INFECTION AND IMMUNITY, Aug. 2009, p. 3485–3490 Vol. 77, No. 8
0019-9567/09/$08.00+0 doi:10.1128/IAI.01573-08

Streptococcus mitis Phage-Encoded Adhesins Mediate Attachment to α2-8-Linked Sialic Acid Residues on Platelet Membrane Gangliosides▽

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Received 26 December 2008/Returned for modification 31 January 2009/Accepted 28 May 2009

The direct binding of bacteria to human platelets contributes to the pathogenesis of infective endocarditis. Platelet binding by *Streptococcus mitis* strain SF100 is mediated in part by two bacteriophage-encoded proteins, PblA and PblB. However, the platelet membrane receptor for these adhesins has been unknown. In this study, we demonstrate that these proteins mediate attachment of bacterial cells to sialylated gangliosides on the platelet cell surface. Desialylation of human platelet monolayers reduced adherence of SF100, whereas treatment of the platelets with *N*- or *O*-glycanases did not affect platelet binding. Treatment of platelets with sialidases having different linkage specificities showed that removal of α2-8-linked sialic acids resulted in a marked reduction in bacterial binding. Preincubation of SF100 with ganglioside GD3, a glycolipid containing α2-8-linked sialic acids that is present on platelet membranes, blocked subsequent binding of this strain to these cells. In contrast, GD3 had no effect on the residual binding of platelets by strain PS344, an isogenic Δ*pblA* Δ*pblB* mutant. Preincubating platelets with specific monoclonal antibodies to ganglioside GD3 also inhibited binding of SF100 to platelets, but again, they had no effect on binding by PS344. When the direct binding of *S. mitis* strains SF100 and PS344 to immobilized gangliosides was tested, binding of PS344 to GD3 was reduced by 70% compared to the parent strain. These results indicated that platelet binding by SF100 is mediated by the interaction of PblA and PblB with α2-8-linked sialic acids on ganglioside GD3.

Among the viridans group streptococci, *Streptococcus mitis* is a leading cause of infective endocarditis (14). The binding of these microorganisms to human platelets appears to be a central process in the pathogenesis of this disease (20). Platelet binding by *S. mitis* strain SF100, an endocarditis isolate, was previously shown to be mediated in part by proteins PblA and PblB (3, 4). The genes encoding these proteins reside within the temperate bacteriophage SM1, a member of the *Siphoviridae* family (23). PblA and PblB are unusual because neither protein has strong similarity to known bacterial adhesins, but instead they resemble structural components of bacteriophages. Disruption of *pblA* and *pblB* results in phage particles lacking tails, further indicating that these proteins are involved in phage morphogenesis (4). Expression of the SM1 phage-encoded holin and lysin results in the permeabilization of a subpopulation of SF100 cells, thereby releasing PblA and PblB into the surrounding medium. Following this, both PblA and PblB attach to choline residues within the cell walls of viable bacteria, where they mediate the binding of viable bacteria to platelets. This novel method of adhesin expression on the *S. mitis* cell surface directly links bacterial platelet binding activity to disease pathogenesis, as deletion of *pblA* and *pblB* results in a significant loss of virulence as measured in an animal model of endocarditis (20).

The interaction of bacteria with sialylated oligosaccharides on host molecules may be a factor in the colonization of the oral cavity by viridans group streptococci by allowing them to adhere to salivary glycoproteins (27). Sialic acid is commonly found as a terminal sugar on oligosaccharides on host glycoproteins and glycolipids. These oligosaccharides serve as receptors for bacterial adhesion (13, 21, 25, 26), in addition to acting as a hydrolysable source of metabolic sugars (7). For example, the serine-rich repeat glycoprotein GspB of *Streptococcus gordonii* recognizes α2-3-linked sialic acid oligosaccharides present on host receptors and mediates adhesion of *S. gordonii* to salivary mucin (27). While GspB may allow *S. gordonii* to colonize the oral cavity in a commensal fashion, it can also contribute to the virulence of the organism in the setting of infective endocarditis by promoting attachment of bacterial cells to platelets via the platelet glycoprotein GP1b (5, 26, 29).

Gangliosides are sialylated glycosphingolipids present in the outer leaflet of the plasma membrane of all mammalian cells. They serve as receptors for a variety of bacteria and bacterial products, such as *Haemophilus influenzae*, *Helicobacter pylori*, *Neisseria meningitidis*, and cholera toxin (1, 6, 11, 15, 19, 28). Two gangliosides (GM3 and GD3) have previously been shown to be present on platelet membranes (10, 18). GM3 is a monosialylated ganglioside that is located in lipid microdomains and is further sialylated to GD3 during an early stage of platelet activation that precedes calcium and granule release (18). GD3 has also been shown to participate in signaling through the FcλRIIa receptor in platelets (10).

Although platelet binding by SF100 is mediated predominantly by PblA and PblB, the platelet binding site for this interaction has not been characterized. In this study, we demonstrate that α2-8-linked sialic acid residues on platelet mem-

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▽ Published ahead of print on 8 June 2009.

