Structure of *scpB-lmb* Intergenic Region as Criterion for Additional Classification of Human and Bovine Group B Streptococci

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Abstract

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The relative location of *scpB* and *lmb* genes in group B streptococcal (GBS) strains was studied. These genes were found in all the strains isolated from human and only in 9% of strains isolated from dairy cows. The *scpB* gene was located upstream of the *lmb* gene, however, three different structures of *scpB-lmb* intergenic region were identified. These genes could be separated by the 164 bp spacer region (type 1), by this spacer region with insertion sequence IS1548 (type 2) or by this spacer region with intron GBSi1 (type 3). The presence of different insertion sequences (IS861, IS1548, ISSa4 and IS1381) was compared with serological types of GBS strains and the types of *scpB-lmb* intergenic region structure. As a result, 10 different genetic variants were identified in GBS. The genetic variants No. 1 - No. 9 were found in human strains while the variant No.10 was found only in bovine strains. The mutually exclusive presence of insertion sequences IS1548, ISSa4 and the type 3 structure of *scpB-lmb* intergenic region was demonstrated. The structure of *scpB-lmb* intergenic region was found to be useful criterion for additional classification of GBS strains.

Streptococcus agalactiae, classification, insertion sequence, intron

Streptococcus agalactiae (group B streptococcus, GBS) is an important human and animal pathogen that causes invasive infections in neonates as well as mastitis of the dairy cows (Regan at al. 1996; Keefe 1997). Recently the numerous genes encoding for the potential virulence factors were identified in GBS (Jones et al. 2000). In particular, the genes *scpB* and *lmb* encoding for C5a peptidase and laminin binding protein, respectively, were considered to be involved in GBS virulence (Chmouryguina et al. 1996; Spellerberg et al. 1999). Previously we analyzed a large collection of GBS strains and found that the genes *scpB* and *lmb* were present in all the strains of human origin. At the same time these genes were found only in a few strains of bovine origin (Dmitriev et al. 2002a; Dmitriev et al. 2002b). After pulsed field gel electrophoresis we found that the genes *scpB* and *lmb* were closely located in GBS genome (Dmitriev et al. 2002a; Dmitriev et al. 2002b). The goal of the present study was to further investigate the precise relative location of *scpB* and *lmb* genes in GBS strains.

Materials and Methods

Bacterial strains

A total of 169 epidemiologically unrelated GBS strains were analyzed. Among them, 79 strains were isolated from the pregnant women in Beijing and Guangzhou (P. R. China) in 1996-2000, 12 strains were isolated from pregnant women in Saint-Petersburg (Russia) in 1989-1995 and 78 strains were isolated from the dairy cows in different regions of Eastern Slovakia in 1999-2002. Bacteria were grown either in Todd-Hewitt broth or on 1.5% sheep blood agar at 37 °C overnight. After serological typing it was found that most of bovine strains were nontypeable. On the contrary, the human strains belonged to different serological types (Ia, Ia/c, Ib/c, II, II/c, II/R, III, III/R, V) and only 3 of 91 human strains (3.3%) were non-typeable (Table 2).

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Table 1
Primers used in the present study

Primer	Primer sequence (5' – 3')	Gene	Annealing temperature
274	GTAAAACGACGGCCAGTG, forward	pGEM-7Zf(+)	47 °C
275	CAGGAAACAGCTATGACCATG, reverse	pGEM-7Zf(+)	47 °C
P2	ACAATGGAAGGCGCTACTGTTC, forward	*scpB*	49 °C
P3	ACCTGGTGTTTGACCTGAACTA, reverse	*scpB*	50 °C
S0	GACGCAACACACGGCAT, forward	*lmb*	50 °C
S1	TGATAGAGCACTTCCAAATTTG, reverse	*lmb*	48 °C
0221-109	GTTATGAGCACTTTCTTCTTGGGAT, forward	*scpB*	51 °C
0221-110	AGCTGGTTACAACTGACATGCCT, reverse	*lmb*	51 °C
0408-152	GTTAAGCTTAGAAGATCTCCTCATGG, forward	IS1381	49 °C
0408-153	CCTTGATAACCACTGTCTGCCA, reverse	IS1381	49 °C
0206-127	TTGCGCAGTTGAATTGGATAG, forward	IS1548	49 °C
0206-130	TTCTCTAACTTCAATCTGTCCCCTA, reverse	IS1548	49 °C
0206-131	ATGACAGAGCTCGAGCGACTT, forward	IS861	49 °C
0206-132	TATCAGCCTTCTTACCAACCTCA, reverse	IS861	49 °C
0408-150	CTGAGCTAGGTATTAAGTCGCAAC, forward	ISSa4	49 °C
0408-151	AGCGTGTCTCAATGGTTCGT, reverse	ISSa4	49 °C

