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SHORT REPORT
Changing prevalent T serotypes and *emm* genotypes of *Streptococcus pyogenes* isolates from streptococcal toxic shock-like syndrome (TSLS) patients in Japan

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SUMMARY

We surveyed T serotypes and *emm* genotypes of *Streptococcus pyogenes* isolates from streptococcal toxic shock-like syndrome (TSLS) patients. T1 (*emm1*) remained dominant through 1992 to 2000, but the dominant T3 (*emm3.1*) strains from 1992 to 1995 disappeared during 1996–2000. Strains of several *emm* genotypes emerged during 1996–2000, indicating alterations in the prevalent strains causing TSLS.

Streptococcus pyogenes (group A streptococcus) 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 these symptoms have been reported, including pyrogenic exotoxins (SpeA, SpeB and SpeC) 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 of M protein serotypes have been identified, and a molecular approach to identification of *emm* (M protein) genes has also been documented. For example, *emm1*, *emm2* and *emm3* genes encode the M1, M2 and M3 proteins, respectively. In addition, the *emm3.1* and *emm22.2* belong to the *emm3* and *emm22* alleles, respectively (http://www.cdc.gov/ncidod/biotech/strep/emmtypes.html).

T serotypes of *S. pyogenes* have also been important markers in the epidemiological investigation of *S. pyogenes* infections and more than 25 different T serotypes have been described [3, 4]. The combination of T serotype and *emm* genotype allows the identification of strain diversity [5].

From the late 1980s, streptococcal TSLS caused by *S. pyogenes* became a serious problem in both

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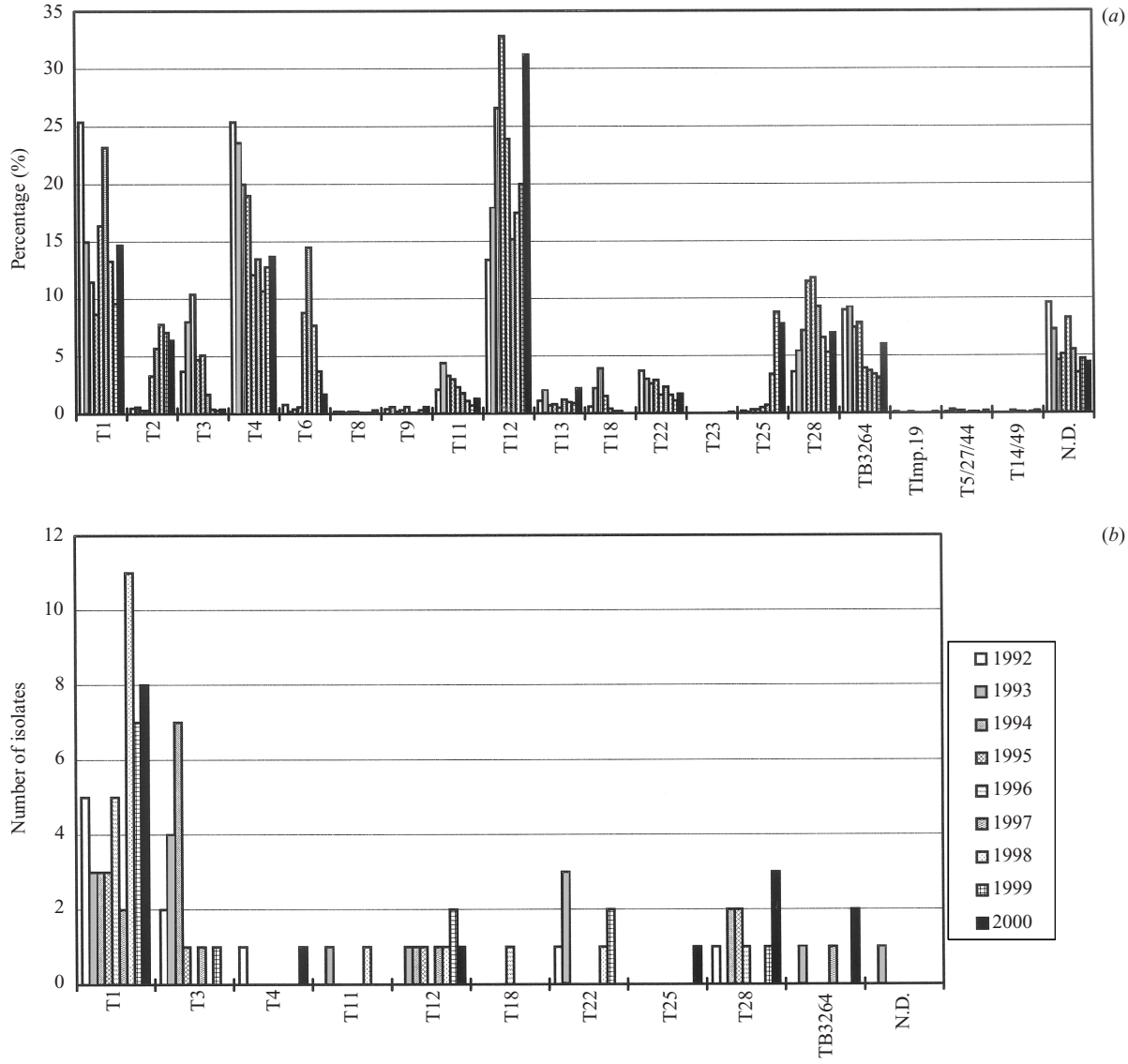

