JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1997, p. 3220–3224 Vol. 35, No. 12
0095-1137/97/$04.00+0
Copyright © 1997, American Society for Microbiology

Characterization of Group A *Streptococcus* Strains Recovered from Mexican Children with Pharyngitis by Automated DNA Sequencing of Virulence-Related Genes: Unexpectedly Large Variation in the Gene (*sic*) Encoding a Complement-Inhibiting Protein

LUIS M. PEREA MEJIA,[1] KATHRYN E. STOCKBAUER,[2] XI PAN,[2] ALEJANDRO CRAVIOTO,[1] AND JAMES M. MUSSER[2]*

Departamento de Salud Pública, Facultad de Medicina, Universidad Nacional Autónoma de México, Ciudad Universitaria, D.F., Mexico,[1] and Section of Molecular Pathobiology, Department of Pathology, Baylor College of Medicine, Houston, Texas[2]

Received 10 June 1997/Accepted 28 August 1997

Sequence variation was studied in several target genes in 54 strains of group A *Streptococcus* (GAS) cultured from children with pharyngitis in Mexico City. Although 16 distinct *emm* alleles were identified, only 4 had not been previously described. Virtually all bacteria (31 of 33 [94%] with the streptococcal pyrogenic exotoxin gene (*speA*) had *emm1*-related, *emm3*, or *emm6* alleles. The gene (*sic*) encoding an extracellular GAS protein that inhibits complement function was unusually variable among isolates with the *emm1* family of alleles, with a total of seven variants identified. The data suggest that many GAS strains infecting Mexican children are genetically similar to organisms commonly encountered in the United States and western Europe. Sequence variation in the *sic* gene is useful for rapid differentiation among GAS isolates with the *emm1* family of alleles.

Group A *Streptococcus* (GAS) strains remain an important cause of human morbidity and mortality worldwide (26). The recent resurgence of GAS infections in the United States, Europe, and elsewhere (6, 19, 20, 22, 23) has stimulated renewed interest in the molecular epidemiology and virulence gene variation of this pathogen. Historically, strains have been subdivided based on serologic reactivity of the M protein, an α-helical coiled-coil protein that projects outward from the bacterial cell wall (9). M protein is antiphagocytic and is an important GAS virulence factor (9). Serological studies have identified more than 100 distinct M protein serotypes among GAS strains recovered from healthy carriers and diseased humans. The molecular basis of M protein serologic diversity is due to sequence variation occurring in the hypervariable amino terminus part of the molecule. In 1994, Whatmore et al. (38) reported the DNA sequences of the region of the *emm* gene encoding the part of the M protein conferring serotype specificity. The same year, automated DNA sequencing of a hypervariable region of the streptokinase gene was used as a molecular epidemiologic tool to rapidly and unambiguously determine GAS strain relationships in a putative outbreak of invasive disease (24). Subsequent studies (5, 7, 36, 37) demonstrated that sequencing of the hypervariable region of *emm* could also be used to delineate strain relationships in invasive disease case clusters. Recently, the *emm* sequencing approach has been extended to analyze GAS isolates recovered during longitudinal surveys of invasive disease episodes in specific geographic areas (4).

The results of serologic studies conducted with anti-M protein antisera have shown that a large percentage of strains recovered from individuals in localities outside of the United States and western Europe are nontypeable (12, 13, 34). This observation, together with limited data available from *emm* gene sequencing (30), has suggested that GAS strains circulating in areas of the world not yet extensively sampled are genetically distinct. In this study, we used automated DNA sequencing of several virulence-associated genes to characterize 54 GAS strains recovered from children with recent episodes of pharyngitis in Mexico City. Strains from Mexican children were chosen for analysis in part because data suggest that diseases such as acute rheumatic fever and rheumatic heart disease are important causes of human morbidity in Mexico (1, 2, 17, 31). We found that the great majority of isolates had alleles of the target genes that had been previously described. The gene (*sic*) encoding an extracellular GAS protein recently shown to inhibit complement function (3) was found to be unusually variable among isolates with the *emm1* family of alleles.

MATERIALS AND METHODS

Bacterial strains. The analysis was based on 54 GAS organisms cultured from children with GAS pharyngitis in Mexico City in the 1990s. Three of the isolates were recovered from children who were contacts of patients with acute rheumatic fever. Pharyngeal specimens were cultured on blood agar plates, and group A beta-hemolytic streptococci were identified by conventional diagnostic laboratory techniques. The organisms were frozen in brain heart infusion broth with 15% glycerol and stored at −70°C. After subculture onto solid media, the strains were transported to the laboratory of J.M.M. for analysis. Chromosomal DNA was isolated by previously described methods (14).

