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ORIGINAL ARTICLE

Streptococcal pyrogenic exotoxin G gene in blood and pharyngeal isolates of *Streptococcus dysgalactiae* subspecies *equisimilis* has a limited role in pathogenesis

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Superantigen

Background: *Streptococcus dysgalactiae* subspecies *equisimilis* (SE) causes human infections that clinically resemble infections due to *Streptococcus pyogenes* (SP). SE expresses several virulence determinants initially identified in SP, including genes encoding streptococcal pyrogenic exotoxins. SE isolates from patients with toxic shock syndrome were found to harbor a gene designated *spegg*, which is similar to the SP pyrogenic exotoxin-G gene, termed *speG*. Other streptococcal pyrogenic exotoxins known to exist in SP were not detected.
Methods: To determine the prevalence of the superantigen gene, *spegg*, we examined 65 invasive SE from patients presenting from 1989 to 2008 with bacteremia secondary to a variety of illnesses including two patients who fulfilled the criteria for toxic shock syndrome, in comparison with 46 noninvasive pharyngeal isolates. All isolates were tested for the presence of *spegg* by polymerase chain reaction. Forty-four of the 65 blood isolates were also characterized by *emm* typing.
Results: *spegg* was identified in 49.2% and 69.5% of the blood and pharyngeal isolates, respectively. *emm* typing revealed the presence of 13 distinct types. There was no association between clinical presentation and the presence of *spegg*. We found an association between the presence of *spegg* and the *emm* type ($p < 0.001$). The *emm* types *stG*485

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and *stG*840 were more frequent among *spegg* positive isolates, and *stG*4222, *stG6*, and *stG*166b were associated with *spegg* negative isolates.
Conclusion: We found a high prevalence of *spegg* in invasive and noninvasive SE isolates, associated with specific *emm* types. Our finding suggests that this gene does not have a role in the pathogenesis of bacteremia.

Introduction

In recent years, *Streptococcus dysgalactiae* subspecies *equisimilis* (SE) has become increasingly recognized as causing a number of human infections that clinically resemble infections due to *Streptococcus pyogenes* (SP).[1,2] SE accounts for 5–8% of human streptococcal diseases.[3] Several cases of severe invasive disease and streptococcal toxic shock syndrome (STSS) associated with SE have been reported.[4,5] Unlike SP-induced STSS, underlying conditions have been noted in most patients with STSS related to SE. These patients had cardiopulmonary disease, alcoholism, diabetes mellitus, malignancy, lymphatic drainage disorders, and renal or hepatic failure.[6] Bacteremia due specifically to SE appears to be increasing in incidence.[7] SE expresses several virulence determinants initially identified in SP. They express a hyaluronic acid capsule, M protein, streptolysins O and S, streptokinase, C5a peptidase, and binding proteins for fibronectin, plasminogen, and immunoglobulin.[8] Besides these factors, SP strains express a large number of distinct streptococcal pyrogenic exotoxins.[8,9] These toxins belong to a family of T cell mitogens, or superantigens (SAgs),[10,11] and are capable of inducing T-lymphocyte proliferation that can result in a massive release of cytokines. In this manner, the streptococcal pyrogenic exotoxins are thought to contribute to the pathogenesis of severe invasive disease and STSS.[9] Most reported SAg genes are located on integrated prophage genomes, a situation that is putatively conductive to genetic transfer among different SP *emm* types as well as other streptococcal species.[12–15] Some SAgs, such as SMEZ, SPE-A, SPE-G, and SSA, show allelic variations, which are characterized by single or multiple amino-acid replacement.[16] At present, SP pyrogenic exotoxin A, C, G, and M genes (*speA*, *speC*, *speG*, and *speM*, respectively) as well as *ssa*, *smeZ* superantigen genes, have been found in human pathogenic SE.[3,13,17] As noted, SE possesses genes that encode molecules similar to SAgs. For example, *S. dysgalactiae* harbors a gene encoding a protein similar to SPE-G, which has been designated in different ways, such as *spegg* or $speG^{dys}$.[3,18,19] Hashikawa et al[19] analyzed the prevalence of SAgs in 12 clinical isolates of *S. dysgalactiae* from STSS cases by polymerase chain reaction (PCR) and found that only *spegg* was detected in seven isolates, with none of the other superantigen genes being detected in any of the strains. The purpose of our study was to determine the presence of *spegg* genes in a collection of invasive SE bacteremia isolates, and in comparison with noninvasive SE isolates, in order to identify the virulence mechanisms associated with *emm* types and severity of disease.

