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Wall Teichoic Acid-Dependent Adsorption of Staphylococcal Siphovirus and Myovirus[▽]

Guoqing Xia,[1]* Rebecca M. Corrigan,[2] Volker Winstel,[1] Christiane Goerke,[3] Angelika Gründling,[2] and Andreas Peschel[1]

Cellular and Molecular Microbiology Section, Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Elfriede-Aulhorn-Strasse 6, D-72076 Tübingen, Germany[1]; Section of Microbiology, Imperial College London, South Kensington Campus, London SW7 2AZ, United Kingdom[2]; and Medical Microbiology and Hygiene, Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Elfriede-Aulhorn-Strasse 6, D-72076 Tübingen, Germany[3]

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The molecular interactions between staphylococcal phages and host cell surfaces are poorly understood. Employing *Staphylococcus aureus* teichoic acid mutants, we demonstrate that wall teichoic acid (WTA), but not lipoteichoic acid, serves as a receptor for staphylococcal siphovirus and myovirus, while only the siphovirus requires glycosylated WTA.

The horizontal transfer of virulence and resistance genes by bacteriophages has a profound impact on the pathogenicity and environmental adaptation of *Staphylococcus aureus* and other major human pathogens. The host range of a specific phage is largely determined by its capacity to adsorb to cognate receptor structures on the bacterial cell surface. Understanding the molecular determinants of host specificity is also critical for the design of phage therapies, which are increasingly regarded as an alternative strategy to combat antibiotic-resistant bacteria (15). However, while the receptors for many coliphages have been investigated in detail, the nature of host receptors has remained unknown for most phages infecting Gram-positive pathogens.

The vast majority of known bacteriophages belong to the order *Caudovirales* or tailed phages, which are composed of an icosahedral head filled with double-stranded DNA and a thin tail. The tailed phages can be further classified into three major families based on tail morphology: *Podoviridae* with a very short tail, *Siphoviridae* with a long, noncontractile tail, and *Myoviridae* with a long, contractile, double-sheathed tail (1). Staphylococcal phages can be assigned to the major serogroups A, B, D, and F. Serogroups A, B, and F are siphoviruses, which differ in tail length, head size, and head shape. Serogroup D phages, on the other hand, belong to the family *Myoviridae* with double-sheathed, contractile tails (3, 14).

Phage tail tip proteins and/or phage tail fiber proteins are most often involved in recognition of and adsorption to specific components at the host cell surface (11). Many Gram-positive cell envelopes are modified with a unique anionic glycopolymer, the peptidoglycan-anchored wall teichoic acid (WTA), which is one of the most abundant molecules at the bacterial surface (16). Most *S. aureus* strains express polyribitol phosphate WTA substituted with *N*-acetylglucosamine (GlcNAc) and D-alanine (20). We have recently identified the *S. aureus* WTA glycosyltransferase TarM and demonstrated that depletion of TarM leads to a phage-resistant phenotype (21). We concluded that α-GlcNAc glycoepitopes expressed on *S. aureus* WTA serve as an adsorption receptor for serogroup B phages such as ϕ11. This study inspired us to further explore the adsorption receptors of staphylococcal phages belonging to other serogroups or morphogroups.

To study the role of wall teichoic acids in staphylococcal phage adsorption, we first created the mutant RN4220Δ*tagO* (Δ*tagO*) (Table 1), which is deficient in WTA. Δ*tagO* was constructed by replacing the *tagO* gene, which is required for the first step of WTA biosynthesis, with an erythromycin re-

TABLE 1. Bacterial strains and phages used in this study

Strain or phage	Description	Source or reference(s)
S. aureus strains		
RN4220	Restriction deficient, no capsule, no prophage, transformable strain	10, 17
4S5	RN4220 Δ*spa* Δ*ltaS*	6
RN4220Δ*tagO*	RN4220 Δ*tagO*	This study
K6	RN4220, transposon insertion in *tarM*	21
cK6	K6 complemented with pRB474-*tarM*	21
ctagO	Δ*tagO* complemented with pRB474-*tagO*	This study
SA113	Derivative of NCTC 8325 harboring prophages ϕ11, ϕ12, and ϕ13	8
SA113Δ*tagO*	SA113 Δ*tagO*; no wall teichoic acids	18
Phages		
ϕ47	*Siphoviridae*, serogroup A	NCTC[a]
ϕSa2mw	*Siphoviridae*, serogroup A	C. Goerke
ϕ13	*Siphoviridae*, serogroup F	G. Bierbaum
ϕ77	*Siphoviridae*, serogroup F	NCTC
ϕK	*Myoviridae*, serogroup D	G. Bierbaum
ϕ812	*Myoviridae*, serogroup D	S. Moineau

[a] NCTC, National Collection of Type Cultures.

