Lmb, a Protein with Similarities to the LraI Adhesin Family, Mediates Attachment of *Streptococcus agalactiae* to Human Laminin

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**Streptococcus agalactiae** is a leading cause of neonatal sepsis and meningitis. Adherence to extracellular matrix proteins is considered an important factor in the pathogenesis of infection, but the genetic determinants of this process remain largely unknown. We identified and sequenced a gene which codes for a putative lipoprotein that exhibits significant homology to the streptococcal LraI protein family. Mutants of this locus were demonstrated to have substantially reduced adherence to immobilized human laminin. The nucleotide sequence of the gene was subsequently designated *lmb* (laminin binding) and shown to be present in all of the common serotypes of *S. agalactiae*. To determine the role of Lmb in the adhesion of *S. agalactiae* wild-type strains to laminin, a recombinant Lmb protein harboring six consecutive histidine residues at the C terminus was cloned, expressed, and purified from *Escherichia coli*. Preincubation of immobilized laminin with recombinant Lmb significantly reduced adherence of the wild-type strain O90R to laminin. These results indicate that Lmb mediates the attachment of *S. agalactiae* to human laminin, which may be essential for the bacterial colonization of damaged epithelium and translocation of bacteria into the bloodstream.

The expression of cell surface receptors determines adhesive properties of streptococci, which include binding to eukaryotic extracellular matrix (ECM) proteins, epithelial cells, and endothelial cells, as well as to other bacteria. The LraI (lipoprotein receptor antigen I) family of surface-associated lipoproteins is involved in the coaggregation of *Streptococcus gordonii* with *Actinomyces naeslundii*, the adherence of *S. sanguis* to the salivaary pellicle, the binding of *S. parasanguis* to a platelet fibrin matrix (14, 37), and the adherence of *S. pneumoniae* to type II pneumocytes (3). Previously identified members of this family are PsaA from *S. pneumoniae*, FimA from *S. parasanguis*, SsaB from *S. sanguis*, EfaA from *Enterococcus faecalis*, ScbA from *S. cristai*, and ScaA from *S. gordonii*. Proteins of this family appear to serve a dual role in adhesion and transport; they are located in ABC transporter-type operons and code for lipoproteins. Similarities between the deduced proteins of *lraI* genes and MntC, an Mn$^{2+}$ transporter of *Synechocystis*, have been described (1), and recently Mn$^{2+}$ transporter activity was demonstrated for PsaA of *S. pneumoniae* (5) and ScaA of *S. gordonii* (17). It has been proposed that the LraI proteins together with other proteins constitute a large family of metal transporters (5). With regard to pathogenicity, PsaA of *S. pneumoniae* and FimA of *S. parasanguis* have been shown to be essential for virulence in animal models (3, 37), and immunogenic properties were demonstrated for EfaA (19), FimA (37), and PsaA (35), indicating their potential use as vaccine candidates.

*S. agalactiae* (group B streptococcus [GBS]) is one of the most important neonatal pathogens, causing 1.8 cases of septicemia or meningitis per 1,000 live births (40). Despite adequate antimicrobial therapy, mortality rates still range between 5 and 30% (38). In addition, recent studies have found an increasing number of serious infections in adults (7, 8). Several virulence factors that contribute to the pathogenesis of the disease have been identified: capsular polysaccharides (39), CAMP factor (9, 28), hemolysin (24), and C proteins (23). The adherence of *S. agalactiae* to immobilized fibronectin has been implicated in the pathogenesis of disease (34), but genetic determinants for the adherence of *S. agalactiae* to ECM proteins have not been identified.

Laminin, a 900-kDa glycoprotein, is a major component of the basement membrane. It is composed of three distinct polypeptide chains (A, B1, and B2) which reversibly assemble to form the macromolecular structure. Functions of laminin include the formation of the basement membrane by interaction with other basement membrane components and the development and maintenance of cellular organization. *S. agalactiae* has been demonstrated to damage the pulmonary epithelium (24), a process that leads to the exposure of underlying basement membrane structures. Thus, the adhesion to basement membrane components may be critical for the bacterial colonization of damaged epithelium and invasion of bacteria into the bloodstream.