TABLE 1. Gangliosides and glycosphingolipids used in this study

Structure	Name
Galβ1-4Glcβ1-ceramide	Lactosyl ceramide
Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-ceramide	Asialo GM1
Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ1-ceramide	GM1
Neu5Acα2-3Galβ1-4Glcβ1-ceramide	GM3
Neu5Acα2-3 Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glcβ1-ceramide	GD1a
Galβ1-3GalNAcβ1-4(Neu5Acα2-8)(Neu5Acα2-3)Galβ1-4Glcβ1-ceramide	GD1b
Neu5Acα2-8Neu5Acα2-3Galβ1-4Glcβ1-ceramide	GD3
Neu5Acα2-3Galβ1-3GalNAcβ1-4(Neu5Acα2-8Neu5Acα2-3)Galβ1-4Glcβ1-ceramide	GT1b
Neu5Acα2-8Neu5Acα2-3Galβ1-3GalNAcβ1-4 (Neu5Acα2-8Neu5Acα2-3)Galβ1-4Glcβ1-ceramide	GQ1b

brane ganglioside GD3 are the principal targets for PblA/PblB-associated binding to human platelets.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The bacterial strains used in this study are *S. mitis* SF100 and its isogenic Δ*pblA* Δ*pblB* variant strain, *S. mitis* PS344 (3). Of note, SF100 and PS344 have no detectable sialidase activity (unpublished data), and both strains grow equally well in broth culture (3). Bacteria were grown statically at 37°C in Todd-Hewitt broth (Difco Laboratories) or on sheep blood agar (Hardy) at 37°C in a 5% CO_2 environment.

Quantitative assay for binding to immobilized platelets. Overnight cultures were diluted 1:10 in fresh Todd-Hewitt broth, incubated for 1 h at 37°C, and then exposed to UV light (λ = 312 nm) for 3 min. The cultures were incubated at 37°C for an additional 4 h, followed by the harvesting of bacteria by centrifugation at 8,200 × *g* for 15 min and washing three times in phosphate-buffered saline (PBS).

The binding of streptococci to human platelets was assessed quantitatively as described previously (20). In brief, washed, fixed human platelets were immobilized in poly-L-lysine-coated, 22-mm-diameter tissue culture wells, producing monolayers of 75 to 90% confluence. To reduce nonspecific adherence, the wells were treated with a casein solution (blocking reagent; Roche) in PBS for 2 h at room temperature. After the blocking solution was removed by aspiration, the wells were inoculated with approximately 1×10^7 CFU of streptococci suspended in 1 ml of PBS and incubated at 37°C for 2 h, with gentle rocking to enhance mixing. The viability of bacterial cells in PBS was tested at 30-min intervals over the 2-h incubation period and was found not to change over time, and there was no difference in survival between the wild-type SF100 cells and PS344 cells. Unbound bacteria were then removed by washing with PBS, and bound bacteria were recovered by scraping and resuspension in 1 ml PBS. The number of organisms bound was determined by plating serial dilutions of the suspension onto blood agar, and binding was expressed as a percentage of the inoculum. All studies were carried out in triplicate on three separate occasions, using platelets from a different human donor on each day. Differences in platelet binding were compared by the unpaired *t* test.

Quantitative assay for binding to deglycosylated platelets. Platelets monolayers were washed three times in PBS and then incubated with a final concentration of 5 mU/ml of *N*-glycanase, *O*-glycanase, sialidase A, sialidase C, or sialidase V (Glyko) for 16 h at 37°C. Platelets were washed a further three times with PBS, and all wells were incubated with a solution of 2% formaldehyde in PBS for 1 h at room temperature to inactivate any remaining enzyme. Control wells were also treated with 2% formaldehyde in PBS. The platelet monolayers were then washed three times with PBS and the binding of bacterial cells to platelets was assayed as described above.

Inhibition of binding to platelets by antibodies. Platelet monolayers were washed three times in PBS and then incubated with a final concentration of 0.5 μg/ml anti-GD3 monoclonal antibody (MAb) 4F6 (immunoglobulin G3 [IgG3]), an isotype control IgG3 antibody MG3-35 or an anti-GP1b IgG1 MAb HIP1 (Abcam) for 1 h at room temperature with gentle agitation. Platelets were subsequently washed three times in PBS, and the binding of bacterial cells to treated platelets was assayed as described above.

Concentration-dependent inhibition of binding to platelets by antibodies. Platelet monolayers were washed three times in PBS and then incubated with 10-fold dilutions of 5 μg/ml of MAb 4F6 starting at a 10^{-2} dilution (0.5 μg/ml) for 1 h at room temperature with gentle agitation. Platelets were washed in PBS, and bacterial binding was assessed as described above.

Inhibition of binding to platelets by gangliosides. Overnight cultures of *S. mitis* were processed as described above and then incubated with a final concentration of 0.35 mM ganglioside (EMD Biosciences) for 1 h at room temperature with gentle agitation. The bacteria were then washed three times in PBS, and the binding of treated bacterial cells to platelets was assayed as described above. The gangliosides used and their structures are listed in Table 1.

