Detection of novel serotype *k* *Streptococcus mutans* in infective endocarditis patients

Infective endocarditis (IE), a microbial infection of the endothelial surface of the heart, is known to be a life-threatening disease in spite of an extremely low rate of incidence (approx. 2–12 cases per 100 000 person-years) (Moreillon & Que, 2004). Recent studies of IE in Japan have found that the most common causative micro-organisms are streptococci (approx. 50%), followed by staphylococci (32–37%) (Nakatani *et al.*, 2003). Dental manipulation is considered to be an important factor for the onset of IE and dentists are generally cautious to prevent the occurrence in IE in patients with cardiac disorders predisposed for IE (Seymour *et al.*, 2000).

Oral streptococci, major members of the oral flora, frequently cause bacteraemia and IE, among which *Streptococcus mutans*, a major causative bacterium of dental caries, has been shown in several reports to be isolated from the blood of patients with IE (Fujiwara *et al.*, 2001; Nomura *et al.*, 2006). Although few studies have demonstrated the presence of *S. mutans* in blood, we recently reported that *S. mutans* DNA was frequently detected in cardiovascular specimens, such as heart valves and atheromatous plaque (Nakano *et al.*, 2006a).

S. mutans was originally classified into three serotypes (*c/elf*) based on its serotype-specific rhamnose glucose polymers (RGPs), which contain a backbone of rhamnose polymers with side chains of glucose polymers (Linzer *et al.*, 1986). However, drastic reductions in the glucose side chains in RGPs were recently identified in blood isolates of *S. mutans*, which were designated new serotype *k* (Nakano *et al.*, 2004a). In the following study, 2–5% of Japanese children tested were shown to possess serotype *k* *S. mutans* in their oral cavities (Nakano *et al.*, 2004b). In addition, a recent study conducted in the UK reported that a non-*c/elf* *S. mutans* strain isolated in 1991 also belonged to

serotype *k* (Waterhouse & Russell, 2006). In the present study, the prevalence of serotype *k* *S. mutans* in extirpated heart valve specimens was analysed using a molecular technique.

Ten patients who underwent heart valve replacement procedures with a diagnosis of IE based on Duke criteria were analysed. The operations were carried out at the Department of Cardiovascular Surgery in Osaka Rosai Hospital, Sakai, Osaka, Japan, during which extirpated heart valves were placed in sterile PBS immediately after collection. All of the procedures in the present study were approved by the Ethical Committee of Osaka Rosai Hospital. Eight of the patients were diagnosed with subacute IE, while the remaining two were diagnosed with acute IE. In addition, four of those ten patients were referred to the Department of Dentistry and Oral Surgery for an oral examination prior to cardiovascular surgery, at which time dental plaque specimens were collected and placed in sterile PBS. Further, 62 subjects (mean age 67.5 years; range 46–84 years; 43 males, 19 females) who underwent valve replacement surgery with a diagnosis of aortic regurgitation, aortic stenosis, mitral regurgitation, mitral stenosis or tricuspid regurgitation were also examined, from whom 62 heart valve and 26 dental plaque specimens were obtained and placed in sterile PBS. These specimens included 52 heart valve and 22 dental plaque samples collected between December 2004 and August 2006 described in our previous report (Nakano *et al.*, 2007), while the 10 additional heart valve and 4 additional dental plaque samples were collected from September to December 2006.

The extirpated heart valves were aseptically cut into small pieces and whole DNA fractions were extracted using a method described previously (Nakano *et al.*, 2004b). PCR was carried out using primers constructed based on the

glucosyltransferase D (*gtfD*) sequence, as described previously (Hoshino *et al.*, 2004). Specimens showing a negative reaction to this set of primers were also analysed by an additional *S. mutans*-specific set of primers based on the *gtfB* sequence (Oho *et al.*, 2000). Seven of the eight subacute IE cases were positive for *S. mutans*, while there were no positive reactions by the specimens from the two acute IE cases. Serotype *k* *S. mutans* species were detected by PCR with AmpliTaq Gold polymerase (Applied Biosystems) using a specific set of primers, as described previously (Nakano *et al.*, 2004b). Samples with visible amplicon bands of 294 bp were regarded as positive for serotype *k*. For reference strains, MT8148 (serotype *c*) and FT1 (*k*) were used.

