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Contribution of Human Thrombospondin-1 to the Pathogenesis of Gram-Positive Bacteria

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Keywords

Human thrombospondin-1 · *Streptococcus pneumoniae* · *Staphylococcus aureus* · Colonization · Dissemination

Abstract

A successful colonization of different compartments of the human host requires multifactorial contacts between bacterial surface proteins and host factors. Extracellular matrix proteins and matricellular proteins such as thrombospondin-1 play a pivotal role as adhesive substrates to ensure a strong interaction with pathobionts like the Gram-positive *Streptococcus pneumoniae* and *Staphylococcus aureus*. The human glycoprotein thrombospondin-1 is a component of the extracellular matrix and is highly abundant in the bloodstream during bacteremia. Human platelets secrete thrombospondin-1, which is then acquired by invading pathogens to facilitate colonization and immune evasion. Gram-positive bacteria express a broad spectrum of surface-exposed proteins, some of which also recognize thrombospondin-1. This review highlights the importance of thrombospondin-1 as an adhesion substrate to facilitate colonization, and we summarize the variety of thrombospondin-1-binding proteins of *S. pneumoniae* and *S. aureus*.

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Introduction

Streptococcus pneumoniae (the pneumococcus) and *Staphylococcus aureus* are commensals of the human upper respiratory tract. At least once in a lifetime, every human being is asymptomatically colonized with both bacteria. These facultative pathogens can affect other organs and invade deeper tissues. The occupation of normally sterile niches of the human body with the bacteria leads to local infections such as sinusitis, otitis media, and abscesses, or to life-threatening diseases like pneumonia, meningitis, or sepsis. A strong interaction between the bacterium and respiratory epithelial cells is a prerequisite for a successful colonization. Bacterial binding to the epithelial lineage occurs predominantly indirectly via components of the extracellular matrix (ECM), but also directly to cellular receptors. Thus, the multifaceted interactions are ensured mostly by bacterial surface proteins. These proteins can act as adhesins and are often referred to as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) of the host. Besides colonization, several MSCRAMMs of *S. pneumoniae* and *S. aureus* exert multiple other functions, including immune evasion or immune modulation of the host to facilitate the dissemination of the pathogen [1–4].

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Human thrombospondin-1 (hTSP-1 or THBS-1) is a high-molecular-mass glycosylated protein. As a matricellular protein, it does not contribute to the structural integrity of the ECM but regulates ECM function by interacting with multiple ligands including proteins, cytokines, proteases, and cells. This homotrimeric protein was first isolated from activated platelets as a thrombin-sensitive protein in 1971 [5]. It is synthesized by the progenitor cells megakaryocytes and is mainly stored in high amounts in α-granules of platelets, with an estimated copy number of 101.000 hTSP-1 molecules per platelet [6]. Due to platelet activation, hTSP-1 gets released subsequently and is found in its soluble form or bound to the platelet membrane. The plasma concentration of hTSP-1 in healthy individuals commonly ranges between 20 and 300 ng/mL, but it achieves its maximum level of 30 μg/ml at sites of platelet clot formation [7]. Human TSP-1 is reported to also be synthesized and secreted by a variety of other cell types including endothelial cells, monocytes, macrophages, fibroblasts, smooth muscle cells, dendritic cells, and B cells, and it gets incorporated into the ECM [8–12].

Human TSP-1 is a member of the family of oligomeric glycoproteins, which is divided into 2 subgroups depending on the oligomerization status and size. Subgroup A contains the homotrimeric TSP-1 and TSP-2, and subgroup B includes the much smaller homopentameric TSP-3, TSP-4, and TSP-5/COMP. The *THBS-1* gene is located on the human chromosome 15:39.58–39.6 and is encoded in 22 exons with a size of about 20 kb. The mature homotrimer has a size of 420 kDa. Each monomeric polypeptide chain contains 1,152 amino acids, and has a modular organization formed by: a globular N-terminus followed by a coiled-coil oligomerization domain, a von Willebrand factor C module, 3 properdin-like type-I repeats, 3 epidermal growth factor-like type-II repeats, 8 calcium-binding type-III repeats, and a globular C-terminal domain (Fig. 1) [13]. The amino-terminal region is composed of groups of basic amino acids and is characterized by its function to bind heparin and various other ligands. This fraction of hTSP-1, which comprises the globular domain up to and including the type-I repeats, varies within the TSP family. The carboxy-terminal part, referred to as the signature domain, contains the type-II repeats, the type-III repeats, and the globular C-terminal domain. This part is conserved among proteins of the thrombospondin family with an identity of 53–82% [14].

The Biological Functions of Matricellular Thrombospondin-1

The distribution of hTSP-1 is more important in embryonic tissue than in adult tissue [15]. In general, the expression of hTSP-1 is enhanced in proliferating cells rather than in quiescent cells and is induced during tissue remodeling and lesion formation [15, 16]. Of >80 hTSP-1 ligands, 35 have been identified along with their binding sites within the hTSP-1 molecule (Fig. 1). The interacting components are a heterogeneous group of proteinaceous and nonproteinaceous nature. Due to the multidomain organization of hTSP-1, the glycoprotein is involved in multiple and partly opposing biological processes, amongst others, hemostasis, angiogenesis, focal adhesion, the proliferation and migration of cells, immune regulation, endocytosis, and apoptosis.

