

Genome Analysis Reveals Pili in Group B *Streptococcus*

Peter Lauer,^{1*} Cira D. Rinaudo,¹ Marco Soriani,¹
Immaculada Margarit,¹ Domenico Maione,¹ Roberto Rosini,¹
Anna Rita Taddei,² Marirosa Mora,¹ Rino Rappuoli,¹
Guido Grandi,¹ John L. Telford^{1†}

Group B *Streptococcus* (GBS) is the major cause of neonatal sepsis in developed countries. Maternal opsonic antibodies to surface polysaccharide structures can cross the placenta and correlate with protection of the child (1). While screening the genomes of multiple GBS strains (2), we identified two surface exposed antigens, GBS80 (TIGR annotation SAG0645) and GBS104 (SAG0649), that mediated complement-dependent, opsonophagocytic killing of virulent GBS bacteria and conferred passive protection against GBS challenge in a mouse maternal immunization model (3). The genes coding for these two

strains on reducing SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblotted them with antisera specific for the GBS80 and GBS104 proteins (6). In addition to bands corresponding to the predicted molecular weights of the monomeric proteins, both antisera revealed a ladder of bands ranging from 150 kD to beyond the resolution of the 3 to 8% gradient gels used (Fig. 1B).

Immunogold electron microscopy of GBS80 (6) revealed pilus-like structures extending from the bacterial surface (Fig. 1C). In a strain carrying a plasmid that overexpressed GBS80 (6), antisera to GBS80 stained ex-

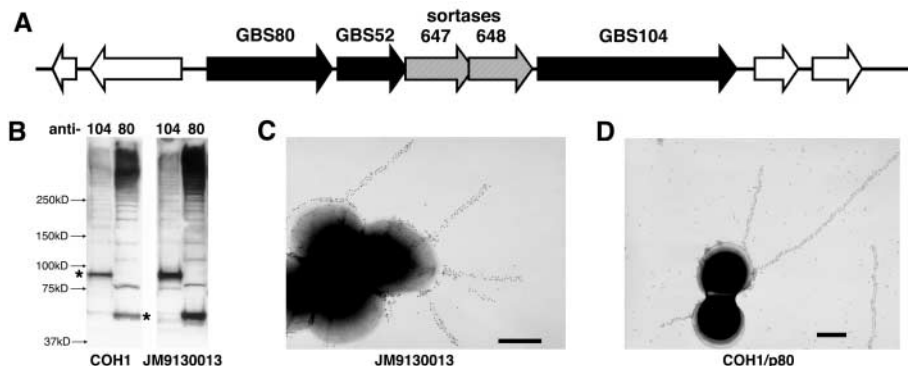


Fig. 1. (A) Schematic representation of the operon containing GBS80 and GBS104. (B) Immunoblots of total protein extracts of GBS bacteria with antisera specific for GBS80 and GBS104. The antisera used are given above the lanes, and the strains used are below the lanes. The positions of the monomeric forms of GBS80 (53 kD) and GBS104 (91 kD) are marked by asterisks. (C) Immunogold labeling and transmission electron microscopy of GBS80 in strain JM9130013, showing long pilus-like structures. (D) Immunogold staining of GBS80 in a strain of GBS (COH1) transformed with a plasmid (pGBS80) capable of overexpressing the protein. Scale bars, 500 nm.

proteins are part of an operon containing five genes (Fig. 1A). GBS80, GBS104, and a third protein, GBS52 (SAG0646), contain the LPXTG motif found in surface proteins usually attached to the cell wall peptidoglycan. The other two genes (SAG0647 and SAG0648) code for sortase enzymes similar to those known to catalyze the covalent linkage of LPXTG motif proteins to the peptidoglycan (4).

Mouse antisera raised against recombinant GBS80 and GBS104 proteins stained the surface of intact GBS bacteria of strain COH1 (serotype III) and strain JM9130013 (serotype VIII) in flow cytometry (5). We separated total protein extracts of bacteria from these

extremely long structures on the bacterial surface (Fig. 1D). Antisera specific for GBS104 also stained the pilus-like structures, but much less intensely (5). Neither immunogold labeling nor the high molecular weight ladder was observed with GBS80 antisera in a strain lacking the GBS80 gene; however, staining was recovered when we transformed this strain with a plasmid expressing GBS80 (fig. S1). Deletion of the genes coding for sortases 647 and 648 revealed that both are required for the correct assembly of the pilus (5). Thus, the high molecular weight covalent polymers detected by SDS-PAGE corresponded to pilus-like structures, and the length of the pili

appeared to depend on the level of expression of GBS80.

Numerous pili or fimbriae are essential virulence factors and protective antigens in Gram-negative bacteria (e.g., *Neisseria meningitidis* and *N. gonorrhoeae*), where they are involved in adhesion of the bacteria to eukaryotic cells (7). In Gram-positive bacteria, pili have been described in *Corynebacterium diphtheriae*, where they are formed by covalent polymerization of pilin subunits catalyzed by particular sortase enzymes (8). Pilus-like structures have also been detected in some other Gram-positive bacteria (9); however, very little is known about their function, and they have not been described in any of the most important species of *Streptococcus* that are pathogenic to humans: GBS, Group A *Streptococcus*, and *Streptococcus pneumoniae*.

The presence in GBS of pilus-like structures composed of antigens that confer protection in a mouse model of maternal immunization suggests that pili may play an important role in the virulence of Gram-positive bacteria as well as in Gram-negative. These macromolecular structures, which are as long as the bacteria, may not have been detected by conventional approaches in Group B *Streptococcus* because they are not readily visible in electron microscopy of samples prepared by standard negative staining techniques. Genome surveys may therefore reveal other important features of pathogens hitherto missed by classical methodologies.

References and Notes

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Supporting Online Material

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Materials and Methods

Fig. S1

References and Notes

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¹Chiron Srl, Via Fiorentina 1, 53100 Siena, Italy. ²Centro Interdipartimentale di Microscopia Elettronica, University of Tuscia, Viterbo, Italy.

*Present address: Cerus Corporation, 2411 Stanwell Drive, Concord, CA 95420, USA.

†To whom correspondence should be addressed.
E-mail: john_telford@chiron.com