Concentration-dependent inhibition of binding to platelets by GD3. Overnight cultures of *S. mitis* were processed as described above and then incubated with serial 10-fold dilutions of 3.5 mM GD3 (10^0 to 10^{-4} dilution) for 1 h at room temperature. The binding of bacteria to platelets was then assessed as detailed above.

Direct binding of bacteria to immobilized gangliosides. Thirty-five micromoles of each ganglioside was added to a flat well in a 96-well microtiter dish and dried for 16 h at 37°C. The wells were then blocked with 200 μl of casein solution for 2 h at room temperature.

Bacteria were grown as described above, harvested, washed in PBS, and suspended in PBS to 1×10^7 CFU/ml. Fifty microliters of each bacterial suspension was added to each well and allowed to adhere for 1 h at room temperature with gentle agitation. Plates were subsequently washed three times with 200 μl PBS. Adherent bacteria were detached from the plate by incubation of the wells with trypsin for 10 min at room temperature, and the trypsin reaction was stopped by the addition of 50 μl fetal bovine serum to each well. The number of organisms bound was determined by plating serial dilutions of the bacterial suspension onto blood agar, and binding was expressed as a percentage of the inoculum. All studies were carried out in triplicate. Differences in binding were compared by the unpaired *t* test.

Binding of PlbA and PbB to immobilized gangliosides. Asialo GM1 and GD3 affinity binding studies were performed as described previously (12). In brief, 2 mM of GD3 or asialo GM1 was bound separately to 200 μl of prewashed octyl-Sepharose CL 4B (GE Healthcare) in a solution of methanol-water (1:1, vol/vol) and 0.1 M KCl for 4 h at room temperature. The Sepharose beads were washed extensively with PBS and then incubated for 1 h with clarified supernatants of SF100 or PS344 cultures, with rotation at room temperature. The beads were then washed repeatedly with PBS to remove unbound material until no protein could be detected in the recovered fluid, as measured by UV light absorbance. Octyl-Sepharose CL 4B served as a control for nonspecific adsorption. Bound proteins were eluted with 6 M urea.

All samples were added to lithium dodecyl sulfate sample buffer (Invitrogen) and separated on 3 to 8% Tris-acetate polyacrylamide gels (Invitrogen). The separated proteins were transferred to nitrocellulose membranes (Invitrogen), blocked with 1× blocking reagent (Roche), and probed with anti-PblA antibodies or anti-PblB antibodies. Bound antibodies were detected with anti-goat IgG horseradish peroxidase (Sigma) and SuperSignal chemiluminescent substrate (Pierce).

RESULTS

Desialylation of platelets results in a reduced platelet binding by *S. mitis*. In pilot studies examining the platelet receptor for *S. mitis* SF100, several proteins known to be receptors for other viridans group streptococci and gram-positive pathogens were tested for bacterial binding. *S. mitis* SF100 did not bind to fibrinogen, fibronectin, collagen, or the platelet glycoprotein GPIb or GPIIb/IIIa (data not shown). In addition to these protein targets, we also examined the contribution of surface-exposed carbohydrates to platelet binding by SF100.

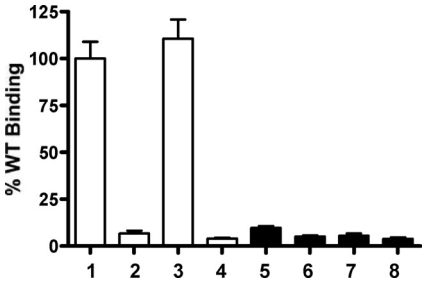

FIG. 1. Desialylation of human platelets reduces binding by *S. mitis*. Binding of SF100 (□) to untreated platelets (lane 1), platelets pretreated with sialidase A (lane 2), platelets treated with *N*- and *O*-glycanase (lane 3), and platelets treated with *N*- and *O*-glycanase and sialidase A (lane 4). Binding of PS344 (■) to untreated platelets (lane 5), platelets pretreated with sialidase A (lane 6), platelets treated with *N*- and *O*-glycanase (lane 7), and platelets treated with *N*- and *O*-glycanase and sialidase A (lane 8). Values are expressed as a percentage of wild-type binding to untreated platelets (mean ± SD).

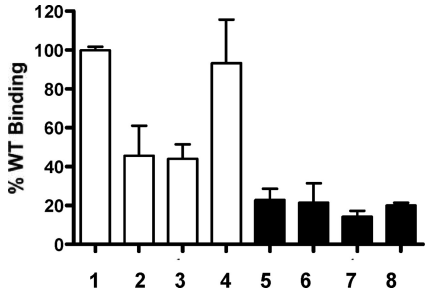

FIG. 2. Cleavage of α2-8-linked sialic acid from platelet surfaces results in a reduction of *S. mitis* binding. Binding of SF100 (□) to untreated human platelets (lane 1) or platelets pretreated with sialidase A (lane 2), sialidase V (lane 3), or sialidase C (lane 4). Binding of PS344 (■) to platelets (lane 5) or platelets pretreated with sialidase A (lane 6), sialidase V (lane 7), or sialidase C (lane 8). Values presented are expressed as a percentage of wild-type binding to untreated platelets (mean ± SD). Data represent three experiments performed in triplicate on different occasions from a different donor each time. Note that these donors are different from those shown in Fig. 1.

