



Dual actions of group B *Streptococcus* capsular sialic acid provide resistance to platelet-mediated antimicrobial killing

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Circulating platelets have important functions in thrombosis and in modulating immune and inflammatory responses. However, the role of platelets in innate immunity to bacterial infection is largely unexplored. While human platelets rapidly kill *Staphylococcus aureus*, we found the neonatal pathogen group B *Streptococcus* (GBS) to be remarkably resistant to platelet killing. GBS possesses a capsule polysaccharide (CPS) with terminal α 2,3-linked sialic acid (Sia) residues that mimic a common epitope present on the human cell surface glycoalyx. A GBS mutant deficient in CPS Sia was more efficiently killed by human platelets, thrombin-activated platelet releasate, and synthetic platelet-associated antimicrobial peptides. GBS Sia is known to bind inhibitory Sia-recognizing Ig superfamily lectins (Siglecs) to block neutrophil and macrophage activation. We show that human platelets also express high levels of inhibitory Siglec-9 on their surface, and that GBS can engage this receptor in a Sia-dependent manner to suppress platelet activation. In a mouse i.v. infection model, antibody-mediated platelet depletion increased susceptibility to platelet-sensitive *S. aureus* but did not alter susceptibility to platelet-resistant GBS. Elimination of murine inhibitory Siglec-E partially reversed platelet suppression in response to GBS infection. We conclude that GBS Sia has dual roles in counteracting platelet antimicrobial immunity: conferring intrinsic resistance to platelet-derived antimicrobial components and inhibiting platelet activation through engagement of inhibitory Siglecs. We report a bacterial virulence factor for evasion of platelet-mediated innate immunity.

platelets | group B *Streptococcus* | innate immunity | sialic acid | Siglec

Group B *Streptococcus* (GBS, *Streptococcus agalactiae*) causes sepsis and meningitis in human newborns and infants (1). In most developed countries, public health interventions such as universal culture screening of pregnant women or risk factor-based decision algorithms guide intrapartum antibiotic prophylaxis to reduce neonatal GBS transmission (2). Nevertheless, the incidence of GBS infection in infants below 3 mo of age is \sim 0.53 cases per 1,000 births in European countries, 0.67 cases per 1,000 births in the Americas, and at least twice as high in less developed countries not practicing intrapartum antibiotic prophylaxis, with overall case fatality at \sim 7 to 10% (3). Invasive GBS infections are increasingly reported in nonpregnant adult populations, especially the elderly, diabetics, and immune-compromised individuals (4).

GBS systemic dissemination indicates intrinsic resistance to clearance by innate immune defenses operating in the bloodstream. The best-studied GBS virulence factor is its capsular polysaccharide (CPS), represented by 10 unique serotypes (Ia, Ib, II to IX) that differ antigenically, but which all share a critical terminal sialic acid (Sia) residue α 2,3- linked to an underlying galactose within the repeating structure. This GBS Sia motif is identical to a common epitope prominently displayed on surface

glycoproteins and glycolipids of all mammalian cells, an example of bacterial molecular mimicry of its human host. CPS Sia is a critical determinant for GBS survival in vivo (5), interfering with host complement pathways to block C3b deposition, impairing opsonophagocytosis (6), and reducing generation of chemotactic C5a, limiting phagocyte recruitment (7). Sia mimicry also allows GBS to functionally engage inhibitory Sia-binding Ig-like lectins (Siglecs) (8), key immunoregulatory receptors expressed by leukocytes including neutrophils and macrophages (9). Inhibitory Siglecs (e.g., human Siglec-5, Siglec-7, or Siglec-9; murine Siglec-E) recognize the ubiquitous Sia epitopes of host cells as “self-associated molecular patterns,” prompting recruitment of Src homology region 2 domain-containing phosphatases (SHP) to their intracellular immunotyrosine-based inhibitory motif (ITIM) domain(s), thus limiting cellular activation under homeostatic conditions (10). GBS Sia mimicry allows the pathogen to bind inhibitory Siglecs, suppressing oxidative burst, proinflammatory cytokine release, neutrophil extracellular trap (NET) production, and phagocytosis, cumulatively enhancing GBS survival in human blood and virulence in murine infection models (11, 12).

Significance

Platelets have an important role in blood clotting, but also function in immune responses, including the release of antimicrobial peptides that eliminate pathogens such as *Staphylococcus aureus*. Here we observe that group B *Streptococcus* (GBS), a leading cause of sepsis and meningitis in human newborns, is not killed by platelets. The key to GBS platelet resistance is a polysaccharide capsule that coats the bacterial surface and contains sialic acid (Sia), a common sugar present on all human cells. GBS Sia blocks platelet-derived antimicrobial factors, and also engages an inhibitory receptor (Siglec-9) on the platelet surface to block platelet activation. Platelet resistance is a novel consequence of GBS “molecular mimicry” of its host and contributes to its propensity to produce bloodstream infection.