Human TSP-1 affects angiogenesis, which is exerted by different domains of the glycoprotein. The interaction of the type-I repeats with CD36 is considered an important negative regulator of angiogenesis, and also induces the apoptosis of endothelial cells [17–19]. The antiangiogenic activity of hTSP-1 is avoided by the interaction with the histidine-rich glycoprotein within the CD36-binding region [20]. The major antiangiogenic site of TSP-1 was thought to be localized within the type-I repeats. However, the type-III repeats also diminish angiogenesis by preventing the binding of fibroblast growth factor 2 (FGF2) to the endothelial cells [21]. The interaction of hTSP-1 with growth factors has opposite effects on cell proliferation. Binding of the hepatocyte growth factor (HGF) results in the inhibition of angiogenesis [22]. In contrast, hTSP-1 is the major activator of latent transforming growth factor β (TGF-β), which stimulates angiogenesis [23, 24]. Additionally, activated TGF-β mediates the formation of the ECM and the immune response. The opposite functions of hTSP-1 in proliferation and angiogenesis can be partly explained by its conformational state in the secreted form or ECM-incorporated form [25, 26]. Furthermore, existing discrepancies are dependent on the cell type and tissue studied.

Cell adhesion to the ECM is crucial to maintain the integrity of tissues. Human TSP-1 mediates the adhesion and chemotaxis of different cell types via the N-terminal domain by binding to sulfatides, proteoglycans, and the integrins $\alpha_3\beta_1$, $\alpha_4\beta_1$, and $\alpha_6\beta_1$ [27–30]. The interaction with proteoglycans leads to endocytosis of soluble hTSP-1, which is triggered by binding to the low-density lipoprotein receptor-related protein (LRP) [31]. The N-terminal binding of calreticulin results in focal adhesion disassembly and further to cell migration [32].

