus* was detectable in any Δ*tagO*-instilled noses (**Table 1**). One and two days after instillation, nasal colonization in Δ*tagO*-instilled rats was 90.7 ± 1.4% and 98.3 ± 0.3% lower, respectively, compared with rats instilled with wild-type bacteria (mean ± s.d. of at least ten rats), indicating that WTA deficiency resulted in rapid elimination of the bacteria from cotton rat noses. All animals treated with the complemented mutant were colonized after 7 d, albeit at a lower level than with wild-type bacteria. This can be explained by the lack of antibiotic selective pressure in the nose, resulting in loss of the complementing plasmid pRBtagO. Accordingly, pRBtagO exhibited only limited *in vitro* stability in the absence of antibiotics (**Supplementary Fig. 1** online). All recovered bacteria retained the plasmid, indicating that the presence of WTA is a prerequisite for continued nasal colonization. The *S. aureus*

Table 1 *S. aureus* nasal colonization of cotton rats 7 d after bacterial instillation

Strain	Number of rats colonized/number tested (number of experiments)	Mean CFU recovered per colonized naris	Median CFU recovered per colonized naris
Wild-type	15/15 (3)	6,011	6,207
Δ*tagO*	0/15 (3)[a]	0	0
Δ*tagO* (complemented)	5/5 (1)[a]	173	146
Δ*dltA*	4/10 (2)[a]	30	33

[a]In one experiment, bacteria were instilled in PBS containing antibiotics as described in **Supplementary Note** online. CFU, colony-forming units.

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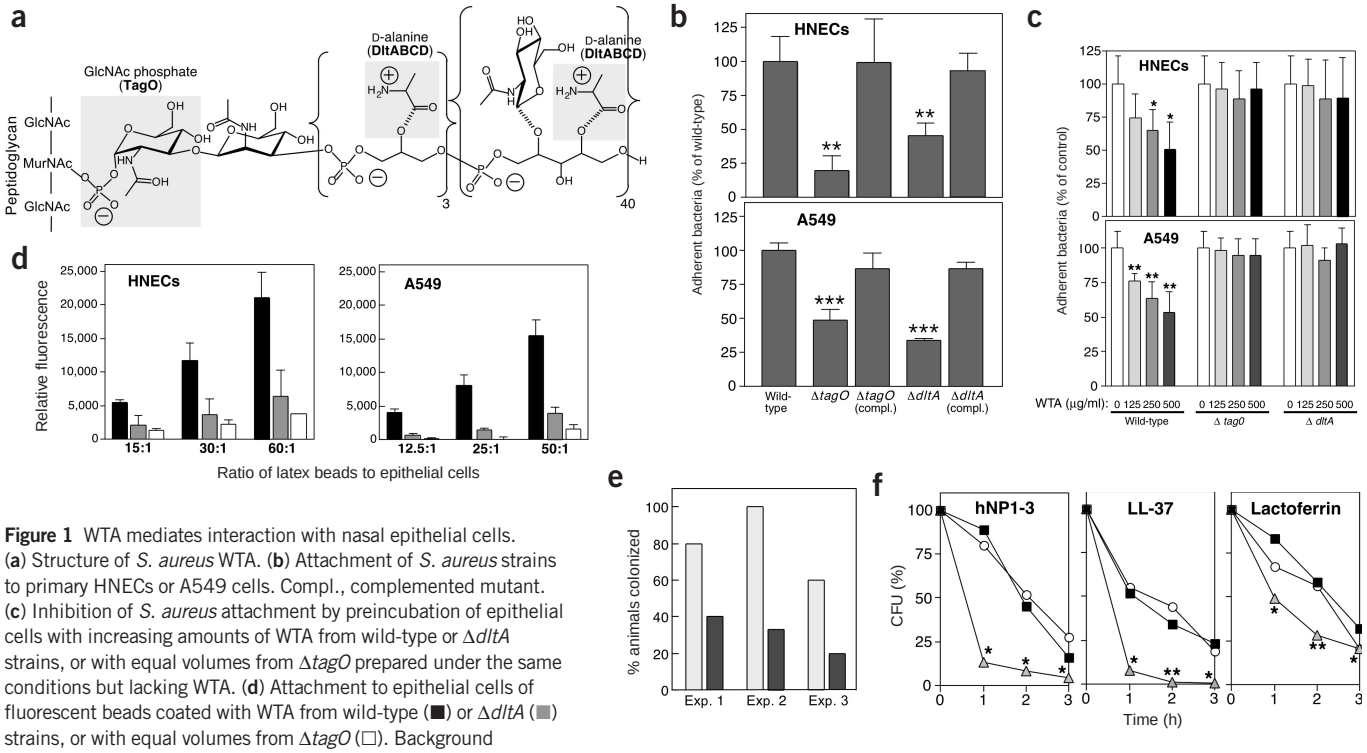

