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%) (Nakano 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, Roversy 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
In the 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 bacteremia 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; \( P < 0.0001 \) (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\(_s\) 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 RGP\(_s\) 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-c\(_\text{elf}\) 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.

### Acknowledgements

This study was supported by the 21st Century COE program entitled ‘Origination of Frontier BioDentistry’ at Osaka University Graduate School of Dentistry supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan, and a Grant-in-Aid for Young Scientists (A), 18689050, from the Ministry of Education, Culture, Sports, Science and Technology. Hideo Yoshioka, Kazuhiro Taniguchi, Atsuo Amano, and Takashi Ooshima

| Table 1. Distribution of serotype k S. mutans in subacute IE and non-IE patients |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Heart valves                       | Dental plaque                   |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| S. mutans | Serotype k | S. mutans | Serotype k |
| Subacute IE (\(n=8\)) | 7/8 (88%) | 3/7 (71%)* | 4/4 (100%) | 25/26 (96%) |
| Non-IE (\(n=62\)) | 37/62 (60%) | 3/37 (8%) | 3/4 (75%)† | 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.

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