Fig. 1. Distribution of T serotypes; (*a*) the ratio of pharyngitis isolates (%, the number of isolates in each T serotype/the number of total isolates in the year), (*b*) the number of isolates from TSLS patients in each T serotype for each year.

developed and developing countries. Symptoms such as pharyngitis, fever and pain may suddenly develop and progress very rapidly in some patients to soft tissue necrosis, acute kidney failure, adult respiratory distress syndrome (ARDS), disseminated intravascular coagulopathy (DIC) and multiorgan failure (MOF), leading to shock and death. The first defined case of TSLS in Japan was reported in 1992 [6], and the strains of T3 M3 and T1 M1 serotypes were dominant in causing TSLS during 1992–1995 in Japan [7]. Dominance of these M types was also observed in the United States and United Kingdom [8, 9]. In this study, we describe the current epidemiological features of TSLS isolates in Japan in comparison with the characteristics of isolates from pharyngitis patients.

A total of 18 219 strains (group A streptococci) for T serotyping were isolated in 3041 co-operative hospitals located all over Japan during the period of 1996–2000 and serotyped in the Prefectural Institutes of Public Health. This information was sent to the National Institute of Infectious Diseases (reference centre) from branch offices of *S. pyogenes* centre (seven branch offices are located in the Prefectural Institutes of Public Health of Yamagata (1996–7) – Fukushima (1998–2000), Kanagawa, Toyama, Osaka, Yamaguchi, Oita and Tokyo). Almost all strains were isolated from throat-swabs of paediatric patients who suffered from pharyngitis, but a minority was isolated from respiratory secretions (sputum, tracheal aspirates, etc). The dominant serotypes were T12,

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T1 and T4, which accounted for more than half of the isolates each year. This distribution was similar to that found in 1992–5 [7] but the number of T3 isolates (1·8%) decreased in 1996–2000 compared with 1992–5 (5·3%) [7].

Information on streptococcal TSLS patients and T serotypes of the causative pathogens during the period of 1992–2000 was also sent to the National Institute of Infectious Diseases from the branch offices of reference centre and co-operative hospitals. The diagnostic criteria of TSLS were principally based on those described by the Working Group on Severe Streptococcal Infections (1993) [10]. A total of 99 *S. pyogenes* isolates (44 from 1992–5 and 55 from 1996–2000) were cultured predominantly from the blood of TSLS-patients. The T serotypes of 29 of the 44 isolates in 1992–5 were reported previously [7]. The number of T-serotyped isolates each year is shown in Figure 1 and the ratio of each serotype compared between periods of 1992–5 and 1996–2000 is given in Figure 2. T1 isolates were constantly dominant, accounting for 14 of 44 (33%) cases in 1992–5 and 33 of 55 (60%) cases in 1996–2000. However, the ratio of T3 isolates reduced dramatically from 33% in 1992–5 to 4% in 1996–2000. T4 isolates (2% in Fig. 2), which dominated among pharyngitis cases, were less frequent in TSLS. T18 and T25 serotypes were isolated from TSLS patients in 1996–2000, but were absent in 1992–5. These results indicate that serotypes of TSLS isolates have changed between the survey periods.