DNA sequence analysis. Strategies for analysis of sequence variation in genes encoding M protein, streptococcal pyrogenic exotoxin A (*speA*), and streptococcal pyrogenic exotoxin B (*speB*) have been described previously (14, 23, 25, 28, 38). Sequence variation in the *sic* gene (3) encoding a streptococcal protein that inhibits human complement function in vitro was also studied (Fig. 1). The structural gene and upstream noncoding sequence were amplified with the primers SIC.1 (5′-TAAGGAGAGGTCACAAACTA-3′) and SIC.2 (5′-TTACGTT GCTGATGGTGTAT-3′). Two internal primers used for sequencing were SIC.3 (5′-GATGAGACAGAAGATAAAAC-3′) and SIC.4 (5′-CATATTCCTAAAC CTGAGAA-3′).

* Corresponding author. Mailing address: Section of Molecular Pathobiology, Department of Pathology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030. Phone: (713) 798-4198. Fax: (713) 798-4595. E-mail: jmusser@path.bcm.tmc.edu.

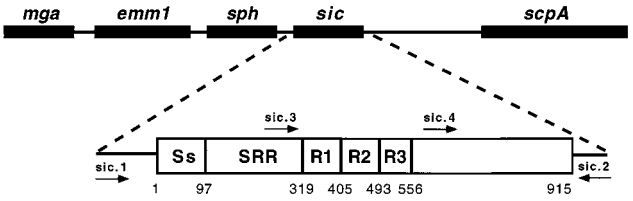

FIG. 1. Schematic of the *sic* gene. The diagram shows the location of the 915-bp *sic* gene in the chromosome of an M1 strain of GAS as described previously (3). The gene is located in the Mga regulon that encodes several virulence genes. *mga* encodes a regulatory protein, *emm1* codes for serotype M1 protein, *sph* encodes protein H, and *scpA* codes for streptococcal C5a peptidase. Ss, signal sequence; SRR, amino-terminal short repeat region; R1 to R3, tandem repeats. The numbers below the *sic* gene are base pair designations. sic.1 through sic.4 denote oligonucleotide primers used for *sic* gene amplification (see Materials and Methods).

All sequence data were obtained with an Applied Biosystems model 377 automated DNA sequencer. The sequence data were assembled and edited electronically with the EDITSEQ, ALIGN, and MEGALIGN programs (DNASTAR, Madison, Wis.) and were compared with published sequences for *emm*, *speA*, *speB*, and *sic* (3, 11, 14, 28, 38). For *emm* sequences that were not identical to one previously described, we determined the closest related *emm* allele by comparing the sequence with sequences from a database of approximately 100 *emm* alleles (38).

RESULTS

Sequence variation in *emm*. A total of 16 distinct *emm* alleles were identified, including 11 that had been previously described (Table 1). The most abundantly represented alleles would encode M1, M3, and M6 serotype proteins. Three distinct *emm1*-related alleles were found, including *emm1.0*, *emm1.3*, and a newly identified variant, designated *emm1.8*. Compared to reference allele *emm1.0*, the *emm1.8* allele was characterized by nucleotide substitutions resulting in an amino acid replacement located at position 23 (E→D) of the deduced mature amino acid sequence (10, 25). The *emm3* allele in this study was identical to allele *emm3.1*, deposited in GenBank under accession no. U40231 (8). Based on phylogenetic analysis, two of the three other strains with previously unidentified *emm* variants had sequences related to *emm3* and *emm10/12*, and the sequence in the third isolate was not closely allied with previously identified variants. Interestingly, all three *emm1* family organisms recovered from individuals who were contacts of patients with acute rheumatic fever had the *emm1.3* allele.

TABLE 1. Characteristics of 54 GAS strains from children in Mexico with pharyngitis

emm sequence[a]	No. of isolates	No. (%) of isolates *speA* positive
emm1[b]	16	12 (75)
emm3	13	12 (92)
emm4	4	0
emm5	1	0
emm6	7	7 (100)
emm10/12	3	0
emm22	1	0
emm72	1	0
emm75	2	0
emm78	1	0
emm4245	2	1 (50)
Nontypeable	3	1 (33)

[a] See data presented in reference 38.
[b] Twelve isolates had allele *emm1.0*, 3 had *emm1.3*, and 1 had a newly recognized variant, designated *emm1.8*.

