Immunobiology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Review

The staphylococcal surface-glycopolymer wall teichoic acid (WTA) is crucial for complement activation and immunological defense against *Staphylococcus aureus* infection

Kenji Kurokawa[a], Kazue Takahashi[b], Bok Luel Lee[c,*]

[a] *Faculty of Pharmaceutical Sciences, Nagasaki International University, Sasebo, Nagasaki, Japan*
[b] *Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA*
[c] *College of Pharmacy, Pusan National University, Jangjeon Dong, Kumjeong Ku, Busan 46241, South Korea*

ARTICLE INFO

Article history:
Received 17 April 2016
Received in revised form 8 June 2016
Accepted 9 June 2016
Available online xxx

Keywords:
MBL
Complement
Staphylococcus aureus
Wall teichoic acid
Classical pathway
Opsonophagocytosis

ABSTRACT

Staphylococcus aureus is a Gram-positive bacterial pathogen that is decorated by glycopolymers, including wall teichoic acid (WTA), peptidoglycan, lipoteichoic acid, and capsular polysaccharides. These bacterial surface glycopolymers are recognized by serum antibodies and a variety of pattern recognition molecules, including mannose-binding lectin (MBL). Recently, we demonstrated that human serum MBL senses staphylococcal WTA. Whereas MBL in infants who have not yet fully developed adaptive immunity binds to *S. aureus* WTA and activates complement serum, MBL in adults who have fully developed adaptive immunity cannot bind to WTA because of an inhibitory effect of serum anti-WTA IgG. Furthermore, we showed that human anti-WTA IgGs purified from pooled adult serum IgGs triggered activation of classical complement-dependent opsonophagocytosis against *S. aureus*. Because the epitopes of WTA that are recognized by anti-WTA IgG and MBL have not been determined, we constructed several *S. aureus* mutants with altered WTA glycosylation. Our intensive biochemical studies provide evidence that the β-GlcNAc residues of WTA are required for the induction of anti-WTA IgG-mediated opsonophagocytosis and that both β- and α-GlcNAc residues are required for MBL-mediated complement activation. The molecular interactions of other *S. aureus* cell wall components and host recognition proteins are also discussed. In summary, in this review, we discuss the biological importance of *S. aureus* cell surface glycopolymers in complement activation and host defense responses.

Contents

Abbreviations: WTA, wall teichoic acid; LTA, lipoteichoic acid; MBL, mannose-binding lectin; MASPs, MBL-associated serine proteases; PMNs, polymorphonuclear leukocytes; IVIGs, intravenous IgG.

* Corresponding author at: Global Research Laboratory of Insect Symbiosis, College of Pharmacy, Pusan National University, Busan 46241, South Korea.
E-mail address: brlee@pusan.ac.kr (B.L. Lee).

http://dx.doi.org/10.1016/j.imbio.2016.06.003
0171-2985/

1. Introduction

Staphylococcus aureus is a commensal bacterium in the human nasal cavity and on the skin; approximately 30% of healthy individuals are carriers of this bacterium. *S. aureus* is also a human pathogen that causes infectious diseases of the skin and soft tissues, pneumonia, sepsis, and other disorders in immunocompromised individuals (e.g., after surgery) (Fournier and Philpott, 2005; Lowy, 1998). Clinical problems associated with this pathogen include the following: *S. aureus* has acquired resistance to the majority of clinically used antibiotics; methicillin-resistant *S. aureus* strains (MRSAs) show multiple drug resistance and have spread through hospitals and communities all over the world; and an effective vaccine has not yet been developed (DeLeo et al., 2009; Fowler and Proctor, 2014).

Most Gram-positive bacteria, including *S. aureus,* incorporate membrane- or peptidoglycan- attached carbohydrate-based polymers into their bacterial cell envelopes. These cell wall glycopolymers have highly diverse structures and play important roles in modulating the major bacterial envelope constituents (Neuhaus and Baddiley, 2003). Other important roles of bacterial glycopolymers in host-cell adhesion, inflammation, and immune activation have also been described in recent years (Cobb et al., 2004; Pluddemann et al., 2006). Therefore, identifying and biochemically characterizing highly conserved bacterial ligand molecules that are recognized by host defense proteins and determining the molecular cross-talk between pathogens and host defense proteins are important for developing new antibiotics, vaccines, and diagnostics for use in the fight against severe infectious diseases.

