Streptococcus Pneumoniae Teichoic Acids

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Abstract: *Teichoic acids (TAs) are one of the key virulence factors of S. pneumoniae, as well as one of the key components of the bacterial cell wall. The complexity of TAs structure and biosynthesis stems from its diverse functions and roles, including their involvement in the regulation of bacterial virulence and bacterial division, it also plays a crucial role in bacterial transformation. In this paper, the basic structure and biosynthesis of teichoic acid in S. pneumoniae were described. Meanwhile, the functions of TAs in the pathogenic mechanism of S. pneumoniae, the regulatory factors of biosynthesis, and the effects in vaccines are summarized. Finally, we also provide a perspective for future research. This review may provide a foundation for further studies to provide the pathogenic mechanism of TAs in S. pneumoniae and a theoretical basis for the design of vaccines.*

Keywords: Streptococcus pneumoniae; Teichoic acids; biosynthetic; vaccine

1. Introduction

Streptococcus pneumoniae is a gram-positive conditionally pathogenic bacterium, when *Streptococcus pneumoniae* evades host defenses, it can cause local or systemic infections such as community-acquired pneumonia, otitis media, septicemia, and meningitis[1, 2]. The World Health Organization has ranked *S. pneumoniae* as the fourth most common microorganism that can cause infections. Currently, the growing phenomenon of multi-drug resistance has led to the weakening of the efficacy of commonly used antimicrobial drugs in the clinical management of *S. pneumoniae* infections, and the limitations of non-serotypic vaccines forcing serious challenges in the prevention and treatment of *S. pneumoniae* infections.[3, 4]. Bacterial virulence factors are one of the main pathogenic factors of *S. pneumoniae*. Therefore, in-depth study of virulence factors of *S. pneumoniae* is of great significance for the prevention and treatment of *S. pneumoniae* infection. The crucial virulence factors of *S. pneumoniae* include: Teichoic acids (TAs)[5], Capsular polysaccharide (CPS)[6], Pneumolysin(Ply)[7] and so on[8, 9], Among them, TAs is a key and conserved virulence factor. This review will concentrate on the biosynthesis, function and pathogenic mechanism of TAs in *S. pneumoniae*, the regulation of TAs biosynthesis, and the application of TAs in vaccine research.

2. *Streptococcus pneumoniae* biosynthesis of teichoic acids

TAs biosynthesis in *S. pneumoniae* is mediated by multiple cytoplasmic membrane-associated proteins[10]. The main steps include: synthesis of TAs precursor molecules, polymerization of TAs repetitive units (RUs), transmembrane transport of RUs, and ultimately anchoring of TA glycan chains to the surface of the bacterial wall to form TAs[11]. Wherein the repeat unit synthesis of TAs is first catalyzed by Spr1654 to catalyze PLP-dependent amino addition to the C-6 atom of UDP-4-keto-4-deoxy-d-GlcNAc to form UDP-AATGal and serves as the initial sugar for the synthesis of the TAs repeat unit[12]. And then the SPR1645/1655, SPR0091/0092 synthesize the Glc-AATGal-PP-upr precursor molecule and immobilize it on the cell membrane[13, 14]. Subsequently, TarI and TarJ of the lic1 locus complete the synthesis of the CDP-ribitol and Rib-P was transferred to Glc-AATGal-PP-upr by SPR1125(licD3) to form Rib-P-Glc-AatGal-PP-UPR[15]. Eventually, the SPR1123/1124 are responsible for linking GalNAc to the glycan to form the repeat unit backbone of TAs[16]. Nicolas Gisch et al. revealed that TAs glycan chains from AATGal β-glycosidic linkages to lipid anchors can form LTA, whereas WTA is formed by alpha linking from AATGal to MurNAc-phosphate in peptidoglycan[17]. Researches

show that the WTA and LTA of *S. pneumoniae* have the similar polysaccharide skeleton.

3. Biological functions of *S. pneumoniae* teichoic acid

3.1 Provides anchoring sites for choline-binding proteins

S. pneumoniae TAs is decorated with a large number of phosphorylcholine residues. The bacterial wall surface proteins, collectively known as choline-binding proteins (CBP) family proteins, are non-covalently bound to the phosphocholine of the teichoic acid and anchored to the TAs[18-20]. CBP is the family of polypeptides found in pneumococci and related species and some of their associated phages, it is mainly involved in immune escape, virulence and host pathogenicity[11, 21]. Part of CBPs also mediate carbohydrate and glucose metabolism (GAPDH and RafE) or control cell division (LytB and StkP)[17, 20, 22]. the choline binding proteins PspC and PspA[23, 24] during the formation of competent of *S. pneumoniae* are exposed to the bacterial surface. In summary, *S. pneumoniae* TAs provides anchoring sites for choline-binding proteins.