Sequence variation in *speA*. Thirty-three organisms had the *speA* gene encoding SpeA, a superantigen that has been implicated in virulence (22, 23) (Table 1). Virtually all (94%) bacteria with the *speA* gene had the *emm* gene encoding serotype M1, M3, or M6 protein. Among the 16 isolates with the *emm1* family of alleles, the *speA* gene was detected by PCR in 12 organisms. Three random *emm1* isolates were chosen for *speA* sequencing, and all had the *speA2* allele. Similarly, 12 of 13 organisms with the *emm3* allele had the *speA* gene, and sequence analysis of three organisms identified the *speA3* allele. All seven organisms with the *emm6* allele had the *speA* gene. Sequence analysis of three randomly chosen organisms showed that all had the *speA4* allele (28). Among the two other organisms with the *speA* gene, one had the *emm4245* allele, and one had an *emm* allele that has not been previously described. Sequence analysis showed that both organisms had the *speA3* allelic variant.

Sequence variation in *speB*. The *speB* gene encodes a cysteine protease that is a critical GAS virulence factor (11, 18, 21). Essentially all organisms have this gene (21, 22). Previous studies have documented that *speB* is well conserved and that *speB* is invariant among members assigned to the same GAS clone (14, 23, 25). We therefore chose to sequence *speB* only from three of the strains with a previously undescribed *emm* allele. The *speB* gene in MGAS 4879 was identical to a published sequence designated allele *speB1* (14). The *speB* sequences in the other two isolates (MGAS 4874 and MGAS 4883) were identical to each other and matched those of allele *speB3* (14).

Sequence variation in *sic*. Compared to the relatively limited variation in *emm* and *speA*, there was substantial allelic polymorphism in *sic* in M1 strains (Fig. 2). Seven variants were identified among the 16 strains containing this gene. None of the *sic* sequences in this Mexican strain sample was identical to the sequence of the *sic* gene deposited in GenBank under accession no. X92968. Variation was due to nucleotide substitutions distributed throughout the entire length of the gene, and insertions and deletions were located mainly in the SRR and R motif regions (Fig. 2). Ten strains had identical 9-bp inserts at position 684 (GGCTTTGAT [Gly-Phe-Asp]) (Table 2). Three strains had an 87-bp insert located in the R3 motif, and one strain had two contiguous 87-bp insertions in R3. These 87-bp inserts were apparent duplications of nucleotides 493 through 580, encoding amino acids 165 through 194. These inserts are reminiscent of structural alterations that can arise from slipped-strand mispairing events during replication (10, 16). Interestingly, 10 of the 11 single-nucleotide substitutions identified would result in amino acid replacements in the Sic protein. The three organisms recovered from patients with acute rheumatic fever had a total of five *sic* polymorphisms that were not found in other isolates in the sample (Fig. 2).

Lack of effect of laboratory passage on *sic* variation. Because the analysis showed that allelic variation in *sic* was unusually high, we were concerned that some of the polymorphism might be accumulating in the course of laboratory passage. To test this hypothesis, we passaged two strains (MGAS 4861 and MGAS 4872) three times each in the laboratory and then characterized the *sic* gene in the resulting derivative strain. For each passage, a single colony was picked, streaked onto a new blood agar plate, and grown overnight at 37°C. The *sic* sequences in the parental and laboratory-passaged organisms were identical.