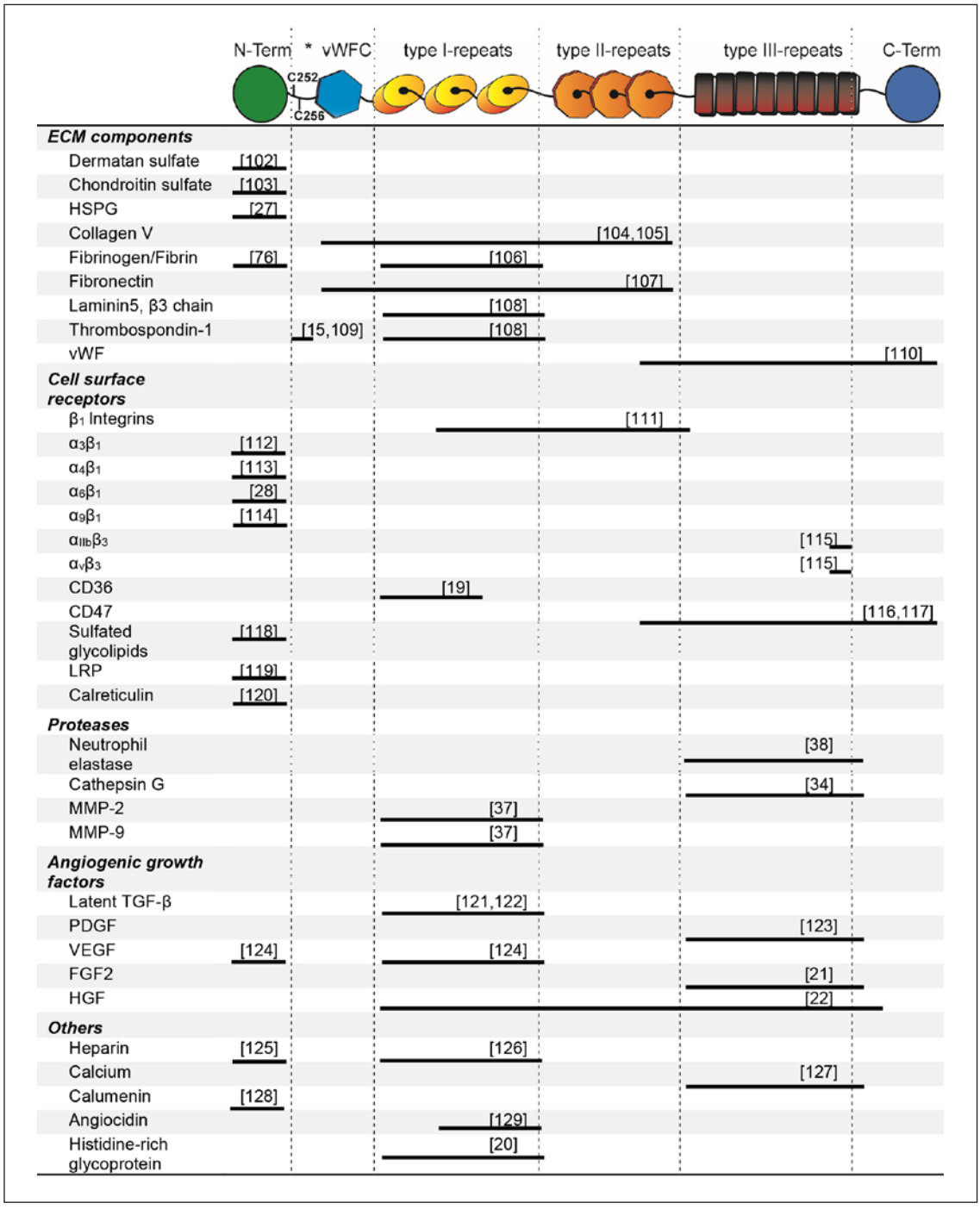

Fig. 1. Functional domains of hTSP-1 with interacting ligands. Adapted from Bonnefoy et al. [130]. The asterisk represents the coiled-coil oligomerization domain of hTSP-1.

Overall, many of these interactions are dependent on the conformational state of hTSP-1, which is regulated by calcium-ions, heparin, and heparan-sulfate [26]. Furthermore, the binding of various hTSP-1 ligands is inhibited by heparin and is highly susceptible to calcium concentration [33]. For example, the type-III repeats contain a cryptic binding motif for cathepsin G and neutrophil elastase, which becomes exposed after a drastic structural

change within hTSP-1 induced by a low calcium concentration [34].

As a matricellular glycoprotein, hTSP-1 is involved in the organization of the ECM which is continuously processed by proteases. Based on the multiple biological functions of hTSP-1, its extracellular and intracellular proteolytic degradation is also tightly controlled. The sensitivity of hTSP-1 towards the proteolytic activity of the proteases differs, depending on the origin of the glycoprotein. Endothelial cell-derived hTSP-1 has been shown to be degraded by plasmin, cathepsin G, and leukocyte elastase [35]. In contrast, platelet-derived hTSP-1 is known to inhibit the activity of several proteases, including plasmin, neutrophil elastase, cathepsin G, and matrix metalloproteinases (MMP-2 and MMP-9) [34, 36–38]. After secretion of hTSP-1 by platelets, the glycoprotein becomes a substrate for thrombin and factor XIIIa [26]. The intracellular degradation of hTSP-1 occurs in the lysosome after endocytosis [39].

For more than 2 decades, the role of hTSP-1 during the pathogenesis of Gram-positive bacteria has been investigated. So far, several surface-exposed proteins of *S. pneumoniae* and *S. aureus* have been identified to interact directly with hTSP-1 to promote adhesion and colonization [40–43]. Furthermore, these staphylococcal proteins are able to activate human platelets, the main source of hTSP-1 [44, 45].

S. pneumoniae and *S. aureus* Target Matricellular hTSP-1

The contact of *S. pneumoniae* and *S. aureus* to host tissue, predominantly to components of the ECM, is a prerequisite for the establishment of a stable colonization. The initial loose attachment occurs via glycoconjugates on the host surface, while the adhesion requires stronger and specific interactions to host proteins [46]. Pneumococci and *S. aureus* can bind directly to host cellular receptors, or, in most cases, the pathogens utilize matrix proteins as molecular bridges.

S. pneumoniae has been shown to exploit cell-bound hTSP-1 to promote in vitro adherence to and invasion into different host cells [47]. Pneumococcal adhesion to epithelial and endothelial cells was diminished by addition of the glycosaminoglycans heparin and heparan-sulfate as inhibitors. Likewise, hTSP-1-mediated pneumococcal adhesion to epithelial cells was reduced after heparitinase treatment. In addition, blocking of hTSP-1-binding integrins had no effect on pneumococcal adherence, suggesting that cell-bound glycosaminoglycans and proteoglycans function as hTSP-1 receptors. The hTSP-1-binding component on the pneumococcal surface was thought to involve peptidoglycan [47]. However, in subsequent studies, Binsker et al. [40, 41] identified 3 pneumococcal proteinaceous virulence factors with hTSP-1-binding activity (Table 1). One of the candidates, the pneumococcal adhesion and virulence factor B (PavB), is covalently incorporated into the peptidoglycan due to expression of an LPNTG motif. PavB is distributed in 100% of all tested pneumococcal isolates [48, 49]. The protein consists of a 42-amino acid (aa) signal peptide followed by repetitive sequences, designated as SSURE (Streptococcal SUrface REpeat) domains, whose number varies from 5 to 9 repeats, depending on the pneumococcal strain [48, 50]. The SSURE domains vary in length and sequence and can be separated into 3 groups. The first repeat consists of 150 aa and differs from the remaining repeats but shows a high interstrain conservation. The core repeats exhibit a high intrastrain- and interstrain-specific conservation and contain 152 aa residues. The last repeat is truncated, thereby consisting of 136 aa residues, and is conserved in different pneumococcal strains [48]. A flexible linker region is situated between the SSURE domains and the anchoring motif and is composed of proline-rich repeats. The SSURE units harbor fibronectin- and plasminogen-binding activity, and PavB-binding strength positively correlates with the number of its repeats (Table 1) [48, 51].