In this paper, we describe the identification of a putative lipoprotein with homology to the streptococcal LraI family in GBS. We show that mutants of the genetic locus are deficient in adherence to immobilized laminin and that the recombinant protein inhibits adherence of the wild-type strain to human laminin.

**MATERIALS AND METHODS**

**Bacterial strains.** The *Escherichia coli* and *S. agalactiae* strains used in this study are listed in Table 1. *E. coli* DH5α served as the host for recombinant plasmid pG1-1 harboring chromosomally integrated *pG1-1* vectors. *E. coli* BL21 was used for the expression of recombinant protein from plasmid pET21a, and *E. coli* XL1-Blue MRF and XLOLR (Stratagene, Heidelberg, Germany) were used as hosts for phages Lambda ZAP Express and ExAssist, respectively.

*S. agalactiae* isolates were cultured on Columbia agar (Oxoid, Basingstoke, England) supplemented with 5% sheep blood, in Todd-Hewitt broth (THB) (Oxoid) or in THB supplemented with 0.5% yeast (THY) at 37°C. Mutant strains harboring chromosomally integrated *pG1-1* vectors were maintained in medium containing 5 mg of erythromycin per liter at a temperature of ≥37°C.
Growth rates of wild-type and mutant strains were determined by measuring optical density at 600 nm (OD$_{600}$) in THY or THY supplemented with MnCl$_2$.

**General DNA techniques.** Standard recombinant DNA techniques were used for nucleic acid preparation and analysis. PCR was carried out with strains 872 Spellenberg et al. Infec. Immun.

**Plasmids**

- pUC18
- pG1 host5
- pET21a
- pBS1876
- pBS1817
- pBS1815

**Strains**

- E. coli DH5α
- XLI-Blue MRF
- BL21(DE3)
- S. agalactiae R268
- O90R (ATCC 12386)
- ATCC 12400
- ATCC 27591
- 18 RS 26
- CNCTC 13/63
- CNCTC 1/82
- M 1A-0008
- 92-085
- 87-603
- JM9-130013
- Lmb-k1
- Lmb-k2

**TABLE 1. Bacterial strains and plasmids used**

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DNA fragments were separated according to size by a salt gradient technique (12). Fractions containing fragments 2 to 9 kb in length were ligated with BamHI-digested λ arms and packaged by using a Gigapack II packaging kit (Strategene). Further processing and plaque lifting were done according to the manufacturer’s instructions. The library was screened by hybridization with PCR products at 65°C overnight. The PCR products were labeled by adding Dig-dUTP (Boehringer) at a final concentration of 5 μM to the reaction mixture. Detection of positive plaques by CSPD (Serva) was done as instructed by the manufacturer.

**Construction of lmb mutants.** Plasmid pG1 host5 was used for targeted genetic mutagenesis of lmb. Two mutants of the wild-type strain O90R (Lmb-k1 and Lmb-k2) were created by plasmid insertion at nucleotides 495 and 777, respectively, of the lmb gene. Internal fragments of the lmb gene were amplified by PCR with primers 5’-ACC GTC TGT AAA TGA TGT GG-3’ and 5’-GAT TGA CGT TGT CTG C-3’. The resulting PCR products were labeled by adding Dig(digoxigenin)-dUTP (Boehringer) at a final concentration of 5 μM. Hybridizing fragments were visualized by diatomaceous earth (Stratagene). Further processing and plaque lifting were done according to the manufacturer’s instructions. Plasmid and PCR products were sequenced on an ABI 373 automated DNA sequencer, using an ABI PRISM dye terminator cycle sequencing kit (PE Applied Biosystems, Weiterstadt, Germany) as instructed by the manufacturer. Plasmid DNA was isolated and purified by using a Qiaprep Spin Miniprep kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Plasmid and PCR products were sequenced on an ABI 373 automated DNA sequencer, using an ABI PRISM dye terminator cycle sequencing kit (PE Applied Biosystems, Weiterstadt, Germany) as instructed by the manufacturer. Plasmid DNA was isolated and purified by using a Qiaprep Spin Miniprep kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Plasmid and PCR products were sequenced on an ABI 373 automated DNA sequencer, using an ABI PRISM dye terminator cycle sequencing kit (PE Applied Biosystems, Weiterstadt, Germany) as instructed by the manufacturer.

