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Short communication

Epidemiologically and clinically relevant Group B *Streptococcus* isolates do not bind collagen but display enhanced binding to human fibrinogen

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Abstract

Group B *Streptococcus* (GBS) is the leading cause of neonatal septicemia and meningitis. Pili appendages were shown to play a critical role in bacterial adhesion and colonization of human tissues. Recently it was claimed that binding of the pilus-associated adhesin PilA to collagen is a critical, initial step in promoting interactions with the α2β1 integrin expressed on brain endothelial cells. Here we show that strain NCTC10/84 used in this study is not representative for GBS isolates and question the importance of collagen as a critical extracellular matrix component for GBS infections of the central nervous system.
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Keywords: GBS; *Streptococcus*; Pili; Collagen; Meningitis

1. Introduction

Streptococcus agalactiae (also known as Group B *Streptococcus*, GBS) is the leading cause of neonatal infections in the industrialized world. The first systematic review and meta-analysis of neonatal Group B Streptococcal disease shows that five serotypes (Ia, Ib, II, III and V) are responsible for the global burden of disease [1]. Among those, serotype III is of major importance in neonatal diseases whereas serotype V is mainly involved in adult invasive diseases. Several epidemiological studies, including a recent report from our laboratory [2], pinpoint a single GBS clone of capsular serotype III, designated ST17 according to its sequence type (ST) as being responsible for the vast majority (>80%) of meningitis cases [3–8]. The ST17 clone is now worldwide referred to as the hypervirulent GBS clonal complex 17 (CC-17) and COH1, a representative strain of this group, has been sequenced recently [9].

Surface-exposed pili has emerged as appealing host colonizing factors as well as vaccine antigen candidates. We and others have initially characterized pili in *S. agalactiae* [10–12] and have demonstrated their role in adhesion to host cells and in biofilm formation. Two genomic pilus islands (PI-1 and PI-2) have been identified in GBS. Of note, the PI-2 locus exists in two variant forms: PI-2a (73%) being present in the vast majority of GBS strains belonging to all serotypes and PI-2b (27%) being restricted to ST17 and a few other clinical isolates. The overall organization of each PI locus is similar as it contains three genes that encode cell wall anchored LPXTG proteins and two genes encoding class C sortases involved in pilus assembly. The PI-2a pilus is composed of the major backbone protein (PilB) decorated with the tip adhesin (PilA) and attached to the bacterial cell wall with the pilus-anchoring subunit (PilC) [10,13]. In non-ST17 strains, lack of PilA was associated with a significant decrease in the ability of bacteria to bind to human epithelial

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[10,14] as well as to endothelial (hBMECs) cells [15]. Interestingly PilA contains a Von Willebrand type A factor domain (VWA), a domain known to tightly bind to collagens present on the vessel wall and to control platelet adhesion to the damaged vasculature. This VWA was shown to be mandatory for adhesive properties of PilA [14]. A recent report from Banerjee et al. proposed that binding of the pilus-associated adhesin PilA to collagen is a critical initial step in promoting interactions with the α2β1 integrin expressed on brain endothelial cells. Activation of this host receptor was reported to cause focal adhesion kinase (FAK) activation and downstream phosphatidylinositol-3 phosphate kinase (PI3K) and MAPK signaling, resulting in host chemokine expression and neutrophil recruitment during infection and bacterial entry into the central nervous system (CNS) [16]. Banerjee et al. have used the GBS strain NCTC10/84 which is a clinical isolate of capsular serotype V, a serotype not normally associated with meningitis. On the other hand, Al Safadi et al. proposed that human fibrinogen is the extracellular matrix (ECM) component critical for CNS infections caused by CC-17 isolates [17]. In this brief report, we tested the binding capacity of randomly selected clinical GBS isolates to bind to collagen and fibrinogen. Our results question the choice of GBS strain NCTC10/84 as a relevant representative to study GBS-host interactions, in particular CNS invasion.

