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ORIGINAL ARTICLE

Characterization of aortic aneurysms in cardiovascular disease patients harboring *Porphyromonas gingivalis*

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OBJECTIVE: ***Porphyromonas gingivalis*** **was recently shown to cause intimal hyperplasia in a mouse model by a novel cholesterol-independent mechanism, suggesting to be a pathogen-specific feature of cardiovascular diseases. The aim of this study was to characterize the clinical and histopathological features of aortic aneurysms in cardiovascular disease patients harboring oral** ***P. gingivalis*****.**

SUBJECT AND METHODS: Aortic aneurysm specimens were collected from 76 Japanese patients who underwent surgery, of whom dental plaque specimens were also collected from 31 patients. Bacterial DNA was extracted from each specimen to detect ***P. gingivalis*** **by polymerase chain reaction. Histopathological analyses of the aortic aneurysm specimens, including immunohistochemical staining for embryonic myosin heavy chain isoform (SMemb) and S100 calcium-binding protein A9 (S100A9), were also performed.**

RESULTS: The number of aneurysms occurring in the distal aorta was significantly higher in subjects positive for ***P. gingivalis*** **in dental plaque compared with those who were negative. The expressions of S100A9 and SMemb were also significantly greater in the subjects positive for** ***P. gingivalis*** **in dental plaque. On the other hand, there were no significant differences in adipocellular accumulation between the groups.**

CONCLUSIONS: These results suggest that aortic aneurysms in patients harboring oral ***P. gingivalis*** **have greater expression of S100A9 and proliferative smooth muscle cells, which was different from the present patients without oral** ***P. gingivalis*****.**

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Introduction

Chronic marginal periodontitis occurs worldwide and is among the most prevalent infectious diseases in humans (Pihlstrom *et al*, 2005). This inflammatory disorder is caused by interactions of a small subset of periodontal pathogens that are harbored in dental plaque, a complex microbial biofilm. Periodontal inflammation often leads to superficial ulcers in the periodontal pocket, where capillaries are exposed to microbial biofilms (D'Aiuto *et al*, 2004). It is well known that periodontal pathogens are translocated and released from periodontal pockets into the bloodstream, and transient bacteremia has been found to develop after preventive dental procedures and periodontal therapy, including tooth brushing (Sconyer *et al*, 1973; Caroll and Sebor, 1980; Schlein *et al*, 1991; Kinane *et al*, 2005; Forner *et al*, 2006a), subgingival irrigation (Waki *et al*, 1990), periodontal debridement (Heimdahl *et al*, 1990; Messini *et al*, 1999; Kinane *et al*, 2005; Forner *et al*, 2006b), and tooth extraction (Heimdahl *et al*, 1990; Veréis *et al*, 2001), as well as in relation to chewing (Everett and Hirschmann, 1977; Forner *et al*, 2006a), with the frequency ranging from 17% to 100% in individuals with periodontitis. Therefore, it has been suggested that periodontitis is associated with several systemic diseases, including cardiovascular diseases, preterm low birth weight, rheumatoid arthritis, and diabetes mellitus (Beck *et al*, 1996; Offenbacher *et al*, 1999; Ebersole *et al*, 2002; Scannapieco *et al*, 2003). However, there is little definitive evidence regarding the role of periodontitis in systemic diseases.

Porphyromonas gingivalis is known to be a major causative agent of periodontitis (Pihlstrom *et al*, 2005) and is gaining increasing attention for its possible

association with cardiovascular diseases, as several epidemiological studies have indicated involvement of the bacterium in the development of atherosclerosis and aneurysms (Scannapieco *et al*, 2003; D'Aiuto *et al*, 2004). Furthermore, recent *in vitro* and *in vivo* animal experiments have shown that the bacterium possesses several properties known to be related to the pathogenesis of atherosclerosis (Kuramitsu *et al*, 2001; Li *et al*, 2002). However, it remains unclear how *P. gingivalis* is involved in the development of atherosclerosis and aneurysms, because no systematic studies have been performed in human subjects.

Porphyromonas gingivalis has long fimbriae, filamentous appendages on the bacterial surface, and is classified into six genotypes based on the diversity of the *fimA* genes encoding each fimbria subunit (FimA). We previously demonstrated that bacterial clones with types II, IV, and Ib *fimA* are invasive, whereas types I, III, and V are non-invasive (Amano *et al*, 1999; Nakagawa *et al*, 2002b). However, it is unknown whether these genotypic variations are related to atherosclerosis or aneurysms.