3.2 Involved in regulating bacterial growth and division

Deletion of the *S. pneumoniae* TAs ligase TacL, decreases LTA content thereby affecting bacterial growth[25]. Veening et al. reported that bacterial virulence was attenuated after *tacL* deletion, while did not affect its growth[5], this conclusion remains controversial. The *S. pneumoniae* WTA ligase LytR, a key molecule in TAs biosynthesis, similarly affects bacterial growth and also involved in bacterial division and autolysis[26]. It was shown that *S. pneumoniae* TAs and StkP anchor LytB to the PG layer and localize LytB in the septa to control its division[27]. Solation of the bacteriophage requires the involvement of the choline-binding protein LytA (N-acetylcytidylyl-l-alanine amidase)[28, 29]， also leads to cell lysis by mediating peptidoglycan hydrolysis[30]. The above studies indicate that TAs biosynthesis-related genes and choline-binding proteins are involved in the growth and division processes.

3.3 Involved in host adhesion and colonization

Invasive pneumococcal disease (IPD) is a disease caused by the spread of *Streptococcus pneumoniae* from the nasopharynx to the lungs, blood, and brain[31]. Bacterial colonization is a primary condition for the development of IPD, TAs is one of the major factors for the colonization of *S. pneumoniae* in the host upper respiratory tract[32, 33]. Two bacterial phenotypes are produced when *S. pneumoniae* interacts with the host, with the surface of the transparent phenotype, which adheres more strongly to the nasopharynx, has abundant teichoic acid on its surface, while after entering the blood has less TAs[34-36]. Significantly increased levels of choline phosphate and choline-binding protein A (CbpA) on the surface of hyaline phenotypes have been reported[18, 37]. and CbpA is structurally related to PspA and mediates adhesion to cytokine-activated lung cells, contributing to *S. pneumoniae* colonization of the nasopharynx[18]. TAs can bind to host platelet activating factor receptors to mediate bacterial adhesion and invasion. These results suggest that TAs of *S. pneumoniae* is involved in host adherence and colonization.

3.4 Involved in biofilm formation

*B*iofilm formation is considered as an important factor in bacterial colonization and infection[38, 39]. It has been found that choline binding protein anchored to cell wall teichoic acid of *S. pneumoniae* plays an important role in biofilm formation[40, 41], When pneumococci were incubated in the presence of high concentrations of choline or ethanolamine, some of the choline-binding proteins such as LytA, LytB, and LytC were all inhibited and released from the cell surface, while a significant reduction was observed in biofilm formation [42]. Furthermore, deletion of the genes encoding pneumococcal surface protein A or the putative adhesins PcpA and CbpA resulted in diminished biofilm formation[43]. Trappetti et al. suggested that lic operons, which involved in choline metabolism also contribute to biofilm formation[44, 45]. The above studies suggest that TAs of *S. pneumoniae* may largely mediate its biofilm formation.

3.5 Involved in S. pneumoniae genetic transformation

Reported by Veening's team in 2023[46]， TAs synthesis-related genes have a crucial role in the formation of competent of *S. pneumoniae*. For example, LytR-mediated biosynthesis of WTA protects *S. pneumoniae* cells from cleavage by lyases. And when tacL-dependent expression was induced, the early

gene *ssBb* transcriptional activity of *S.pneumoniae* transformation showed a dependent increase. the *S. pneumoniae* plic1 promoter was repressed during competence formation[47]. However, how the balance between LTA and WTA to protect the host is regulated by *S. pneumoniae* during competent formation needs to be further investigated. Moreover, Deletion of licD2, the gene for TAs anabolic resulted in a decrease in TAs content and a significant decrease in transformation ability of *S. pneumoniae*[16]. It has been reported that the transparent phenotype has a higher transformation efficiency, due to the presence of fewer CPS and more LTA on the surface of the bacterial during phase transition[34]. Studies have shown that colonized bacteria in the nasopharynx of mice showed high levels of natural transformation, whereas bacteria during sepsis were transformed less efficiently[39, 48]. Therefore, *S. pneumoniae* TAs plays a crucial role in the genetic transformation.

