Superantigen genes in group A streptococcal isolates and their relationship with *emm* types

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Superantigens are important virulence factors in the pathogenesis of invasive disease caused by group A streptococcus (GAS). There has been a recent re-emergence of this disease worldwide. A number of novel superantigens have been described recently. This study investigated 107 isolates of GAS for possession of each of the 11 currently known superantigen genes to determine the prevalence, co-occurrence and genetic restriction amongst different *emm* types of GAS. The results were compared with those in previously published studies. Superantigen genes were not randomly distributed amongst GAS isolates. Certain combinations of superantigen genes were more common and the majority of *emm* types showed restricted superantigen profiles. This is the first prevalence study of GAS isolates to include the complete range of known superantigen genes and their restriction amongst *emm* types. This study contributes to the understanding of the relationship between superantigen genes and *emm* types, and highlights the importance of comprehensive studies in different populations.

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INTRODUCTION

Over the past 20 years, there has been a significant increase in the incidence of invasive disease caused by group A streptococcus (GAS) (Stevens *et al.*, 1989; Johnson *et al.*, 1992). Superantigens are believed to be important virulence factors of this pathogen. They are extracellular protein toxins that are pyrogenic, increase host susceptibility to endotoxic shock, suppress immunoglobulin production and have mitogenic activity for specific T-cell subsets (Curtis, 1996). This activity leads to extensive immune activation and a massive release of pro-inflammatory cytokines, such as tumour necrosis factor- α , interleukin-6, interleukin-2 and gamma interferon, which can cause shock and widespread organ damage. With the recent sequencing of the complete genomes of the *emm1*, *emm3*, *emm6*, *emm18* and *emm28* isolates of GAS, a number of novel superantigens have been discovered (Ferretti *et al.*, 2001; Beres *et al.*, 2002; Smoot *et al.*, 2002a; Nakagawa *et al.*, 2003; Banks *et al.*, 2004; Green *et al.*, 2005). A total of 11 superantigens have been identified in GAS to date: streptococcal pyrogenic exotoxin (SPE) A, SPEC, SPEG, SPEH, SPEI, SPEJ, SPEK, SPEL,

SPEM, streptococcal mitogenic exotoxin (SME) Z and streptococcal superantigen (SSA).

Most GAS superantigen-encoding genes are associated with bacteriophages, except for *speG*, *speJ* and *smeZ* (Proft *et al.*, 2000, 2003; Ferretti *et al.*, 2001; Proft, 2003). The documented prevalence of bacteriophage-encoded superantigens varies widely geographically and temporally. GAS superantigens have also been associated with isolates of particular *emm* and M types. The *emm*-type-specific associations are hypothesized to reflect a selective influence of surface M protein on bacteriophage entry (Mylvaganam *et al.*, 2000). The superantigen genes *speG*, *speJ* and *smeZ* are believed to be chromosomally encoded. This is supported by the reported 100 % prevalence of these genes in many studies (Proft *et al.*, 2000, 2003; McCormick *et al.*, 2001; Smoot *et al.*, 2002a; Proft, 2003) and a genome sequencing study that showed no association of these genes with mobile elements in an M1 strain (Ferretti *et al.*, 2001). However, *smeZ* and *speG* appear to have undergone horizontal transfer between species (Sachse *et al.*, 2002; Igwe *et al.*, 2003; Hashikawa *et al.*, 2004). In addition, not all studies have found a 100 % prevalence rate for these genes.

This paper describes a study of GAS isolates collected during a short period within one metropolitan area. One

Abbreviation: GAS, group A streptococcus.

hundred and seven isolates were *emm*-typed and tested for possession of each of the 11 superantigen genes. A previous study by us looked at the relationship between superantigens and virulence of GAS in the same collection of isolates (Rogers *et al.*, 2007). This study focused on superantigen prevalence and the relationship of superantigens to *emm* type. It is one of the most comprehensive studies of superantigen gene prevalence to date.