To examine the difference in *emm* genotypes between the two surveys, we determined nucleotide sequences of the *emm* genes, which encoded the M proteins, of all the 99 TSLS isolates (Fig. 2). The major *emm* genotypes during 1992–5 were *emm1* (14/44; 33%), *emm3.1* (14/44; 33%) and *emm28* (6/44; 14%) while in 1996–2000 they were *emm1* (33/55; 60%), *emm12* (5/55; 9%) and *emm28* (5/55; 9%). Furthermore, *S. pyogenes* carrying the other *emm* types (*emm11*, *emm18*, *emm75* and *emm81*), which were not isolated from TSLS patients in 1992–5, appeared in these patients in 1996–2000. The TSLS isolates with *emm1*, *emm4*, *emm12*, *emm18*, *emm22.2* and *emm28*, expressed T1, T4, T12, T18, T22 and T28, respectively. On the other hand, T11 and T25-serotype strains of TSLS patients carried *emm89* and *emm75*, respectively. In addition, *emm89* isolates of TSLS patients were grouped into T serotypes of T11 and TB3264 (Fig. 2B).

We found 99 cases of TSLS by *S. pyogenes* during 1992–2000 in Japan. The ratio of T serotypes and

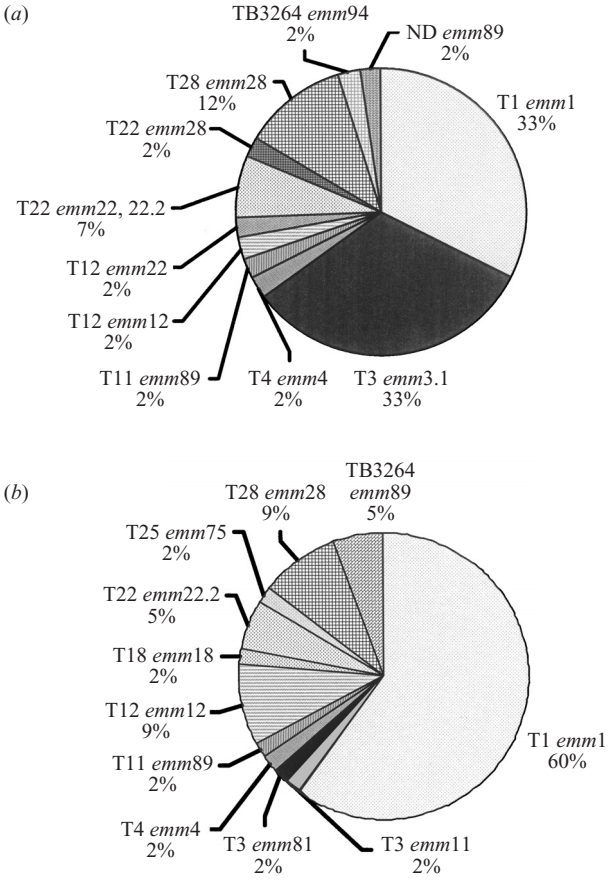

Fig. 2. Comparison of T serotype and the *emm* genotype of *S. pyogenes* strains from TSLS patients in 1992–5 (44 strains) (*a*) and 1996–2000 (55 strains) (*b*). The *emm* genotypes were determined as described by Beall et al. [5].

emm genotypes of TSLS isolates in 1996–2000 clearly changed in comparison with that in 1992–5; T3 *emm3.1* isolates were not found in 1996–2000, and other T and *emm* types (T3 *emm11*, T3 *emm81*, T18 *emm18*, T25 *emm75* and TB3264 *emm89*), which did not appear in 1992–5, emerged in 1996–2000 (Fig. 2).

The frequency of T3 serotype strains from pharyngitis patients increased rapidly during 1993–4 in Japan, and T3 strains predominated from TSLS patients [7]. However, after 1996, the incidence of T3 strains isolated from both pharyngitis and TSLS patients suddenly decreased due to unknown reasons. A similar phenomenon was observed with the T25 serotype; the isolation of T25 strains from pharyngitis patients was more common in 1999 and 2000, and a T25 serotype strain was first isolated from a TSLS patient in 2000 (Fig. 1). We recently showed [11] that T3 serotype strains newly emergent during 1993–4 had acquired foreign phage DNA carrying a new superantigen gene which may be associated with the

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pathogenesis of TSLS. Such an alteration may also have occurred in the T25 strains. The rapid increase and decrease in specific strains among pharyngitis patients may be related to fluctuation of strain serotypes in TSLS.

The *emm* genotypes of T3 isolates from TSLS patients were *emm3.1*, *emm11* and *emm81* and interestingly, the *emm3.1* accounted for all T3 isolates from TSLS patients in 1992–5. In contrast T3 isolates from TSLS patients in 1996–2000 were *emm11* and *emm81*, but not *emm3.1* (Fig. 2). The combination of T3 and *emm11* has not been reported to our knowledge. We do not know how T3 strains acquired the *emm11* gene. However, this suggests that the combination of the T serotypes and the *emm* genotypes may be converted by genetic rearrangement and strains causing TSLS are undergoing genetic change over time.

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