2. Material and methods

2.1. Bacterial adherence to ECM components

GBS and *Streptococcus gallolyticus* were grown in Todd-Hewitt (TH) broth whereas recombinant *Lactococcus lactis* was grown in M17 1% glucose containing erythromycin at 5 μg/ml. Polystyrene 96-wells plates (NUNC) were coated with collagen I (from rat tail, BD Biosciences) or collagen IV (from human cell culture, Sigma–Aldrich C6745) at 50 μg/ml (100 μl per well) and incubated overnight at 4 °C. After saturation of non-specific sites with Superblock Buffer (Thermo Scientific), approx. 10^8 bacterial colony forming units (cfu) were added and the plates were incubated for 2 h at 37 °C. Non-adherent bacteria were removed using the ELx50 Microplate Strip Washer (BioTek). Adherent bacteria were stained with 0.1% crystal violet for 30 min, washed twice with PBS, and air-dried for 15 min. The stained bacteria were resuspended for quantification in ethanol/acetone (80:20). The plate was scanned and absorbance at 595 nm was measured. Similar experiments were performed with human fibrinogen purchased from Sigma–Aldrich (from human plasma F3879).

2.2. Hemolysin assay

Approximately 2×10^8 cfu of GBS cells were collected from liquid cultures at exponential phase, washed once with phosphate-buffered saline (PBS), and resuspended in 1 ml of PBS containing 0.2% glucose. Serial dilutions (2-fold) of this suspension in PBS containing 0.2% glucose were mixed with an equal volume of 1% sheep erythrocytes (RBC) in the same buffer and incubated at 37 °C for 1 h. After incubation, unlysed RBC and bacteria were removed by centrifugation and hemoglobin content of the supernatant was assessed by measuring absorbance at 420 nm (A420). The hemolytic titer of each strain was determined as the reciprocal of the greatest dilution producing 50% hemoglobin release compared to control samples in which all RBC were lysed by sodium dodecyl sulfate (SDS 0.2%).

3. Results and discussion

3.1. Binding of clinical GBS isolates to collagen and fibrinogen

We investigated the ability of 20 GBS strains isolated from invasive neonatal disease (10 non-ST17 and 10 ST17 strains that were randomly selected) to bind to collagen type I, type IV, and fibrinogen. We found no significant binding to collagen I and IV as compared to the positive control *S. gallolyticus*, a collagen-binding species causing endocarditis [18] (Fig. 1A and data not shown). Furthermore, recombinant strains of *L. lactis* overexpressing either PilA or the entire PI-2a pilus of GBS strain NEM316 did not bind to collagen I or IV, unlike *L. lactis* overexpressing the collagen-binding Pil1 of *S. gallolyticus* (Fig. 1A and data not shown). These results indicate that the PilA protein is most likely not directly responsible for collagen binding.

In contrast, all 20 GBS strains were able to bind to human fibrinogen at various levels in a dose-dependent manner. All ST17 isolates displayed higher binding as compared to non-ST17 strains (Fig. 1B). The representative assay shown in Fig. 1B was conducted as blind test. The isolates marked by an asterisk were predicted as being ST17 strains based on their increased fibrinogen binding phenotype. This prediction was later indeed confirmed to 100%. These results are in perfect agreement with a recent study [17] suggesting that the higher fibrinogen binding ability of CC-17 strains may participate in their enhanced invasiveness for neonates as compared to strains of other CCs.

3.2. GBS strain NCTC10/84 is an atypical clinical isolate

The GBS strain NCTC10/84 (also called 1169-NT1) is of capsular serotype V and was isolated from blood of a septic neonate [19]. Its genome has not been sequenced yet. We determined that strain NCTC10/84 belongs to the ST-26 (http://pubmlst.org/sagalactiae/), a worldwide very rare sequence type [20,21] although it is representing 15% of GBS isolates from the Central African Republic and Senegal [22].