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From the host perspective, bacterial clearance from the bloodstream is commonly attributed to neutrophils, the most abundant circulating leukocyte, or complement-mediated lysis. However, the complex outer membrane of Gram-negative bacteria, the target of complement attack, is absent in Gram-positive GBS, whose highly cross-linked peptidoglycan and thick cell wall are not permeabilized by the membrane attack complex. Recently, increased attention has focused on the innate immune functions of another abundant cell type in circulating blood, the platelet (13, 14). These tiny (2 μ M to 3 μ M) anucleated cells play a central role in coagulation but are also recruited and activated in response to microbial pathogens (15). Platelets express formyl peptide receptors, Toll-like receptors (TLRs), and chemokine receptors to sense pathogen-associated molecular patterns and display directed chemotaxis (16, 17), whereupon they can engage microbes and undergo degranulation to release small cationic platelet microbicidal proteins (PMPs) and kinocidins with direct antimicrobial activities (18, 19).

A direct contribution of platelets in defense against GBS infection has not been described. In stark contrast to another leading invasive Gram-positive bacterial pathogen of humans, *Staphylococcus aureus*, we find GBS to be highly resistant to platelet killing in vitro and in vivo. Our mechanistic analyses identify dual roles of GBS CPS Sia to suppress platelet activation and block killing by PMPs, revealing an unexpected function of Siglec receptors in regulating platelet immunity, and pinpointing a specific bacterial determinant that counteracts platelet-mediated host defense.

Results

GBS Displays Intrinsic Resistance to Platelet-Mediated Killing. A role for circulating platelets in antimicrobial defense, although not commonly studied, is increasingly appreciated (13, 15, 20). Consistent with recent studies (21, 22), induction of thrombocytopenia in mice with anti-CD41 antibody impaired bloodstream clearance of a USA300 strain of methicillin-resistant *S. aureus* (MRSA) following i.v. challenge (Fig. 1A). In contrast, anti-CD41 treatment did not affect bloodstream clearance of a wild-type (WT) strain of invasive serotype III GBS strain under identical challenge conditions (Fig. 1A). Strikingly, when coincubated with freshly isolated human platelets at a 1:10 ratio, MRSA were rapidly killed, with viable cfu reduced 80% within 2 h, while GBS proliferated to reach eightfold higher cfu levels than the input inoculum during the same time period (Fig. 1B). Likewise, resistance to human platelet killing was observed in a panel of five additional GBS strains of differing CPS serotype (SI Appendix, Fig. S1A). Similarly, thrombin-activated releasates from platelets (ranging from 0.8×10^7 to 5.0×10^8 cells) showed dose-dependent bactericidal activity against MRSA, in marked contrast to dose-dependent promotion of GBS growth from the same releasates (Fig. 1C). This finding likely reflects enriched nutritional content of releasates prepared from greater platelet numbers, yet simultaneously devoid of anti-GBS bactericidal/static activity. In sum, GBS appears intrinsically resistant to platelet killing.

GBS Capsular Sia Increases Resistance to Platelets and Platelet-Derived Antimicrobial Peptides. The surface CPS of GBS is a major virulence factor that promotes resistance to bloodstream clearance (23), with terminally exposed Sia residues that interfere with complement C3b deposition and opsonophagocytosis (6). We noted that a serotype 14 strain of *Streptococcus pneumoniae*, which shares the same capsule structure as serotype III GBS except its terminal Sia, was sensitive to killing by human platelets (SI Appendix, Fig. S1B). To determine its contribution to platelet resistance, we employed an isogenic mutant GBS strain lacking Sia (Δ Sia) secondary to a mutation in the *neuA* gene encoding CMP-Neu5Ac synthase (24). In contrast to the resistant WT GBS strain, bloodstream survival of the platelet-sensitive Δ Sia GBS mutant