METHODS

Isolate collection and culture. A total of 107 GAS isolates were studied, comprising 67 isolates randomly selected from the 'Burden of Superficial GAS Diseases in Victoria Study' at the Royal Children's Hospital Melbourne, Australia, between August 2001 and December 2002, and 40 isolates collected during the same period as part of the 'Invasive GAS Surveillance Study' in metropolitan Melbourne. To prevent confounding from epidemiologically related isolates, those from related family members were excluded. Isolates were cultured on horse blood agar at 37 °C overnight with 5% CO₂. β -Haemolytic colonies were typed by latex agglutination to detect the presence of Lancefield group A antigen.

Purification and extraction of DNA. DNA was extracted by mechanical agitation as preliminary experiments (data not shown) showed that PCR amplification using DNA produced by this method was significantly more sensitive than using DNA prepared by lysis (heating at 100 °C for 10 min; Beall *et al.*, 1996). In brief, supernatants from overnight cultures at 37 °C in 10 ml brain heart infusion broth were resuspended in lysis buffer. DNA was extracted by agitation in a FastPrep FP120 cyclor with glass beads. DNA was purified using conventional phenol/chloroform extraction and 2-propanol precipitation, and treated with RNase A. The pellet was

resuspended in 50 μ l dH₂O. PCR detection of the *speB* gene was used to assess contamination in negative controls and also as a positive control for all isolates (Dmitrieva *et al.*, 2002).

Verification of species of *Streptococcus pyogenes*. Isolates in which *speB* was not detected were subjected to repeat agglutination testing and subsequent 16S rRNA gene sequencing with primers 63f (5'-CAGGCCTAACACATGCAAGTC-3') and 1387r (5'-GGGCGG-TGTGTACAAGGC-3') (Marchesi *et al.*, 1998).

***emm* gene typing.** Specific regions of the *emm* gene were amplified using the Qiagen HotStarTaq system and a Centers for Disease Control and Prevention protocol (www.cdc.gov/ncidod/biotech/strep/protocols.htm) with minor modifications. Cycling conditions were 15 min at 95 °C, 30 cycles of 45 s at 95 °C, 30 s at 40 °C and 120 s at 72 °C, and extension at 72 °C for 10 min. Products were purified using a QIAquick PCR purification kit (Qiagen) and sequenced with 2.5 pmol of the primer *emmseq2* (5'-TATTCGCTTAGAAAATTAACAGG-3'). *emm* types were identified using the CDC *emm* database.

PCR testing for superantigens. Individual PCR amplifications were undertaken to detect the genes for each of the 11 superantigens and *speB* as a positive control. To minimize the chance of allelic variation affecting results, primers were designed or selected following analysis of all published sequences and targeting of a conserved site within alleles (Table 1). Products of primers that have not been published previously were sequenced to verify that the correct gene was being targeted.

Each 50 μ l reaction contained 20 pmol of each of the forward and reverse primers, PCR buffer (10 mM Tris/HCl, pH 8.3; 50 mM KCl), 1.5 mM MgCl₂, 200 μ M each dNTP, 2.5 U AmpliTaq Gold DNA polymerase and 10 μ l genomic DNA (diluted 1:10 in dH₂O). Cycling conditions were 10 min at 95 °C, 35 cycles of 30 s at 94 °C, 30 s at 50 °C and 60 s at 72 °C, and extension for 2 min at 72 °C.

Table 1. Primers used for superantigen gene amplification

Bold indicates primers designed for this study.

Superantigen	Forward primer (5'→3')	Reverse primer (5'→3')	Amplicon size (bp)
<i>speA</i>	CCAAGCCAACCTTCACAGATC	CCCTTCATGATTGTGTACCCC	309
<i>speC</i>	CTATGGTGGAATTACACC	CAATTCCTGGAAAGTTACG	140
<i>speG</i> *	CTGGATCCGATGAAAATTTAAAGATTTAA	AAGAATTCGGGGGAGAAATAG	743
<i>speH</i> *	TTGGATCCAATCTTATAATACAACC	CCACTTCCTGAGCGGTTACTTTCCG	399
<i>speI</i> †	GGATCCATGAGTAGTGTGGGAGTTATTAA	AAGCTTTTATTTATTAATTTAACTAAG	690
<i>speJ</i>	CTATGGTGGAATTACACC	CATGTTTATTGCCATTGATCGC	247
<i>speK</i> ‡	GCGGATCCGATACGTACAATACAAATG	GCGAATTC AATAGCATTCAACCA	798
<i>speL</i> §	GCAGCCATATGGAAGAGACTATTCCTCTTA- AGGATATATACTCTC	GGGGATCCTTAATTTCTTTGTTGTGAATA- AATAGAC	703
<i>speM</i> §	GCAGCCATATGTTTTTCAGATGCTGTGTTGG	GGGGATCCTTAATTTTGTAGAAAAATCTTCGT- TTAAGTA	661
<i>ssa</i>	TGAGGTAATTGGGGAGATGA	CTAATTCCTGAACAGTGACTTG	621
<i>smeZ</i> *	TGGGATCCTTAGAAGTAGATAATA	AAGAATTCCTTAGGAGTCAATTC	376
<i>speBl</i>	GGATCCCAACCAAGTTGTTAAATCTCT	AACGTTCTAAGGTTTGATGCCTACAA	774
<i>speJ</i> – Profit†	GGATCCGATAGTGAAAAATTAAAGCG	GAATTCGCTCCTATCTTATTAGTCC	650