* Corresponding author. Mailing address: Cellular and Molecular Microbiology Section, Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Elfriede-Aulhorn-Strasse 6, D-72076 Tübingen, Germany. Phone: 49-7071-29-81514. Fax: 49-7071-29-3435. E-mail: guoqing.xia@med.uni-tuebingen.de.

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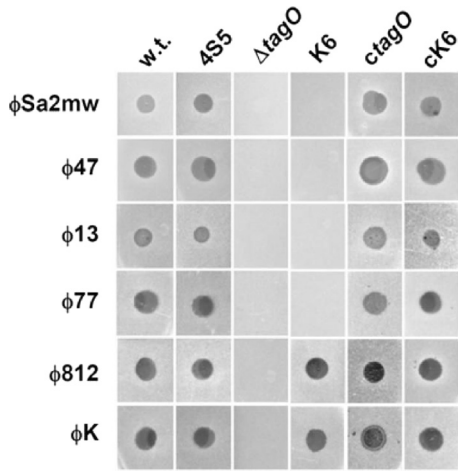

FIG. 1. WTA-dependent phage infection of *S. aureus*. Phage lysates from serogroup A (ϕ47, ϕSa2mw), serogroup F (ϕ13, ϕ77), and serogroup D phages (ϕK and ϕ812) were spotted onto lawns of wild-type (w.t.) or cell wall mutant *S. aureus* RN4220. Macroplaque formation indicates successful adsorption and infection by phages. The bacterial strains used include a Δ*tagO* mutant (deficient in wall teichoic acid), mutant strains K6 (no GlcNAc modification of wall teichoic acid) and 4S5 (no lipoteichoic acids), and *ctagO* and cK6, which are *tagO*- and *tarM*-complemented strains, respectively, and produce wild-type WTA.

sistance cassette as described previously (18). The mutant was complemented with the plasmid pRB474-*tagO*, which was constructed by subcloning the *tagO* gene into the *Escherichia coli-S. aureus* shuttle expression vector pRB474 (4). The loss of WTA in Δ*tagO* was verified by no detectable phosphate contents in WTA preparations. Of note, the WTA mutants Δ*tagO* and K6 were constructed in the genetic background of *S. aureus* strain RN4220, which is free of capsule (17), prophages, and restriction mechanisms (10). Since this strain is devoid of all of these pre- and postadsorption factors and mechanisms that might lead to phage resistance (12), impaired plaque formation on the mutant lawn indicates impaired adsorption and plaque formation suggests successful adsorption and infection.

We then challenged wild-type RN4220 and the RN4220-derived mutants with staphylococcal phages of serogroup A (ϕ47 and ϕSa2mw), serogroup F (ϕ13 and ϕ77), and serogroup D (ϕK and ϕ812). Briefly, 10 μl of phage lysate containing approximately 10^7 PFU was spotted onto soft agar containing test bacteria as described previously (21). All of the phages tested formed plaques on the bacterial lawn of wild-type RN4220 but failed to form plaques on the WTA-deficient Δ*tagO* mutant (Fig. 1), indicating that the infection is dependent on WTA. This observation was further verified by the fact