We previously demonstrated the presence of the *P. gingivalis* bacterial DNA in aortic aneurysm specimens (Nakano *et al*, 2006), indicating the possibility that aortic aneurysms and atherosclerosis are associated with *P. gingivalis* harbored in the oral cavity. In addition, we recently reported that human aortic smooth muscle cells were drastically transformed from the contractile to proliferative phenotype by infection with *P. gingivalis*, resulting in increased cell proliferation *in vitro* (Inaba *et al*, 2009). That transformation of aortic smooth muscle cells from contractile to proliferative phenotype was shown to be accompanied by upregulation of the expression of S100 calcium-binding protein A9 (S100A9) (Inaba *et al*, 2009), which is generally known to be a proinflammatory mediator in acute and chronic inflammation (Lagasse and Clerc, 1988; Gebhardt *et al*, 2006). In addition, attenuation of S100A9 expression by small interfering RNAs dramatically inhibited cell transformation and secondary growth (Inaba *et al*, 2009).

Atherosclerosis is generally known to be initiated by the formation of cholesterol-rich lesions in the arterial wall, with the cholesterol ester-rich macrophages, termed foam cells, considered to be important for development of those lesions (Ross, 1993). On the other hand, we found that intravascular *P. gingivalis* infection of murine aortas is associated with transformation of intimal smooth muscle cells from the contractile to proliferative phenotype, resulting in the development of intimal hyperplasia *in vivo* in the absence of hypercholesterolemia (Hokamura *et al*, 2010). In that study, we showed that S100A9 expression was upregulated in intimal tissues concurrently with the development of intimal hyperplasia (Hokamura *et al*, 2010). These findings led us to investigate the pathogenic mechanisms of *P. gingivalis*-related cardiovascular diseases in humans.

In this study, we investigated clinical and histopathological differences with regard to aortic aneurysms obtained from patients with and without oral *P. gingivalis*. Our results showed that aortic hyperplasia in the aneurysms of patients positive for *P. gingivalis* in the oral cavity was characteristically associated with a significant expression of S100A9 and local inflammation. These findings provide new evidence for the involvement of *P. gingivalis* in smooth muscle hyperplasia that induces human aortic aneurysmal dilatation.

Subjects and methods

Study design

This investigation conformed to the principles outlined in the Declaration of Helsinki. All of the study protocols were approved by the Ethics Committee of Osaka Rosai Hospital (Sakai, Osaka, Japan), and all patients were informed of the protocols and gave written approval prior to their participation in the study. Seventy-six Japanese patients who underwent surgery for aortic aneurysms at the Department of Cardiovascular Surgery in Osaka Rosai Hospital from December 2004 to June 2008 were analyzed. The subjects in this study were diagnosed as having a thoracic aortic aneurysm (TAA), abdominal aortic aneurysm (AAA), or thoracoabdominal aortic aneurysm (TAAA), and further classified into two categories based on the location of the involved aorta. Aneurysms formed between the ascending aorta and the aortic arch were defined as 'aneurysms of the proximal aorta,' whereas those located between the descending aorta and abdominal aorta were defined as 'aneurysms of the distal aorta.'

Detection of Porphyromonas gingivalis in aortic aneurysm specimens

The distribution of *P. gingivalis* in 45 aneurysm specimens was determined by polymerase chain reaction (PCR) with bacterial species-specific and universal primer sets, as previously described (Nakano *et al*, 2006). In addition, genotyping of *P. gingivalis* long fimbriae (*fimA*) was also performed for *P. gingivalis*-positive specimens by PCR, as previously described (Amano *et al*, 1999; Nakagawa *et al*, 2002b). The detection limit of the PCR systems for *P. gingivalis* and *fimA* genes were approximately 5–10 bacterial cells per sample (Amano *et al*, 1999). In addition, immunochemical staining for bacterial bodies of *P. gingivalis* on tissue sections (3-μm thick) prepared from the main part of the aneurysmal lesion was carried out using rabbit antiserum against recombinant FimA proteins of *P. gingivalis*, as previously described (Nakagawa *et al*, 2002b). Primary staining was visualized using a Vectastain ABC kit and 3,3′-deaminobenzidine substrate kit (Vector Laboratories, Co. Ltd., Burlingame, CA, USA) according to the instructions of the supplier.

Classification of patients by Porphyromonas gingivalis detection

Both aortic aneurysm and dental plaque specimens were further collected from 31 subjects (Table 1). Supra- and sub-gingival dental plaque specimens were collected from the mesial and buccal sites of all teeth with sterile Gracey curettes, and then PCR detection of *P. gingivalis* in the aneurysm and dental plaque specimens was performed. Subjects negative for *P. gingivalis* in dental