in vivo was increased upon CD41-induced thrombocytopenia (Fig. 1D). Compared with the WT parent GBS strain, the Δ Sia mutant had reduced proliferation on exposure to human platelets (Fig. 1E) or platelet releasate (Fig. 1F), while plasmid complementation of *neuA* in the Δ Sia mutant restored growth to WT GBS levels (Fig. 1E and F). Similarly, treatment of WT GBS with purified sialidase from *Arthrobacter ureafaciens* (AUS) increased susceptibility to killing by human platelets (Fig. 1G) and platelet releasate (SI Appendix, Fig. S2A), and increased platelet killing of an isogenic Δ Sia mutant in the GBS serotype Ia background compared with its WT parent strain was also noted (SI Appendix, Fig. S3A). The releasate of thrombin-activated platelets contains several cationic antimicrobial peptides (18) including platelet factor-4 (PF-4) (25) and human β -defensin 1 (HBD-1) (19). Loss of capsular Sia expression by genetic mutation or AUS treatment increased GBS sensitivity to killing by both PF-4 (Fig. 1H and SI Appendix, Fig. S2B) and HBD-1 (Fig. 1I and J). Together, these results indicate that the Sia component of the GBS polysaccharide augments resistance to antimicrobial factors released by activated platelets, but cannot in and of itself fully explain the dramatic overall resistance of GBS to platelet killing (Fig. 1B), nor how platelet numbers fail to influence on GBS clearance in vivo (Fig. 1A).

GBS Capsular Sia Subverts Platelet Antimicrobial Activity by Inhibiting Platelet Activation. Platelet-mediated antimicrobial defense requires not only molecular effectors of bacterial killing (e.g., PF-4, HBD-1, and others) but also activation processes by which the platelets respond to bacterial encounter by receptor-linked signaling (e.g., TLR2, adenosine receptors), leading to degranulation and release of these effectors at the focus of infection (14, 17). We hypothesized that GBS Sia may further subvert platelet defense by blocking normal activation kinetics. P-selectin is an α -granule component exposed on the platelet surface during granule fusion with the outer membrane, whereupon it modulates activated platelet interactions with leukocytes and endothelium. The glycoprotein IIb/IIIa complex, recognized by monoclonal antibody PAC-1, is a platelet activation marker (~80,000 copies per platelet) that binds fibrinogen, von Willebrand factor, and other matrix and clotting system components. By flow cytometry, platelet expression of P-selectin or activated glycoprotein IIb/IIIa was significantly greater upon challenge with the GBS Δ Sia mutant compared with the WT parent strain in both the serotype III (Fig. 2A) and serotype Ia (SI Appendix, Fig. S3B) backgrounds. Corroborating the genetic deletion study, enzymatic removal of Sia from WT GBS capsule using AUS restored platelet activation to the level seen with the Δ Sia mutant (Fig. 2B). Representative transmission electron microscopy of human platelets incubated for 3 h with WT GBS showed that most platelets retained their discoid shape with normal features of a resting platelet, including microtubular coils, glycogen store, and an open canalicular system (Fig. 2C). In contrast, pronounced evidence of processing degranulation, degeneration, and necrosis is seen in platelets incubated with the isogenic Δ Sia mutant (Fig. 2C).

GBS Capsular Sia Inhibits Platelet Activation by Interacting with Siglec-9 Expressed on the Platelet Surface. We previously reported a mechanism whereby GBS capsular Sia suppresses the activation of myeloid cells, that is, neutrophils and macrophages, by engagement of inhibitory CD33-related Siglec receptors on the leukocyte cell surface (reviewed in ref. 26). Specifically, GBS binds the most-abundant Siglec on human neutrophils, Siglec-9, in a Sia-dependent manner to suppress neutrophil innate immune phenotypes including oxidative burst, phagocytosis, extracellular trap formation, and bacterial killing (11). Further genetic studies in a knockout mouse lacking Siglec-E, a murine paralog of human Siglec-9, confirmed that GBS blunts macrophage immune responses in a Sia- and Siglec-dependent manner to promote its own survival (12). Recently, it was reported that Siglec-7 was present