Figure 1 WTA mediates interaction with nasal epithelial cells. (**a**) Structure of *S. aureus* WTA. (**b**) Attachment of *S. aureus* strains to primary HNECs or A549 cells. Compl., complemented mutant. (**c**) Inhibition of *S. aureus* attachment by preincubation of epithelial cells with increasing amounts of WTA from wild-type or Δ*dltA* strains, or with equal volumes from Δ*tagO* prepared under the same conditions but lacking WTA. (**d**) Attachment to epithelial cells of fluorescent beads coated with WTA from wild-type (■) or Δ*dltA* (■) strains, or with equal volumes from Δ*tagO* (□). Background adherence of beads incubated without WTA was subtracted. (**e**) Inhibition of nasal colonization by preinstillation of cotton rat nares with WTA. Ten, six, or twenty animals pretreated with PBS (■) or WTA dissolved in PBS (■) were used in experiments 1, 2 and 3, respectively. The percentage of animals containing more than ten colony-forming units (CFU) of *S. aureus* per nose are shown. Further differences in experimental settings are described in **Supplementary Note**. (**f**) Susceptibilities of *S. aureus* wild-type (■), Δ*tagO* (○) and Δ*dltA* (▲) strains to nasal antimicrobial peptides. Data represent means of three to five independent experiments (**b**–**f**) and s.d. (**b**–**d**). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ by two-tailed Student *t*-test, compared with wild-type (**b**,**f**) or control (**c**) samples.

SA113 *dltA* mutant (Δ*dltA*), which lacks the D-alanine modifications in teichoic acids[10], was also abrogated in its capacity to colonize cotton rat noses. Only four of ten rats were colonized with Δ*dltA*, with very low bacterial numbers. (**Table 1**)

The Δ*tagO* and Δ*dltA* mutants adhered considerably less efficiently to primary human nasal epithelial cells (HNECs) and to the human airway epithelial cell line A549 than did wild-type *S. aureus* (**Fig. 1b**). Preincubation of the cells with WTA isolated from wild-type *S. aureus* caused a dose-dependent reduction in the number of cell-bound, wild-type *S. aureus* (**Fig. 1c**), in accordance with a previous report[11]. No such reduction was observed when WTA from Δ*dltA*, or samples from Δ*tagO* prepared by the same method but lacking WTA, were used. Latex beads coated with wild-type WTA showed a strong, dose-dependent increase in binding to HNECs or A549 compared with noncoated beads, whereas coating with samples from Δ*tagO* or Δ*dltA* caused no binding or very weakly increased binding (**Fig. 1d**). These results suggest a specific interaction between wild-type WTA and epithelial cells, which is further substantiated by a pronounced reduction in the capacity of *S. aureus* to colonize cotton rat noses upon preinstillation of the noses with WTA (**Fig. 1e**).

Wild-type and Δ*tagO S. aureus* induced similar levels of interleukin-8 in HNECs (**Supplementary Fig. 1 online**), indicating equal inflammatory capacities of these strains. Δ*tagO* did not show any reduction in its capacity to bind to immobilized fibronectin. The antimicrobial activity of human defensin hNP1-3, cathelicidin LL-37 and lactoferrin, found in nasal secretions[12,13], was not affected by the lack of WTA in Δ*tagO* (**Fig.1f**). Only Δ*dltA* was considerably more susceptible to all three substances than the wild-type strain, confirming previous studies on the role of teichoic acid D-alanine in resistance to antimicrobial peptides[14] and suggesting that increased inactivation of Δ*dltA* by nasal host defenses may contribute to this strain's reduced capacity to colonize cotton rat nares.

WTA is the first factor identified as essential for nasal colonization by *S. aureus*. Several lines of evidence indicate that WTA mediates specific interaction with airway epithelial cells. Many lectin-like receptors interact with *S. aureus* and purified teichoic acids or similar polymers[15]. It is tempting to speculate that receptors of this type mediate *S. aureus* attachment to nasal cells. Staphylococcal species are quite diverse in WTA structure[4], and we hypothesize that these differences may have a role in the tropism for a certain host organism and certain areas of skin or mucous membranes. The linkage unit between peptidoglycan and WTA is invariant, however, and *tagO* homologs are found in most Gram-positive bacteria, including *Listeria*, enterococci, streptococci, bacilli and clostridia. Thus, TagO represents an interesting target for new antimicrobial substances that may impede the ability of bacteria to colonize the host. WTA may also be considered as a new target for vaccination.