Table 1 Summary of patients examined in this study

Group	No.	Age/Gender	*Porphyromonas gingivalis* detection by PCR[a]	*Features of aortic aneurysm*		
				Diagnosis[b]	*Classification*[c]	*Size (mm)*
Oral Pg-negative	1	75/F	–	TAA (ascending)	Proximal	60
	2	77/M	–	TAA (arch)	Proximal	60
	3	65/M	–	TAA (ascending)	Proximal	45
	4	71/F	–	TAA (arch)	Proximal	53
	5	68/F	–	AAA	Distal	65
	6	68/M	–	TAA (ascending)	Proximal	60
	7	68/F	–	TAA (ascending)	Proximal	50
	8	79/M	–	AAA	Distal	90
	9	74/M	–	TAA (arch)	Proximal	55
	10	57/M	–	AAA	Distal	55
	11	82/M	–	TAA (arch- descending	Distal	70
	12	76/M	–	TAA (arch)	Proximal	45
	13	76/M	–	TAA (arch)	Proximal	50
	14	68/M	–	TAA (ascending)	Proximal	45
Oral Pg-positive	15	64/M	+ (Ib)	TAAA	Distal	60
	16	80/F	+ (I)	AAA	Distal	75
	17	70/M	+ (IV)	AAA	Distal	40
	18	67/M	+ (I)	TAAA	Distal	55
	19	60/M	+ (IV)	AAA	Distal	40
	20	59/M	+ (I)	TAA (ascending-arch)	Proximal	60
	21	70/M	+ (Ib)	AAA	Distal	65
	22	61/M	+ (II)	AAA	Distal	60
	23	77/M	+ (II)	AAA	Distal	55
	24	73/M	+ (II)	AAA	Distal	35
	25	78/F	+ (II)	AAA	Distal	50
	26	78/M	+ (IV)	TAA (arch)	Proximal	45
	27	78/M	+ (IV)	AAA	Distal	45
	28	63/M	+ (IV)	TAA (ascending)	Proximal	42
	29	73/M	+ (II)	TAA (arch)	Proximal	51
	30	67/F	+ (III/IV)	AAA	Distal	48
	31	72/M	+ (II)	TAA (arch-descending	Distal	67

[a]'+' and '–' indicate *P. gingivalis* detected and not detected, respectively. Parentheses indicate the *fimA* genotype of *P. gingivalis*.
[b]Ascending, arch, and descending indicate the ascending aorta, aortic arch, and descending aorta, respectively.
[c]'Distal' and 'Proximal' indicate aneurysms in the distal and proximal aorta, respectively.

plaque were classified as Oral Pg-negative, while those with *P. gingivalis* detected in dental plaque were classified as Oral Pg-positive. In addition, genotyping of *P. gingivalis fimA* was performed, as described in the previous section. Dental plaque specimens were also tested for five other periodontal bacteria, including *Prevotella intermedia*, *Treponema denticola*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans*, and *Campyrobacter rectus*, using a PCR method as previously described (Nakano *et al*, 2006).

Histopathological evaluations

Hematoxylin–eosin staining of tissue sections was performed and pathological features, including proliferation of smooth muscle cells, accumulation of adipose cells, and infiltration of inflammatory cells, in the aneurysmal lesions were scored as follows: 0 (none), 1 (mild), 2 (moderate), and 3 (severe). All scoring evaluations were performed a double-blind fashion by three investigators, including one pathologist. Averaged scores from the three investigators were then plotted.

S100A9 and SMemb expressions in aneurysm specimens

Immunohistochemical staining for S100A9 in the aneurysmal lesions was performed using anti-S100A9 monoclonal (Monosan Co. Ltd., Uden, Netherlands) and polyclonal (Santa Cruz Co. Ltd., Santa Cruz, CA, USA) antibodies. Immunohistochemical staining for the embryonic isoform of the myosin heavy chain, a phenotypic marker of proliferative smooth muscle cells (Aikawa *et al*, 1995) in the aneurysmal lesions was performed using the anti-embryonic isoform of the myosin heavy chain (SMemb) polyclonal antibody (Yamasa, Co. Ltd., Choshi, Japan) as described previously (Aikawa *et al*, 1995). Detection of signals was performed as described in the section above. Immunohistochemical marker expression was evaluated by scoring as follows: 0 (none), 1 (mild), 2 (moderate), and 3 (severe). All scoring evaluations were performed in a double-blind fashion by three investigators, including one pathologist. Averaged scores from the three investigators were then plotted.

Statistical analysis

All data are presented as the mean ± s.d. Statistical analyses were performed using the computational packages StatView 5.0 (SAS Institute Inc., Cary, NC, USA) and Prism 4 (GraphPad Software Inc., San Diego, CA, USA). Fisher's exact probability test was utilized for comparisons of the location of aneurismal

lesions and distribution of six periodontal bacterial species between the Oral Pg positive- and -negative groups, as well as high- and low-virulence *fimA* genotypes in *P. gingivalis*-positive specimens. The size of the lesions, histopathological features, and expression of S100A9 and SMemb in Oral Pg-negative and Oral Pg-positive groups were compared using an unpaired Student's *t*-test. In addition, the correlation of S100A9 monoclonal, polyclonal, and SMemb polyclonal antibody staining scores for sections from the same cases in all aneurysm specimens were evaluated by regression analysis.

Results

Detection of Porphyromonas gingivalis in aneurysm specimens

PCR analyses revealed that eight of the 76 aneurysm specimens (10.5%) were positive for *P. gingivalis*, all of which were classified by invasive *fimA* genotypes (Figure 1a, $P < 0.01$). On the other hand, *P. gingivalis* bacterial bodies were immunochemically detected with FimA-specific antiserum in one of the eight PCR-positive specimens (Figure 2). In addition, immunochemical staining for *P. gingivalis* was negative in the PCR-negative specimens.

