JOURNAL OF BACTERIOLOGY, Nov. 2002, p. 6384–6386 Vol. 184, No. 22
0021-9193/02/$04.00+0 DOI: 10.1128/JB.184.22.6384–6386.2002

NOTES

Structural Heterogeneity of the Streptococcal C5a Peptidase Gene in *Streptococcus pyogenes*

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Received 14 June 2002/Accepted 22 July 2002

The 3′ ends of the genes for the C-terminal region of C5a peptidase from 15 *Streptococcus pyogenes* isolates were analyzed by PCR. Amplicons were found to differ in size. DNA sequence analysis revealed that the differences between PCR fragment sizes accorded with the number of R repeats in the C5a peptidase gene.

Streptococcus pyogenes (group A streptococci [GAS]) is the cause of a number of contagious human diseases, including pharyngitis, skin infections, and scarlet fever, as well as generalized infections like necrotizing fasciitis and toxic shock syndrome. It is well documented that GAS, together with group B streptococci (GBS) and group G streptococci, express on their surfaces streptococcal C5a peptidase (SCP) (1, 2, 3, 6). This enzyme is related to serine proteases and specifically cleaves the C5a complement component (3, 7), resulting in the inactivation of C5a and the destruction of its ability to act as an anaphilatoxin and a strong chemoattractant (11, 12, 13, 14, 16). Thus, SCP is considered an important virulence factor of pathogenic streptococci. However, virulent gene heterogeneity, which usually reflects the degree of host response, has so far not been demonstrated for SCP genes from different GAS strains (*scpA*). Moreover, *scpA* has been reported to be highly homologous to SCP genes from GBS (*scpB*) (4, 5); the genes were found to differ only by 51 bp. This region was characterized with so-called R repeats that encode the C-terminal part of SCP (3, 4).

In order to investigate the degree of *scpA* heterogeneity, several GAS isolates belonging to different M types and ribotypes were selected for the present study. SCP genes from GAS were analyzed by PCR and sequence analysis.

Bacterial strains and DNA techniques. GAS strains were isolated from patients. GAS strain SF370 serotype M1 and GBS strain 090R serotype Ia were obtained from the collection of the University of Oklahoma. Sera from the PHLS Respiratory and Systemic Infection Laboratory (Colindale, United Kingdom) were used to perform serotyping.

Chromosomal DNAs from GAS and GBS strains were extracted with phenol-chloroform and ethanol precipitation according to standard methods (15). Ribotyping of the strains was carried out by digestion with *Pvu*II and *Eco*RI (P and E ribotypes) and by using the *rrs* gene, labeled with digoxigenin, as a 16S rRNA probe (Roche, Mannheim, Germany). The sizes of DNA fragments were estimated with the computer program SEQAID.

Chromosomal DNA was used as a template for amplification by PCR (9, 20). The 5′-ACAATGGAAGGCTCTACTG TTC-3′ (forward) and 5′-ACCTGGTGTTTGACCTGAACT A-3′ (reverse) primers corresponded to the 3′ end of the SCP gene.

Sequencing was accomplished by the sequencing facility of the Microbiology Department of the University of Oklahoma. Computer analysis of DNA and amino acid sequences was performed with the Genetics Computer Group sequence analysis package (program manual for the Wisconsin Package, version 8). The analysis of the amino acid sequence for the region rich in proline, glutamic acid, serine, and threonine

TABLE 1. Sizes of SCP genes amplified by PCR and PEST analysis of the C-terminal parts of their products

Strain[a] (isolate no.)	16S rRNA gene profile[b]		Size of amplicon fragment (bp)	No. of PEST-positive sequences with a PEST score of:	
	*Pvu*II	*Eco*RI		9.91[c]	4.75[d]
090R	NA	NA	255	2	0
M1 (SF370)	NA	NA	357	2	1
M1 (11)	P3	E3	306		
M5 (8)	P5	E4	255	0	0
M6 (4)	P1	E1	357		
M6 (18)	P1	E2	306	1	1
M11 (7)	P1	E5	357		
M11 (19)	P1	E1	306		
M22 (12)	P3	E3	357		
M64 (13)	P3	E3	306		
M68 (24)	P1	E2	396	0	0
M83 (14)	P3	E3	357	1	2
M83 (20)	P3	E3	357		
M87 (15)	P3	E3	357		
M89 (2)	P2	E2	357		
M89 (10)	P2	E2	357		

[a] All strains are GAS with the exception of strain 090R, which is a GBS.
[b] NA, not analyzed.
[c] Amino acid sequence, PEQDGSGQTPD.
[d] Amino acid sequence, PEQDGSGQAPD.

