Wall Teichoic Acid-Dependent Adsorption of Staphylococcal Siphovirus and Myovirus

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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 tips 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 phospho-
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 ΔtagO was verified by no detectable phosphate contents in WTA preparations. Of note, the WTA mutants ΔtagO 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, 10^7 PFU was spotted onto soft agar 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 ΔtagO mutant (Fig. 1), indicating that the infection is dependent on WTA. This observation was further verified by the fact

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 ΔtagO mutant (deficient in wall teichoic acid), mutant strains K6 (no GlcNAc modification of wall teichoic acid) and 4S5 (no lipoteichoic acids), and tagO and cK6, which are tagO- and tarM-complemented strains, respectively, and produce wild-type WTA.

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.
also in good agreement with previous observations that phages infecting other Gram-positive bacteria, such as Listeria and Bacillus phages, used the WTA glycopeptide as an adsorption receptor (2, 5, 19, 22). Of note, although adsorption of serogroup D phages is dependent on WTA, neither the glycopeptide nor the alanyl modification of WTA seems to be essential for adsorption since these phages infected both the K6 mutant and the alanyl modification of WTA appears to be essential for adsorption since these phages infected both the K6 mutant and the alanyl-modified WTA (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 φ5LT 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 glycopeptidases 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, ΔltaS 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 φ47 and 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 SA113tagO was comparable to that released by wild-type SA113, indicating that depletion of WTA does not 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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