		SRR (II)	SRR (II)	SRR (III)	SRR (III)	SRR (III)	SRR (IV)	SRR (IV)	SRR	SRR	R 2	R 2	R 3	R 3					
		141	141	161	165	165	212	220	244	249	474	479	492	506	648	650	659	691	721
MGAS strain	*emm* allele	15 bp insert	15 bp del.	GGA> GAA G - E	93 bp del.	45 bp del.	GGC> GAC G - D	CAA> AAA Q - K	GAA> CAA E - Q	12 bp del.	GTA> GTG V - V	ATT> ACT I - T	87 bp insert	GAA> GGA E - G	9 bp insert	GAA> GGA E - G	TTT> TCT F - S	GCC> ACC A - T	CCA> ACA P - T
4867	1.0	X						X			X	X		X	X	X	X	X	X
4869	1.0	X						X			X	X		X	X	X	X	X	X
4972	1.0	X						X			X	X		X	X	X	X	X	X
4876	1.0	X						X			X	X		X	X	X	X	X	X
4877	1.0	X						X			X	X		X	X	X	X	X	X
4882	1.0	X						X			X	X		X	X	X	X	X	X
4892	1.0	X						X			X	X		X	X	X	X	X	X
4918	1.0	X						X			X	X		X	X	X	X	X	X
4898	1.8	X						X			X	X		X	X	X	X	X	X
4909	1.0	X						X			X	X		X		X	X	X	X
4910	1.0	X						X			X	X		X		X	X	X	X
4861	1.0	X						X			X	X	X	X		X	X	X	X
4914	1.0		X		X						X	X	X	X	X	X	X	X	X
4840	1.3			X		X	X	X	X		X	X		X		X		X	
4842	1.3			X		X	X	X	X		X	X	X	X		X		X	
4839	1.3			X		X	X	X	X	X	X	X	XX	X		X		X	X

FIG. 2. Variation in the *sic* gene and SIC protein identified in M1 strains of *Streptococcus pyogenes* recovered from Mexican children with pharyngitis. The figure shows a compilation of structural variation found in the 16 strains characterized. Strains MGAS 4840, MGAS 4842, and MGAS 4839 were recovered from children with pharyngitis who were contacts of patients with acute rheumatic fever. The numbers refer to the nucleotide sequence position designated in reference 3. The single-letter amino acid abbreviations are used. Strain MGAS 4839 has two 87-bp insertions beginning at nucleotide 492. SRR, amino-terminal short repeat region; R 2 and R 3, tandem repeats.

DISCUSSION

This study is the first to characterize nucleotide variation in virulence-related genes of GAS strains recovered from patients in Mexico. Our analysis was motivated by four factors. First, results from several studies have demonstrated that a large percentage of GAS strains recovered from areas of the world not yet extensively sampled are frequently serologically nontypeable with antisera directed against known M types (12, 13, 34). This suggests that strains circulating in areas outside of, for example, the United States and western Europe, may contain a large pool of new M types, and hence *emm* sequences, awaiting discovery. Second, there is a relative lack of ready availability of the large battery of typing sera necessary to identify GAS M types. Because M serologic specificity is encoded by a region of the *emm* gene that is less than 400 bp (38), an automated DNA sequencing strategy is ideally suited for strain characterization. Third, with the exception of extremely limited data (23, 25), little information is available bearing on the issue of virulence gene polymorphism outside of regions such as the United States and western Europe. Fourth, inasmuch as Texas and Mexico share a 1,248-mile border, and there are extensive historical, cultural, and economic ties between the two populations, it is reasonable to hypothesize that GAS strains circulating in Texas may be carried to Mexico, and vice versa. The affinity between Texas and Mexico is illustrated by the occurrence in 1993 of a recorded 114 million northbound border crossings (35). In principle, GAS strain migration could be an important contributor to temporal variation in disease frequency and severity. However, our study showed that in general, most of the GAS strains analyzed were closely similar, if not identical, to previously identified clones or subclones. These data suggest that the GAS strains commonly circulating in Mexico City and the United States are not, as a population, genetically differentiated from one another. These preliminary data will need to be confirmed by a more extensive

TABLE 2. Structural changes in the *S. pyogenes sic* gene and Sic protein caused by insertions and deletions

Nucleotide position[a]	Length (bp)	Amino acids
Insertion		
141	15	SGDDW
492	87	PPYGGALGTGYEKRDDWGGPGTVATDPYT
648	9	GFD
Deletion		
141	15	PEDDW
165	45	GLSKYDRSGVGLSQY
165	93	GLSKYDRSGVGLSQYGWSQYGWSSDKEEWPE
249	12	WPED

[a] Designated according to the numbering system described in reference 3.

analysis of GAS strains recovered from patients in areas outside of the Mexico City region.