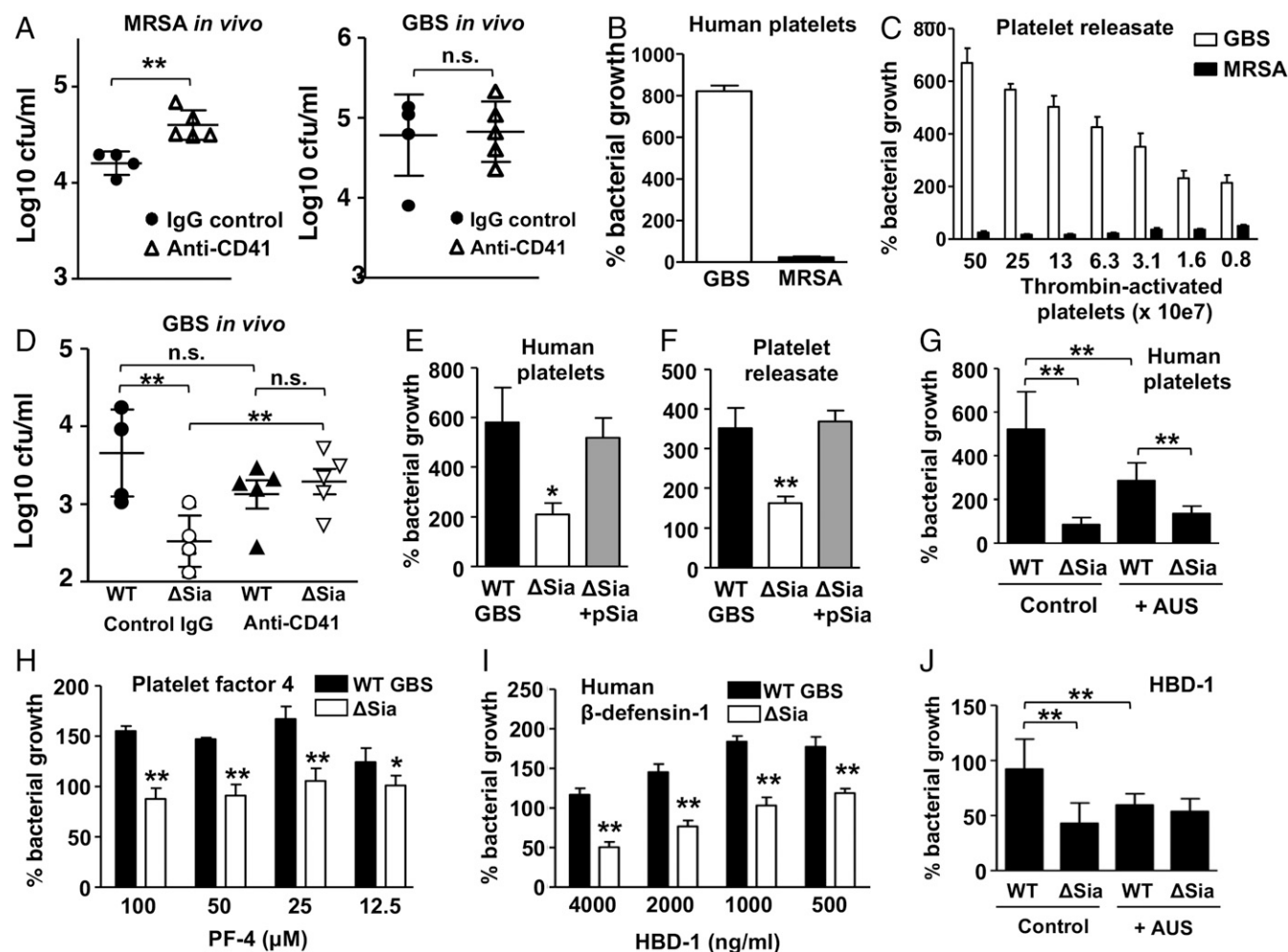


Fig. 1. Sialylated bacterial pathogen GBS displays intrinsic resistance to platelet-mediated killing. (A) Impaired clearance of MRSA from the bloodstream of mice rendered thrombocytopenic by anti-CD41 antibody treatment compared with IgG isotype control; similar treatment did not influence bloodstream counts of GBS. (B) GBS proliferates when coincubated in the presence of freshly isolated human platelets, whereas MRSA is killed. (C) GBS proliferates in a dose-dependent manner when exposed to thrombin-activated platelet releasate, whereas the same releasates have antimicrobial activity against MRSA. (D) In contrast to the resistant WT parent GBS strain, in vivo bloodstream survival of an isogenic Sia capsule-deficient (Δ Sia) mutant is increased upon CD41-induced thrombocytopenia. (E and F) Compared with WT GBS, the Δ Sia mutant has reduced proliferation on exposure to (E) human platelets or (F) platelet releasate; complementation of Sia capsule expression to the mutant restores WT growth levels. (G) Treatment of WT GBS with AUS sensitizes it to human platelet killing. (H and I) Increased susceptibility of Δ Sia mutant GBS to killing by the platelet-derived antimicrobial peptides (H) PF-4 and (I) HBD-1. (J) AUS treatment of WT GBS renders it more susceptible to HBD-1. For in vivo experiments, A and D show means \pm SD ($n = 4$ or 5 per group); n.s., nonsignificant; $^{***}P < 0.01$. For ex vivo and in vitro experiments, C and E through J, means \pm SD ($n = 3$ per experiment, mean of at least three independent experiments) are shown; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the GBS WT-infected group.

