ELSEVIER
CrossMark
Institut Pasteur
Microbes and Infection 17 (2015) 360–368
www.elsevier.com/locate/micinf

Original article

Group B *Streptococcus* pili mediate adherence to salivary glycoproteins

Jane L. Brittan, Angela H. Nobbs*

School of Oral & Dental Sciences, University of Bristol, Lower Maudlin Street, Bristol, BS1 2LY, UK

Received 13 November 2014; accepted 30 December 2014
Available online 7 January 2015

Abstract

Group B *Streptococcus* (GBS) is a leading cause of neonatal sepsis, pneumonia and meningitis, and is responsible for a rising number of severe invasive infections in adults. For all disease manifestations, colonisation is a critical first step. GBS has frequently been isolated from the oropharynx of neonates and adults. However, little is understood about the mechanisms of GBS colonisation at this site. In this study it is shown that three GBS strains (COH1, NEM316, 515) have capacity to adhere to human salivary pellicle. Heterologous expression of GBS pilus island (PI) genes in *Lactococcus lactis* to form surface-expressed pili demonstrated that GBS PI-2a and PI-1 pili bound glycoprotein-340 (gp340), a component of salivary pellicle. By contrast, PI-2b pili did not interact with gp340. The variation was attributable to differences in capacities for backbone and ancillary protein subunits of each pilus to bind gp340. Furthermore, while GBS strains were aggregated by fluid-phase gp340, this mechanism was not mediated by pili, which displayed specificity for immobilised gp340. Thus pili may enable GBS to colonise the soft and hard tissues of the oropharynx, while evading an innate mucosal defence, with implications for risk of progression to severe diseases such as meningitis and sepsis.
© 2015 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

Keywords: *Streptococcus agalactiae*; Bacterial pili; Human gp340 protein; Bacterial adhesion; Agglutination

1. Introduction

Streptococcus agalactiae (Group B *Streptococcus* [GBS]) is the leading cause of neonatal sepsis, pneumonia and meningitis in the US and Europe, and those infants that survive often suffer severe neurological sequelae, resulting in long-term disease burden [1]. GBS also causes significant morbidity and mortality among adults, and there has been an increasing incidence of serious invasive disease since the 1970s [2].

For both neonatal and adult disease, colonisation is a critical step in GBS pathogenesis. Maternal carriage of GBS within the genitourinary (GU) tract is responsible for the majority of neonatal disease via vertical transmission. Many studies have thus focused on GBS colonisation of this site. Several GBS adhesins have been characterised that promote attachment to components of the extracellular matrix [3–5], while BibA, α-protein and pili bind directly to cervical or vaginal epithelial cells [6–10]. Pili are a family of adhesins that assemble on the bacterial cell surface as long, filamentous structures composed of covalently-linked protein subunits [11]. The genes encoding pili are located within 2 distinct loci, denoted pilus islands 1 and 2 (PI-1 and PI-2), the latter having 2 allelic variants (PI-2a and PI-2b). However, all conform to the same basic organisation [12]. Each pilus island (PI) comprises 3 genes that encode structural proteins with a characteristic LPXTG anchoring motif recognised by sortase transpeptidases: backbone (Bkb) subunit, forming the polymeric shaft of the pilus; ancillary subunit 1 (An1), which often serves as the functional tip; and ancillary subunit 2 (An2), which typically serves as the C-terminal anchor following attachment to peptidoglycan in the cell wall by sortase A (Supplementary Fig. S1). A study of 289 clinical GBS isolates circulating in the US and Italy in 2009 found that all isolates contained one (often two) PI. The most prevalent PI

* Corresponding author. Tel.: +44 117 3424779; fax: +44 117 3424313.
E-mail address: angela.nobbs@bristol.ac.uk (A.H. Nobbs).

http://dx.doi.org/10.1016/j.micinf.2014.12.013
1286-4579/© 2015 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

combination found across the isolates was PI-1 with PI-2a, followed by PI-2a alone and then PI-1 with PI-2b [13].

Aside from the GU tract, the oropharynx serves as an additional reservoir of GBS. Carriage rates of 4–12% have been reported in the young adult population [2,14], with oropharyngeal isolates matching multilocus sequence types typically associated with disease in non-pregnant adults. Oral motor dysfunction in the elderly has also been associated with increased numbers of GBS in dental plaque [15]. For neonates, the throat has been identified as the second most frequent colonisation site by GBS [16] and, critically, is regarded as the route via which neonatal meningitis is initiated [17]. Oropharyngeal colonisation thus has important implications for both adult and neonatal GBS disease, yet underlying mechanisms remain poorly understood.