Materials and methods

Bacterial isolates

In this study, we investigated 65 human blood isolates of SE from patients admitted to our hospital from 1989 through 2008, and 46 human SE pharyngeal isolates obtained from ambulatory patients with sore throat. Bacteremic patients presented in a variety of ways, including cellulitis, septic arthritis, and septic shock. Two patients fulfilled the criteria for STSS. All isolates used in this study were identified by β-hemolysis as group G streptococci, and Lancefield antigen typing according to the manufacturer's instructions (PathDox Strep Grouping; DPC Diagnostic Products Corporation, Los Angeles, CA, USA). These isolates had been stored at −70°C in the laboratory collection. All isolates displayed large colonies and did not belong to the *S. anginosus* group.

Typing of M protein gene (*emm*)

A total of 44 SE randomly chosen blood isolates were available for *emm* typing. The M protein types of the SE strains were determined according to the M protein gene (*emm*) typing protocol by the sequencing of the *emm* gene (http://www.cdc.gov/ncidod/biotech/strep/protocol_emm-type.htm).

Detection of the superantigenic gene spegg by PCR

Crude chromosomal DNA extracts were prepared using mutanolysin and hyaluronidase as previously described.[20] The presence of *spegg* gene was determined using PCR. The forward primer was designed on the basis of the respective DNA sequences deposited in the NIH GenBank database, and a reverse primer was used as published previously[21]: spegg-F 5′-CACTTGAGTATGAAAATGTAGAG-3′ and spegg-R 5′-CTAGTGCGTTTTTAAGTAGATA-3′. PCR was carried out by denaturation over 5 minutes at 95°C, followed by 30 cycles of 30 seconds at 95°C, 30 seconds at 47.4°C and 1 minute at 72°C with a final extension at 72°C for 7 minutes. Validation of PCR product was made by sequencing three out of 32 positive isolates.

Statistical evaluation

Fisher's exact test and chi-square tests were used for statistical analysis. The statistical software used was Sigma Plot 16.0 software (SPSS Inc., Chicago, IL, USA). A p value less than 0.05 was considered significant.

Results

Results of the molecular characterization of all SE studied, and the clinical source of the bloodstream isolates are shown in Table 1. Of the 65 SE blood isolates and 46 pharyngeal isolates tested, 32 isolates in each group (49.2% and 69.5%, respectively) were positive for *spegg* amplification, yielding products of the expected size (578 bp). The difference between the blood isolates and the pharyngeal isolates was statistically significant ($p < 0.05$). The PCR product of one of the *spegg* positive blood isolates was subjected to sequencing. Alignment of these sequences against the NIH GenBank database, using Basic Local Alignment Search Tool (BLAST), revealed a nucleotide identity of 98% with the known *spegg* gene (accession no. AB105080.1). The M protein type was determined according to the *emm* typing protocol. As Table 2 shows, of the 44 SE blood isolates available for *emm* typing, we found 13 *emm* types. The *emm* type distribution among *spegg* positive and *spegg* negative blood isolates differed significantly, using the Fisher's exact test ($p = 0.00004$). *stG840* and *stG485* were the most prevalent *emm* types among *spegg* positive isolates (12/24), whereas *stG4222*, *stG6*, and *stG166b* were present mostly on *spegg* negative isolates. Among the invasive isolates, we could not find a correlation between presence of *spegg* and the clinical source of the isolates.

Table 2 Correlation between *emm* types and presence of *spegg*

emm types[a]	No. of isolates	
	spegg positive	*spegg* negative
stc36	2	0
stc74a	2	0
stG10	2	1
stG166b	0	4
stG245	0	2
stG4222	0	4
stG480	2	0
stG485	7	0
stG507	1	0
stG5420	0	1
stG6	1	6
stG6792	2	0
stG840	5	2

[a] The *emm* type distribution among *spegg* positive and *spegg* negative blood isolates differed significantly ($p = 0.00004$).