on activated human platelets and, when cross-linked by Sia ligands, accelerated platelet senescence through apoptosis (27). By flow cytometry, we corroborated the presence of Siglec-7 on activated human platelets yet observed even higher expression of Siglec-9 (Fig. 3A), the same Siglec exploited by GBS to suppress neutrophil immune functions. In addition, an anti-Siglec-9 blocking antibody significantly reduced GBS binding to human platelets in a plate-based assay, an effect not seen with Siglec-7 or Siglec-5 blocking antibodies (Fig. 3B). The Sia-dependent and Siglec-9-dependent binding of GBS to human platelets was confirmed by flow cytometry using the Δ Sia mutant and the anti-Siglec-9 blocking antibody (Fig. 3C). Engagement of Sia ligands by inhibitory Sigslecs leads to tyrosine phosphorylation of their cytoplasmic tail, whereupon ITIMs recruit SHP phosphatases, important negative regulators of immune and inflammatory receptor signaling (9). In line with this, platelets exposed to Sia-expressing WT GBS exhibited more Siglec-9 phosphorylation compared with those challenged with the Δ Sia mutant (Fig. 3D

and E). To confirm the inhibitory effect of platelet activation, we employed a recent technique to selectively modify GBS Sia through mild sodium metaperiodate oxidation, followed by aldehyde quenching using 4-methyl-3-thiosemicarbazide (28). Recapitulating effects seen with the Δ Sia mutant, chemical modification of Sia blocked Siglec-9 binding to WT GBS (Fig. 3F) and increased platelet activation in response to WT GBS challenge (Fig. 3G). We conclude that the regulatory role of Siglec-9 to control immune cell responsiveness is not restricted to myeloid cells, but rather extends to platelets. Molecular mimicry of host Sia epitopes by the pathogen GBS allows it to suppress platelet activation and release of bactericidal effectors.

Mice Lacking Inhibitory Siglec-E Show Increased Platelet Activation upon GBS Bloodstream Infection. GBS interacts with murine Siglec-E, the inhibitory receptor most closely related to human Siglec-9 (26). We confirmed expression of Siglec-E on murine platelets (Fig. 4A) and determined that efficient GBS binding to murine

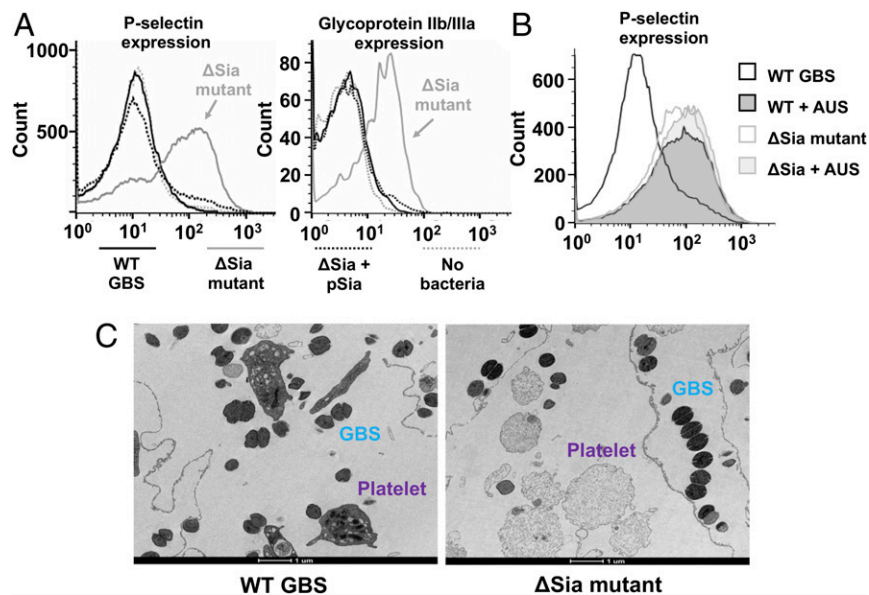


Fig. 2. GBS capsular Sia subverts platelet antimicrobial activity by inhibiting platelet activation. (A) Shown by flow cytometry, platelet expression of P-selectin or PAC-1 (activated GP IIb/IIIa) is greater upon challenge with the ΔSia mutant compared with the WT parent GBS strain. (B) Enzymatic removal of the Sia from the WT GBS capsule by AUS increases platelet activation to the level seen in the ΔSia mutant. (C) Representative transmission electron microscopy of human platelets incubated for 3 h with WT GBS shows most platelets retaining their unactivated discoid shape, whereas pronounced evidence of processing degranulation, degeneration, and necrosis is seen in platelets incubated with the isogenic ΔSia mutant. Plots and images are representative of at least three independent experiments.