The second identified hTSP-1-binding protein is the pneumococcal surface protein C (PspC, also known as CbpA or SpsA), which is a highly abundant virulence factor and encoded by >75% of all analyzed pneumococcal strains [52–56]. The different names reflect the numerous biological functions of the surface protein, which arose from the different allelic forms of PspC. Sequence comparison of the *pspC* gene in 43 pneumococcal strains revealed the expression of a modular protein, composed differentially in each strain [57]. The polymorphic PspC variants are classified into 2 groups, depending on the surface anchoring in the cell envelope of *S. pneumoniae*, and into 11 further subgroups based on the organization of functional and structural domains [57]. The classical PspC proteins (group I; subgroups 1–6) harbor a C-terminal choline-binding module (CBM) responsible for the noncovalent attachment to phosphorylcholine moieties within cell wall-associated teichoic acids. In contrast, the PspC-like proteins (group II; subgroups 7–11) contain an LPXTG sortase A-motif required for the covalent anchoring to peptidoglycan. The N-terminal α-helical region of

Table 1. Pneumococcal and staphylococcal hTSP-1-binding proteins

Protein	Platelet activation (hTSP-1 secretion)	Interaction with other host receptors	Pathogenic function
S. pneumoniae			
PavB	No [40]	Fibronectin	Involved in adherence / colonization [48, 50, 51]
		Plasminogen	ECM degradation and transmigration [48]
PspC (CbpA, SpsA)	No [40]	Vitronectin	Inhibition of complement cascade [60]
		Secretory component	Transcytosis across epithelial lineage [54, 61]
		Factor H	Adhesion to and invasion into host cells;
		C4bBP	Inhibition of complement cascade [58, 131, 132]
		Laminin receptor	Inhibition of complement cascade [62]
			Invasion of the cerebrospinal fluid [63]
Hic (PspC 11.4)	No [41]	Vitronectin	Inhibition of complement cascade [59]
		Factor H	
S. aureus			
AtlA	Yes [45]	Vitronectin	Adherence to extracellular matrix/
		Fibronectin	plasma proteins [43,133]
			Binding to host cell integrin $\alpha_5\beta_1$ [133]
		Fibrinogen	Adherence to extracellular matrix [133]
		Hsc70	Invasion into endothelial cells [133]
Eap	Yes [44, 45]	Vitronectin	Adherence to extracellular matrix/
		Collagen I	plasma proteins [42]
		Fibronectin	
		Fibrinogen	
		C4b	Inhibition of complement cascade [134]
		ICAM-1	Inhibition of neutrophil recruitment [135]
S. epidermidis			
AtlE	Yes [45]	Vitronectin	Invasion into endothelial cells [43, 133]
		Hsc70	

PspC is characterized by high variability with regard to size and sequence among the PspC proteins. Classical PspC proteins are composed of a 37-aa leader peptide, followed by the factor H-recognition sequence (aa 38–158) and either 1 or 2 separate repeat domains (R_1 and R_2) [58]. The proline-rich region, which precedes the CBM, is highly homologous among the different PspC groups with an identity of the aa sequence of 80–100% [57]. In the N-terminal part, instead of distinct repeat domains, PspC-like proteins contain regions of predicted α-helical conformation.

The factor H-binding inhibitor of complement (Hic; PspC 11.4) is a representative of the PspC-like proteins and has been identified as the third pneumococcal hTSP-1-binding protein. So far, 8 allelic variants of the PspC subgroup 11 (PspCs 11.1–8) are characterized by a 100% DNA and protein sequence homology among their N- and C-terminal domains. However, a high variation in length and aa composition of the proline-rich region between the PspC 11 variants has been determined [57, 59]. Hic is a 68.3-kDa protein that includes a 37-aa signal peptide, followed by a stretch of 6 predicted α-helical regions, 25 proline-rich repeats, and an LPSTG motif required for the covalent anchorage to peptidoglycan [41, 57].

PspC and Hic were shown to bind the fluid-phase components Factor H and vitronectin, whereas classical PspC proteins interact additionally with the complement component C4b-binding protein, the free secretory component (SC), the SC of secretory IgA or polymeric Ig receptors, and the laminin receptor of endothelial cells (Table 1) [54, 58–63].

It has been identified that the hTSP-1-binding proteins PavB and PspC contribute to pneumococcal adherence to human lung epithelial cells in an hTSP-1-dependent manner [40]. Pneumococci deficient in both adhesins are significantly impaired in adherence compared to the isogenic parent strain. In vitro binding studies under static and flow conditions confirmed a direct interaction

between recombinant PavB or PspC with immobilized hTSP-1. The use of truncated PavB and PspC constructs allowed the identification of the hTSP-1-binding site within the pneumococcal surface proteins. The strength of the interaction between the human glycoprotein and PavB correlated with the number of the SSURE units present in the recombinant protein fragment [40]. This observation is in accordance with the known interaction between PavB and human fibronectin [48, 51]. It has been suggested that the binding site for hTSP-1 in different PspC proteins is within the R domains and is most efficient when 2 R domains are present [40].