Previous studies have shown that strains grouped together based on the *emm* allele generally have restricted variation in other characterized virulence-related genes (23, 25). In addition, strains of serotype M1 have generally been refractory to extensive subdifferentiation by molecular strategies (19, 20, 22, 23, 25, 27, 29, 32). Hence, the discovery of a substantial level of allelic variation in the *sic* gene among strains with the *emm1* family of alleles was unexpected. In recent years, M1 strains have been the most common serotype recovered from patients with invasive GAS disease in the United States and western Europe (26). They have also been important causes of disease in New Zealand and Australia (6, 19). Based on sequence analysis of the *emm* gene and pulsed-field gel electrophoresis, it was demonstrated that many strains recovered from patients with invasive episodes represented the same subclone (25). Comparative sequencing of the genes encoding streptokinase, streptococcal pyrogenic exotoxin B, pyrogenic exotoxins A and C, and C5a peptidase has generally failed to identify allelic variation that could serve as a useful strategy for additional differentiation among M1 isolates (25). The variation present in *sic* provides a convenient molecular tool for obtaining further insights into the molecular epidemiology of M1 organisms, associations of subclones with specific auxiliary virulence factors, such as pyrogenic exotoxins, and the relationship between distinct subclones and disease character. We will report elsewhere on the utility of *sic* sequencing for extensive M1 strain differentation among strains from intercontinental sources (33).

Although our study did not investigate the molecular mechanism responsible for *sic* variation, we infer that at least two processes are driving allelic diversity: nucleotide substitutions and insertion and deletion events. It is also a formal possibility that horizontal gene transfer and recombination processes (15) are contributing to *sic* allelic variation. Importantly, 10 of the 11 nucleotide substitutions would result in amino acid replacements in the Sic protein. Moreover, the insertion and deletion events we identified would also result in expression of structurally distinct molecules. Taken together, these observations indicate that positive Darwinian selection is contributing to generation of structural variation in the Sic protein. Selection could be due to antibody pressure, but thus far, no evidence has been presented that the protein is either expressed in vivo during human infection or that patients generate an immune response to Sic. However, inasmuch as Sic has a typical leader sequence characteristic of secreted proteins and it binds to human proteins in vitro, it is reasonable to speculate that there might be a premium on structural variation. Additional studies will be required to address this issue.

ACKNOWLEDGMENTS

We thank Romeo Rodriguez and Silvia Giono for providing some of the strains.

This study was supported by Public Health Service grant AI-33119 and Fogarty International Center grant TW-37004-03S1. J.M.M. is an Established Investigator of the American Heart Association.