Detection of periodontal bacteria in dental plaque specimens

PCR-analysis of 31 dental plaque specimens revealed that 17 were positive for *P. gingivalis* (54.8%), whereas the remaining 14 showed no positive reaction. The former and latter were categorized into the Oral Pg-positive and -negative groups, respectively, as shown in Table 1. In addition, invasive clones (types II, IV, and Ib *fimA*) of *P. gingivalis* were detected in dental plaque specimens at significantly greater rates than non-invasive clones (types I, III and V *fimA*; Figure 1b, $P < 0.01$). On the other hand, analyses of the distribution of five other periodontal bacterial species showed that the detection rate of *P. intermedia* in the Oral Pg-positive group was significantly greater than that in Oral Pg-negative group (Figure 3, $P < 0.01$). By contrast, the distribution rates of the other four species (*T. denticola*, *T. forsythia*, *A. actinomycetemcomitans* and *C. rectus*), were not significantly different between the Oral Pg-positive and -negative groups.

Clinical features

The average ages of the subjects in the Oral Pg-positive and -negative groups were almost the same (70.2 ± 7.1 vs 71.3 ± 6.6). On the other hand, proximal aorta involvement occurred at a significantly higher incidence in the Oral Pg-negative group than the Oral Pg-postive group (71.4% vs 23.5%; $P < 0.05$), while the incidence of distal aorta involvement was significantly higher in the Oral Pg-positive group than the Oral Pg-negative group (76.5% vs 28.6%; $P < 0.05$). In addition, the size of the lesions in the Oral Pg-negative group (mm) tended to be slightly larger (57.3 ± 12.0 vs 52.5 ± 10.9), although the difference was not significant.

Histopathological features

The score for smooth muscle cell proliferation in the Oral Pg-positive group was 2.35 ± 0.79, which was higher than that in the Oral Pg-negative group (1.87 ± 0.91), although not statistically significant ($P = 0.1937$). Moreover, the adipose cell accumulation score in the Oral Pg-positive group was 1.13 ± 0.99, which was lower than that in the Oral Pg-negative group (1.68 ± 0.14), although it was not statistically significant ($P = 0.1464$). There was no difference with regard to the infiltration of inflammatory cells between the groups.

S100A9 and SMemb expressions in aneurysm specimens

The level of SMemb expression differed greatly between aneurysmal lesions isolated from Oral Pg-positive and -negative patients (Figure 4a,b). Furthermore, the scores for SMemb expression in the Oral Pg-positive

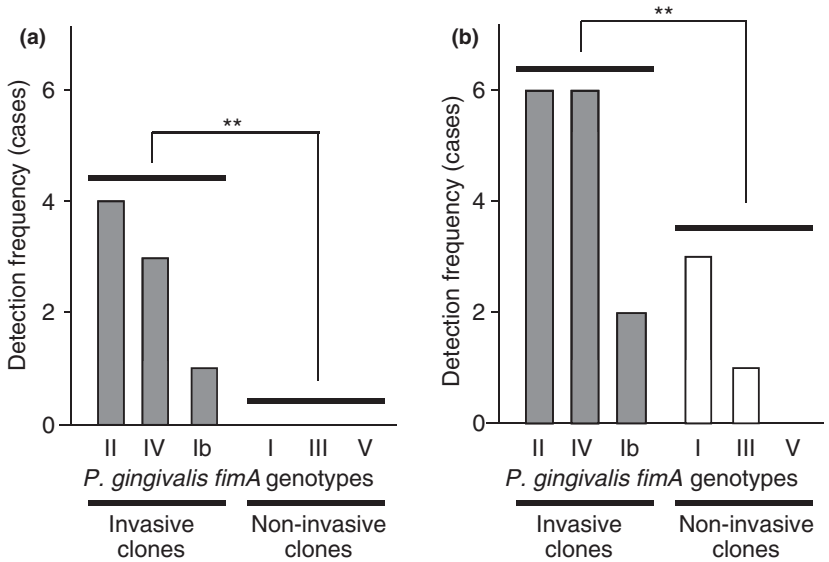

Figure 1 Classification of *fimA* genotypes in *Porphyromonas gingivalis* detected subjects. Determination of *fimA* genotypes was carried out using (**a**) *P. gingivalis*-positive aneurysm tissues and (**b**) dental plaque specimens from 25 patients. One of the dental plaque specimens showed two *fimA* genotypes, thus a total of 26 were analyzed. Fisher's exact probability test was used to compare the distribution of invasive and non-invasive *fimA* clones (**$P < 0.01$). Gray columns represent invasive genotypes of *P. gingivalis*