General DNA techniques

Most of the molecular genetic procedures were carried out according to previously published protocols (Maniatis et al. 1982). PCR was carried out for 30 cycles consisted of denaturation at 94 °C for 30 s, annealing at 47-51 °C (depending on the primers composition) for 1 min and elongation at 72 °C for 1 min. Sequencing of PCR products was performed using ABI Prism™ 377 Perkin-Elmer Sequencer and Big Dye Terminator Kit (Applied Biosystems). The primers for cloning and sequencing are listed in Table 1.

Table 2
Comparative analysis of the structure of *scpB-lmb* intergenic region and the presence of different insertion sequences in GBS strains

Genetic variant	Presence of different insertion sequences in GBS strains				Structure of *scpB–lmb* region	Number of the strains	Serotype
	IS1381	IS861	ISSa4	IS1548			
No 1	—	—	—	—	Type 1	7 (human)	Ia, II, III, V
No 2	+	—	—	—	Type 3	4 (human)	Ib/c, II/c, V, NT
No 3	+	—	—	—	Type 1	13 (human)	Ia, Ia/c, Ib/c, II/c, III, NT
No 4	—	+	—	—	Type 1	5 (human)	III
No 5	—	+	—	—	Type 3	7 (human)	III/R
No 6	+	+	—	—	Type 3	9 (human)	Ia/c, II/R
No 7	+	+	—	—	Type 1	12 (human)	Ia, Ia/c, Ib/c, II/c, NT
No 8	+	+	+	—	Type 1	21 (human)	II, II/c
No 9	+	+	—	+	Type 2	13 (human)	III, III/R
No 10	+	—	+	—	Type 1	7 (bovine)	III, NT
Total number						98	

Computer analysis

The nucleotide sequences of the genes were accessed through the GenBank database (http://www.ncbi.nlm.nih.gov/entrez). The sequences of the primers were designed using the computer program "OLIGO". Computer analysis of DNA was accomplished using BLAST (http://www.ncbi.nlm.nih.gov/BLAST). The 100 bp ladder (BioLabs) and λ-*Hin*dIII fragments were used as DNA molecular size standards when the sizes of PCR products were analyzed.

Nucleotide sequence deposition

The sequences data reported in this paper were deposited in the GenBank Nucleotide Sequence Database under accession numbers AY464086, AY464087 and AY464088.

Results and Discussion

Cloning and sequencing of the *scpB-lmb* intergenic region

Previously we demonstrated that the virulence genes *scpB* and *lmb* were present in all human GBS strains as well as in few bovine GBS strains (Dmitriev et al. 2002a). These genes were probably located on the 11 kb fragment of GBS genome (Dmitriev et al. 2002b). In the present study, PCR analysis of 169 epidemiologically unrelated GBS strains for the presence of *scpB* and *lmb* genes was performed. As expected, it was found that all 91 human strains and only 7 of 78 bovine GBS (9%) possessed both *scpB* and *lmb* genes. In this study two possibilities of the relative location of *scpB* and *lmb* genes were analyzed.

According to the first possibility, the *lmb* gene could be located upstream of the *scpB* gene. If true, PCR analysis employing the forward primer for the 3'-end of *lmb* gene and the reverse primer for the 5'-end of *scpB* gene could produce an amplifying fragment. In our experiments, the time of PCR elongation step was extended to 3 min, because the size of *scpB-lmb* intergenic region could be relatively large. However, both human and bovine GBS strains did not reveal any PCR products (data not shown).