The major cell envelope-associated glycopolymers in *S. aureus* are highly diverse and are species- or strain-specific (Weidenmaier and Peschel, 2008). The cell wall of most *S. aureus* cells contains the following five major components: capsular polysaccharides, wall teichoic acid (WTA), lipoteichoic acid (LTA), peptidoglycan, and lipoproteins. Of these, WTA, LTA, and peptidoglycan are not easily purified due to their structural similarity and complexity (Navarre and Schneewind, 1999). Furthermore, purification of the specific host defense proteins that recognize these glycopolymers and determination of their binding specificities for their ligands are quite difficult. However, with recent advances in the analytical techniques used in glycobiology and the availability of mutant strains made possible by developments in genetics and microbiology, our knowledge of the molecular structures and the genes encoding *S. aureus* bacterial cell wall glycopolymers such as WTA and LTA has increased (Weidenmaier and Peschel, 2008). The common structure of WTAs is an *N*-acetylmannosamine (ManNAc)-(β-1 → 4)-*N*-acetylglucosamine (GlcNAc)-1-phosphate disaccharide with one to three glycerol phosphates attached to the C4 hydroxyl of the ManNAc residue (the "linkage unit") followed by a much longer chain of glycerol- or ribitol- phosphate repeats (the "main chain") (Fig. 1). The linker unit of WTA is covalently linked to the peptidoglycan via a phosphodiester bond between the GlcNAc-1-phosphate and the C6 position of the *N*-acetylmuramic acid (MurNAc) of the peptidoglycan. The hydroxyl groups of the glycerol- or ribitol-phosphate repeats are bound to cationic D-alanine esters and monosaccharides such as glucose or GlcNAc. It has been suggested that WTA is an important ligand in the adsorption of several bacteriophages to *S. aureus* (Winstel et al., 2013) and in the adherence of *S. aureus* to nasal epithelial cells (Baur et al., 2014; Weidenmaier et al., 2004). In contrast, LTAs are generally composed of glycerol-phosphate repeating units and are connected to glycolipids (Fischer, 1994). Although LTA plays a crucial role in cell division and membrane integrity (Grundling and Schneewind, 2007) and WTA is dispensable for cell viability, *S. aureus* cannot survive with the deletion of both WTA and LTA (Oku et al., 2009), indicating that teichoic acids are important for fitness and cell wall maintenance in *S. aureus*. Additionally, *S. aureus* cells synthesize lipoproteins that are membrane proteins anchored by characteristic N-terminal lipid-modified structures (Nakayama et al., 2012a). Therefore, mutant strains of *S. aureus* that are deficient in cell surface components are useful in analyzing pathogen-host interactions. Table 1 summarizes the typical *S. aureus* mutants used in this field and the critical roles of various cell surface components in host interactions.

Recent studies regarding innate immunity in organisms ranging from insects to mammals have clearly demonstrated that microbial pattern recognition receptors (PRRs) expressed on macrophages, dendritic cells, and mast cells and soluble pattern recognition proteins in body fluid recognize pathogen-associated molecular patterns (PAMPs) that are expressed on the bacterial cell wall surface (Ferrandon et al., 2007; Takeuchi and Akira, 2010). These interactions result in the secretion of antimicrobial proteins and increase vascular permeability and the recruitment and activation of neutrophils by inducing the secretion of inflammatory cytokines and chemokines such as TNF-α, IL-6, IL-8, and complement-derived peptides (Hoffmann et al., 1999; Kawai and Akira, 2010; Medzhitov and Janeway, 1997). In insects, peptidoglycan recognition proteins (PGRPs), which occur in both soluble and membrane-bound forms, sense bacterial peptidoglycans and initiate the proteolytic cascade for the activation of the Toll/IMD pathways to induce antimicrobial peptide secretion (Ferrandon et al., 2007; Iwanaga and Lee, 2005). As part of cellular innate immunity, phagocytosis of invading pathogens occurs through interaction of the host scavenger receptors of phagocytes with PAMPs (Meister, 2004). In mammals, the MD2-Toll-like receptor 4 (TLR-4) complex senses the lipopolysaccharides (LPSs) of Gram-negative bacteria and is involved in endotoxin-mediated toxicity. TLR-2/1 and TLR-2/6 heterodimers sense the N-terminal lipid-modified structures of bacterial lipoproteins and induce the production of inflammatory cytokines (Takeuchi et al., 2000). Also, nucleotide-binding oligomerization domain proteins (NODs) are known to recognize bacterial peptidoglycans (Caruso et al., 2014). Peptidoglycans and LTAs have been thought of as TLR-2 ligands (Michelsen et al., 2001); however, recent studies have shown that the major ligands of TLR-2 are lipoproteins. The *S. aureus* lgt deletion mutant [*lgt* encodes the first enzyme, Lgt (lipoprotein diacylglycerol transferase), in the bacterial lipoprotein biosynthesis pathway], which lacks the lipid-modified structure of lipoproteins, is unable to induce TLR-2-dependent inflammatory cytokine secretion (Kurokawa et al., 2009; Nakayama et al., 2012b). Lipoprotein-mediated inflammatory cytokine secretion is also involved in bone destruction through the augmentation of osteoclast differentiation and activation (Kim et al., 2013).

In bacterial cells, the synthesis of polypeptides begins with a formylated methionine, whereas in human cells, protein synthesis begins with an unmodified methionine. Formylated peptides from bacteria have been shown to activate formyl peptide receptor 1, a G protein-coupled receptor (GPCR), and to induce the chemo-

Please cite this article in press as: Kurokawa, K., et al., The staphylococcal surface-glycopolymer wall teichoic acid (WTA) is crucial for complement activation and immunological defense against *Staphylococcus aureus* infection. Immunobiology (2016), http://dx.doi.org/10.1016/j.imbio.2016.06.003