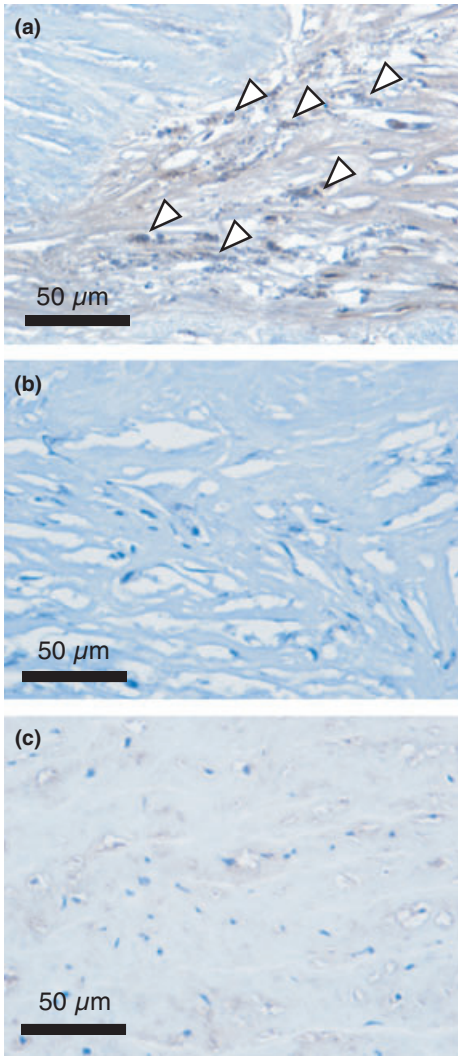

Figure 2 Immunochemical detection of *Porphyromonas gingivalis*-bacterial bodies in aortic aneurysm specimens. Representative images from a PCR-positive *P. gingivalis* specimen (AAA extirpated from 81-year-old male patient) stained with (**a**) antiserum against *P. gingivalis* fimbriae and (**b**) with non-specific serum. White arrowheads show *P. gingivalis*-bacterial bodies. In addition, (**c**) a *P. gingivalis*-negative specimen (TAA extirpated from 69-year-old female patient) stained with antiserum against *P. gingivalis* fimbriae is shown

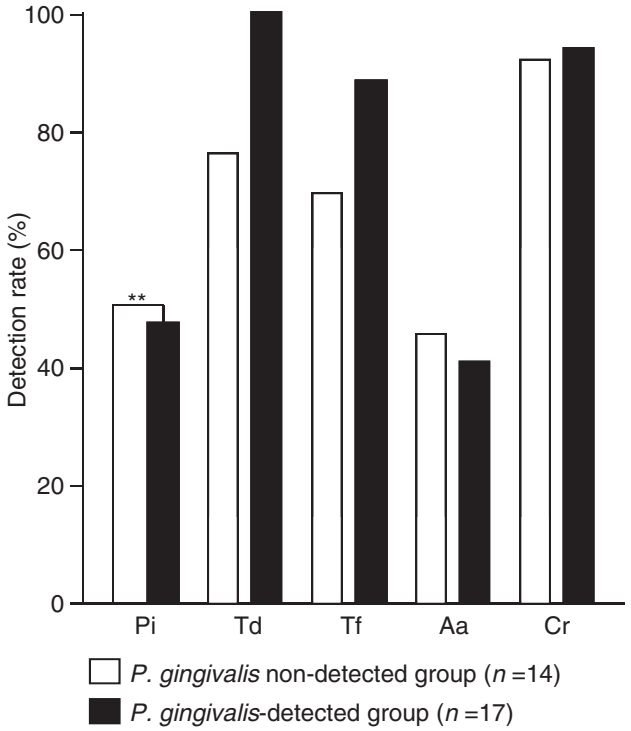

Figure 3 Distribution of five periodontal bacterial species in dental plaque specimens from Oral Pg-positive and -negative groups. Pg; *P. gingivalis*, Pi; *P. intermedia*, Td; *T. denticola*; Tf; *T. forsythia*, Aa; *A. actinomycetemcomitans*, Cr; *C. rectus*. There were statistically significant differences between the groups (**$P < 0.01$; Fisher's exact probability test)

group were significantly greater than those in the Oral Pg-negative group ($P < 0.0001$; Figure 5a). Immunochemical staining for S100A9 in aneurysmal lesions is shown in Figure 4c,d. S100A9 expression in the Oral Pg-negative group was weak and primarily observed in macrophage-like inflammatory cells around areas of adipose cell accumulation. By contrast, the expression of S100A9 in aneurysmal lesions from Oral Pg-positive patients was observed in proliferating smooth muscle cell layers within hyperplasic lesions. It is notable that the expression levels of S100A9 were shown to be significantly greater in the Oral Pg-positive group by both monoclonal and polyclonal antibody staining ($P = 0.0002$ and $P < 0.0001$, respectively; Figure 5b,c). Immunochemical staining revealed that areas positively stained for SMemb and S100A9 were significantly overlapped (Figure 4). Examinations of the correlation between S100A9 and SMemb expressions in all specimens ($n = 76$) indicated that there was a positive correlation between S100A9 monoclonal and polyclonal antibody scores ($P < 0.0001$; Figure 6a). In addition, S100A9 expression scores were positively correlated with SMemb expression scores ($P < 0.0001$, Figure 6b,c).