Likewise, it has been found that the binding site for human vitronectin and the SC is located in the R domains of pneumococcal PspC [60, 61]. Interestingly, interaction studies using hSTP-1 and vitronectin simultaneously revealed a competitive behavior of both glycoproteins in binding to PspC (unpubl. data). Similarly, a direct interaction between hTSP-1 and pneumococcal Hic was shown in complementary protein-protein interaction studies [41]. The strongest binding between hTSP-1 and different recombinant Hic proteins was observed with the construct containing the complete α-helical regions (aa 38–245). This interaction differs from the Hic binding of human Factor H and vitronectin, in which the specific binding regions are narrowed down to the regions aa 39–92 and aa 151–201, respectively. The identification of the hTSP-1-binding domain for the pneumococcal proteins in hTSP-1 has been narrowed down using recombinant PavB, PspC, and Hic proteins as competitors. Interestingly, PspC and Hic seem to target the same hTSP-1-domain, which is distinct from PavB [41]. The interaction of the pneumococcal adhesins with hTSP-1 are charge-dependent and can be inhibited by the glycosaminoglycans heparin and, with the exception of PavB, also chondroitin sulfate A. As a result, it is suggested that these interactions take place in the N-terminal domain and/or type-I repeats of hTSP-1. Remarkably, the heparin-binding domain of various human glycoproteins such as vitronectin and fibronectin seem to be involved in the interactions with these proteinaceous virulence factors of *S. pneumoniae* and they display a common motif [51, 59, 60].

In 1991, Herrmann et al. [64] showed enhanced binding of *S. aureus* to hTSP-1-coated artificial surfaces. Staphylococcal binding to hTSP-1 was calcium-dependent, as calcium was shown to change the conformation of hTSP-1. Interestingly, the *S. aureus*-hTSP-1 interaction was almost completely blocked by heparin, suggesting involvement of the heparin-binding domain of hTSP-1. Furthermore, it was assumed that the hTSP-1 receptor on the bacterial surface is not of a proteinaceous nature. Comparable to the pneumococcal adherence, *S. aureus* binding to epithelial cells was significantly enhanced after hTSP-1 incubation, and peptidoglycan was suggested to be involved in hTSP-1-binding [47]. However, ligand overlay immunoblots identified 60-kDa and 72-kDa staphylococcal surface-associated protein capable of binding to hTSP-1 [65].

It was subsequently identified in complementary protein-protein interaction studies that the secreted surface-associated protein Eap interacts with hTSP-1 [42]. Eap (also referred to as Map, p70) is a member of the secretable expanded repertoire of adhesive molecules (SERAM) family of staphylococcal surface proteins and is expressed by >98% of the tested clinical *S. aureus* isolates [66]. Eap is a modular organized protein consisting of 4–6 tandem (EAP) repeats, depending on the *S. aureus* strain. It has been shown that single domains of Eap are able to bind hTSP-1. However, at least 2 domains of Eap are crucial for staphylococcal adherence to and invasion of host endothelial cells. Interestingly, Eap was shown to interact with further human matrix proteins such as vitronectin and fibronectin (Table 1) [42].

Kohler et al. [43] identified the major autolysin Atl as another hTSP-1-binding protein using 2-dimensional SDS-PAGE with isolated surface proteins of *S. aureus* and subsequent hTSP-1 ligand overlay blot. Like Eap, the surface-associated protein Atl belongs to the SERAM family and is highly conserved among all *S. aureus* strains [67]. Interestingly, the Atl proteins of *S. aureus* and *S. epidermidis* are identical in their domain organization, share a high similarity in protein sequence and, consequently, are functionally interchangeable [68]. Atl is defined by a modular organization, consisting of a propeptide and an N-acetylmuramyl-l-alanine amidase, followed by 3 repeating units and an endo-β-N-acetylglucosaminidase. Following secretion, Atl associates via its repeats to the teichoic acids and peptidoglycan. The protein is cleaved proteolytically after the propeptide and after the second repeat, which results in the 2 separate biologically active enzymes, amidase and glucosaminidase, responsible for the cell wall turnover [69–71]. Remarkably, the binding site for hTSP-1 was located in the first 2 repeats, R_1R_2, and is distinct from the binding site for the teichoic acids based on an increased hTSP-1-binding after preincubation of staphylococci with the recombinant repeat domains [43]. Complementary protein-protein interaction studies revealed that the binding activity of the 2 connected repeats R_1R_2 to immobilized hTSP-1 is higher than

that of the single-repeat domain, R_1. It is then plausible to suggest that the repeats R_1R_2 within Atl represent the minimum domain necessary for binding to hTSP-1. Interestingly, and in accordance with the other hTSP-1-binding proteins of *S. aureus* and *S. pneumoniae*, Atl binds human vitronectin via its repeats R_1R_2. Vitronectin and hTSP-1 are able to competitively inhibit each other in binding to Atl R_1R_2 [43].