Molecular techniques are considered to be valuable tools for the diagnosis of IE. Further, it is known that bacterial DNA can still be detected after bacterial cultures become sterile, since it is sufficiently stable and can be amplified by PCR for long periods after the bacteria are no longer viable (Gauduchon *et al.*, 2003), indicating that DNA detection should not be used as a tool for monitoring treatment in IE patients. However, Ravery *et al.* (2005) showed that a PCR assay was more likely to show positive results for patients whose histology is indicative of IE and when bacteria are observed in histological preparations. Taken together, a positive reaction to *S. mutans* using the present PCR method showed that *S. mutans* DNA from viable or non-viable organisms was present in the heart valve at the time of extirpation, although detection of *S. mutans* does not necessarily mean that it is the pathogenic bacterial species in all cases of IE.

Using the same method as in the present study, *S. mutans* was detected in 63.4–68.6% of heart valves extirpated from patients with a diagnosis other than IE (Nakano *et al.*, 2006a, 2007). In the

Table 1. Distribution of serotype *k* *S. mutans* in subacute IE and non-IE patients

	Heart valves		Dental plaque	
	<i>S. mutans</i>	Serotype <i>k</i>	<i>S. mutans</i>	Serotype <i>k</i>
Subacute IE (<i>n</i> =8)	7/8 (88 %)	5/7 (71 %)*	4/4 (100 %)	3/4 (75 %)†
Non-IE (<i>n</i> =62)	37/62 (60 %)	3/37 (8 %)	25/26 (96 %)	5/25 (20 %)

* $P=0.0009$ and † $P=0.0525$ by Fisher's exact probability test, as compared with serotype *k* distribution in heart valve and dental plaque samples, respectively, from the non-IE patients.

present study, 60 % of the heart valves extirpated from patients without IE showed a positive reaction for *S. mutans* (Table 1). It is considered that transient or prolonged bacteraemia is caused by an oral infection, with professional dental treatments and daily oral practices such as tooth brushing and flossing, as well as food chewing, possibly inducing dissemination of oral bacteria into the bloodstream. Therefore, it is possible that the *S. mutans* DNA identified in the present cases was derived from incidental dissemination into the bloodstream and may not have been pathogenic for IE. However, another possibility is that the viable *S. mutans* organisms might have soon become virulent for IE if the heart valves had not been extirpated.

Recently, the serotype distribution of *S. mutans* organisms detected in heart valve specimens was shown to be totally different from that of those taken from the oral cavity (Nakano *et al.*, 2007). In that study, serotype *k* was detected in only 9.1 % of the *S. mutans*-positive specimens. In spite of the limited number of IE cases in the present study, serotype *k* was positive in 71 % of the IE cases, which was a significantly higher rate of incidence than in those from the non-IE patients (Fisher's exact probability test; $P<0.001$) (Table 1). This result indicates that serotype *k* *S. mutans* may be associated with the development of IE.

Serotype *k* *S. mutans* strains have a drastic reduction of glucose side chains in RGP and their common feature is a lower susceptibility to phagocytosis by human polymorphonuclear leukocytes (Nakano *et al.*, 2004a). Two crucial pathogenic steps in the development of IE are vegetation formation by attachment to impaired endothelial surfaces and platelet aggregation (Moreillon & Que, 2004), with

serotype-specific RGPs known to contribute to such platelet aggregation (Chia *et al.*, 2004). In addition, alterations of the major protein antigens on the cell surface have been frequently identified in serotype *k* strains (Nakano *et al.*, 2006b), which might be correlated with the pathogenesis of IE.

Oral bacterial species such as *S. mutans* invade the bloodstream from the oral cavity, thus it is important to analyse the bacterial profiles of oral specimens from IE patients. In surveys of Japanese children conducted using conventional serological and sensitive PCR methods, the distribution of serotype *k* *S. mutans* was 2 % and 5 %, respectively, in oral specimens (Nakano *et al.*, 2004a, b). In addition, non-*clf* *S. mutans* organisms were recently detected using a conventional method in 85 subjects (age range 5–38 years old) living in a suburban area of Tokyo, with a prevalence of 10.6 % reported (Hirasawa & Takada, 2003). It is notable that serotype *k* *S. mutans* was detected at a much higher frequency in the dental plaque specimens from the present subacute IE patients (75 %) than in those from the non-IE patients (20 %) (Table 1), suggesting that those with IE may possess serotype *k* *S. mutans* in the oral cavity at a higher frequency.

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Chia, J. S., Lin, Y. L., Lien, H. T. & Chen, J. Y. (2004). Platelet aggregation induced by serotype polysaccharides from *Streptococcus mutans*. *Infect Immun* **72**, 2605–2617.

Fujiwara, T., Nakano, K., Kawaguchi, M., Ooshima, T., Sobue, S., Kawabata, S., Nakagawa, I. & Hamada, S. (2001). Biochemical and genetic characterization of serologically untypable *Streptococcus mutans* strains isolated from patients with bacteremia. *Eur J Oral Sci* **109**, 330–334.