We first assessed the impact of treating platelet monolayers with sialidase A or *N*-glycanase and *O*-glycanase on platelet binding by *S. mitis* SF100 and its isogenic Δ*pblA* Δ*pblB* variant strain in order to determine whether *S. mitis* adheres to glycosylated residues on the surface of platelets. Sialidase A is a recombinant sialidase from *Arthrobacter ureafaciens* that specifically cleaves nonreducing terminal branched and unbranched sialic acids from glycoproteins and glycolipids. *N*-Glycanase is a recombinant glycosidase from *Elizabethkingia meningoseptica* that cleaves asparagine-linked oligosaccharides from glycoproteins. *O*-Glycanase is a recombinant glycosidase from *Streptococcus pneumoniae* that releases unmodified oligosaccharides attached to a serine or threonine residue in glycoproteins.

As is shown in Fig. 1, desialylation of platelets significantly reduced the binding of *S. mitis* SF100 to platelets ($P < 0.0001$), whereas it had a negligible effect on the residual background level of binding by PS344. In contrast, pretreatment of the platelets with *N*- and *O*-glycanases had no impact on SF100 or PS344 binding to platelets. These results indicate that the primary host receptor for *S. mitis* SF100 is sialylated and that the phage proteins PblA and PblB mediate adherence of SF100 to sialic acid residues. The inability of *N*- and *O*-glycanase treatment to reduce *S. mitis* platelet binding also indicates that the sialylated glycans to which *S. mitis* is binding are more likely to form part of a glycolipid rather than a glycoprotein, although it is possible that more-complex O-linked carbohydrates may partially contribute to the interaction of *S. mitis* SF100 with platelets.

Enzymatic removal of α2-8-linked sialic acid residues from platelets reduces *S. mitis* platelet binding. To further characterize the type of sialic acid linkages bound by *S. mitis* SF100, platelets were treated with sialidases with differing linkage specificities. Sialidase V is a sialidase from *Vibrio cholerae* which cleaves α2-3-, α2-6-, and α2-8-linked sialic acids from oligosaccharides. Sialidase C is a recombinant sialidase from *Clostridium perfringens* which cleaves α2-3- and α2-6-linked sialic acids. Pretreatment of platelet monolayers with sialidase A or sialidase V resulted in a significant reduction in SF100 binding to platelets ($P = 0.0241$ or 0.0018, respectively) (Fig. 2). In contrast, treatment of platelet monolayers with sialidase C had no significant effect on platelet binding by SF100. In control studies, none of the sialidases tested altered the already low levels of binding by PS344. These results indicate that α2-8-linked sialic acid constitutes the main platelet receptor for SF100.

Antibody to ganglioside GD3 blocks *S. mitis* SF100 platelet binding. Ganglioside GD3 is the only reported molecule containing α2-8-linked sialic acid on the platelet membrane surface. To elucidate whether this was the receptor for *S. mitis* SF100, platelets were preincubated with an IgG3 MAb to GD3. The ability of SF100 cells to adhere to the antibody-treated platelets was then tested. As shown in Fig. 3A, the MAb 4F6 reduced the adherence of SF100 to platelets by 45% ($P = 0.0052$). In contrast, this antibody did not affect the residual binding of *S. mitis* strain PS344. Additionally, treatment of platelets with a MAb to the glycosylated platelet membrane protein GPIb (IgG1 MAb HIP1) as a control for nonspecific binding of SF100 to sialylated proteins did not block binding of strain SF100 to platelets. Treatment of platelets with an isotype control IgG3 MAb did not affect *S. mitis* binding these cells (data not shown). Additionally, the ability of anti-GD3 MAb 4F6 to block the interaction of *S. mitis* strain SF100 to platelets was concentration dependent and saturable (Fig. 3B). These data confirm that the ganglioside GD3 is a receptor for *S. mitis* platelet binding and that the phage proteins PblA and PblB mediate binding to this receptor.

Preincubation of *S. mitis* SF100 with ganglioside GD3 blocks platelet binding. To more fully assess the role of gangliosides in platelet binding, *S. mitis* SF100 and PS344 were incubated with a variety of differentially glycosylated gangliosides (Table 1) and subsequently tested for their abilities to adhere to platelets. Pretreatment of SF100 with asialo GM1 or GM1 did not significantly affect the binding of this strain to platelets (Fig. 4). However, treatment of SF100 with GM3 reduced binding by 35%. Moreover, treatment of SF100 with GD3, which is the ganglioside in platelet membranes containing α2-8-linked sialic acid, caused a 45% reduction in platelet binding ($P = 0.0031$). GD3 also blocked the interaction of *S. mitis* SF100