The Role of hTSP-1 during Hemostasis

Human TSP-1, accounting for 25% of the protein secreted by platelets, is a major protein component of platelet α-granules from where it gets rapidly released during platelet activation at sites of vascular injury. Human TSP-1 contributes to platelet aggregation by binding directly to the platelet surface in a calcium-dependent manner [72, 73]. Platelets express a variety of surface receptors for hTSP-1, including CD36, CD47, and several integrins, which target different domains of the glycoprotein. Human TSP-1 further participates in hemostasis by stabilizing platelet-fibrinogen associates, by binding to platelet-bound fibrinogen and influencing the structure of the fibrin clot [74–76]. Moreover, hTSP-1 increases the sensitivity and reactivity of platelets towards agonists such as thrombin [77]. Furthermore, the trimer hTSP-1 acts as endogenous lectin in platelet aggregation and thrombus formation by the agglutination of platelets and erythrocytes [78–80]. Platelets from a patient with severe bleeding disorder were deficient in intact hTSP-1, and the aggregation activity of platelets induced by collagen could be restored by adding exogenous hTSP-1 [81]. Besides von Willebrand factor (vWF), hTSP-1 of the subendothelium might serve as alternative substrate for platelet adhesion under the physiological high-shear rates found in stenosed coronary arteries [82].

Pneumococcal and Staphylococcal Interactions with Soluble Platelet-Derived hTSP-1

Invasive pneumococcal and staphylococcal strains can overcome the epithelial barrier to invade deeper tissues and enter the bloodstream. They therefore come into contact with platelets, which are the most abundant cells after red blood cells, with a concentration ranging from 150 to 400 × 10^9 platelets/L in healthy humans [83, 84]. The classical physiological function of platelets is the immediate binding to the exposed subendothelium of damaged blood vessels, aggregation, and thrombus formation to prevent excessive bleeding [85, 86]. In addition, platelets mediate further cross-talk to the cells of the blood and the vessel wall [87]. Due to their high number in the circulatory system and the expression of immune receptors, platelets can be considered as the first responding innate immune cells towards invading bacteria. Platelet activation is a common observation in septic patients, and it has therefore been suggested as biomarker for the development of sepsis [88].

Rennemeier et al. [47] and Kohler et al. [43] uncovered that, most probably, only Gram-positive bacteria such as *S. pneumoniae*, *S. aureus*, or other streptococci like *S. pyogenes*, as well as *Listeria monocytogenes*, are able to acquire soluble, nonimmobilized hTSP-1 on their surface. Hitherto, the surface proteins of *S. pyogenes* and *L. monocytogenes*, which interact with hTSP-1, have not been deciphered. It has been established that the pneumococcal adhesins PavB, PspC, and Hic that mediate binding to immobilized hTSP-1 are also involved in the acquisition of soluble hTSP-1 to the bacterial cell surface envelope [40, 41]. Different *S. pneumoniae* strains deficient in PavB, PspC, or both, showed an additive loss in their hTSP-1-binding capacity. Human TSP-1-binding was reduced up to 85% for the double mutants deficient in PavB and PspC, suggesting that these adhesins are major hTSP-1-binding proteins on the pneumococcal surface. Similar observations were made using a deletion mutant of the PspC-like protein Hic, which is expressed by the clinically relevant serotype 3 pneumococcus. Lack of Hic in the cell wall of *S. pneumoniae* A66 resulted in an impaired hTSP-1 acquisition of 40%. Although TSP-1-binding for the adhesins PavB, PspC, and Hic was shown, platelet activation activity for these virulence factors could not be proven by using recombinant proteins or *S. pneumoniae* strains lacking the adhesins [40, 41].

Only a few studies regarding pneumococci-platelet interaction exist, and they are partly contradictory. In 1971, Clawson and White [89] observed, for the first time, in vitro platelet aggregation caused by heat-inactivated serotype 8 but not serotype 24 pneumococci. In 2010, Keane et al. [90] suggested that the aggregation of platelets is independent of the serotype and secreted products of *S. pneumoniae*. Furthermore, pneumococcal platelet aggregation is induced by encapsulated and nonencapsulated strains and involves the TLR2 receptor [90]. However, a further study reported that encapsulated pneumococcal strains failed to aggregate human platelets [91]. The same study also indicated that platelet activation by encapsulated and nonencapsulated *S. pneumoniae* strains