Gauduchon, V., Chalabreysse, L., Etienne, J., Celard, M., Benito, Y., Lepidi, H., Thivolet-Bejui, F. & Vandenesch, F. (2003). Molecular diagnosis of infective endocarditis by PCR amplification and direct sequencing of DNA from valve tissue. *J Clin Microbiol* **41**, 763–766.

Hirasawa, M. & Takada, K. (2003). A new selective medium for *Streptococcus mutans* and the distribution of *S. mutans* and *S. sobrinus* and their serotypes in dental plaque. *Caries Res* **37**, 212–217.

Hoshino, T., Kawaguchi, M., Shimizu, N., Hoshino, N., Ooshima, T. & Fujiwara, T. (2004). PCR detection and identification of oral streptococci in saliva samples using *gtf* genes. *Diagn Microbiol Infect Dis* **48**, 195–199.

Linzer, R., Reddy, M. S. & Levine, M. J. (1986). Immunochemical aspects of serotype carbohydrate antigen of *Streptococcus mutans*. In *Molecular Microbiology and Immunology of Streptococcus mutans*, pp. 29–38. Edited by S. Hamada, S. Michalek, H. Kiyono, L. Manaker & J. R. McGhee. Amsterdam: Elsevier Science Publishers.

Moreillon, P. & Que, Y. A. (2004). Infective endocarditis. *Lancet* **363**, 139–149.

- Nakano, K., Nomura, R., Nakagawa, I., Hamada, S. & Ooshima, T. (2004a).** Demonstration of *Streptococcus mutans* with a cell wall polysaccharide specific to a new serotype, *k*, in the human oral cavity. *J Clin Microbiol* **42**, 198–202.
- Nakano, K., Nomura, R., Shimizu, N., Nakagawa, I., Hamada, S. & Ooshima, T. (2004b).** Development of a PCR method for rapid identification of new *Streptococcus mutans* serotype *k* strains. *J Clin Microbiol* **42**, 4925–4930.
- Nakano, K., Inaba, H., Nomura, R., Nemoto, H., Takeda, M., Yoshioka, H., Matsue, H., Takahashi, T., Taniguchi, K. & other authors (2006a).** Detection of cariogenic *Streptococcus mutans* in extirpated heart valve and atheromatous plaque specimens. *J Clin Microbiol* **44**, 3313–3317.
- Nakano, K., Tsuji, M., Nishimura, K., Nomura, R. & Ooshima, T. (2006b).** Contribution of cell surface protein antigen PAc of *Streptococcus mutans* to bacteremia. *Microbes Infect* **8**, 114–121.
- Nakano, K., Nemoto, H., Nomura, R., Homma, H., Yoshioka, H., Shudo, Y., Hata, H., Toda, K., Taniguchi, K. & other authors (2007).** Serotype distribution of *Streptococcus mutans*, a pathogen of dental caries, in cardiovascular specimens from Japanese patients. *J Med Microbiol* **56**, 551–556.
- Nakatani, S., Mitsutake, K., Hozumi, T., Yoshikawa, J., Akiyama, M., Yoshida, K., Ishizuka, N., Nakamura, K., Taniguchi, Y. & other authors (2003).** Current characteristics of infective endocarditis in Japan: an analysis of 848 cases in 2000 and 2001. *Circ J* **67**, 901–905.
- Nomura, R., Nakano, K., Nemoto, H., Fujita, K., Inagaki, S., Takahashi, T., Taniguchi, K., Takeda, M., Yoshioka, H. & other authors (2006).** Isolation and characterization of *Streptococcus mutans* in heart valve and dental plaque specimens from a patient with infective endocarditis. *J Med Microbiol* **55**, 1135–1140.
- Oho, T., Yamashita, Y., Shimazaki, Y., Kushiya, M. & Koga, T. (2000).** Simple and rapid detection of *Streptococcus mutans* and *Streptococcus sobrinus* in human saliva by polymerase chain reaction. *Oral Microbiol Immunol* **15**, 258–262.
- Rover, C., Greub, G., Lepidi, H., Casalta, J. P., Habib, G., Collart, F. & Raoult, D. (2005).** PCR detection of bacteria on cardiac valves of patients with treated bacterial endocarditis. *J Clin Microbiol* **43**, 163–167.
- Seymour, R. A., Lowry, R., Whitworth, J. M. & Martin, M. V. (2000).** Infective endocarditis, dentistry and antibiotic prophylaxis; time for a rethink? *Br Dent J* **189**, 610–616.
- Waterhouse, J. C. & Russell, R. R. (2006).** Dispensable genes and foreign DNA in *Streptococcus mutans*. *Microbiology* **152**, 1777–1788.