According to another possibility, the *scpB* gene could be located upstream of the *lmb* gene. In order to analyze it, the forward primer 0221-109 for the 3'-end of *scpB* gene and the reverse primer 0221-110 for the 5'-end of *lmb* gene were designed (Table 1) and used in PCR. As a result, the PCR fragments were obtained in all human GBS as well as in bovine GBS strains with *scpB* and *lmb* genes. However, the sizes of PCR fragments were found to be different. Size of the smallest PCR product was about 400 bp. Other PCR fragments were larger and their sizes were approximately equal to 1.7 kb and 2.3 kb. All these data taken together demonstrated that *scpB* gene was located upstream of the *lmb* gene in GBS strains. However, it was obviously that the structure of *scpB-lmb* intergenic region could be different. Three GBS strains, i.e., 98-D60C, 97-3 and G-85V, that produced 400 bp, 1.7 kb and 2.3 kb PCR products, respectively, were used for further analysis. These 400 bp, 1.7 kb and 2.3 kb amplifying fragments were isolated from the agarose gel, cloned into the pGEM-7Zf(+) cloning vector and sequenced using the primers 274 and 275 (Table 1).

Different structures of the *scpB-lmb* intergenic region

After the sequencing of 400 bp PCR product it was found that *scpB* and *lmb* genes were separated by the spacer region. The size of spacer region was equal to 164 bp and such structure of *scpB-lmb* intergenic region was named as type 1 (Fig.1). After analysis of the *Entrez* database it was found that 164 bp spacer region was present in group A and B streptococci. Nucleotide sequence of this spacer DNA did not reveal any significant homology with other bacterial DNA genomes. In our study, the type 1 structure of *scpB-lmb* intergenic region was found in 58 of 91 human GBS strains tested (Table 2). As to the bovine GBS, the type 1 structure was found in all 7 strains that possessed *scpB* and *lmb* genes (Table 2). The type 1 structure was also found when the serotype III GBS genome was sequenced (Glaser et al. 2002).

The sequencing of the larger PCR product (about 1.7 kb) revealed the presence of the identical 164 bp spacer region between *scpB* and *lmb* genes. In addition to this region, the complete insertion sequence IS1548 (1316 bp) discovered in group A and B streptococci (Hoe et al. 1999; Tettelin et al. 2002) was found to be inserted in this spacer region. Insertion of IS1548 occurred 9 bp upstream of the putative promoter for *lmb* gene. Such structure of *scpB-lmb* intergenic region was named as type 2 (Fig. 1). This structure was found in 13 of 91 human strains (Table 2). The insertion of IS1548 resulted in the duplication of the target sequence that corresponds to the genetic features of the insertions of IS elements (Mahillon and Chandler 1998). In this study the target sequence was identified as TAAAAGATGG.

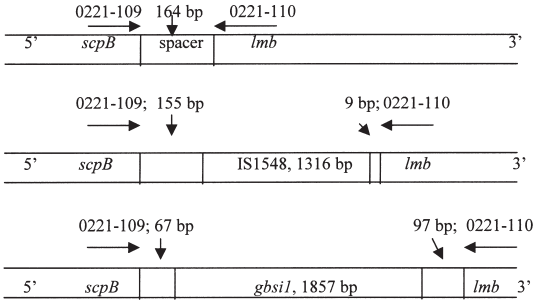

Fig. 1. Different structures of *scpB-lmb* intergenic region

The sequencing of the largest PCR product (about 2.3 kb) also revealed that *scpB* and *lmb* genes were separated by 164 bp spacer region. However, instead of IS1548, the intron GBSi1 (1857 bp) previously identified in group B streptococci (Granlund et al. 2001), was found within this spacer. Insertion of GBSi1 occurred 97 bp upstream of the putative promoter for *lmb* gene (Fig.1). Such structure of *scpB-lmb* intergenic region was named as type 3 (Fig.1) and it was found in 20 of 91 GBS human strains tested. Nucleotide sequence of *gbsi1* gene did not reveal any significant homology with other bacterial DNA sequences, however, it demonstrated the 99% homology with *gbsi1* genes sequenced in other GBS strains (Granlund et al. 2001; Tettelin et al. 2002; Bohnsack et al. 2002; Luan et al. 2003).

