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The Genetic Properties of *Streptococcus pyogenes emm49* Genotype Strains Recently Emerged among Severe Invasive Infections in Japan

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Streptococcus pyogenes (group A streptococcus; GAS) is one of the most common human pathogens. It causes a wide array of infections, the most frequent of which is acute pharyngitis (strep throat). Many streptococcal virulence factors involved in GAS-based diseases have been reported, including pyrogenic exotoxins and M protein. M protein, which is an important virulence factor of *S. pyogenes*, protects *S. pyogenes* from phagocytosis by polymorphonuclear leukocytes (1, 2). More than 90 M protein-derived serotypes have been identified, and a molecular approach to the identification of *emm* (M protein) genes has also been documented (http://www.cdc.gov/ncidod/biotech/strep/emmtypes.html). For example, *emm1*, *emm2*, and *emm3* genes encode the M1, M2, and M3 proteins, respectively. *emm* genotyping is also a useful tool for epidemiological investigation.

Since the late 1980s, streptococcal toxic shock-like syndrome (TSLS) caused by *S. pyogenes* has become a serious problem in both developed and developing countries. Its characteristic symptoms progress very rapidly and are fulminant from their onset. Patients can develop necrosis of the soft tissue, acute kidney failure, adult respiratory distress syndrome (ARDS), disseminated intravascular coagulopathy (DIC), and multiorgan failure (MOF) within scores of hours, leading to shock and death. The first defined case of TSLS in Japan was reported in 1992 (3), and the strains of the *emm1* genotype have been found to be dominant in causing TSLS in Japan (4). Since 2000, a total of five *emm49* genotype *S. pyogenes* strains have been isolated from severe invasive GAS patients in Japan, but had not been isolated before 1999 (Table 1); 1 case was reported in 2000, 3 cases in 2002, and 1 case in 2003. T serotypes of all the isolates were grouped into the nontypable.

We examined whether or not five isolates carry pyrogenic exotoxin genes *speA*, *speB*, and *speC*, by PCR with specific primers. All of the isolates carried *speB* gene. None of the isolates carried the *speC* gene. Four of five isolates had the *speA* gene (Table 1). *Sma*I- or *Sfi*I-digested pulsed field gel electrophoresis (PFGE) profiles of the isolates were examined

Table 1. Characteristics of *emm49* genotype *Streptococcus pyogenes* isolates

Strain No.	Year isolated	Place isolated (Prefecture)	Mutation(s) in *emm49* gene from base 89 to base 563[1)]	Possession of *spe* gene		
				speA	*speB*	*speC*
NIH147	2000	Osaka	153 T to C, 183 C to T	−	+	−
NIH200	2002	Tokyo	333 T to C	+	+	−
NIH211	2002	Ehime	333 T to C	+	+	−
NIH226	2002	Hiroshima	333 T to C	+	+	−
NIH230	2003	Tokyo	333 T to C	+	+	−

[1)]: The nucleotide location is based on accession No. M23689 (6). The *emm49* gene with two mutations, 153 T to C and 183 C to T, was designated *emm49.3*.

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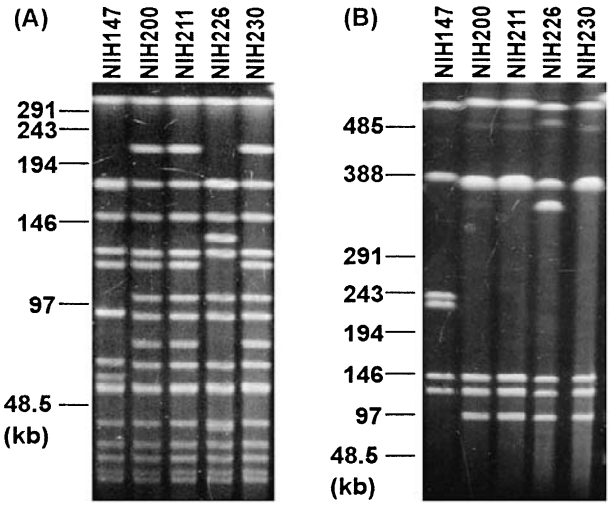

Fig. 1. Ethidium bromide staining of (A) *Sma*I- or (B) *Sfi*I-digested genomic DNAs of *emm49* type *Streptococcus pyogenes* isolates after PFGE separation. Sizes of lambda concatemers are shown at the left. Strain number is indicated on each lane.

as described previously (5). NIH200, NIH211, and NIH230 strains were isolated from patients unrelated each other; however, their PFGE profiles were indistinguishable from one another (Fig. 1). The PFGE profiles of NIH147 and NIH226 were different in several fragments from the above strains, but their profiles as a whole seemed to be similar each other. These results suggest that clonal or possibly related strains of the recently emerged *emm49* genotype *S. pyogenes* have been spreading to cause severe invasive diseases. Much attention should be paid to the prevalence of the strains.

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