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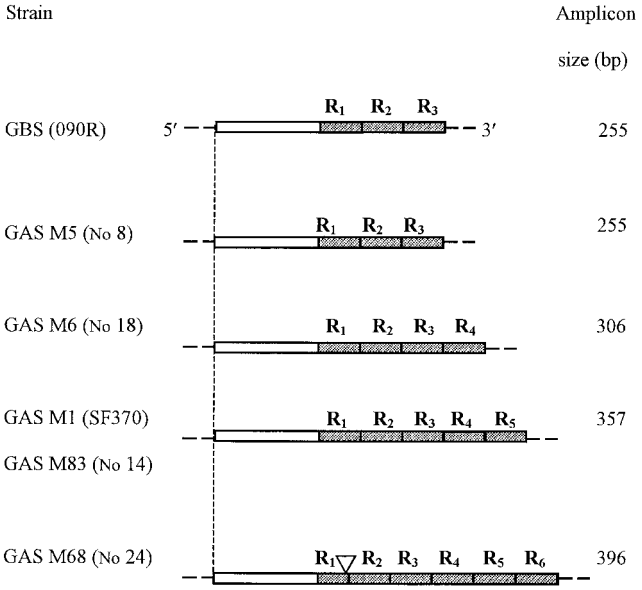

FIG. 1. Comparison of the 3′ ends of C5a peptidase genes. DNA sequence analysis revealed that SCP genes from GBS strain 090R and GAS strains M1 (isolate SF370), M5 (isolate 8), M6 (isolate 18), M68 (isolate 24), and M83 (isolate 14) differed from each other in their numbers of 51-bp R repeats. Symbols: shaded box, R repeats; ▽, 12-bp deletion.

(PEST-positive region) was conducted with the PEST FIND program (PC/Gene).

Amplification of SCP genes. The 3′ ends of *scpA* genes from 15 GAS strains and that of *scpB* from one GBS (090R) strain were amplified by PCR. All GAS examined revealed four different amplicon sizes that corresponded to the C-terminal partof SCP. The amplicon size of *scpB* was found to be 255 bp, as previously reported (8). The amplicon sizes of *scpA* genes ranged from 255 to 396 bp (Table 1). The amplicon sizes of *scpA* genes from strains of similar M types and different E ribotypes (strains M1, M6, and M11) were different. On the other hand, the amplicon sizes of *scpA* genes for strains of similar M types belonging to similar P and E ribotypes were identical (strains M83 and M89). The *scpA* amplicon sizes of 306 and 357 bp were found to be the most common. One strain (M68) among all GAS strains examined produced the 396-bp amplicon fragment. The amplified region of *scpA* from strain M5 was the smallest of all and equal in length to the 255-bp *scpB* fragment.

DNA sequencing and sequence analysis. Amplified fragments of *scpA*s from GAS of types M1 (isolate SF370), M5 (isolate 8), M6 (isolate 18), M68 (isolate 24), and M83 (isolate 14) (*scpA1*, *scpA5*, *scpA6*, *scpA68*, and *scpA83*, respectively) were cloned in *Escherichia coli* (DHIB) with subsequent sequence analysis. DNA sequence analysis revealed the highest degree of homology between *scpA83* and *scpA1* (97%) and *scpA5* and *scpB* (90%), which accorded with their similarity in amplicon size. A comparison of analyzed fragments of *scpB*s and *scpA*s from five GAS strains is schematically presented in Fig. 1. As shown in Fig. 1, all *scpA* genes examined differed from each other by 51-bp sequences, which resulted in various numbers of R repeats corresponding to 17 amino acids. Only one R repeat in *scpA68* contained a 12-bp deletion.

It was previously reported that the proline-rich C terminus of SCP carried several PEST-positive regions (4). The PEST regions were found to be sensitive to proteolysis, which led to rapid destruction of the proteins (17, 18, 19). The data from a deduced amino acid analysis of the C-terminal part of SCPs from GAS strains revealed significant differences in PEST scores (Table 1). SCPs from different isolates either did not comprise any PEST-positive regions (strains M5 and M68) or comprised several regions with a significant PEST score. Every 17-amino-acid repeat sequence except the last one contained a 6-residue motif (PDKKPE). It has been reported that the lysine pairs (labeled "KK") in the sequence of some proteins might play a role in proteolysis (10). The 6-residue sequence in the proline-rich region of SCP may also serve as a target for proteolysis.

Heterogeneity demonstrated in the *scpA* region encoding the C terminus of SCP can be explained by selective pressure from the host to delete the protease-sensitive regions from the surface-expressed proteins. This may be important when the bacteria (like GBS) have a tendency for long-lasting colonization in the protease-rich milieu. Another explanation might reflect the fact that the cleavage sites for eukaryotic proteases can be important for the secretion of SCP from the cell surface.

We are grateful to J. Ferretti for valuable help in DNA sequencing. We also thank A. Dmitriev for helpful discussions.

This work was supported by Public Health Service grant AI19304, NIH grant TW00188, and RFFI grant 00-04-49360a.

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