*Proft *et al.* (1999).

†Proft *et al.* (2001).

‡Proft *et al.* (2003).

§Smoot *et al.* (2002b).

||Chatellier *et al.* (2000).

Staphylococcus aureus and *Streptococcus pneumoniae* genomic DNA were used as negative controls.

Eight isolates negative for *speJ* were retested with the primers used by Proft *et al.* (2001) (Table 1). Samples in which the *smeZ* gene did not amplify under the above conditions were retested with 2.5 mM MgCl₂ in the reaction mixture and an annealing temperature of 53 °C, using the conditions used by T. Proft (personal communication).

Statistical analysis. Statistical pair-wise analysis was undertaken with χ^2 and Fisher's exact tests by comparing observed and expected associations.

RESULTS AND DISCUSSION

Prevalence of superantigen genes

The reported prevalence of superantigen genes in GAS isolates varies considerably. Laboratory technique is a possible source of false variation. Our pilot study showed diminished sensitivity for gene detection by PCR using DNA extraction by boiling compared with agitation. Allelic variation in primer-binding sites may also affect gene detection. However, there are also likely to be genuine geographical differences in superantigen prevalence, as well as differences over time.

In the present study, clearly defined, dense bands were detected for all superantigen genes, except for *smeZ*, which for some isolates was associated with faint bands. As expected, the putative chromosomally encoded superantigens *speG* and *smeZ* were the most prevalent, detected in 90 and 95 % of isolates, respectively. This contrasts with previous studies where these superantigens were found in 100 % of isolates using PCR-based detection (Proft *et al.*, 2000, 2003).

Isolates in which these superantigens were not detected were found to be restricted to certain *emm* types. In accordance with previous studies (Murakami *et al.*, 2002; Vlamincx *et al.*, 2003), this study found *speG* to be absent from *emm4* isolates. This suggests that *emm4* isolates may contain an allele with mutations in the primer-binding sites.

Polymorphisms in the primer-binding sites may also explain the variable and occasionally absent amplification in a number of isolates of the *smeZ* gene, which is known to be highly polymorphic (Proft *et al.*, 2000). However, variation in the binding sites of the *smeZ* primers used in the present study has not been documented previously. The present study and two previous studies (Schmitz *et al.*, 2003; Vlamincx *et al.*, 2003) found the absence of *smeZ* in *emm3* isolates. A variation in binding sites in *emm3* isolates would be consistent with evidence that *smeZ* alleles are linked to *emm* type (Proft *et al.*, 2000). Interestingly, the *smeZ* gene and *emm3* isolates have been linked independently to streptococcal toxic shock syndrome (Beres *et al.*, 2002; Vlamincx *et al.*, 2003).

Despite also being putatively chromosomally encoded, *speJ* was found in only 51 % of isolates, consistent with some

previous studies (Schmitz *et al.*, 2003; Vlamincx *et al.*, 2003). However, this contrasts with the results of Proft and co-workers, who found a 100 % prevalence for *speJ*, suggesting this superantigen to be chromosomally encoded (Proft, 2003; Proft *et al.*, 2003). This discrepancy was not attributable to the use of different primers, as results were unchanged in the present study when testing was repeated using the same primers as used by Proft and co-workers.