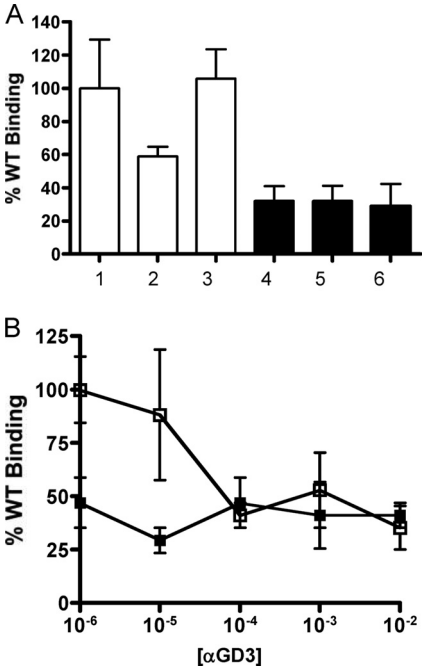

FIG. 3. Antibodies to ganglioside GD3 block *S. mitis* binding to platelets. (A) Binding of SF100 (□) to untreated platelets (lane 1), platelets pretreated with a murine MAb to ganglioside GD3 (lane 2), or a murine MAb to platelet glycoprotein GPIb (lane 3) and binding of PS344 (■) to untreated platelets (lane 4) platelets pretreated with a murine MAb to ganglioside GD3 (lane 5) or a murine MAb to platelet glycoprotein GPIb (lane 6). (B) Concentration-dependent inhibition of *S. mitis* (□) and PS344 (■) binding to platelets by a murine MAb to GD3. Values presented are expressed as a percentage of wild-type binding to untreated platelets (mean ± SD).

with platelets in a concentration-dependent manner, as shown in Fig. 4B.

Further analysis of the ability of different gangliosides to block SF100 platelet binding revealed that preincubation of SF100 with gangliosides containing α2-8-linked sialic acids (Fig. 4) blocked binding to platelets. Like GD3, ganglioside GD1a (Fig. 4) is also disialylated, but both sialic acids are α2-3

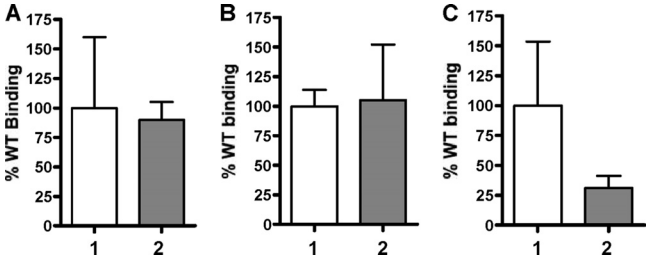

FIG. 5. Binding of *S. mitis* to immobilized gangliosides. Strains SF100 (□) and PS344 (■) were assayed for binding to lactosyl ceramide (A), GM3 (B), and GD3 (C). Values are expressed as a percentage of wild-type binding to ganglioside (mean ± SD).

linked. Preincubation of SF100 with GD1a did not result in a significant inhibition of platelet binding. In parallel studies, none of the gangliosides tested affected the residual binding of PS344 to human platelets. These data further indicate that binding of SF100 to platelets is specific for the α2-8-linked sialic acids in ganglioside GD3 and that this interaction is mediated by PblA and PblB.

Direct binding of *S. mitis* SF100 to ganglioside GD3 requires PblA/PblB. To assess the direct interaction of strains SF100 and PS344 with gangliosides, we compared the adherences of these strains to gangliosides immobilized on microtiter dishes. As shown in Fig. 5A, there is no significant difference in the relative adherence of either strain to lactosylceramide, a structural precursor to GM3 and GD3 that does not contain sialic acid. Similarly, no significant difference was seen in the binding of SF100 versus PS344 to ganglioside GM3 (Fig. 5B). However, the binding of strain PS344 to the ganglioside GD3 was reduced by 70% compared to that of the wild-type strain SF100 ($P = 0.0223$) (Fig. 5C). These data show that SF100 binds directly to ganglioside GD3 and that this binding is mediated by the phage proteins PblA and PblB.

PblA and PblB proteins bind directly to ganglioside GD3. In order to verify that PblA and PblB proteins bind directly to GD3, clarified cell culture supernatants of *S. mitis* SF100 were mixed with either GD3 or asialo GM1 immobilized on octyl-Sepharose CL-4B. The Sepharose was then extensively