Discussion

The increasing number of genome sequencing projects has led to the identification of a substantial number of streptococcal SAgs in SP[21,22] and other streptococcal species. Recombinant SPE-G from SP has been characterized and shown to be a potent superantigen for the human host.[21] In earlier studies, *speG* was found on all analyzed SP isolates, suggesting a genomic origin.[15,23] There is an 87% similarity of the gene *spegg* of SE to *speG* of SP, suggesting that SE harboring this gene have the potential to produce SAgs and cause sepsis and toxic shock.[3] Hashikawa et al[19] analyzed the prevalence of SAgs in 12 clinical isolates of *S. dysgalatiae* from STSS cases by PCR and found that only *spegg* was detected (7/12 isolates), with none of the other 12 superantigen genes being detected in any of the strains. The fact that not all isolates of STSS patients had *spegg* suggests that there are other, as yet unidentified, superantigen genes in SE. The authors did not find an association between the presence of *spegg* and the *emm* type of isolates, suggesting that there is no selection of virulent SE clones.[19] Brandt et al[18] reported that six of the 46 SE isolated from human invasive infections were positive for *spegg*; however, in contrast to SP, none of them expressed mRNA in the reverse transcriptase-PCR assay, and they had no mitogenic activity in their culture supernatants, suggesting that they lack the ability to induce proinflammatory responses or T-cell activation.[18] Zhao et al[16] showed that recombinant SPEGG (rSPEGG) from bacteria isolated from human STSS did not stimulate human peripheral blood mononuclear cells effectively, suggesting that *spegg* plays a limited role in the severity of invasive SE infection in humans. In our study, only 32 of 65 (49.2%) SE isolates from invasive human infections were *spegg* PCR-positive, identical to a previously reported *spegg* allele from SE (GenBank accession no. AB105080.1). Although we did not examine *spegg* expression in SE blood isolates, its higher rate in pharyngeal isolates suggests that this gene has a limited role in causing invasive infection such as bacteremia. Recently, high and similar rates of *speG* were found among invasive and noninvasive SP isolates from Germany (93.8% and 89%, respectively).[24]

The M protein encoded by *emm* is known to be a major virulence factor of SP that inhibits the activation of the alternative complement pathway and impedes phagocytosis by polymorphonuclear leukocytes.[8] Shulman et al[25] described an *emm*-specific invasive index, based on analyses of more than 3000 SP isolates in the United States, and hypothesized that the observed variations in the invasiveness could probably be caused by differences in virulence factors, including exotoxins. Ekelund et al[26] found a strong association between certain *emm* types and the SAg genes detected among invasive isolates of SP. These findings were

Table 1 Clinical source and molecular characterization of blood and throat isolates

Clinical source	No. of isolates	
	spegg positive	*spegg* negative
Blood isolates ($N = 65$)		
Cellulitis	22	21
Septic arthritis	0	2
Toxic shock syndrome	2	0
Pneumonia	1	0
Line sepsis	1	0
Leg abscess	0	1
Unknown	6	7
Total	32	33
Pharyngeal isolates ($N = 46$)	32	14

in concordance with the previously reported SAg linkage to certain *emm* types (especially *emm*-1).[23] It is possible that the M protein also plays an important part in SE pathogenesis, although its role remains largely unexplored. In a study of 116 invasive and noninvasive strains of SE clinical isolates from Portugal, the *emm* types *stG10* and *stG2078* were found to be significantly more associated with invasive SE disease. The *emm* types *stG6792* and *stG166b* were found only among noninvasive isolates.[27] In a recent study by Anand et al,[28] *stG643.0* was the predominant *emm* type (28.6%) among SE pharyngeal strains. Nontypeable SE strains were shown to possess at least one SAg gene, although the number of SAg genes was lower in nontypeable than in typeable strains. It is thought that host factors as well as bacterial traits other than the M protein may contribute to the frequency of invasive disease. This is supported by the finding of *emm* type variability among invasive isolates reported elsewhere.[6,19,27] We found that the *emm* type distribution differed significantly between the *spegg* positive and the *spegg* negative blood isolates, with *stG*485 being significantly more frequent among *spegg* positive isolates (Table 2), probably reflecting the correlation between superantigen genes and certain *emm* types. The most common *emm* types observed in SE blood isolates were *stG840*, *stG485*, and *stG6*. Because we did not examine *emm* types in noninvasive SE isolates, no conclusion can be deduced on *emm* differences between invasive and noninvasive isolates.

In summary, we found a moderately high prevalence of *spegg* in invasive as well as in noninvasive SE. Similar to that reported in previous studies, our finding suggests that the ability of SE to cause invasive disease is probably not associated with the presence of this gene.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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