The reported prevalences of *speA* and *speC* are the most variable, perhaps attributable to the fact that they have been the most studied superantigens. The prevalence of *speA* in the present study was higher than that in previous Australian studies (DeVecchio *et al.*, 2002; Norton *et al.*, 2004). This could be due to three and two base mismatches, respectively, between the reverse primers used in these previous studies and the recently described alleles *speA4* and *speA5*.

The prevalence of *ssa* in the present study was similar to that found in two other recent studies (Jing *et al.*, 2006; Darenberg *et al.*, 2007), but lower than the prevalence reported in earlier studies (Proft, 2003; Proft *et al.*, 2003; Schmitz *et al.*, 2003; Descheemaeker *et al.*, 2000). This may be explained by the lower proportion of *ssa*-containing *emm3* isolates. In contrast, the prevalence of the recently discovered superantigen genes *speK*, *speL* and *speM* was similar to that found in New Zealand (Proft *et al.*, 2003). These three recently discovered superantigens have been associated with acute rheumatic fever (Smoot *et al.*, 2002b; Proft *et al.*, 2003).

The prevalence of superantigens in the present study is compared with previously published data in Table 2. A comparison of superantigen prevalence between invasive and non-invasive isolates from the present study has been published previously (Rogers *et al.*, 2007).

Random or non-random association between superantigen genes?

To determine whether there was a non-random association between different toxin genes (due, for example, to gene co-location or *emm* restriction), pair-wise statistical analysis was undertaken. Some combinations of superantigen genes were detected at rates that differed to that predicted if they occurred independently (Table 3). For example, *speH* was detected in 18.6 % of isolates and *speI* in 16.8 %. The observed co-detection rate (16.8 %) was significantly higher than the expected rate (3.1 %) ($P=0.0008$). In contrast, the observed co-detection rate (1.9 %) for *speA* and *speC* was significantly lower than the expected rate (17.1 %) ($P=0.0001$).

As found in previous studies, in the present study *speL* and *speM* were always co-detected (Igwe *et al.*, 2003; Proft *et al.*, 2003). This result was expected, as the genes are in adjacent positions on the same bacteriophage (ϕ *speL/M*) (Smoot *et al.*, 2002a). In contrast, the presence of *speH* was not always predictive of the presence of *speI*, despite their reported

Table 2. Comparison of superantigen gene prevalence (%) in studies from different geographical locations

Reference	No. of isolates	Location	<i>speA</i>	<i>speC</i>	<i>speG</i>	<i>speH</i>	<i>speI</i>	<i>speJ</i>	<i>speK</i>	<i>speL</i>	<i>speM</i>	<i>ssa</i>	<i>smeZ</i>
This study*	107	Melbourne	35 (26–44)	50 (40–59)	90 (84–95)	19 (11–26)	17 (10–24)	51 (42–61)	9 (4–15)	8 (3–14)	8 (3–14)	22 (15–30)	95 (91–99)
Luca-Harari <i>et al.</i> (2008)	278	Denmark	40	58	99	0.7	0	0				14	
Darenberg <i>et al.</i> (2007)	1519	Sweden	11	64	90	20		24				16	96
Creti <i>et al.</i> (2007)	207	Italy	36	38									
Jing <i>et al.</i> (2006)	86	China	52	51								23	82
Bianco <i>et al.</i> (2006)	182	Italy	19.2										
Ekelund <i>et al.</i> (2005a)	553	Denmark	24.8	23.5		23.0	3.6					30.7	
Ekelund <i>et al.</i> (2005b)	493	Denmark	34	62		14	5					44	
Creti <i>et al.</i> (2005)	259	Italy	48	132									
Norton <i>et al.</i> (2004)	109	Australia	6.4	20									
Proft <i>et al.</i> (2003)	40	New Zealand	31	42	100	26	16	100	15	5		32	92
Proft (2003)	19	England	36.8	15.8	100	26.3	10.5	100				31.6	100
Schmitz <i>et al.</i> (2003)	292	European	54.4	33.9		15.4		32.8				23.6	30.8
Vlaminckx <i>et al.</i> (2003)	170	Holland	45	42									32
DeVecchio <i>et al.</i> (2002)	75	Northern Territory, Australia	7	24									
	39	Sydney, Australia	15	51									
Haukness <i>et al.</i> (2002)	80	USA	75	85									
Murakami <i>et al.</i> (2002)	316	Japan	67	76	78	40						40	
Nandi <i>et al.</i> (2002)	60	India	8										
Descheemaeker <i>et al.</i> (2000)	500	Belgium	24.8	53.6								29.8	
Mylvaganam <i>et al.</i> (2000)	42	Norway	59.5									100	
Bessen <i>et al.</i> (1999)	160	Worldwide	23	46									
Hsueh <i>et al.</i> (1997)	25	Taiwan	4	96									
Chaussee <i>et al.</i> (1996)	117	Worldwide	44	34									
Reda <i>et al.</i> (1996)	80 clones	Worldwide										12.5	
Norrby-Teglund <i>et al.</i> (1994)	8	Scandinavia		100									
Black <i>et al.</i> (1993)	62	USA	56	27									
Leggiadro <i>et al.</i> (1993)	31	USA	26	58									
Cleary <i>et al.</i> (1992)	67	USA	57										
Tyler <i>et al.</i> (1992)	152	Canada	33.6	28.9									
Musser <i>et al.</i> (1991)	108	USA	47.2										
Yu & Ferretti (1991)	500	Worldwide		50									
Yu & Ferretti (1989)	446	Worldwide	25										