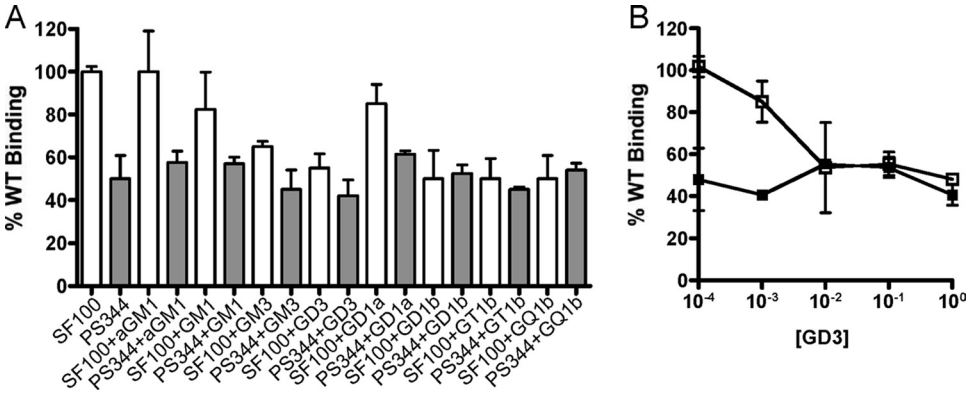

FIG. 4. Effect of gangliosides on platelet binding by *S. mitis*. (A) Strains SF100 (□) and PS344 (■) were incubated with buffer or gangliosides and then tested for binding to platelets. Gangliosides tested were as follows: asialo GM1, GM1, GM3, GD3, GD1a, GD1b, GT1b, and GQ1b. (B) Strains SF100 (□) and PS344 (■) were incubated with 10-fold dilutions of ganglioside GD3 and then tested for binding to platelets. Values are expressed as a percentage of wild-type binding to platelets (mean ± SD).

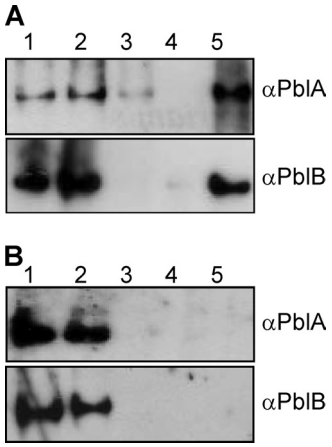

FIG. 6. Direct binding of PblA and PblB proteins to GD3. Clarified culture supernatants of SF100 were passed over GD3 Sepharose (A) or asialo GM1 Sepharose (B) in order to assess the direct binding of PblA and PblB. Lane 1, supernatant; lane 2, flowthrough; lane 3, first column wash; lane 4, final column wash; lane 5, eluate. Protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes and probed with antibodies to PblA (αPblA) or PblB (αPblB).

washed, and then any proteins bound to GD3 or asialo GM1 were recovered by treatment with urea. As is shown Fig. 6, both PblA and PblB were affinity purified with GD3 Sepharose. In contrast, neither PblA nor PblB bound to asialo GM1 immobilized on Sepharose (Fig. 6B) or to octyl-Sepharose CL-4B alone (data not shown). These findings indicate that PblA and PblB proteins interact directly with GD3 and that this interaction is specific.

DISCUSSION

The adhesion of bacteria to host tissues and cell surfaces is a critical first step in the initiation of infection. The binding of pathogenic bacteria to platelets is thought to play a role in the virulence of these bacteria in infectious endocarditis (8, 11, 17, 22, 24). Studies from our laboratory have shown that platelet binding by *S. mitis* strain SF100 is in part mediated by two surface proteins, PblA and PblB (3, 4, 19). Both proteins are encoded within a temperate phage of the *Siphoviridae* family, ϕSM1 (23), and indeed deletion of the genes encoding PblA and PblB was shown to reduce the virulence of *S. mitis* in a rabbit model of infectious endocarditis (20). Until now, however, the platelet receptor for PblA- and PblB-mediated binding has been unknown. In exploratory studies we performed, *S. mitis* SF100 did not appear to interact with any of the commonly identified protein ligands for bacteria (e.g., fibronectin and fibrinogen) that have been linked to platelet binding or the major platelet receptors GPIb/IIIa or GPIb (data not shown). Accordingly, we expanded our search for the platelet receptor to potential carbohydrate structures. Several bacteria are known to adhere to carbohydrate residues on the surfaces of host cells (9, 16), including *S. gordonii*, *Helicobacter pylori*, and *Escherichia coli*, among others. The results herein indicate that α2-8-linked sialic acid of ganglioside GD3 is the likely target receptor for the phage-encoded proteins PblA and PblB. However, while we have shown here that PblA and PblB can interact directly with GD3, further investigation is needed to identify whether both proteins can bind individually to GD3. It remains possible that PblA and PblB form a protein complex that mediates attachment of *S. mitis* SF100 to GD3. This hypothesis is supported by previous studies suggesting that expression of both proteins is needed for stability of the proteins and full platelet binding activity (3).

Our studies using selective sialidases indicate that α2-8-linked sialic acid is the primary receptor for *S. mitis* binding to platelets. The fact that carbohydrates released by *N*- and *O*-glycanases are not involved in the adherence of *S. mitis* SF100 to platelets further suggests that it is unlikely that the sialic acids implicated as the bacterial receptor are linked to proteins. Instead, our studies using a variety of mono-, di-, tri-, and tetrasialylated gangliosides indicate that *S. mitis* SF100 has a tropism for gangliosides containing α2-8-linked disialic acid and, in particular, GD3. Of note, GD3 is the only reported disialylated ganglioside in the membranes of platelets. Confirmation of the interaction of *S. mitis* SF100 to platelet membrane GD3 was provided by the ability of a MAb to GD3 to block binding to platelets. This is the first observation of a pathogenic bacterium binding directly to sialylated gangliosides on the host platelet surface.