It is interesting to note that Doran et al. previously reported that the beta-haemolysin/cytolysin (β-h/c) of GBS activates specific signaling pathways in human brain microvascular endothelial cells (HBMEC) resulting in IL-8 release, neutrophil recruitment and enhanced virulence [23]. The β-h/C toxin was identified as the principal factor responsible for blood-

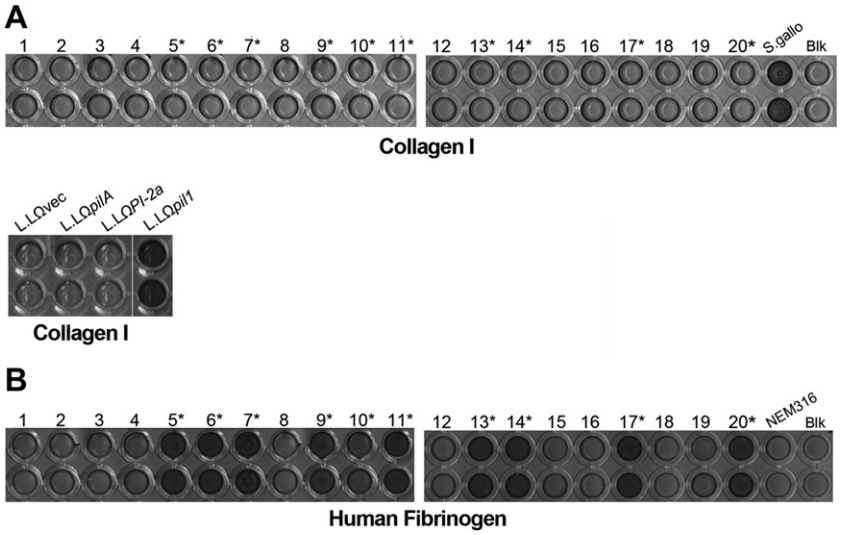


Fig. 1. A – Adherence of GBS wild type strains and *Lactococcus* recombinant strains to immobilized collagen I (Col I). Microtiter wells were coated with 5 μg of Col I and 10^7 cfu were added. Adherent bacteria were detected using crystal violet staining. The two horizontal rows represent sample duplicates. Upper panel – 20 GBS neonatal isolates 10 of which are ST17* and 10 non-ST17 (for details see Table 1). *Streptococcus gallolyticus* (*S. gallo* strain UCN34) is used as positive control. Lower panel – Adherence of *L. lactis* NZ9000/pOri23 and its various derivatives. Similar results were obtained with human collagen IV (not shown). B – Adherence of GBS wild type strains to immobilized human fibrinogen (3 μg per well).

brain barrier activation triggered by the GBS strains A909, COH1, and NCTC10/84. We thus compared the hemolytic activity of the seven sequenced GBS strains (NEM316, 2603V/R, A909, COH1, H36B, 515, and 18RS21) and 20 additional GBS isolates from neonatal disease that we have previously used [2]. Most clinical isolates belonging to different ST groups, including ST17, were less hemolytic than our reference strain NEM316 WT (Fig. 2, Table 1). The hemolysin-negative mutant NEM316Δ*cylE* and the hyperhemolytic mutant NEM316Δ*covSR* were used as negative and positive controls, respectively. Strikingly, GBS NCTC10/84 appeared to be as hemolytic as the hyperhemolytic mutant NEM316Δ*covSR* (Fig. 2B, Table 1). In view of these two relevant characteristics i) the epidemiological rarity and ii) a hyperhemolytic phenotype, we conclude that NCTC10/84 is an atypical GBS strain.