**Phage techniques.** A Lambda ZAP Express library of strain O90R was created as described by Podbielski et al. (29). Briefly, 200 μg of genomic DNA was digested with 0.2 U of Sau3A (Boehringer) for 30 min at 37°C. The resulting DNA fragments were separated according to size by a salt gradient technique. Fractions containing fragments 2 to 9 kb in length were ligated with BamHI-digested λ arms and packaged by using a Gigapack II packaging kit (Strategene). Further processing and plaque lifting were done according to the manufacturer’s instructions. The library was screened by hybridization with PCR products at 65°C overnight. The PCR products were labeled by adding Dig-dUTP (Boehringer) at a final concentration of 5 μM to the reaction mixture. Detection of positive plaques by CSPD (Serva) was done as instructed by the manufacturer.

**Expression of Lmb in E. coli.** The lmb gene was cloned into the pET21a expression vector (Novagen, Madison, Wis.) in E. coli BL21(DE3) (Novagen) for high-level expression and purification over a Ni$_2$-column. To construct the pET21a::lmb vector, nucleotides coding for amino acids 19 to 306 were amplified by PCR using primers 5’-GCC GAC GAT ATG TGT GAT GAG AAC ACC A-3’ and 5’-GCC GCG CTG CAG CTT AAA TCT GAA GAC CAC TTC C-3’ (the newly introduced restriction sites Ndel and XhoI) were underlined). Resulting PCR products were the vector were digested with BamHI and XhoI and ligated, and transformed into E. coli. Chromosomal integration into strain O90R was performed as previously described (21). To confirm correct chromosomal insertion of the plasmid, genomic DNA of mutant Lmb-k1 and wild-type strain O90R was digested with the restriction endonuclease EcoRI or XhoI and probed with a nucleotide probe directed to the duplicated fragment of lmb. PCR with primers annealing to vector sequences and genomic nucleotide sequence upstream or downstream of the duplication site followed by DNA sequencing of PCR products was used to confirm chromosomal insertion for both mutants.

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Lmb MEDIATES ATTACHMENT TO LAMININ

FIG. 1. Amino acid sequence alignment of LraI proteins (S. pneumoniae PsA, S. parasanguinis FimA, S. sanguis SibA, S. gordoni StacA, S. crista SchA, and E. faecalis EfaA) and Lmb. Alignment and determination of consensus sequence were performed with the MultiAlin program (http://www.toulouse.inra.fr). Amino acid residues of Lmb matching the consensus sequence are shown in boxes. Highly conserved residues (consensus level of 90%) are represented as capital letters in yellow; important residues (consensus level of 75%) are shown in red. Parameters: gap weight, 2; gap length weight, 2; mismatches, 4.

the expression of recombinant protein, E. coli BL21(DE3) (Novagen) harboring the pET22b::Lmb construct was grown to an OD600 of 0.6 in Luria-Bertani medium, and protein expression was induced by 1 mM isopropyl-1-thiogalactopyranoside (IPTG) for 2 h. Cells were pelleted, resuspended in 1 ml of lysis buffer (50 mM Tris-HCl [pH 8.0], 300 mM NaCl, 20 mM imidazole), and sonicated. Recombinant Lmb was purified from lysed cells by passage over a commercial nickel affinity matrix (Ni-NTA [nickel nitrilotriacetic acid] Spin kit; Qiagen) and eluted under native conditions according to the manufacturer’s instructions. The eluate was subjected to 8 to 25% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using the Phast system (Pharmacia LKB, Uppsala, Sweden) and visualized by silver staining.