REFERENCES

1. **Abdalá, A. L., M. C. A. Castañeda, C. N. González, A. R. Cárdenas, J. R. Manzur, and L. C. Rodríguez.** 1995. Evaluación ecocardiográfica en pacientes con fiebre reumática con corea de Sydenham. Pediatria **62:**44–47.
2. **Abdalá, A. L., R. Sandoval, L. Lastra, C. Vidales, L. Carbajal, and F. D. Clavijo U.** 1980. Comportamiento clínico de 200 casos de fiebre reumática. Acta. Pediatr. Mex. **1:**83–89.
3. **Akesson, P., A. G. Sjoholm, and L. Bjorck.** 1996. Protein SIC, a novel extracellular protein of *Streptococcus pyogenes* interfering with complement function. J. Biol. Chem. **271:**1081–1088.
4. **Beall, B., R. Facklam, T. Hoenes, and B. Schwartz.** 1997. Survey of *emm* gene sequences and T-antigen types from systemic *Streptococcus pyogenes* infection isolates collected in San Francisco, California; Atlanta, Georgia; and Connecticut in 1994 and 1995. J. Clin. Microbiol. **35:**1231–1235.
5. **Beall, B., R. Facklam, and T. Thompson.** 1996. Sequencing *emm*-specific PCR products for routine and accurate typing of group A streptococci. J. Clin. Microbiol. **34:**953–958.
6. **Carapetis, J. R., R. Robins-Browne, D. Martin, T. Shelby-James, and G. Hogg.** 1995. Increasing severity of invasive group A streptococcal disease in Australia: clinical and molecular epidemiological features and identification of a new virulent M-nontypeable clone. Clin. Infect. Dis. **21:**1220–1227.
7. **Cockerill, F. R., III, K. L. MacDonald, R. L. Thompson, F. Roberson, P. C. Kohner, J. Besser-Wiek, J. M. Manahan, J. M. Musser, P. M. Schlievert, J. Talbot, B. Frankfort, J. M. Steckelberg, W. R. Wilson, M. T. Osterholm, and the Investigation Team.** 1997. An outbreak of severe invasive group A streptococcal disease associated with high carriage rates of the invasive clone among school-aged children. JAMA **277:**38–43.
8. **Dale, J. B., M. Simmons, E. C. Chang, and E. Y. Chang.** 1996. Recombinant, octavalent group A streptococcal M protein vaccine. Vaccine **14:**944–948.
9. **Fischetti, V. A.** 1989. Streptococcal M protein: molecular design and biological behavior. Clin. Microbiol. Rev. **2:**285–314.
10. **Harbaugh, M. P., A. Podbielski, S. Hugl, and P. P. Cleary.** 1993. Nucleotide substitutions and small-scale insertion produce size and antigenic variation in group A streptococcal M1 protein. Mol. Microbiol. **8:**981–991.
11. **Hauser, A. R., and P. M. Schlievert.** 1990. Nucleotide sequence of the streptococcal pyrogenic exotoxin type B gene and relationship between the toxin and the streptococcal proteinase precursor. J. Bacteriol. **172:**4536–4542.
12. **Jamal, F., S. Pit, D. R. Johnson, and E. L. Kaplan.** 1995. Characterization of group A streptococci isolated in Kuala Lumpur, Malaysia. J. Trop. Med. Hyg. **98:**343–346.
13. **Kaplan, E. L., D. R. Johnson, P. Nanthapisud, S. Sirilertpanrana, and S. Chumdermpadetsuk.** 1992. A comparison of group A streptococcal serotypes isolated from the upper respiratory tract in the USA and Thailand: implications. Bull. W. H. O. **70:**433–437.
14. **Kapur, V., S. Topouzis, M. W. Majesky, L.-L. Li, M. R. Hamrick, R. J. Hamill, J. M. Patti, and J. M. Musser.** 1993. A conserved *Streptococcus pyogenes* cysteine protease cleaves human fibronectin and degrades vitronectin. Microb. Pathog. **15:**327–346.
15. **Kehoe, M. A., V. Kapur, A. Whatmore, and J. M. Musser.** 1996. Horizontal gene transfer among group A streptococci: implications for pathogenesis and epidemiology. Trends Microbiol. **4:**436–443.
16. **Levinson, G., and G. A. Gutman.** 1987. Slipped-strand mispairing: a major mechanism for DNA sequence evolution. Mol. Biol. Evol. **4:**203–221.
17. **Loredo-Abdalá, A., J. J. S.-G. De Quevedo, and L. Carbajal-Rodriguez.** 1991. Fiebre reumática. Perfil clínico de una enfermedad persistente. Gac. Med. Mex. **127:**227–232.
18. **Lukomski, S., S. Sreevatsan, C. Amberg, W. Reichardt, M. Woischnik, A. Podbielski, and J. M. Musser.** 1997. Inactivation of *Streptococcus pyogenes* extracellular cysteine protease significantly decreases mouse lethality of serotype M3 and M49 strains. J. Clin. Invest. **99:**2574–2580.
19. **Martin, D. R., and L. A. Single.** 1993. Molecular epidemiology of group A streptococcus M type 1 infections. J. Infect. Dis. **167:**1112–1117.
20. **Muotiala, A., H. Seppala, P. Houvinen, and J. Vuopio-Varkila.** 1997. Molecular comparison of group A streptococci of T1M1 serotype from invasive and noninvasive infections in Finland. J. Infect. Dis. **175:**392–399.
21. **Musser, J. M.** 1997. Streptococcal superantigen, mitogenic factor, and pyrogenic exotoxin B expressed by *Streptococcus pyogenes*, p. 281–310. *In* D. Y. M. Leung, B. T. Huber, and P. M. Schlievert (ed.), Superantigens. Molecular biology, immunology, and relevance to human disease. Marcel Dekker, Inc., New York, N.Y.
22. **Musser, J. M., A. R. Hauser, M. H. Kim, P. M. Schlievert, K. Nelson, and R. K. Selander.** 1991. *Streptococcus pyogenes* causing toxic-shock-like syndrome and other invasive diseases: clonal diversity and pyrogenic exotoxin expression. Proc. Natl. Acad. Sci. USA **88:**2668–2672.
23. **Musser, J. M., V. Kapur, S. Kanjila, U. Shah, D. M. Musher, N. L. Barg, K. H. Johnston, P. M. Schlievert, J. Henrichsen, D. Gerlach, R. M. Rakita, A. Tanna, B. D. Cookson, and J. C. Huang.** 1993. Geographic and temporal distribution and molecular characterization of two highly pathogenic clones of *Streptococcus pyogenes* expressing allelic variants of pyrogenic exotoxin A (scarlet fever toxin). J. Infect. Dis. **167:**337–346.
24. **Musser, J. M., V. Kapur, J. E. Peters, C. W. Hendrix, D. Drehner, G. D. Gackstetter, D. R. Skalka, P. L. Fort, J. T. Maffei, L.-L. Li, and G. P. Melcher.** 1994. Real-time molecular epidemiologic analysis of an outbreak of *Streptococcus pyogenes* invasive disease in US Air Force trainees. Arch. Pathol. Lab. Med. **118:**128–133.
25. **Musser, J. M., V. Kapur, J. Szeto, X. Pan, D. S. Swanson, and D. R. Martin.** 1995. Genetic diversity and relationships among *Streptococcus pyogenes* strains expressing serotype M1 protein: recent intercontinental spread of a subclone causing episodes of invasive disease. Infect. Immun. **63:**994–1003.
26. **Musser, J. M., and R. M. Krause.** The revival of group A streptococcal diseases, with a commentary on staphylococcal toxic shock syndrome. *In*