4. Mechanism of teichoic acid pathogenesis in *S. pneumoniae*

4.1 Promotes adhesion of the bacterium to host cells

S. pneumoniae TAs binds to receptors on the host cell surface, thereby facilitating attachment and invasion of *S. pneumoniae* into host cells. The choline binding protein CbpA and of PspA anchoring to TAs of *S. pneumoniae*, CbpA acts as an adhesin, and PspA enhances the invasiveness of bacteria and promotes the adhesion of *S. pneumoniae* to host cells[24, 49]. Furthermore, studies have reported that P-Cho residues exert a toxic effect by mediating the adhesion of platelet-activating factor receptors[50, 51]. Our previous study reported that *tacL* is highly conserved as an in vivo inducible expression gene of *S. pneumoniae*, and deletion of which resulted in a significant reduction in *S. pneumoniae* adhesion and invasive capacity[5, 52]. These results indicate that TAs of *S. pneumoniae* promotes adhesion of the bacterium with to host cells.

4.2 Interferes with the host immune system

S. pneumoniae can evade attack by the host immune system through a variety of mechanisms leading to persistence of infection. the TAs surface proteins can evade the host immune system by inhibiting complement-mediated immune responses, including the choline-binding protein PspA prevents complement from mediating the clearance and phagocytosis of *S. pneumoniae* by host cells, and directly reduces complement deposition[42, 53]. PspC inhibits complement-dependent phagocytosis of *S. pneumoniae*[23, 54]. Other choline-binding proteins have been found to play a role in inhibiting complement activation, including the hydrolase LytA inhibits complement deposition through both classical and alternative pathways[55]. LytC inhibits the binding of C3b to *S. pneumoniae*[56]. P-Cho residues act as anchors for choline-binding proteins on TAs, and choline-binding proteins attached to the surface have immune protective potential. It is not known whether this mechanism is directly related to the phosphocholine moiety of TAs or mediated through choline binding proteins attached to it. These studies suggest that TAs of *S. pneumoniae* can be interfered with the immune system, thereby reducing the effectiveness of the host immune response.

4.3 Induction of host inflammatory response

S. pneumoniae TAs can directly induce the host cells to produce inflammatory factors, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β)[10, 57]. Studies have found that higher concentrations of TAs in cerebrospinal fluid (CSF) can lead to the proliferation of *S. pneumoniae* and release of cell wall components to stimulate the inflammatory response of the host[58, 59]. Clinical outcome of *S. pneumoniae* meningitis is not optimistic, when TAs of CSF concentration is increased[60]. *S. pneumoniae* have the dlt gene locus for D-alanine modification of TAs[61],and it affects the host inflammatory response by altering signal transduction in a Toll-like receptor 2 (TLR2) -dependent manner[62]. When pneumococci are attacked by antimicrobial peptides or lysozyme, the D-alanylation of the bacterial wall LTA is increased, which enhances the TLR2-mediated inflammatory response[63]. The above studies suggest that TAs facilitating invasion and infection by *S. pneumoniae* through mechanisms inducing an inflammatory response in the host.

5. Regulation of teichoic acid biosynthesis

5.1 Key components of teichoic acid synthesis

The LytR protein (SPD1741) belongs to a member of the LytR-CPS-PsR (LCP) family of proteins that have been shown to be involved in the anchoring of cell wall polysaccharides to peptidoglycans in a variety of bacteria[64]. When *lytR* is deleted, TAs content decrease [65, 66]. TacL is an essential gene responsible for lipoteichoic acid biosynthesis, and when *tacL* is deleted, TAs biosynthesis is significantly reduced[67]. The transaminase Spr1654 was found to catalyze the PLP-dependent attachment of amino to the C-4 atom of UDP-4-keto-6-deoxy-D-glcnAc to form UDP-AATGal in *S. pneumoniae*[11, 68]. UDP-AATGal is the primary substrate of the TA synthesis pathway, thus SPD-1654 acts as an essential aminotransferase in teichoic acid biosynthesis in *S. pneumoniae*, affecting teichoic acid biosynthesis[69]. The above studies have shown that deletion of key molecules responsible for TAs biosynthesis in *S. pneumoniae* can affect TAs content.