RESULTS

Identification of Lmb. Fragments of chromosomal DNA from GBS strain R268 were amplified by PCR using degenerate primers directed toward the conserved glycine-rich G1 and G2 blocks of bacterial sensor proteins, which resemble nucleotide binding domains (27). This method has previously been used to amplify the nucleotide sequence of cell surface-asso-
Comparison of the deduced amino acid sequence with sequences of previously identified members of the LraI adhesin family revealed 47% homology and 27% identity with PsaA of *S. pneumoniae*. Similarities to the other LraI proteins were between 36 and 46% (Fig. 1). In addition, there was 30% identity with Adc, a novel Zn transporter of *S. pneumoniae* which presumably binds Zn through a histidine-rich region of the protein. Lmb, however, does not possess a similar domain. Jenkinson (14) proposed four common structural domains of the LraI proteins: a 20-residue hydrophobic leader sequence that is cleaved off by signal peptidase II; two transmembrane domains, B1 and B2; and the α region, which is assumed to be exposed to the cell surface and comprises the solute binding region of the protein. These domains appear to be conserved in the *lmb* gene product (Fig. 2). Interestingly, amino acid residues 152 to 197 of Lmb, corresponding to the α domain of the LraI family, exhibit 50% homology to the human laminin B2 chain (Fig. 2).

**Presence and expression of the lmb gene in various GBS serotypes.** To determine the distribution of *lmb* in various *S. agalactiae* strains, a fragment of the gene was amplified by PCR and used as a hybridization probe for a Southern blot of GBS serotypes Ia, Ib, Ic, and II to VIII. Hybridization of the probe with chromosomal DNA could be detected as a single band in all of the serotypes tested (Fig. 3); immunofluorescence tests performed on the different serotypes confirmed the expression of the protein in these strains.

**Transcription analysis of the lmb locus.** Several of the homologous streptococcal *lraI* genes were reported to be polycistronically transcribed (3, 10, 13, 18). To analyze transcription of the *lmb* locus in GBS, RT-PCR was performed with RNA isolated from strain R268. For the RT reaction, we used a reverse primer which anneals to nucleotides 191 to 174 of the start codon. The subsequent PCR amplified a 930-bp product consisting of the last 716 nucleotides of *lmb*, the intergenic region, and the first 191 nucleotides of the second open reading frame (Fig. 4), which supports the hypothesis that *lmb* and the second open reading frame are transcribed together. To confirm that the specific PCR product originated from mRNA, controls were performed on a portion of the RNA preparation that had not been subjected to an RT reaction.

**Subcellular localization of Lmb.** Based on the homology of the deduced protein with other members of the LraI adhesin family and the presence of a putative signal peptidase II recognition site, we hypothesized that Lmb is localized at the cell surface (Jaeger et al., 1994). Several lines of evidence support this hypothesis. Lmb contains this atypical recognition sequence (32).

**Nucleotide and protein sequence analysis.** Nucleotide sequence analysis identified transport proteins in gram-positive organisms (2). The resulting PCR products were purified and cloned into plasmid pUC18. Nucleotide sequences of the inserts were determined by automated DNA sequencing. Comparison of the deduced amino acid sequence with the GenBank database entries revealed that 3 of 42 clones harbored overlapping fragments of a gene with significant homology to the streptococcal LraI family revealed 47% homology and 27% identity with PsaA of *S. pneumoniae*. Similarities to the other LraI proteins were between 36 and 46% (Fig. 1). In addition, there was 30% identity with Adc, a novel Zn transporter of *S. pneumoniae* which presumably binds Zn through a histidine-rich region of the protein. Lmb, however, does not possess a similar domain. Jenkinson (14) proposed four common structural domains of the LraI proteins: a 20-residue hydrophobic leader sequence that is cleaved off by signal peptidase II; two transmembrane domains, B1 and B2; and the α region, which is presumed to be the solute binding domain. (C) Amino acid sequence alignment of the α region of Lmb with the laminin B2 chain. The alignment was performed with the BLASTp program at the National Center for Biotechnology Information web site.