R. M. Krause (ed.), Emerging infections, in press. Academic Press, Inc., San Diego, Calif.
27. **Mvlvaganam, H., B. Bjorvatn, T. Hofstad, R. Hjetland, E. A. Hoiby, and S. E. Holm.** 1994. Small-fragment restriction endonuclease analysis in epidemiologic mapping of group A streptococci. J. Med. Microbiol. **40:**256–260.
28. **Nelson, K., P. M. Schlievert, R. K. Selander, and J. M. Musser.** 1991. Characterization and clonal distribution of four alleles of the *speA* gene encoding pyrogenic exotoxin A (scarlet fever toxin) in *Streptococcus pyogenes*. J. Exp. Med. **174:**1271–1274.
29. **Norgren, M., A. Norrby, and S. E. Holm.** 1992. Genetic diversity in T1M1 group A streptococci in relation to clinical outcome of infection. J. Infect. Dis. **166:**1014–1020.
30. **Relf, W. A., D. R. Martin, and K. S. Sriprakash.** 1992. Identification of sequence types among the M-nontypeable group A streptococci. J. Clin. Microbiol. **30:**3190–3194.
31. **Rodriguez, R. S., A. L. Abdala, and A. Vizcaino.** 1974. Evaluacion de la parametasona en la etapa aguda de la carditis reumatica. Bol. Med. Hosp. Infant. Mex. **31:**1149–1163.
32. **Seppala, H., J. Vuopio-Varkila, M. Osterblad, M. Jakhola, M. Rummukainen, S. E. Holm, and P. Huovinen.** 1994. Evaluation of methods for epidemiologic typing of group A streptococci. J. Infect. Dis. **169:**519–525.
33. **Stockbauer, K. E., D. Grigsby, L. M. Perea Mejia, X. Pan, A. Cravioto, and J. M. Musser.** Hypervariability generated by natural selection in an extracellular complement inhibiting protein made by serotype M1 strains of group A streptococcus. Proc. Natl. Acad. Sci. USA, in press.
34. **Tran, T. P. O., D. R. Johnson, and E. L. Kaplan.** 1991. The presence of M protein in non-typeable group A streptococcal upper respiratory tract (URT) isolates from South-East Asia. J. Infect. Dis. **169:**658–661.
35. **United States Immigration and Naturalization Service.** 1994. Statistical yearbook of the Immigration and Naturalization Service—1993. U.S. Government Publishing Office, Washington, D.C.
36. **Upton, M., P. E. Carter, M. Morgan, G. F. S. Edwards, and T. H. Pennington.** 1995. Clonal structure of invasive *Streptococcus pyogenes* in Northern Scotland. Epidemiol. Infect. **115:**231–241.
37. **Upton, M., P. E. Carter, G. Orange, and T. H. Pennington.** 1996. Genetic heterogeneity of M type 3 group A streptococci causing severe infections in Tayside, Scotland. J. Clin. Microbiol. **34:**196–198.
38. **Whatmore, A. M., V. Kapur, D. J. Sullivan, J. M. Musser, and M. A. Kehoe.** 1994. Non-congruent relationships between variation in *emm* gene sequences and the population genetic structure of group A streptococci. Mol. Microbiol. **14:**619–631.