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Molecular Epidemiology of Group A Streptococci T Serotype 1

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Group A streptococcus (GAS) serotype T1/M1 has been commonly associated with streptococcal infections in Japan (1). Pulsed-field gel electrophoresis (PFGE) analysis of T1 isolates obtained from patients in Toyama Prefecture revealed that the PFGE pattern in 1987-1988 differed from that in 1991-1993 (2). Clonal changes of M1 strains on the basis of PFGE were also observed in the United States during the late 1980s (3) and in New Zealand during the early 1980s (4). In the present study, we examined the PFGE patterns of T1 GAS strains from four geographically distant areas in Japan during 1983-1998, with a view to clarify the communicability of the organisms.

A total of 308 T1 GAS isolates from patients without complications were examined. *Sma*I PFGE analysis was performed by using CHEF-DRII (Bio-Rad Laboratories, Hercules, Calif., USA). Southern hybridization analysis was performed employing the Gene Images Random-Prime Labeling and Detection System (Amersham Pharmacia Biotech, Buckinghamshire, UK) using probes of PCR amplified *speA* (393 bp), *speB* (1,113 bp), and *speC* (540 bp).

Fourteen different PFGE patterns were found (Fig. 1). A computer-generated dendrogram revealed two large clusters, A and B (data not shown). Southern hybridization analysis also showed the clear difference between patterns A and B isolates (Fig. 2). The *speA* probe hybridized with none of the DNA fragments of the pattern A isolates but with a ~110 kb fragment of all the pattern B isolates. The *speB* probe hybridized with a ~160 kb fragment of all the pattern A isolates and a ~200 kb fragment of all the pattern B isolates except for pattern B3 isolate that showed a slightly larger hybridizing band. The *speC* probe hybridized with a DNA fragment of patterns A3, A7, and B2. Table 1 shows a common tendency in the three distant areas, Akita, Toyama, and Oita Prefectures, in which pattern A strains predominant before 1990 were gradually replaced by pattern B strains in 3 years from 1990 to 1992. The pattern A strains were present until

1990 in Akita and until 1992 in Toyama and Oita. The second prevalent strains, pattern B, were isolated in these three areas since 1990 but not before. All strains isolated in Okinawa Prefecture from 1991 belonged to pattern B. Among pattern A strains, either pattern A2 or A7 or both were dominant depending upon the area. Pattern B1 was dominant among pattern B strains in all the areas. Murase et al. (5) reported that PFGE patterns of T1/M1 strains isolated from patients with pharyngitis in Kanagawa Prefecture changed over 1988-1989, and that almost all strains showed dominant PFGE patterns from 1989 possessed the *speA* gene. All these findings indicate that the *speA*-positive clone of serotype T1/M1 GAS strains spread in Japan during 1989-1990 and replaced the *speA*-negative clone in various areas of Japan.

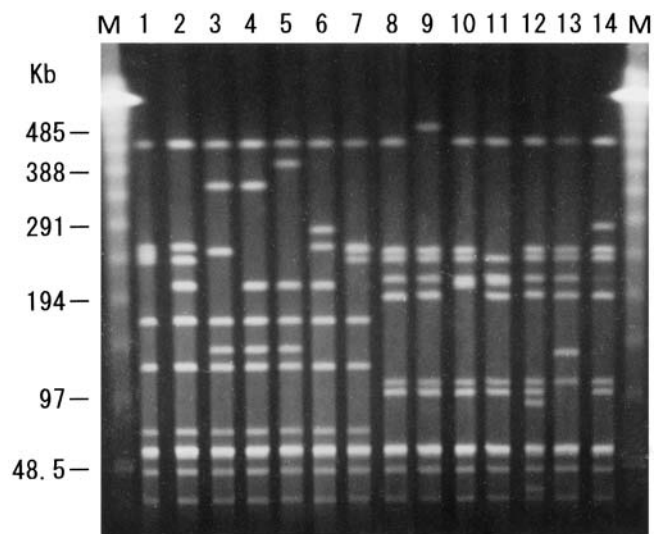


Fig. 1. PFGE patterns of *Sma*I-digested chromosomal DNA of T1 group A streptococci. M: λ DNA ladder, Lane 1: PFGE pattern A1, Lane 2: A2, Lane 3: A3, Lane 4: A4, Lane 5: A5, Lane 6: A6, Lane 7: A7, Lane 8: B1, Lane 9: B2, Lane 10: B3, Lane 11: B4, Lane 12: B5, Lane 13: B6, Lane 14: B7.