*95 % confidence intervals are given in parentheses for the results of this study.

Table 3. Co-detection rates of superantigen genes amongst 107 GAS isolates

Bold depicts statistically significant associations.

Superantigen	Observed/expected frequency (%)									
	<i>speA</i>	<i>speC</i>	<i>speG</i>	<i>speH</i>	<i>speI</i>	<i>speJ</i>	<i>speK</i>	<i>speL</i>	<i>speM</i>	<i>ssa</i>
<i>speA</i>										
<i>speC</i>	2/17*									
<i>speG</i>	35/31	40/44								
<i>speH</i>	0/6†	15/9	19/17							
<i>speI</i>	0/6†	13/8	17/15	17/3*						
<i>speJ</i>	27/18	21/25	51/46	1/10‡	0/9§					
<i>speK</i>	3/3	6/5	9/8	1/2	0/2	3/5				
<i>speL</i>	0/3	2/4	8/8	0/2	0/1	0/4	3/1			
<i>speM</i>	0/3	2/4	8/8	0/2	0/1	0/4	3/1	8/1†		
<i>ssa</i>	6/8	13/11	14/20	1/4	1/4	7/12	3/2	0/2	0/2	
<i>smeZ</i>	31/33	48/47	85/86	19/18	17/16	51/49	6/9	8/8	8/8	20/21

**P* < 0.001; †*P* < 0.05; ‡*P* < 0.01; §*P* < 0.005.

presence on the same bacteriophage (ϕ 370.2) (Ferretti *et al.*, 2001). This result confirmed the findings of previous studies (Proft *et al.*, 2003; Ekelund *et al.*, 2005a) and suggests that *speI* may be lost during integration into the genome or that there is another phage that carries only *speH*.

***emm*-type association with superantigen profiles**

Twenty-six different superantigen profiles (unique combinations of superantigen genes) were present in the 107 isolates and these were distributed amongst 22 different *emm* types. When *emm* types and superantigen profiles were analysed together, the 107 isolates could be divided into 35 genetically distinguishable sets (Table 4).

In those *emm* types for which there were sufficient numbers of isolates represented in the study for further analysis, namely *emm1*, *emm4*, *emm12*, *emm28* and *emm75*, superantigen profiles were relatively restricted. Conserved superantigen profiles in specific *emm* types have been reported previously (Schmitz *et al.*, 2003; Vlamincx *et al.*, 2003; Darenberg *et al.*, 2007; Luca-Harari *et al.*, 2008).