**Fig. 3.** (A) Analysis of the *lmb* gene in various GBS serotypes by Southern hybridization. Genomic DNA was digested with EcoRI and transferred to a nylon membrane. Hybridization was performed with a Dig-dUTP-labeled fragment of *lmb* generated by PCR. Lanes: M, molecular size markers (in nucleotides); 1, GBS strain O90R; 2 to 11, specific serotypes as indicated above the lanes. (B) Southern analysis of GBS strain O90R and the isogenic mutant Lmb-k1. Genomic DNA of the parent (lanes 1 and 2) and (lanes 3 and 4) mutant strains was digested with EcoRI (lanes 1 and 3) or XbaI (lanes 2 and 4). Hybridization was performed with a probe directed to the internal fragment of *lmb* that was used for insertion duplication mutagenesis.
surface of the bacterial cell. The subcellular localization was determined by Western blot analysis with a Lmb-specific antibody after cytoplasmic and membrane fractions of the cells were separated by ultracentrifugation. A single band in the membrane fraction corresponded to the predicted molecular mass of 34 kDa and the size of the recombinant Lmb protein (Fig. 5). Two smaller bands, probably representing degradation products, are present in the lane with the recombinant protein. Preimmune rabbit serum did not react with control blots. The results demonstrate that Lmb is associated with the bacterial membrane fraction. Surface exposure of the protein was investigated with an immunofluorescence test. Anti-Lmb antibodies were used to detect Lmb on the surface of intact bacterial cells, and a fluorescence-labeled secondary antibody was used to visualize the binding of anti-Lmb to the bacteria. Results demonstrate that the protein is located on the surface and that the most intense fluorescence staining is seen at the margins of the cells (Fig. 6).

**Construction of lmb mutants by insertion duplication mutagenesis.** The plasmid pG <sup>H</sup>host<sup>5</sup> (4) was used for targeted genetic mutagenesis of <i>lmb</i>. Two mutants of the wild-type strain O90R (Lmb-k1 and Lmb-k2) were created by insertion duplication mutagenesis at nucleotides 495 and 777, respectively, of the <i>lmb</i> gene (Fig. 2). Correct chromosomal insertion of the plasmid was confirmed by either Southern blot hybridization (Fig. 3) or PCR and subsequent DNA sequencing of both mutants.

**Growth properties of S. agalactiae upon Mn<sup>2+</sup> substitution.** It was recently reported that the reduced growth rates of <i>psaA</i> mutants which could be improved by the addition of micromolar concentrations of Mn<sup>2+</sup> to the culture medium (5). Therefore, growth of the <i>lmb</i> mutants in regular THY and THY medium supplemented with Mn<sup>2+</sup> was determined by OD measurements. Growth rates and final growth densities of the wild-type and mutant strains showed no significant differences if cultured in THY or in THY supplemented with Mn<sup>2+</sup> to final concentrations of 5 and 10 μM (data not shown). These results indicate that the Mn<sup>2+</sup> growth requirements are satisfied in THY, which contains approximately 1 μM Mn<sup>2+</sup>, and that adhesion deficits of <i>lmb</i> mutants are not attributable to impaired growth rates.

**Adherence to ECM proteins.** Since several LtaI proteins were reported to be adhesins and Lmb exhibits homologies to the human laminin B2 chain, we tested the adherence of GBS wild-type and mutant strains to immobilized human placental laminin. Adherence of the two independently derived <i>lmb</i> mutants (Lmb-k1 and Lmb-k2) was significantly less than that of the wild-type strain O90R, in both cases reaching only 25% of the wild-type level (Fig. 7). Reduced adherence of <i>lmb</i> mutants was found with a wide range of bacterial concentrations and was consistent across incubation times with human laminin ranging from 30 to 300 min. To investigate the possibility that binding to the immobilized laminin is mediated by a contaminant of the laminin preparation, we tested the binding of wild-type and mutant strains to collagen IV, which is present in trace amounts in the laminin preparation. Neither the wild-type nor the mutant strain exhibited significant binding to collagen IV. Binding of the wild-type strain to collagen IV was less than 2% of the binding observed for laminin (data not shown). A screen for any major contaminants in the laminin preparation performed by protein gel electrophoresis and subsequent silver staining did not reveal the presence of any unexpected proteins bands (data not shown).