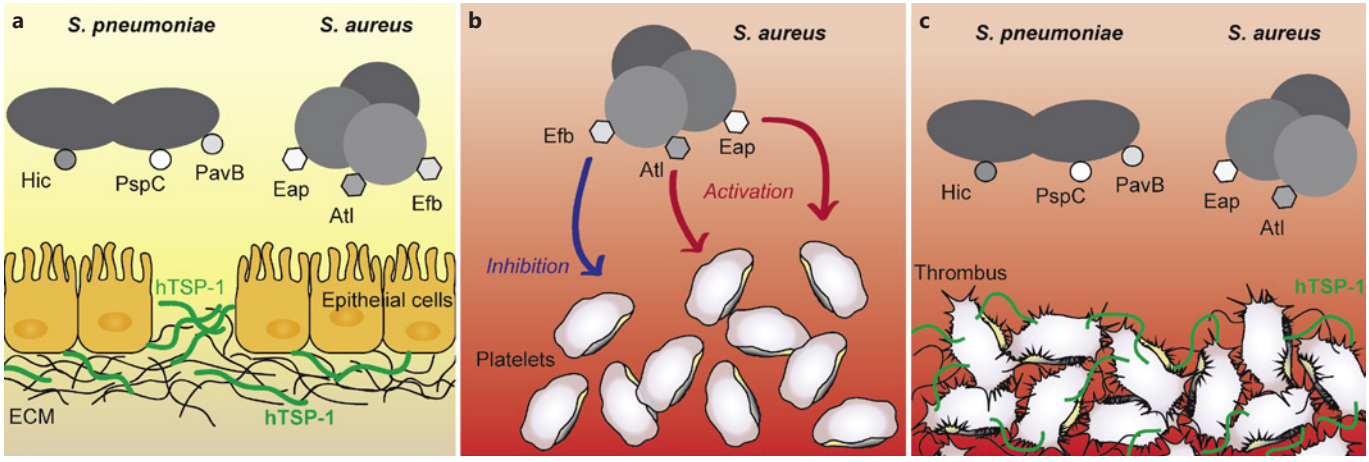

Fig. 2. *S. pneumoniae* and *S. aureus* target hTSP-1 in the ECM and thrombus. **a** Pneumococcal surface proteins PavB, PspC, and Hic, as well as the staphylococcal surface-associated proteins Eap and Atl, interact with immobilized matricellular hTSP-1. The human glycoprotein is located underneath the epithelial lineage, which becomes exposed due to damage of the epithelial barrier and is thereby used as adhesive substrate for bacterial colonization. **b** In the circulation, invading *S. aureus* is able to activate human platelets via the surface proteins Atl and Eap, leading to release of hTSP-1 from the platelet α-granules. Likewise, pneumococci induce platelet activation, albeit with a yet-unknown mechanism. **c** hTSP-1 is incorporated within the forming thrombus, which can be exploited by *S. pneumoniae* and *S. aureus* to mediate further colonization and dissemination within the host.

leads to platelet degranulation, which is independent of TLR2. This observation was confirmed using platelets of wild-type mice and knockout mice deficient in several TLRs, resulting in a comparable response of the platelets to *S. pneumoniae* [91]. Further in vivo animal models demonstrated that platelet depletion leads to enhanced pneumococcal dissemination and increased mortality, and that invasive pneumococcal disease promotes platelet activation and platelet hyperreactivity [92, 93]. So far, only the secreted pore-forming toxin pneumolysin could be identified as a platelet activation agent, which is in contrast to the observations made by Keane et al. [90]. Pneumolysin (Ply) activated human platelets in vitro via induction of intracellular calcium fluxes and P-selectin expression at concentrations similar to those found in severe pneumococcal infections [94]. However, platelet activation was dependent on pore-formation and did not involve an agonist-receptor mediated outside-to-inside signaling cascade. Likewise, Ply generated the production of the platelet-activating factor (PAF) and thromboxane A2 (TxA2) in human neutrophils, leading to a protease-activated receptor 1 (PAR1)-mediated heterotypic neutrophil-platelet aggregation [95]. A recent study identified the binding of the pneumococcal adhesin RrgA to the platelet endothelial cell adhesion molecule (PECAM-1) on endothelial cells, a receptor that is also present on human platelets [96].

Although the mechanism by which *S. pneumoniae* activates human platelets has not yet been fully deciphered, it becomes more evident that platelet activation and subsequent release of hTSP-1 occurs during its pathogenesis. Interestingly, Niemann et al. [97] showed that *S. pneumoniae* adheres to platelet aggregates and is mediated by fibrin and hTSP-1. As a result, the capability of *S. pneumoniae* to target platelet-bound hTSP-1 could be an important dissemination strategy during the manifestation of an invasive disease during pneumococcal infection (Fig. 2).

Likewise, various *S. aureus* lab strains as well as clinical isolates are able to recruit soluble hTSP-1 to the surface [43]. The secreted surface-associated adhesins Atl and Eap are involved in this process. Preincubation of *S. aureus* with the recombinant repeat domains R_1R_2 of Atl resulted in increased hTSP-1-acquisition to the cell surface of *S. aureus*. Remarkably, Atl and Eap are also able to induce platelet activation and the subsequent release of platelet-derived hTSP-1 [45]. The domains of Atl and Eap responsible for platelet activation could be identified. The amidase domain of Atl, which comprises the enzymatic amidase activity and the hTSP-1-binding repeats R_1R_2, was described to be crucial for the activation and aggregation of human platelets. The platelet activation domain within Eap could be narrowed down to the connected Eap repeats 3 and 4 of the *S. aureus* strain Mu50 [45]. Intriguingly, domain 3 of Eap encoded in the Newman strain, which shows a high

similarity to its ortholog in the staphylococcal Mu50, was shown to bind most efficiently to hTSP-1 [42].