Discussion

Recent *in vitro* studies have demonstrated that *P. gingivalis* possesses properties that are consistent with an involvement in the pathogenesis of atherosclerosis. These include the ability to cause low density lipoprotein oxidation, foam cell formation, and platelet aggregation (Kuramitsu *et al*, 2001). In addition, systemic challenge by *P. gingivalis* was reported to accelerate atherogenic plaque formation in mice fed a high cholesterol diet (Li *et al*, 2002). These results strengthened the hypothesis that *P. gingivalis* infection is a risk factor in the development of atherosclerosis. Atherosclerosis is also associated with aortic aneurysm development; however, only 9–16% of patients with atherosclerosis of the abdominal aorta have been reported to develop AAAs (Scott *et al*, 2001; Jaffer *et al*, 2002). This indicates that there must be causative factors other than elevated blood cholesterol that

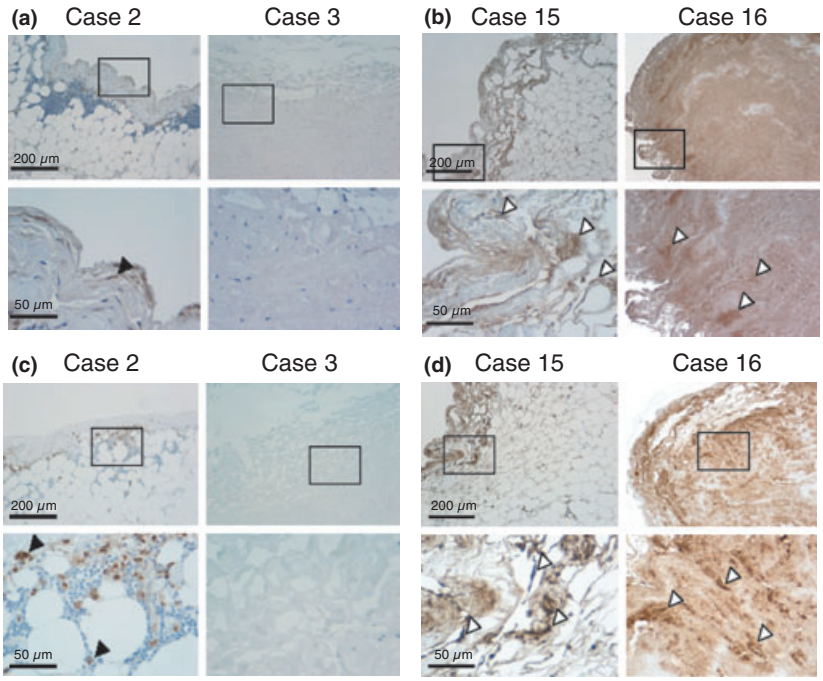

Figure 4 Histopathological appearance of aneurysmal tissues following immunochemical staining with SMemb and S100A9. The polyclonal antibody for the embryonic isoform of the myosin heavy chain (SMemb) was used for immunochemical staining (**a, b**). Brown staining shows SMemb expression. (**a**) Specimens from subjects without *Porphyromonas gingivalis* detected. Black arrowheads indicate proliferative smooth muscle like-cells expressing SMemb. (**b**) Specimens from subjects with *P. gingivalis* detected. White arrowheads indicate proliferated smooth muscle cells expressing SMemb. Upper panels of both (**a**) and (**b**) show low magnifications, while lower panels show higher magnifications of the areas outlined in the low magnification images. The monoclonal antibody for S100A9 was used for immunochemical staining (**c, d**). Brown staining shows S100A9 expression. (**c**) Specimens from subjects without *P. gingivalis* detected. Black arrowheads indicate macrophage-like cells expressing S100A9. (**d**) Specimens from subjects with *P. gingivalis* detected. White arrowheads indicate proliferated smooth muscle cells expressing S100A9. Upper panels of both (**c**) and (**d**) show low magnifications, while lower panels show high magnifications of the areas outlined in the low magnification images