**Influence of recombinant Lmb protein on the adherence of the wild-type strain.** To test the hypothesis that Lmb protein itself interacts with human laminin, a recombinant protein was generated. The recombinant protein could be visualized as a band of approximately 34 kDa upon SDS-PAGE, which is consistent with the predicted molecular mass of Lmb (Fig. 8). To evaluate the influence of Lmb on adherence of the wild-type strain, the immobilized laminin was incubated with recombinant protein prior to adherence assays. Preincubation with recombinant Lmb reduced the adherence of the wild-type strain significantly, to 60% of the initial values (Fig. 7). Interestingly, adherence of the isogenic <i>lmb</i> mutants was slightly increased under the same conditions, possibly because the recombinant protein functions as a bridging molecule between the mutants and laminin.
Influence of Mn$^{2+}$ supplementation on adherence. To investigate if supplementation with Mn$^{2+}$ affected the adherence of bacteria to immobilized laminin, the wild-type strain and mutant strain Lmb-k1 were grown in THB or THB supplemented to a concentration of 10 mM Mn$^{2+}$ and then subjected to the adherence assay in the presence of 10 mM Mn$^{2+}$. Under these conditions, adherence of the mutant strain remained significantly less than that of the wild-type strain (Fig. 7); the level for the mutant reached 34% of the wild-type level, demonstrating that the effect on adherence to immobilized human laminin cannot be circumvented by growing cells in the presence of excess Mn$^{2+}$ or adding Mn$^{2+}$ to the adherence assay.

**DISCUSSION**

Pathogenic bacteria frequently express surface proteins that adhere to components of the mammalian ECM. The interaction of microorganisms with ECM proteins can promote bacterial colonization of damaged tissues. Despite considerable

![Image](http://iai.asm.org/)
understanding of the molecular mechanisms of this process in other streptococcal species, genetic determinants for the adherence of *S. agalactiae* to ECM proteins have not been identified. In this investigation, we characterized the gene encoding a protein of *S. agalactiae* with similarities to the LraI adhesin family that mediates the attachment of GBS to laminin and was subsequently designated *lmb*. Comparison of the deduced amino acid sequence with LraI proteins showed closest homologies to PsaA of *S. pneumoniae*. However, compared to the conservation between proteins of this family present in other streptococci, the gene of GBS is distinct. Within the group of oral streptococci and the genetically related streptococci, the gene of *S. pneumoniae* similarities range between 80 and 93%. The results could reflect the more distant relationship of other streptococcal species, genetic determinants for the adherence of *S. agalactiae* and *S. parasanguis* to saliva-coated hydroxylapatite by purified ScaA or FimA is not due to direct binding of these proteins but rather reflects a requirement for Mn$^{2+}$ (5). To investigate if the effects of an Lmb mutation are influenced by the availability of Mn$^{2+}$, culture of bacterial cells and adherence assays were performed under supplementation with Mn$^{2+}$. Mutants grown in THB containing approximately 1 μM Mn$^{2+}$ and THB supplemented to an Mn$^{2+}$ concentration of 10 μM both exhibited significantly reduced adherence to immobilized laminin, demonstrating that the reduced binding properties cannot be explained by Mn$^{2+}$ deficiency alone. Our results indicate a function of Lmb in the adhesion to laminin that is distinct from the putative role as a Mn$^{2+}$ transporter.

**ACKNOWLEDGMENTS**

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**REFERENCES**