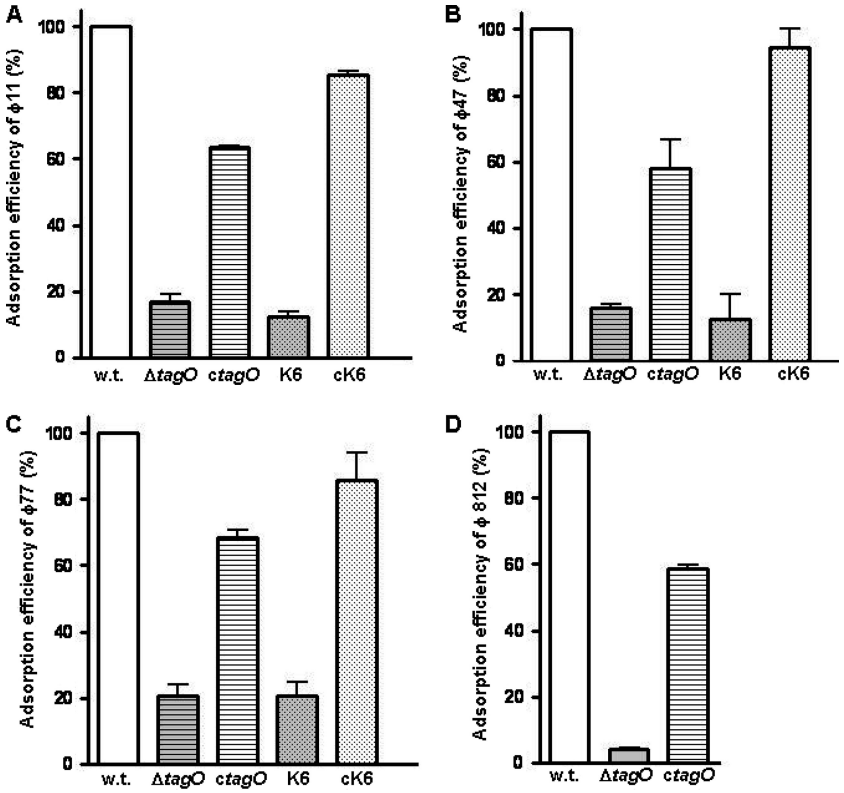

FIG. 2. Phage adsorption to *S. aureus* mutants with altered WTAs in comparison to the wild type (w.t.). *S. aureus* cells (8×10^7 CFU in 200 μl) were incubated with phage ϕ11 (A), ϕ47 (B), or ϕ77 (C) (5×10^7 PFU in 100 μl) at 37°C for 15 min. The bound phage were separated from unbound free phage by centrifugation at 13,000 × *g* for 3 min. Similar adsorption experiments were carried out with myovirus ϕ812 (D), except that 6×10^4 PFU in 100 μl were incubated with 4×10^8 CFU (in 200 μl). Adsorption was calculated by determining the number of PFU of the unbound phage in the supernatant and subtracting it from the total number of input PFU. Adsorption efficiency relative to the adsorption to wild-type strain RN4220, which was set as 100%, is indicated. The data shown are the mean values of three independent measurements. The error bars represent standard deviations.

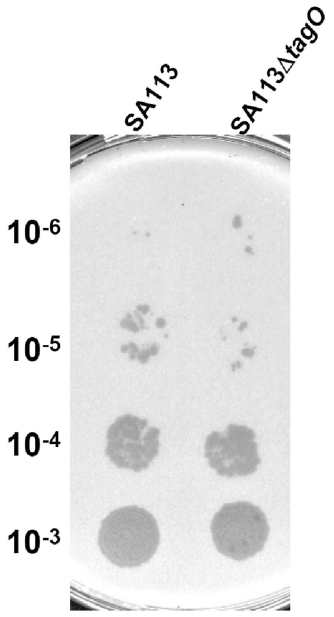

FIG. 3. WTA deficiency does not affect prophage induction and release. Wild-type *S. aureus* strain SA113 and WTA-deficient mutant SA113Δ*tagO* were incubated with mitomycin C at a final concentration of 0.5 μg/ml to induce prophage release. After 6 h of induction, the cultures were centrifuged at 5,000 × *g* for 10 min and then the culture supernatants were serially diluted to 10^{-6}-fold. A 10-μl volume of each dilution (10^{-3} to 10^{-6}) was then spotted onto a lawn of bacterial indicator strain RN4220 and incubated at 37°C overnight.

that the complemented *tagO* mutant (c*tagO*) again becomes susceptible to all phages.

To demonstrate that the impaired phage infection of the *S. aureus* WTA mutant was indeed caused by impaired adsorption, the adsorption rate was determined for ϕ11 (serogroup B), ϕ47 (serogroup A), ϕ77 (serogroup F), and ϕ812 (serogroup D). As shown in Fig. 2, all of the phages showed severely impaired adsorption to the *tagO* mutant, which lacks WTA. Moreover, the adsorption rates of phages infecting the *tagO*-complemented strain (c*tagO*) were restored to around 60% of the adsorption rates of phages infecting the wild-type cells, indicating that successful phage infection and adsorption are dependent on WTA.