The dissemination mechanism suggested for *S. pneumoniae* applies to *S. aureus* as well. Platelet activation induced by *S. aureus* is a common complication during bacteremia, and a manifestation of disseminated intravascular coagulopathy (DIC) and infective endocarditis (IE). A previous study showed that platelet α-granule protein, especially hTSP-1, is required to form *S. aureus*-platelet associates [98]. Therefore, hTSP-1 might function as a crucial element in the establishment of DIC and IE, and it is targeted by staphylococcal surface proteins (Fig. 2).

Human TSP-1 may serve as a substrate for the pneumococcal and staphylococcal adherence-mediating colonization of the ECM of the respiratory epithelium, the subendothelial matrix, or even within the forming thrombus during platelet activation (Fig. 2). Besides its physiologic occurrence, hTSP-1 has also been found on prosthetic devices, such as catheters entering the bloodstream or the cerebrospinal fluid. For example, hTSP-1 was detectable on peritoneal dialysis catheters as well as hydrocephalus shunts and ventricular catheters [99, 100]. This exposed hTSP-1 could serve as an adhesion site for invasive *S. pneumoniae* and *S. aureus* in patients undergoing surgery.

Concluding Remarks and Future Perspectives

The human pathogens *S. pneumoniae* and *S. aureus* have evolved manifold strategies to colonize and invade the human host. To ensure a successful colonization, either on the epithelial layer or in the bloodstream, the binding of human glycoproteins seems to be the crucial element. Thrombospondin-1 is, beside other human glycoproteins such as vitronectin or fibronectin, a notable substrate for bacterial adhesion. So far, 3 pneumococcal and 2 staphylococcal surface proteins have been discovered to interact with hTSP-1. Remarkably, all bacterial adhesins, except Hic, consist of repetitive domains, and the number of repeats determines the efficiency of hTSP-1 binding. The pneumococcal and staphylococcal surface proteins do not share homology of their protein sequence or secondary structure. However, the bacterial proteins seem to bind within the heparin-binding domain and/or the type-I repeats of hTSP-1, as seen by inhibition of binding of the bacterial proteins in the presence of heparin. Additionally, these interactions are charge-dependent.

Interestingly, pneumococcal PspC and Hic as well as staphylococcal Atl and Eap interact with human vitronectin. Like hTSP-1, vitronectin is part of the ECM, and it also functions as a complement inhibitor in the circulatory system. Different studies have shown that the interactions between bacterial proteins and hTSP-1 can be diminished in the presence of vitronectin. The binding affinity of the pneumococcal and staphylococcal surface proteins seems to be higher towards vitronectin than towards hTSP-1. Notably, the heparin-binding domain of vitronectin is also involved in these interactions, as shown for PspC and Hic.

One question arises: Why do the vitronectin-binding proteins also interact with hTSP-1? Pneumococci and staphylococci are versatile pathogens which can cause a broad spectrum of diseases at different sites of the human body, ranging from mild local infections such as otitis media and skin lesions to severe and life-threatening complications like pneumonia, meningitis, and sepsis. In all these niches of the human host, glycoproteins are present and serve as substrates for bacterial adhesion and colonization. Which glycoprotein is preferentially bound is a matter of availability at the site of bacterial infection. Instead of expressing surface proteins, which recognize only 1 human component, the hTSP-1-binding adhesins of *S. pneumoniae* and *S. aureus* are promiscuous. Thus, the surface proteins of the Gram-positive pathogens allow recognition of at least 1 human glycoprotein to ensure colonization at almost every site of the human body.

Studies regarding the recognition of hTSP-1 by the surface proteins of Gram-positive bacteria are still in their infancy. The lack of structural data of different hTSP-1 domains and the difficulty to obtain a heterologous expression limits the study on hTSP-1/bacterial interactions. Furthermore, hTSP-1 is involved in signal transduction between host cells. However, information concerning altered signaling pathways in different human cells, such as epithelial cells or platelets, after bacterial exposure in an hTSP-1-dependent manner is not available so far. All recent investigations have been performed in vitro and have focused predominantly on the identification of binding domains and the impact of hTSP-1 on bacterial adhesion. These initial but interesting findings must be further expanded under in vitro conditions in cell culture and, most importantly, in suitable in vivo animal models to explore the role of hTSP-1 in Gram-positive bacterial infections. A previous study has analyzed the impact of hTSP-1 deficiency in mice on the outcome of *Escherichia coli* sepsis [101]. Interestingly, the absence of hTSP-1 was associated with an improved outcome in murine models of sepsis, explained by the negative regulation of innate immune cells by hTSP-1. As mentioned previously, Gram-negative bacteria are most probably

not able to interact directly with soluble hTSP-1. This highlights the importance of investigating the in vivo role of hTSP-1 in murine disease models using Gram-positive pathogens, such as *S. pneumoniae* and *S. aureus*.

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