In conclusion, the role of the PilA-collagen interaction in the process of CNS invasion by GBS is questionable. We suggest that the unusual hyperhemolytic phenotype of GBS strain NCTC10/84 impacts on the cellular and host responses described in the report by Banerjee et al., precluding any definitive conclusion about the role of the pilus-associated adhesin PilA. The many roles uncovered for GBS pili including bacterial adherence to host cells, biofilm formation, and virulence were attributed to the PI-2a pilus. Importantly, the ST17 hypervirulent clone contains two other pilus loci, PI-1 and PI-2b, whose function(s) remains unveiled. The elucidation of their role is the subject of our ongoing investigations.

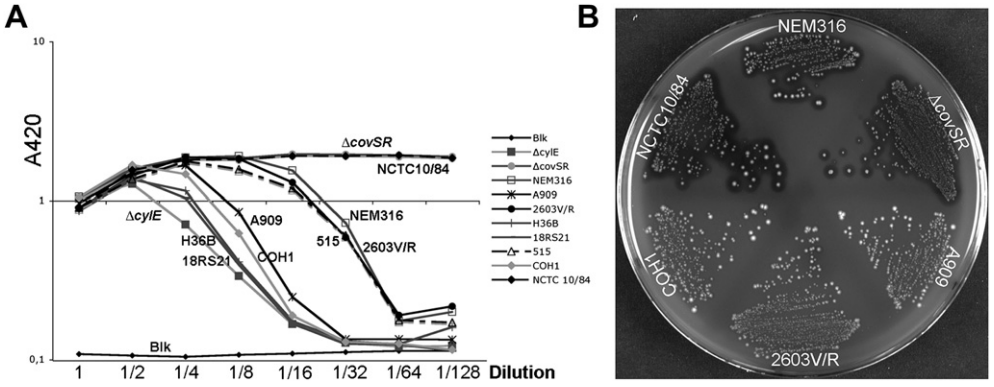


Fig. 2. Hemolytic activity of sequenced GBS strains. The hemolysin-negative mutant Δ*cylE* and the hyperhemolytic mutant Δ*covSR* derived from our reference strain NEM316 were used as negative and positive controls, respectively. A – Hemoglobin release in supernatants quantified by absorbance measurement (A_{420}). B – GBS streaking on horse blood agar plate. The data shown here are representative of at least three independent experiments.

Table 1
Main characteristics of the clinical GBS isolates used in this study.

GBS strains	Capsular serotype	ST cluster	Type of neonatal infection	Hemolytic index (relative value)	Col I or IV binding
Clinical isolates					
1	II	28	EOD/bacteremia	16–32	-
2	Ia	23	EOD/meningitis	8	-
3	Ia	23	EOD/bacteremia	8	-
4	Ia	23	EOD/bacteremia	4–8	-
5	III	17	LOD/meningitis	4–8	-
6	III	17	LOD/meningitis	16–32	-
7	III	17	LOD/meningitis	8–16	-
8	Ia	24	EOD/bacteremia	8–16	-
9	III	17	EOD/bacteremia	4–8	-
10	III	17	LOD/meningitis	4	-
11	III	17	LOD/meningitis	4–8	-
12	Ia	23	EOD/bacteremia	4–8	-
13	III	17	EOD/meningitis	4–8	-
14	III	17	LOD/meningitis	4–8	-
15	V	453	EOD/bacteremia	4–8	-
16	V	1	LOD/bacteremia	4–8	-
17	III	17	EOD/meningitis	4–8	-
18	Ib	10	EOD/meningitis	4–8	-
19	Ia	23	LOD/bacteremia	4–8	-
20	III	17	LOD/meningitis	4–8	-
Reference strains					
2603V/R	V	110	ND	32	-
A909	Ia	7	ND	8–16	-
COH1	III	17	ND	8–16	-
18RS21	II	19	ND	4–8	-
H36B	Ib	6	ND	4–8	-
515	Ia	23	ND	32–64	-
NEM316	III	23	ND	32–64	-
NEM316Δ*covSR*	III	23	ND	>128	-
NEM316Δ*cylE*	III	23	ND	4–8	-
NCTC10/84	V	26	Septicemia	>128	-

ND: not determined; (-): negative result.

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