The oropharynx can be colonised by a multitude of bacterial species that comprise the resident microbiota. Some bacteria bind directly to mucosal epithelium, while others attach to hard or soft tissues through recognition of components of the salivary pellicle. One host molecule common to all sites within the oropharynx is glycoprotein-340 (gp340). Gp340 (also termed salivary agglutinin or DMBT1) is a highly conserved glycoprotein that belongs to the scavenger receptor cysteine-rich (SRCR) superfamily of proteins [18]. It comprises a protein backbone divided into a number of distinct domains, of which SRCR domains predominate, and is heavily glycosylated. In fluid phase, gp340 aggregates a range of bacteria and viruses [19], assisting in microbial clearance. By contrast, when immobilised on a surface, gp340 can promote microbial colonisation. Gp340 recognition enables colonisation of the mouth by a number of oral streptococci, including *Streptococcus mutans*, *Streptococcus gordonii* and pathogenic Group A *Streptococcus* (GAS) [20–22], but interactions of this host molecule with GBS are currently unknown. This study therefore aimed to investigate the potential for GBS to utilise interactions with gp340 to facilitate oropharyngeal colonisation.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The bacterial strains and plasmids used in this study are listed in Supplementary Table S1. Streptococci were routinely grown in Todd Hewitt broth (Oxoid) supplemented with 5 g/l yeast extract (THY) for 16–20 h at 37 °C in a 5% CO_2 incubator. Lactococci were grown in M17 broth (Oxoid) supplemented with 0.5% (w/v) glucose (GM17) at 30 °C in a candle jar. For those strains carrying pMSP7517-derived constructs, heterologous protein expression was induced by addition of nisin to the culture medium at a final concentration of 10 or 100 ng/ml. *Escherichia coli* strains were grown in Luria–Bertani (LB) broth (Becton Dickinson) at 37 °C with shaking. When required, antibiotics were added to the medium at the following concentrations: erythromycin, 5 µg/ml (*Lactococcus lactis*) or 300 µg/ml (*E. coli*); ampicillin, 100 µg/ml.

2.2. Generation of L. lactis heterologous expression strains

Plasmids (listed in Supplementary Table S1) or PCR amplicons were purified using QIAquick Spin Miniprep or PCR Purification kits respectively (Qiagen). Oligonucleotides were synthesised by MWG Eurofins. Chromosomal DNA was extracted using Wizard Genomic DNA Purification Kit (Promega). DNA restriction and modification enzymes were used under the conditions specified by the manufacturer (NEB). DNA was amplified using Expand Long PCR System (Roche).

Primers (listed in Supplementary Table S2) were designed to amplify entire PI loci from *S. agalactiae* NEM316 (PI-1, PI-2a; GenBank accession NC_004368.1) or *S. agalactiae* COH1 (PI-2b; GenBank accession NZ_AAJR00000000.1) chromosomal DNA, which were cloned into vector pGEM-T (Promega) according to manufacturer's instructions (refer to Supplementary Fig. S1 for schematic of cloned PIs). Constructs were then transformed into chemically-induced competent *E. coli* XL1 Blue, prepared using the Hanahan method [23], with ampicillin for selection. Internal restriction endonuclease (RE) sites had to be removed from PI-1 (NcoI, XhoI) and PI-2b (XhoI) loci. This was achieved based upon a circular PCR system [24] using 5′ phosphorylated primers that incorporated base mismatches to disrupt the RE sites. In brief, following PCR amplification of purified pGEM-T constructs carrying PI-1 or PI-2b loci, template was removed by DpnI digestion, and purified amplicons (with RE sites removed) were self-ligated using T4 DNA ligase and transformed into *E. coli* XL1 Blue. All 3 PI loci were then PCR amplified from their respective pGEM-T constructs with unique NcoI/XhoI sites at their termini, and subcloned via these RE sites into vector pMSP7517 [25], which had been similarly digested to remove the *prgB* gene. Each pMSP-PI construct was transformed into *E. coli* K12, recovered, and confirmed by sequencing. Constructs were then electroporated into *L. lactis* NZ9800. *L. lactis* was cultured in GM17 broth to OD_{600} 0.3–0.4, cells were harvested by centrifugation (5000 *g*, 7 min, 4 °C) and resuspended in 50% original volume dH_2O. Plasmid was added to 100 µl *L. lactis* suspension and incubated for 1 h. Cells were then electroporated using a BioRad Genepulser II at 2.5 kV voltage, 25 µF capacitance and 400 Ω resistance. Transformants were recovered in GM17 broth and incubated at 37 °C in a candle jar for 1 h. Cells were then plated onto GM17 agar supplemented with erythromycin and incubated at 30 °C for a further 24–48 h. Expression of pili on the surface of *L. lactis* was verified by whole cell immunoblot analysis.

2.3. Recombinant protein production and purification

Primers (listed in Supplementary Table S2) were designed to amplify Bkb or An1 subunits from PI-1 or PI-2a of *S. agalactiae* NEM316 chromosomal DNA. These were cloned into ligation-independent expression vector pET46-Ek-LIC (Novagen) according to manufacturer's instructions. This

Download English Version:

https://daneshyari.com/en/article/6135650

Download Persian Version:

https://daneshyari.com/article/6135650

Daneshyari.com