platelets requires both bacterial Sia and the presence of Siglec-E (Fig. 4B). Capsular Sia is a critical GBS survival factor in vivo, with capsule-deficient strains so rapidly cleared that the calculated LD50 is up to 10⁵-fold greater than corresponding WT parent strains (6). With that confounding factor in mind, we focused our analysis on short-term effects of Siglec-E expression on platelet activation following WT i.v. GBS infection. Siglec-E knockout (SigE^{-/-}) mice were generated on a C57BL/6 background by gene targeting (SI Appendix, Fig. S4); the SigE^{-/-} animals exhibited normal platelet counts and thrombin-mediated platelet activation (SI Appendix, Fig. S5 and Table S1) similar to WT controls. Consistent with prior findings (12), bacterial counts were significantly reduced in SigE^{-/-} mice compared with the parent strain 4 h after infection (Fig. 4C). However, despite the lower number of bloodstream bacteria, P-selectin expression on platelets of SigE^{-/-} mice was significantly increased vs. WT controls (Fig. 4D). These results strongly suggest that GBS expression can blunt platelet activation in vivo through Siglec-E engagement. Also, whereas

bloodstream clearance of WT GBS was increased in SigE^{-/-} mice compared with WT mice, no difference was seen between WT and SigE^{-/-} mice in clearance of the ΔSia GBS mutant (Fig. 4E), further supporting a specific role of GBS Sia engagement of Siglec-E in modulating platelet immunity.

Discussion

Prominent roles of platelets in driving or influencing immune and inflammatory responses are increasingly recognized (13, 14). However, direct in vivo experimental evidence for an essential platelet function in innate host defense against bloodstream infection remains limited. During the clotting process, human and rabbit platelets release antibacterial substances from their granules that show activities predominantly against Gram-positive bacterial strains such as *S. aureus* (29, 30). The laboratories of Yeaman and coworkers (18, 25, 31, 32) achieved careful biochemical identification and activity testing of several such peptide classes and variants under the rubric of thrombocidins, PMPs, or

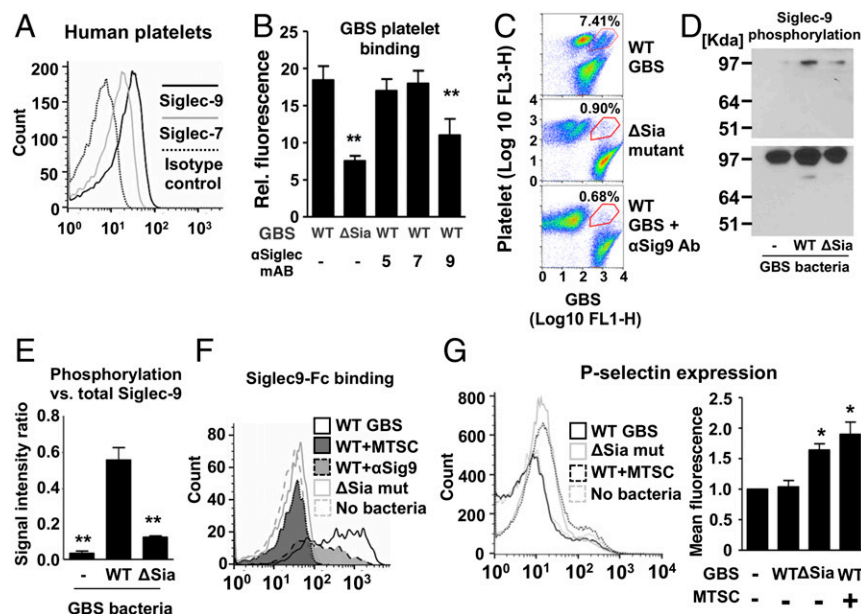


Fig. 3. GBS capsular Sia inhibits platelet activation by interacting with Siglec-9 expressed on the platelet surface. (A) Flow cytometry detects expression of Siglec-9 and Siglec-7 on human platelets. (B and C) Siglec-9 blocking antibody significantly reduces GBS Sia-dependent binding to human platelets as assessed in (B) a plate binding assay and (C) by flow cytometry. (D) Platelets were challenged with GBS strains, then lysed, and protein samples were investigated for phosphorylation (activation) of inhibitory Siglec-9 by immunoprecipitation and Western blot, with densitometric quantification shown in E. (F) Flow cytometry shows that elimination (ΔSia mutant) or chemical modification (MTSC) of Sia reduces Siglec-9 binding to GBS. (G) Flow cytometry shows that elimination (ΔSia mutant) or chemical modification (MTSC) of Sia increases platelet activation marker P-selectin upon exposure to GBS. Means ± SD (n = 3) are shown; *P < 0.05 and **P < 0.01 compared with GBS WT control. Bar graphs are the total of at least three independent experiments. Flow cytometry histograms or Western blot images are representative of at least three independent experiments.