Two previous studies (Descheemaeker *et al.*, 2000; Vlamincx *et al.*, 2003) have suggested that the majority of *emm1* isolates contain *speA*, *speG*, *speJ* and *smeZ* but do not possess *speC*, *ssa* and *speH* (profile D). This is consistent with the present study in which 27 of the 29 *emm1* isolates possessed profile D. The presence of *speC* in *emm1* isolates has also been reported as variable (Ekelund *et al.*, 2005a). In the present study, *emm1* isolates did not possess *speI* (as previously reported; Ekelund *et al.*, 2005a) or *speK*, *speL* or *speM* (not previously been reported). The *speJ* gene was present in 97% of *emm1* isolates in the present study, which contrasts with the variable prevalence reported in two previous studies (Schmitz *et al.*, 2003;

Darenberg *et al.*, 2007). *emm1* isolates have been associated with streptococcal toxic shock syndrome in numerous studies (Musser *et al.*, 1991; Vlamincx *et al.*, 2003).

Other *emm* types that were present in significant numbers also showed conserved superantigen profiles (Table 5), including a number of novel associations in *emm* types previously thought not to be associated with *smeJ* and *smeZ*. All but one of the ten *emm4* isolates possessed profile B. Of the 17 *emm12* isolates, 14 possessed superantigen profile H. Although the remaining three isolates each had a different profile, it was noteworthy that 16 of the 17 isolates possessed *speG*, *speH*, *speI* and *smeZ*. All but two of the 14 *emm28* isolates possessed superantigen profile C. The profile of the remaining two isolates differed only by the addition of *speK*.

In comparison, *emm75* appeared to have a greater diversity in that only four of the eight *emm75* isolates shared a superantigen profile. However, the superantigens in this profile (*speG*, *speL*, *speM* and *smeZ*) were present in three of the remaining four *emm75* isolates. Superantigens commonly associated with *emm75* isolates have not been reported previously.

Why are superantigen profiles restricted amongst different *emm* types?

Conserved superantigen profiles for different *emm* types, especially the presence or absence of bacteriophage-encoded superantigens, support the contention that surface M proteins selectively influence the entry of bacteriophages, including those encoding superantigens (Mylvaganam *et al.*, 2000). For example, *speA* or *speJ* were found together with *speH* or *speI* in the same isolate less frequently than expected. This suggests that the characteristics of the M protein in isolates containing these genes

Table 4. Relationship between *emm* type and superantigen profile

<i>emm</i> type	Superantigen profile	Superantigen											No. of total isolates
		<i>speA</i>	<i>speC</i>	<i>speG</i>	<i>speH</i>	<i>speI</i>	<i>speJ</i>	<i>speK</i>	<i>speL</i>	<i>speM</i>	<i>ssa</i>	<i>smeZ</i>	
1	D	+	–	+	–	–	+	–	–	–	–	+	27
	F	+	–	+	–	–	+	–	–	–	+	+	1
	R	+	–	+	–	–	–	–	–	–	–	+	1
2	X	–	+	+	–	–	–	+	–	–	+	–	1
3	K	+	–	+	–	–	–	+	–	–	+	–	2
	V	+	–	+	–	–	–	+	–	–	–	–	1
4	B	–	+	–	–	–	–	–	–	–	+	+	9
	D	+	–	+	–	–	+	–	–	–	–	+	1
11	G	–	+	+	–	–	–	–	–	–	–	+	1
12	H	–	+	+	+	+	–	–	–	–	–	+	14
	J	–	–	+	+	+	–	–	–	–	–	+	1
	T	–	–	+	+	+	–	–	–	–	+	+	1
	Z	+	+	+	–	–	–	–	–	–	–	–	1
19	Y	–	+	+	+	–	+	+	–	–	–	+	1
22	A	+	–	+	–	–	–	–	–	–	+	+	2
	W	+	+	+	–	–	–	–	–	–	+	+	1
28	C	–	+	+	–	–	+	–	–	–	–	+	12
	P	–	+	+	–	–	+	+	–	–	–	+	2
58	S	–	+	+	+	–	–	–	–	–	–	+	1
68	C	–	+	+	–	–	+	–	–	–	–	+	1
70	J	–	–	+	+	+	–	–	–	–	–	+	1
73	J	–	–	+	+	+	–	–	–	–	–	+	1
75	I	–	–	+	–	–	–	–	–	–	–	+	1
	L	–	–	+	–	–	–	–	+	+	–	+	4
	N	–	–	+	–	–	–	+	+	+	–	+	1
	Q	–	+	+	–	–	–	+	+	+	–	+	2
77	O	–	+	–	–	–	–	–	–	–	–	+	1
87	M	–	+	+	–	–	+	–	–	–	+	+	3
89	C	–	+	+	–	–	+	–	–	–	–	+	2
	I	–	–	+	–	–	–	–	–	–	–	+	1
94	L	–	–	+	–	–	–	–	+	+	–	+	2
102	C	–	+	+	–	–	+	–	–	–	–	+	1
108	E	–	–	+	–	–	+	–	–	–	+	+	4
113	I	–	–	+	–	–	–	–	–	–	–	+	1
ST11014	U	–	–	–	–	–	–	–	–	–	–	+	1