Since the oropharynx is the usual habitat for *S. mitis*, the finding that this organism adheres to α2-8-linked sialic acids on ganglioside GD3 leads to the intriguing possibility that similar carbohydrate structures or gangliosides in the oropharynx serve as the receptors for oral colonization of this site. Further investigation of the ganglioside content of oropharyngeal cells, alongside analysis of the ability of *S. mitis* cells to adhere to these cells, will confirm whether this is the case. It also remains unknown whether other species of viridans group streptococci interact with α2-8-linked sialic acids and whether this interaction is mediated by phage proteins. As for *S. mitis*, it appears that PblA and PblB homologs may be highly prevalent in this organism; all 38 randomly selected strains (of both laboratory and clinical origins) we have tested thus far express these proteins as measured by Western blotting (unpublished data). The finding that *S. mitis* binds to sialic acids opens the possibility for the development of synthetic antiadhesive glycoconjugates as antimicrobial chemotherapeutics.

The question remains as to why phage-encoded proteins would mediate attachment to sialic acids. Interestingly, the capsular polysaccharide of some bacterial species such as group B *Neisseria meningitidis* (2) contains poly-α2-8-linked sialic acids. It is possible that *S. mitis* sialic acid binding may prevent the direct attachment of such pathogens to the host cell surface by preferentially binding the pathogen and sequestering it. Additionally, the occupation of α2-8-linked sialic acid epitopes by *S. mitis* could prevent the deposition of ganglioside-binding toxins, pointing to the possible protective role of *S. mitis* as an oral commensal organism. The different and tissue-specific profiles of exposed sugars on the surfaces of host epithelial cells could explain the changing profile of commensals throughout the oropharynx and gastrointestinal tract, each tissue being preferentially colonized by a commensal organism that the host can tolerate but that also physically inhibits toxin deposition and pathogen infiltration.

ACKNOWLEDGMENTS

This study was supported by the Department of Veterans Affairs and by grants R01 AI041513 and R01 AI057433 from the National Institutes of Health.

We thank Barbara Bensing, Ian Siboo, and Ravin Seepersaud for their helpful scientific and editorial suggestions.