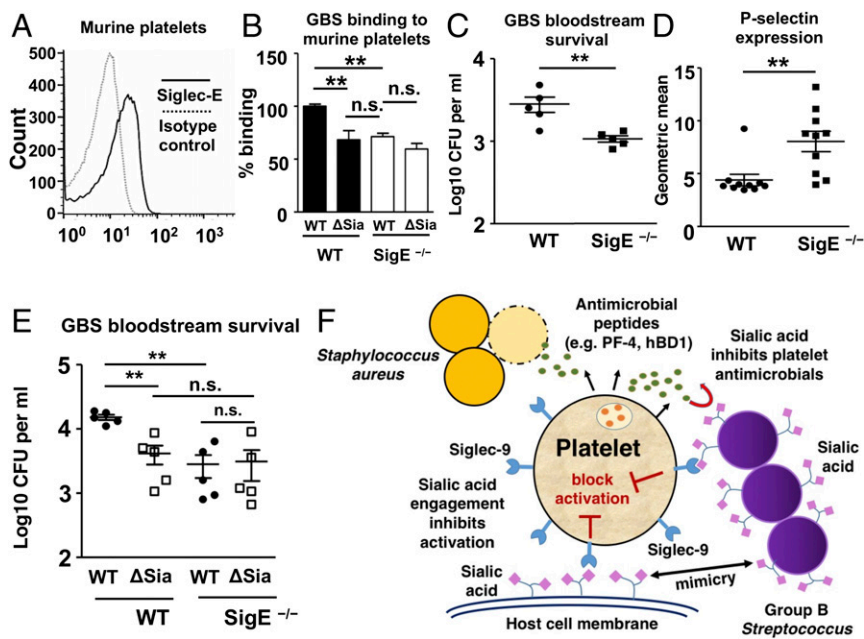


Fig. 4. Mice lacking inhibitory Siglec-E show increased platelet activation upon GBS bloodstream infection. (A) Flow cytometry detects expression of Siglec-E on murine platelets. (B) GBS binds to murine platelets in a Sia-dependent and Siglec-E-dependent manner. (C) Increased bacterial clearance from the blood following GBS i.v. infection in SigE^{-/-} mice compared with WT mice. (D) Increased platelet P-selectin expression following GBS i.v. infection in SigE^{-/-} mice compared with WT mice. (E) The enhanced bloodstream clearance of GBS in SigE^{-/-} mice vs. WT mice is abrogated in studies with a GBS Sia-deficient mutant (Δ Sia). (F) Schematic representation of the dual roles of GBS capsular Sia in counteracting platelet antimicrobial immunity: conferring intrinsic resistance to platelet-derived antimicrobial components and inhibiting platelet activation through engagement of inhibitory Siglecs. Means \pm SD ($n = 3$) are shown; $**P < 0.01$. Figures are representative of at least three independent experiments (for A and B) or a total of two independent experiments (for C–E). (F) Schematic depicting GBS Sia dual roles to counteract platelet immunity: conferring intrinsic resistance to platelet-derived antimicrobial components and inhibiting platelet activation through engagement of inhibitory Siglecs.

platelet kinocidins. Activated platelets further augment *S. aureus* clearance through release of interleukin-1 β , which boosts macrophage phagocytosis, and by a dual action of HBD-1 to stimulate NETs (19). Consistent with a multifaceted role in host defense, antibody-mediated platelet depletion renders mice significantly more susceptible to systemic *S. aureus* infection (21, 22).

Here we report that the prominent human pathogen GBS is remarkably resistant to human platelet killing and that platelet depletion does not influence GBS bloodstream clearance in a murine infection model. Thrombocytopenia and pathological clotting (e.g., disseminated intravascular coagulation), hallmarks of advanced bacterial sepsis, can likewise be observed in severe neonatal GBS infection (33). GBS strains isolated from septic patients, which have an enhanced ability to bind fibrinogen on their surface, preferentially stimulate the aggregation of platelets (34), although this phenomenon appears to be independent of the Sia epitope on serotype III GBS CPS (35). Here we find that terminal Sia in the GBS CPS provides protection against killing by human platelets, their releasates, and two well-characterized platelet-derived antimicrobial peptides, HBD-1 and PF-4. Other groups have used isogenic mutants to demonstrate that terminal Sias present in the lipooligosaccharide of *Campylobacter jejuni* or *Neisseria gonorrhoeae* contribute to resistance against neutrophil-derived cationic host defense peptides (36, 37).