To investigate the requirement of the GlcNAc glycoepitope on WTA for phage infection, the *tarM* mutant K6, which lacks GlcNAc on WTA, was infected with various phages. Interestingly, while serogroup A and F phages were not able to form plaques on K6 lawns, the serogroup D phages were still virulent toward this mutant. In addition, K6 complemented with plasmid-encoded *tarM* was again susceptible to serogroup A and F phages (Fig. 1). This finding leads to the conclusion that the GlcNAc glycoepitope on WTA, introduced by the glycosyltransferase TarM, is required for successful infection by serogroup A and F phages. Further adsorption rate analysis of ϕ11, ϕ47, and ϕ77 (Fig. 2A, B, and C) revealed severely impaired phage adsorption to mutant K6 cells, and the phage adsorption rate was restored to over 80% when the *tarM*-complemented strain (cK6) was infected. This suggested that α-GlcNAc carried by WTA served as the phage adsorption receptor for serogroup B, A, and F phages of *S. aureus*. This is also in good agreement with previous observations that phages infecting other Gram-positive bacteria, such as *Listeria* and *Bacillus* phages, used the WTA glycoepitope as an adsorption receptor (2, 5, 19, 22). Of note, although adsorption of serogroup D phages is dependent on WTA, neither the glycoepitope nor the alanyl modification of WTA seems to be essential for adsorption since these phages infected both the K6 mutant with *tarM* disrupted (Fig. 1) and a Δ*dltA* mutant deficient in alanylation of teichoic acids (data not shown). Thus, the serogroup D phages seem to adsorb to the anionic backbone of WTA.

In a recent study (9), the tail protein ORF636 of ϕSLT was characterized as an adhesion protein for lipoteichoic acid (LTA) of *S. aureus*. By *in silico* analysis, tail proteins that are 99% identical to ORF636 could be identified in the genome of serogroup A phages such as ϕ47 and ϕSa2mw, which require, as shown above, the glycoepitopes of WTA for adsorption. To characterize whether LTA is involved in phage infection, especially phage adsorption, a simple, direct, and convincing method would be a spot assay using an LTA-negative *S. aureus* strain. LTA is synthesized by *ltaS*, an enzyme that was previously shown to be essential for normal cell division and growth (7). However, it has recently been shown that an *ltaS* mutant is viable under osmotically stabilizing conditions (13). By adopting a similar strategy, an *ltaS* knockout mutant was constructed by allelic exchange under conditions that are permissive for growth (broth containing 7.5% NaCl) and the lack of LTA was confirmed by Western blot analysis using a polyglycerolphosphate LTA-specific monoclonal antibody (6; R. M. Corrigan et al., unpublished data). Upon several passages in standard medium without 7.5% NaCl, Δ*ltaS* mutant strain 4S5 regained the ability to grow and divide similar to a wild-type strain. Of note, while LTA was absent from strain 4S5, WTA was still produced by this mutant. We then spotted the phage lysate on the 4S5 lawn and found that all of the phages tested, including serogroup A phages ϕ47and ϕSa2mw, were able to form plaques (Fig. 1), indicating that successful phage infection is independent of LTA.

WTA is extremely abundant on the bacterial surface and might also play a role in phage release. To study whether WTA affects the efficiency of phage release, we did a prophage induction experiment by adding mitomycin C to the culture of *S. aureus* strain SA113, which harbors three prophages in its genome. As shown in Fig. 3, upon mitomycin C induction, the phage titer released by WTA-deficient mutant SA113Δ*tagO* is comparable to that released by wild-type SA113, indicating that depletion of WTA does dot affect phage release.

In summary, our data clearly demonstrate that WTA, but not LTA, is required for siphovirus and myovirus infection of *S. aureus*. While siphoviruses need the GlcNAc on WTA for adsorption, myoviruses seem to adsorb to the backbone of WTA. Further studies are necessary to elucidate how WTA is recognized by staphylococcal phage receptor binding proteins and contributes to the strain and species specificity of staphylococcal phages.

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