Note: Supplementary information is available on the Nature Medicine website.

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Activation of nuclear hormone receptor peroxisome proliferator–activated receptor-δ accelerates intestinal adenoma growth

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We treated *Apc*^{min} mice, which are predisposed to intestinal polyposis, with a selective synthetic agonist of peroxisome proliferator–activated receptor-δ (PPAR-δ). Exposure of *Apc*^{min} mice to the PPAR-δ ligand GW501516 resulted in a significant increase in the number and size of intestinal polyps. The most prominent effect was on polyp size; mice treated with the PPAR-δ activator had a fivefold increase in the number of polyps larger than 2 mm. Our results implicate PPAR-δ in the regulation of intestinal adenoma growth.

PPARs are ligand-activated transcription factors that belong to the nuclear hormone receptor superfamily[1]. Evidence supports a role for the PPAR-δ/β subtype in embryo implantation[2] and development[3], epidermal maturation and wound healing[4,5], and regulation of fatty acid metabolism[6], as well as in repressing the atherogenic inflammatory response[7]. Recent data suggest that PPAR-δ is also important in colorectal cancer (CRC) and is overexpressed in most CRCs[8,9]. PPAR-δ expression and/or activity is increased after loss of the adenomatous polyposis coli (*APC*) tumor suppressor gene or after K-Ras activation. The cyclooxygenase-2 metabolite prostacyclin also increases PPAR-δ activity in CRC cells[8].

Data in support of a direct role for PPAR-δ in CRC are conflicting. Somatic deletion of both *PPARD* alleles from an established CRC cell line results in decreased tumor growth[10]. In contrast, PPAR-δ was shown to be dispensable for polyp formation in *Apc*^{min} mice[3], although this study was limited to a small number ($n = 3$) of *Ppard*$^{-/-}$*Apc*^{min} mice. Notably, previous studies did not test whether PPAR-δ activation with a high-affinity agonist influences intestinal adenomatous polyp growth. We therefore treated *Apc*^{min} mice with the PPAR-δ synthetic ligand GW501516.

GW501516 was shown to be a PPAR-δ subtype-selective ligand using combinatorial chemistry and structure-based drug design[11]. We evaluated this compound for PPAR-δ activation and receptor selectivity in HCT116 CRC cells using a mammalian two-hybrid transfection assay. Exposure of cells to physiological concentrations of GW501516 resulted in a dose-dependent increase in PPAR-δ–GAL-4 transactivation (**Fig. 1a**). Doses of GW501516 up to 10 μM did not transactivate either PPAR-γ or PPAR-α, confirming that GW501516 is a high-affinity, PPAR-δ-selective agonist.

A large percentage of human colorectal polyps have inactivating mutations in the *APC* gene. *Apc*^{min} mice develop multiple intestinal polyps, providing a useful model system for our studies. Expression of PPAR-δ in adenomas and normal tissue from the small and large intestines was determined at the RNA (data not shown) and protein (**Fig. 1b**) levels. PPAR-δ was found mainly in intestinal epithelial cells in both the normal intestine and adenomas. To test the effects of PPAR-δ activation on polyp growth, we treated *Apc*^{min} mice with either vehicle or 10 mg/kg of GW501516. Treatment was limited to 6 weeks because of the development of rectal bleeding and signs of anemia in the ligand-treated mice. Consistent with published reports, the control *Apc*^{min} mice developed an average of 30 small intestine polyps and 1.4 colonic polyps. In contrast, GW501516 treatment led to a twofold increase in polyp number in the small intestine, with no change in the large bowel (**Table 1**). Two previous studies reported that treatment of *Apc*^{min} mice with a PPAR-γ agonist increased polyp formation[12,13], raising the concern that GW501516 could be promoting polyposis by cross-activation of PPAR-γ rather than through direct activation of PPAR-δ. In the previous studies, however, the PPAR-γ ligand affected polyp size only in the colon, not in the small intestine. Our results with GW501516 are exactly the opposite: an increase in polyp size and number in the small intestine but not in the colon. Thus, it is unlikely that the phenotype seen with GW501516 is caused by PPAR-γ activation.

Adenoma size is an independent risk factor for progression to CRC[14]. To determine whether PPAR-δ influences adenoma size, data were stratified for polyp size. Notably, mice treated with the PPAR-δ agonist showed a fivefold increase in polyps larger than 2 mm (**Table 1**), suggesting that PPAR-δ activation primarily

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