5.2 Transcriptional regulators

The specific binding of the promoter of the TAs synthesis related gene lic1 negatively transcriptionally regulates TAs biosynthesis by Mgaspn[70]. It has been reported that the YycF/YycG two-component system participates in various physiological regulations, cell wall metabolism, biofilm formation, and cell division. YycF, as a transcriptional regulator, negatively regulates TAs precursor synthesis by binding specifically to the promoter of lic1. It also was found that the promoter of teichoic acid synthesis gene lic1 in *S. pneumoniae* was directly activated by CiaR[71, 72]. However, whether it regulates teichoic acid biosynthesis needs to be further studied. MarR family proteins are a class of wing-helix-turn-helix (wHTH) DNA-binding proteins[73], comparative homology analysis with the *S. pneumoniae* genome database suggested the presence of two MarR homologues: AdcR, which is zinc-dependent and affects ABC transporters, and FabT, which affects fatty acid biosynthesis[74]. FabT binds sequences specifically to the promoter region of the TAs synthesis biosynthesis-related gene *tacL* and negatively transcriptionally regulates bacterial TAs biosynthesis[75]. These results indicate that *S. pneumoniae* can regulate TAs biosynthesis by using in vivo transcription factors.

5.3 Environmental factors

S. pneumoniae can sense changes in the external environment to regulate the expression of virulence genes, which is the key to its pathogenicity[9, 76]. For example, after the penetration of *S. pneumoniae* from the nasal mucosa into the blood and/or meninges, the pneumococci in the nasopharynx expressed more choline binding protein A (CbpA) than those in the blood[36]. clpP is a negative regulator of the expression of the choline-binding protein cbpA, which could also indirectly explain the reduced teichoic acid content on the bacterial surface after *S. pneumoniae* entry into the blood[77]. In addition, increased host glucose inhibited teichoic acid biosynthesis in *S. pneumoniae* by regulating the expression of MgaSpn, a global transcriptional regulator[78]. The TAs of *S. pneumoniae* also seems to change under acidic conditions, and the choline binding protein LytB is down-regulated after the deletion of acid stress molecule StkP [79], and the expression of LytA anchored to the TAs surface was also regulated[80]. TAs on the surface of the bacterial wall also dynamically regulates the competence of *Streptococcus pneumoniae* to protect the bacteria from cannibalism, but the molecular mechanism is still unclear[46]. These results suggest that TAs biosynthesis is adaptive to different niches and environments in *S. pneumoniae*.

6. Use of TAs in vaccinations

S. pneumoniae TAs is a polysaccharide substance has been recognized as a potential vaccine candidate for Gram-positive bacteria[81]. It was found that the cross-reactive epitope of anti-TAs antibody is the poly-1, 3- (glyceryl phosphate) backbone of LTA in Staphylococcus, Streptococcus and Enterococcus strains. The antibody induced immunity in mice by using the (poly) glyceryl phosphate backbone of TAs[81-83]. Goldenberg and colleagues have shown that a human antibody directed against phosphocholine protects mice against lethal doses of *S. pneumoniae*[84]. Studies have reported that *S. pneumoniae* CbpA has strong active immunity[85], Immunization with purified CbpA provided protection against sepsis caused by *S. pneumoniae* and this protection was mediated by antibodies that cross-reacted with the PspA domain[18, 86]. The above results provide an important theoretical basis for the development

of new pneumococcal vaccines. The research and development of TAs vaccines face so many challenges, among which the cleavage and extraction of teichoic acid are the main problems. The preparation process of teichoic acid vaccine will be further optimized to improve its stability and immune activity.

7. Conclusion

TAs is one of the key virulence factors of *S.pn*, the structure and function, virulence, and biosynthesis of TAs have been extensively studied in the past. TAs can be changed significantly in the adaptation of S.pn to host and environment. However, the molecular mechanisms underlying the adaptive regulation of *S. pneumoniae* under different environmental conditions are still not fully understood. It is of great scientific value to explore the molecular mechanism of *S. pneumoniae* bacterial wall biosynthesis regulation. There is also a need to explore more deeply the regulatory mechanisms between TAs and the immune system, so as to develop safer and more efficient pneumococcal vaccines. In this paper, which allows us to have a deeper and systematic understanding of TAs, which will provide a theoretical basis for the subsequent study of pneumococcal prophylaxis and therapy.

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