Table 5. *emm* type-specific superantigen profiles

<i>emm</i> type	Possesses (according to the present study)			Does not possess (according to the present study)		
	Consistent*	Inconsistent*	Novel†	Consistent*	Inconsistent*	Novel†
<i>emm1</i>	<i>speA, speG, smeZ</i>	<i>speJ</i>		<i>ssa, speH, speI</i>	<i>speC</i>	<i>speK, speL, speM</i>
<i>emm4</i>	<i>speC, ssa, smeZ</i>			<i>speA, speJ</i>	<i>speG, speH</i>	<i>speI, speK, speL, speM</i>
<i>emm75</i>			<i>speG, speL, speM, smeZ</i>			<i>speA, speH, speC, speI, speJ, ssa</i>
<i>emm12</i>	<i>speC, speG, speH</i>	<i>smeZ, speI</i>		<i>speA, speJ</i>	<i>ssa</i>	<i>speK, speL, speM</i>
<i>emm28</i>	<i>speC, speG, speJ</i>	<i>smeZ</i>		<i>speH, ssa</i>	<i>speA</i>	<i>speI, speK, speL, speM</i>

*Consistent/inconsistent with results of previous studies (Descheemaeker *et al.*, 2000; Murakami *et al.*, 2002; Schmitz *et al.*, 2003; Vlaminckx *et al.*, 2003; Ekelund *et al.*, 2005a, b; Jing *et al.*, 2006; Darenberg *et al.*, 2007; Luca-Harari *et al.*, 2008).

†Finding not reported previously.

allow entry of mobile elements containing *speA* and *speI* and prevent entry of bacteriophages containing *speH* and *speI*.

Conserved superantigen profiles could be an evolutionary event. For example, bacteriophages encoding superantigens may have become associated with specific *emm* types at the time of *emm*-type differentiation. However, a number of factors argue against this. Strains before the mid-1980s have tested negative for *speK* (Beres *et al.*, 2002; Ikebe *et al.*, 2002). This superantigen gene was found in all *emm3* isolates in the present study, consistent with the finding that most contemporary *emm3* isolates carry *speK* (Beres *et al.*, 2002; Ikebe *et al.*, 2002) and confirming the dissemination of the *speK*-carrying phage in *emm3* strains in Melbourne. Furthermore, one study showed a progressive change in the proportion of *emm1* isolates carrying *speA* over a 4-year period (Ekelund *et al.*, 2005b), suggesting that variation in prevalence amongst isolates may occur over a relatively short time.

Variation in *emm28* superantigen profiles from different geographical locations suggests that the presence of a superantigen in a given *emm* type may be influenced by more than just M protein. This variation, in association with the dissemination of *speK* in the early 1980s, suggests that the properties of the M protein could change with time. This could be due to the acquisition of other bacteriophages encoding surface proteins, minor nucleotide variations in the M protein itself or changes in the bacteriophage to increase its infective capacity or host range.

Previous studies suggest that there is geographical variation in the association between superantigen gene possession and *emm* type (Table 2), although temporal changes may provide an alternative explanation (Ikebe *et al.*, 2002). This is illustrated by the fact that, in contrast to the results of the present study, *speL* and *speM* were found in *emm4*, *emm22*, *emm28* and 73 % of *emm89* isolates, and *speK* in *emm89* isolates, from New Zealand (Proft *et al.*, 2003).

This study provides detailed data about the prevalence of and relationships between superantigens, and their association with specific *emm* types. The importance and relevance of these relationships needs further investigation by clarifying their role in the outcome of infection with GAS.

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