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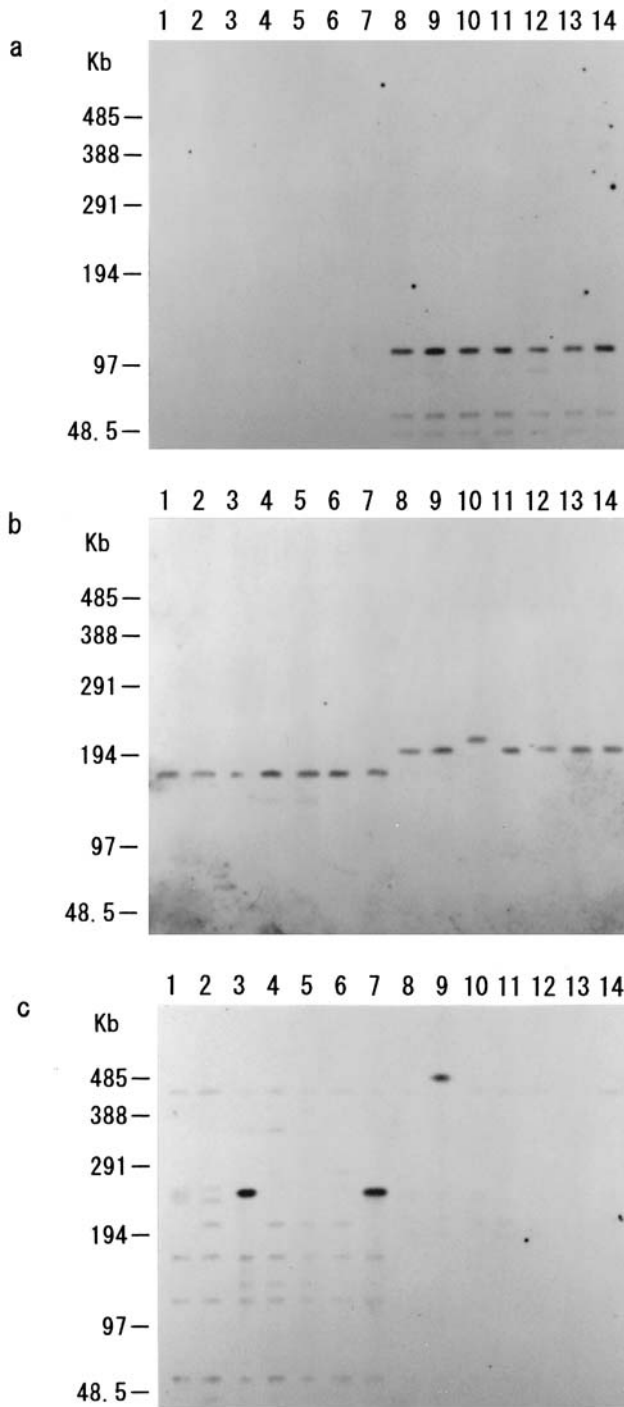


Fig. 2. Southern hybridization analysis of *Sma*I-digested DNA resolved by PFGE. The same membrane was stripped and re-probed with the following probes: *speA* (a), *speB* (b), and *speC* (c).

Table 1. Distribution of Group A Streptococci T serotype 1

Place	PFGE pattern	No. of isolates	1983-1989	1990	1991	1992	1993-1998
Akita	A7	18	13	5			
	B1	20		1	6	5	8
	B6	3					3
Toyama	A1	10	9	1			
	A2	71	54	9	6	2	
	A3	1	1				
	A4	4	4				
	A7	1		1			
	B1	75		3	8	12	52
	B2	1					1
	B3	1					1
Oita	B4	1					1
	B7	2			2		
	A2	21	12	7	2		
	A4	1	1				
	A5	2	1	1			
	A6	1	1				
	A7	9	1	4	3	1	
Okinawa	B1	39		2	6	10	21
	B5	2					2
	B1	24			4	5	15
Total	B4	1			1		
		308	97	34	38	35	104

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