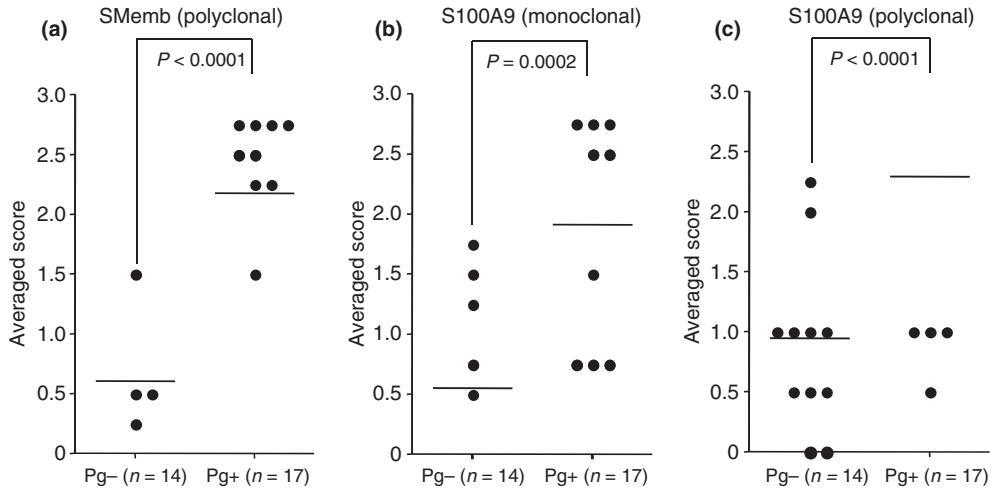

Figure 5 Evaluation of expressions of SMemb and S100A9 in aneurysmal tissues of patients with or without *Porphyromonas gingivalis* in the dental plaque specimens. Scoring for immunochemical detection of SMemb expression in the Oral Pg-positive and -negative groups using (**a**) a polyclonal antibody, and S100A9 expression, using (**b**) monoclonal and (**c**) polyclonal antibodies. Horizontal bars show the mean values for all scores in each group. Each dot indicates an individual specimen

are involved in the formation of aortic aneurysmal lesions.

Molecular approaches are generally used for the detection of bacterial DNA in cardiovascular specimens, because the detection sensitivity is extremely high (Fiehn *et al*, 2005). In this study, *P. gingivalis* was detected by immunochemical means in only one of the specimens shown to be *P. gingivalis*-positive by PCR, likely because of the low sensitivity of immunochemical detection. However, to our knowledge, this is the first

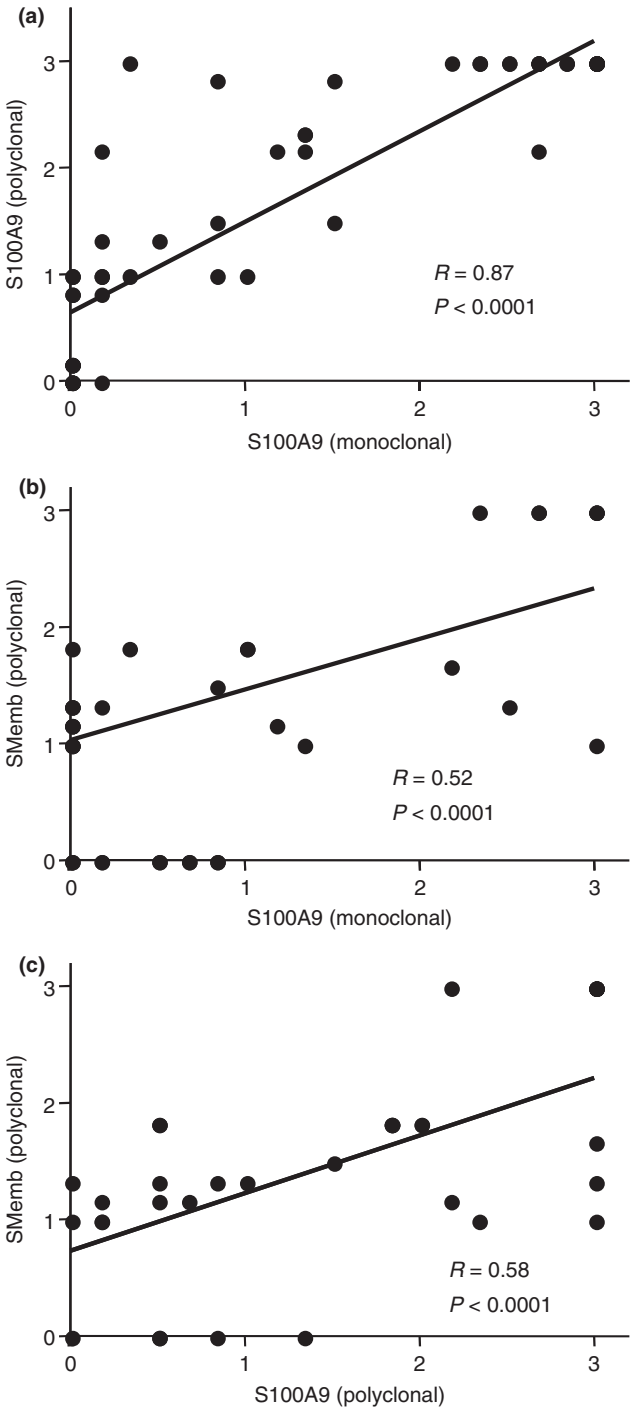

Figure 6 Correlation of S100A9 and SMemb antibody scores. Correlation of scores following (**a**) S100A9 monoclonal and polyclonal antibody staining, (**b**) S100A9 monoclonal and SMemb antibody staining, and (**c**) S100A9 polyclonal and SMemb antibody staining. Results of regression analyses are shown. Each dot indicates an individual specimen