GBS suppressed platelet activation and release of antimicrobial factors through Sia-dependent engagement of the inhibitory receptor Siglec-9. Immunoreceptor-mediated activation of platelets in response to bacterial components has been studied in detail, especially among TLRs, with documentation of TLR-1, TLR-2, TLR-3, TLR-4, TLR-6, TLR-7, and TLR-9 expression on platelets and demonstration of functional signaling outcomes including regulation by MyD88 (16, 38). A role of Siglec-9 and other inhibitory Siglecs (e.g., human Siglec-5, murine Siglec-E) in blunting TLR and MAPK activation pathways has been reported in neutrophils and macrophages (reviewed in ref. 26), and here we observe that their immunoregulatory function extends to the anucleate but granule rich platelets. During bone marrow hematopoiesis, a common myeloid progenitor cell gives rise to neutrophils, macrophages, and megakaryocytes—the producer of platelets. We suspect, as the role of platelets in host defense is increasingly appreciated, further parallels with phagocytic cell signaling and effector pathways are likely to be uncovered.

Platelets contain many potent immunologically active molecules that contribute directly and indirectly to innate and adaptive host defenses (39). Given their abundance in blood, platelets can serve as sentinels for detecting invading microorganisms through TLRs and other receptor pathways (38). Activated platelets release antimicrobial peptides (studied here) but also produce both proinflammatory [e.g., interleukin-1 β (40)] and antiinflammatory [e.g., transforming growth factor-1 (41)] cytokines that can modulate the broader inflammatory/immune response through “cross-talk” with leukocytes (e.g., neutrophils and macrophages) and vascular endothelium (22, 39). To the extent that platelet inhibitory Siglecs counterregulate these platelet activation phenotypes, the ability of GBS Sia to blunt platelet activation via Siglec engagement may propagate a broader immune perturbation that influences the progression of systemic GBS infection. Reciprocally, GBS Sia-mediated inhibition of neutrophils and macrophages (via Siglec-9 and Siglec-5) (11, 12) can suppress release of cytokines that normally promote platelet activation (e.g., IL-1 β , IL-6, and IL-8), magnifying the functional deficit in platelet immunity.

We conclude that GBS Sia has dual roles in counteracting platelet antimicrobial immunity: conferring intrinsic resistance to platelet-derived antimicrobial components and inhibiting platelet activation through engagement of inhibitory Siglecs (schematic in Fig. 4E). We note that, while *S. aureus* was readily killed in vitro by platelets and their releasate, the GBS Δ Sia mutant exhibits only growth inhibition in parallel assays. With the caveat that in vitro assays cannot model the full complexity and/or effectiveness of platelet-mediated immunity in vivo, there are likely to be additional bacterial factors contributing to GBS platelet resistance and host factors contributing to the partial effectiveness of platelets as a bactericidal cell, with Sia a highly significant component, as shown by the multiple complementary approaches of this study. Currently, the Sia epitopes of the GBS capsule are the principal target of GBS vaccines now proposed to enter phase III clinical trials for prevention of early- and late-onset infections in human newborn infants (42). GBS suppression of platelet activation and its intrinsic resistance platelet antimicrobial factors further explain the central role of CPS Sia in bloodstream survival and virulence of this foremost human neonatal pathogen.

Materials and Methods

Mouse studies were conducted in accord with protocols approved by the University of California, San Diego (UCSD) Institutional Animal Care and Use Committee. Blood collection and platelet isolation from healthy adult volunteers under informed consent and subsequent experimentation was approved by the UCSD Human Research Protection Program IRB. Well-characterized human serotype III GBS isolate (COH-1) from a case of human neonatal sepsis and isogenic mutant (Δ Sia) generated by allelic replacement of the *neuA* gene were analyzed in detail. Platelets were isolated by centrifugation from human or murine blood anticoagulated with acid citrate-dextrose buffer (ACD; Sigma Aldrich). Platelet releasates were collected after activation with 0.1 U/mL of bovine thrombin (Sigma-Aldrich) for 30 min. Recombinant PF-4 and HBD-1 were obtained from Bachem Americas and reconstituted in 96-well plates in serial dilutions. To remove or modify GBS

Sia for binding or activation assays, strains were exposed to either sialidase treatment or mild periodate oxidation. Flow cytometry was used for platelet surface P-selectin, PAC-1, and Siglec expression using specific antibodies. The 10- to 12-wk-old WT C57BL/6 and SigE^{-/-} mice were challenged 1×10^8 CFU of bacteria by tail vein injection, then killed 4 h later for blood collection by cardiac puncture. Platelet depletion was achieved with 1 mg/kg of anti-CD41 antibody. Detailed procedures for all studies are described in *SI Appendix, Materials and Methods*.

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