REFERENCES

1. **Aspholm, M., F. O. Olfat, J. Nordén, B. Sondén, C. Lundberg, R. Sjöström, S. Altraja, S. Odenbreit, R. Haas, T. Wadström, L. Engstrand, C. Semino-Mora, H. Liu, A. Dubois, S. Teneberg, A. Arnqvist, and T. Borén.** 2006. SabA is the *H. pylori* hemagglutinin and is polymorphic in binding to sialylated glycans. PLoS Pathog. **2:**e110.
2. **Azmi, F. H., A. H. Lucas, H. L. Spiegelberg, and D. M. Granoff.** 1995. Human immunoglobulin M paraproteins cross-reactive with *Neisseria meningitidis* group B polysaccharide and fetal brain. Infect. Immun. **63:**1906–1913.
3. **Bensing, B. A., C. E. Rubens, and P. M. Sullam.** 2001. Genetic loci of *Streptococcus mitis* that mediate binding to human platelets. Infect. Immun. **69:**1373–1380.
4. **Bensing, B. A., I. R. Siboo, and P. M. Sullam.** 2001. Proteins PblA and PblB of *Streptococcus mitis*, which promote binding to human platelets, are encoded within a lysogenic bacteriophage. Infect. Immun. **69:**6186–6192.
5. **Bensing, B. A., J. A. López, and P. M. Sullam.** 2004. The *Streptococcus gordonii* surface proteins GspB and Hsa mediate binding to sialylated carbohydrate epitopes on the platelet membrane glycoprotein Ibα. Infect. Immun. **72:**6528–6537.
6. **Berenson, C. S., K. B. Sayles, J. Huang, V. N. Reinhold, M. A. Garlipp, and H. C. Yohe.** 2005. Nontypeable *Haemophilus influenzae*-binding gangliosides of human respiratory (HEp-2) cells have a requisite lacto/neolacto core structure. FEMS Immunol. Med. Microbiol. **45:**171–182.
7. **Byers, H. L., K. A. Homer, and D. Beighton.** 1996. Utilization of sialic acid by viridans streptococci. J. Dent. Res. **75:**1564–1571.
8. **Durack, D. T.** 1975. Experimental bacterial endocarditis. IV. Structure and evolution of very early lesions. J. Pathol. **115:**81–89.
9. **Fakih, M. G., T. F. Murphy, M. A. Pattoli, and C. S. Berenson.** 1997. Specific binding of *Haemophilus influenzae* to minor gangliosides of human respiratory epithelial cells. Infect. Immun. **65:**1695–1700.
10. **Ferroni, P., L. Lenti, F. Martini, F. Ciatti, G. M. Pontieri, and P. P. Gazzaniga.** 1997. Ganglioside content of human platelets: differences in resting and activated platelets. Thromb. Haemost. **77:**548–554.
11. **Fitzgerald, J. R., T. J. Foster, and D. Cox.** 2006. The interaction of bacterial pathogens with platelets. Nat. Rev. Microbiol. **4:**445–457.
12. **Garcia-Monco, J. C., R. J. Seidman, and J. L. Benach.** 1995. Experimental immunization with *Borrelia burgdorferi* induces development of antibodies to gangliosides. Infect. Immun. **63:**4130–4137.
13. **Gong, K., D. Y. Wen, T. Ouyang, A. T. Rao, and M. C. Herzberg.** 1995. Platelet receptors for the *Streptococcus sanguis* adhesin and aggregation-associated antigens are distinguished by anti-idiotypical monoclonal antibodies. Infect. Immun. **63:**3628–3633.
14. **Hoen, B., F. Alla, C. Selton-Suty, I. Beguinot, A. Bouvet, S. Briancon, J. P. Casalta, N. Danchin, F. Delahaye, J. Etienne, V. Le Moing, C. Leport, J. L. Mainardi, R. Ruimy, and F. Vandenesch.** 2002. Changing profile of infective endocarditis: results of a 1-year survey in France. JAMA **288:**75–81.
15. **Hugosson, S., J. Angström, B. M. Olsson, J. Bergström, H. Fredlund, P. Olcén, and S. Teneberg.** 1998. Glycosphingolipid binding specificities of *Neisseria meningitidis* and *Haemophilus influenzae*: detection, isolation, and characterization of a binding-active glycosphingolipid from human oropharyngeal epithelium. J. Biochem. **124:**1138–1152.
16. **Imberty, A., and A. Varrot.** 2008. Microbial recognition of human cell surface glycoconjugates. Curr. Opin. Struct. Biol. **18:**567–576.
17. **Loughman, A., J. R. Fitzgerald, M. P. Brennan, J. Higgins, R. Downer, D. Cox, and T. J. Foster.** 2005. Roles for fibrinogen, immunoglobulin and complement in platelet activation promoted by *Staphylococcus aureus* clumping factor A. Mol. Microbiol. **57:**804–818.
18. **Martini, F., S. Riondino, P. Pignatelli, P. P. Gazzaniga, P. Ferroni, and L. Lenti.** 2002. Involvement of GD3 in platelet activation. A novel association with Fcγ receptor. Biochim. Biophys. Acta **1583:**297–304.
19. **Miller-Podraza, H., B. Lanne, J. Angström, S. Teneberg, M. A. Milh, P. A. Jovall, H. Karlsson, and K. A. Karlsson.** 2005. Novel binding epitope for *Helicobacter pylori* found in neolacto carbohydrate chains: structure and cross-binding properties. J. Biol. Chem. **280:**19695–19703.
20. **Mitchell, J., I. R. Siboo, D. Takamatsu, H. F. Chambers, and P. M. Sullam.** 2007. Mechanism of cell surface expression of the *Streptococcus mitis* platelet binding proteins PblA and PblB. Mol. Microbiol. **64:**844–857.
21. **Plummer, C., H. Wu, S. W. Kerrigan, G. Meade, D. Cox, and C. W. I. Douglas.** 2005. A serine-rich glycoprotein of *Streptococcus sanguis* mediates adhesion to platelets via GPIb. Br. J. Haematol. **129:**101–109.
22. **Siboo, I. R., A. L. Cheung, A. S. Bayer, and P. M. Sullam.** 2001. Clumping factor A mediates binding of *Staphylococcus aureus* to human platelets. Infect. Immun. **69:**3120–3127.
23. **Siboo, I. R., B. A. Bensing, and P. M. Sullam.** 2003. Genomic organization and molecular characterization of SM1, a temperate bacteriophage of *Streptococcus mitis*. J. Bacteriol. **185:**6968–6975.
24. **Sullam, P. M., A. S. Bayer, W. M. Foss, and A. L. Cheung.** 1996. Diminished platelet binding in vitro by *Staphylococcus aureus* is associated with reduced virulence in a rabbit model of infective endocarditis. Infect. Immun. **64:**4915–4921.
25. **Takahashi, Y., K. Konishi, J. O. Cisar, and M. Yoshikawa.** 2002. Identification and characterization of *hsa*, the gene encoding the sialic acid-binding adhesin of *Streptococcus gordonii* DL1. Infect. Immun. **70:**1209–1218.
26. **Takamatsu, D., B. A. Bensing, H. Cheng, G. A. Jarvis, I. R. Siboo, J. A. López, J. M. Griffiss, and P. M. Sullam.** 2005. Binding of the *Streptococcus gordonii* surface glycoproteins GspB and Hsa to specific carbohydrate structures on platelet membrane glycoprotein Ibα. Mol. Microbiol. **58:**380–392.
27. **Takamatsu, D., B. A. Bensing, A. Prakobphol, S. J. Fisher, and P. M. Sullam.** 2006. Binding of the streptococcal surface glycoproteins GspB and Hsa to human salivary proteins. Infect. Immun. **74:**1933–1940.
28. **van Heyningen, S.** 1974. Cholera toxin: interaction of subunits with ganglioside GM1. Science **183:**656–657.
29. **Xiong, Y. Q., B. A. Bensing, A. S. Bayer, H. F. Chambers, and P. M. Sullam.** 2008. Role of the serine-rich surface glycoprotein GspB of *Streptococcus gordonii* in the pathogenesis of infective endocarditis. Microb. Pathog. **45:**297–301.

Editor: A. Camilli