study to demonstrate the occurrence of *P. gingivalis* bacterial bodies in aortic aneurysm specimens by immunochemistry findings. The use of better antiserum may increase the sensitivity of this method in subsequent studies. No *P. gingivalis*-positive aortic aneurysm specimens from 31 patients who also had dental plaque specimens were identified by PCR. Therefore, we considered that the bacterial numbers present in the aneurysm specimens could be extremely low or not present, even in specimens from *P gingivalis*-positive subjects. As *P. gingivalis* is a periodontal bacterial species localized in the oral cavity, it is reasonable to consider that *P. gingivalis* detected in blood vessels could be derived from the oral cavity. Therefore, we categorized the groups based on the occurrence of *P. gingivalis* in the oral cavity.

In this study, the clinical and histopathological features of human aortic aneurysm specimens were analyzed for their relationship with oral *P. gingivalis* occurrence. The present results show that aortic aneurysms associated with oral *P. gingivalis* occurrence are markedly different from those in individuals who do not harbor the bacteria. Adipocellular accumulation in the Oral Pg-positive group was not significantly greater than that in the Oral Pg-negative group, supporting the notion of a cholesterol-independent etiology for *P. gingivalis*-related aneurysm formation. In fact, no positive correlation between total cholesterol level and adipocyte accumulation was observed in any of patients with available data (data not shown, $P = 0.2391$). In addition, averaged total cholesterol levels for the Oral Pg-positive and -negative subjects were 201.0 and 206.2 mg/dl, respectively, indicating a cholesterol-independent etiology of the anueurysms.

S100A9 is reportedly expressed in human atherosclerotic lesions, and has been shown to have an influence on atherogenesis and its complications (McCormick *et al*, 2005). We previously observed that the induction of S100A9 by *P. gingivalis* in cultured human aortic smooth muscle cells was associated with a change of these cells into the proliferative phenotype (Inaba *et al*, 2009; Hokamura *et al*, 2010). The proliferative phenotype of smooth muscle cells is known to have lost the ability to contract physiologically, resulting in abnormal expansion of the aorta. Given that increased S100A9 and SMemb expressions occur under non-elevated cholesterol conditions, it is reasonable that a novel cholesterol-independent etiology exists in the formation of aortic aneurysmal lesions associated with *P. gingivalis*. Recently, it was reported that the average total serum cholesterol levels in Asians is lower than that in Westerners (Ueshima *et al*, 2008), indicating that *P. gingivalis* may be a potential new risk factor for cardiovascular diseases in Asian individuals.

Porphyromonas gingivalis clones with types II, IV, and Ib *fimA* have been shown to be involved in the development and deterioration of periodontitis (Amano *et al*, 1999; Nakagawa *et al*, 2002a,b). In addition, subcutaneous injection of strains with these virulent *fimA* genotypes caused greater systemic inflammation than those with non-virulent *fimA* strains (types I, III, and V) in a murine abscess model (Nakano *et al*, 2004). In this study, approximately 85% of the *P. gingivalis*-positive specimens contained invasive clones (types II, IV, and Ib). Therefore, it is possible to speculate that clones with a high virulence for periodontal disease are

also highly virulent in the development of cardiovascular diseases.

Seventy-six patients participated in this study, which enabled us to analyze the clinical and histopathological characteristics of 76 aortic aneurysms specimens. It is unfortunate that dental plaque specimens could be collected from only 31 patients. Nevertheless, the present findings indicate that *P. gingivalis* occurrence is related to aortic aneurysm formation in several cases. Thus, routine dental checkups and oral hygiene procedures should be regarded as important for the detection of patients at high risk for developing aortic aneurysms in addition to other systemic diseases.

As we analyzed human clinical specimens in this study, it should be noted that the effects of other bacterial species may influence the observed mechanism of disease. The detection rate of *P. intermedia* in the Oral Pg-positive group was significantly higher than in the Oral Pg-negative group, indicating that other bacterial species may influence the mechanism of disease. Additional studies focusing on other periodontal species should be performed using *in vitro* and animal experiments.

In conclusion, this study evaluated the clinical and histopathological features of aortic aneurysms associated with *P. gingivalis* occurrence. The major characteristic of aortic tissue associated with *P. gingivalis* occurrence was smooth muscle cell proliferation in association with a high intensity of local aortic inflammation as revealed by the expression of S100A9. Moreover, *P. gingivalis* occurrence was especially associated with aneurysms in the distal aorta. Additional studies should be performed to elucidate the molecular basis of *P. gingivalis*-mediated formation of AAAs, which may support a scenario in which *P. gingivalis* induces an inflammatory milieu within the aorta, resulting in smooth muscle cell-dependent hyperplasia.

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