Occurrence of the different structures of *scpB-lmb* intergenic region among genetic variants of GBS

Recently we proposed that the presence of insertion sequences in GBS strains could be considered as an additional criterion for classification of GBS (Dmitriev et al. 2003). In this study, the PCR analysis of GBS strains for the presence of different insertion sequences (IS1548, IS861, IS1381, IS861) using the primers listed in Table 1 was performed and different genetic variants of GBS were identified (Table 2). These data were compared with the data of serological typing of the strains and the types of *scpB-lmb* intergenic region structure. As a result, the types 1 and 3 structures of the *scpB-lmb* intergenic region were revealed in the strains of various serotypes. At the same time, the type 2 structure was revealed only in the strains of serotypes III and III/R (Table 2).

After the comparative analysis, the strains of serotypes III and III/R were subdivided into several genetic variants (Table 2). This finding confirmed the presence of different genetic lineages among serotypes III and III/R that was previously determined by ribotyping, multilocus enzyme genotyping and long-range mapping (Bohnsack et al. 2002; Chatellier et al. 1996; Quentin et al. 1995). At the same time the results of the present study were in agreement with other publications demonstrated the difference of serotypes

III and III/R in comparison with the strains of other serotypes (Ellis et al. 1996, Hauge et al. 1996).

The strains with IS1548 were characterized by the type 2 structure while the strains with ISSa4 were characterized the type 1 structure. On the contrary, the GBS strains with IS861 as well as the GBS strains with IS1381 could be characterized by the types 1, 2 and 3 structures of *scpB-lmb* intergenic region. After the comparative analysis, the mutually exclusive presence of IS1548, ISSa4 and the type 3 structure of *scpB-lmb* intergenic region was demonstrated (Table 2).

Taken together, 10 different genetic variants were discovered among the strains tested (Table 2). Interestingly, the genetic variants No. 1 - No. 9 were found in human strains while the variant No. 10 was found only in bovine strains. The structure of *scpB-lmb* intergenic region was found to be useful criterion for additional classification of GBS strains. Indeed, the pairs of genetic variants No. 2 and No. 3; No. 4 and No. 5; No. 6 and No. 7; respectively, were differentiated according to the structure of *scpB-lmb* intergenic region (Table 2).

In conclusion, in present study three different structures of *scpB-lmb* intergenic region were revealed. The mutually exclusive presence of insertion sequences IS1548, ISSa4 and the type 3 structure was demonstrated. The structure of *scpB-lmb* intergenic region was found to be useful criterion for additional classification of GBS strains.

Usporiadanie *scpB-lmb* medzigénovej oblasti ako kritérium pre dodatočnú klasifikáciu bovinných streptokokov skupiny B

U kmeňov streptokokov skupiny B (GBS) bolo študované vzájomná umiestnenie *scpB* a *lmb* génov. Tieto gény boli nájdené u všetkých kmeňov izolovaných z šudí a iba u 9% kmeňov izolovaných z hovädzieho dobytka. *scpB* je umiestnený pred *lmb* genóm, avšak boli identifikované 3 rozdielne štruktúry *scpB-lmb* medzigénovej oblasti. Tieto gény môžu byť oddelené buď 164 bp oblasťou (typ 1) alebo oblasťou, v ktorej sa nachádza inzerčná sekvencia IS1548 (typ2) alebo oblasťou s intrónom GBSi1 (typ 3). Prítomnosť rôznych inzerčných sekvencií (IS861, IS1548, ISSa4 and IS1381) bola porovnaná so sérologickym typom GBS kmeňov a typom *scpB-lmb* medzigénovej oblasti. Na základe tohto štúdia bolo identifikovaných 10 rôznych genetických variantov GBS. Genetické varianty No. 1- No. 9 boli nájdené v humánnych kmeňoch, zatiaľčo genetický variant No. 10 bol nájdený iba v bovinných kmeňoch. Bolo dokázané vzájomné vylúčenie prítomnosti inzerčných sekvencií IS1548, ISSa4 a štruktúry *scpB-lmb* medzigénovej oblasti typu 3. štruktúry. Zloženie s*cpB-lmb* medzigénovej oblasti je vhodným kritériom pre ďalšiu charakteristiku kmeňov GBS.

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