

# Oral bacteria in the occluded arteries of patients with Buerger disease

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**Objective:** Recent studies have suggested that infectious organisms play a role in vascular diseases. In this study, to explore a possible link between oral infection and Buerger disease, we investigated whether oral (periodontal) bacteria were present in occluded arteries removed from patients with characteristic Buerger disease.

**Methods:** Fourteen male patients with a smoking history who had developed characteristics of Buerger disease before the age of 50 years were included in this study. Occluded arteries, including superficial femoral (n = 4), popliteal (n = 2), anterior tibial (n = 4), and posterior tibial (n = 4) arteries, were removed and studied. A periodontist performed a periodontal examination on each patient and collected dental plaque and saliva samples from them at the same time. The polymerase chain reaction method was applied to detect whether seven species of periodontal bacteria—*Porphyromonas gingivalis*, *Tannerella forsythensis*, *Treponema denticola*, *Campylobacter rectus*, *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia*, and *Prevotella nigrescens*—were present in the occluded arteries and oral samples. In addition, arterial specimens from seven control patients were examined by polymerase chain reaction analysis.

**Results:** DNA of oral bacteria was detected in 13 of 14 arterial samples and all oral samples of patients with Buerger disease. *Treponema denticola* was found in 12 arterial and all oral samples. *Campylobacter rectus*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythensis*, and *Prevotella nigrescens* were found in 14% to 43% of the arterial samples and 71% to 100% of the oral samples. A pathologic examination revealed that arterial specimens showed the characteristics of an intermediate-chronic-stage or chronic-stage lesion of Buerger disease. All 14 patients with Buerger disease had moderate to severe periodontitis. None of the control arterial samples was positive for periodontal bacteria.

**Conclusions:** This is the first study to identify oral microorganisms in the lesions of Buerger disease. Our findings suggest a possible etiologic link between Buerger disease and chronic infections such as oral bacterial infections. (J Vasc Surg 2005;42:107-15.)

Studies have suggested that infectious organisms such as *Chlamydia pneumoniae*, *Helicobacter pylori*, cytomegalovirus, and oral (periodontal) gram-negative bacteria may play a role in arterial diseases such as atherosclerosis and related disorders. These organisms have been observed in carotid, coronary, and aortic atherosclerotic plaques.<sup>1-4</sup> A previous investigation in our laboratory detected several species of oral bacteria in aneurysmal walls and thrombi of patients with abdominal aortic aneurysms (AAAs).<sup>5</sup> In this study, we focused on Buerger disease from the same point of view.

Buerger disease, described first by Winiwarter in 1878,<sup>6</sup> is a nonatherosclerotic, recurrent, segmental,

inflammatory occlusive vascular disease of unknown etiology. Leo Buerger gave a detailed description of this disease<sup>7</sup> in 1908. It mainly affects medium-sized arteries, veins, and the nerves of both the lower and upper extremities. Buerger disease primarily occurs in male smokers. The incidence among women is less than 5.4% in Japan,<sup>8</sup> but some researchers have reported more frequent occurrences in women in Europe and the United States.<sup>9-11</sup> Infection has been regarded as an important etiologic factor for years, although no microorganisms have ever been identified from vascular lesions. As early as 1928, Allen and Brown<sup>12</sup> mentioned the possibility that infection foci in the mouth were contributory. In the study described here, we searched for a possible link between oral bacteria and Buerger disease because smoking, periodontitis, and Buerger disease seem to be related. Smoking worsens periodontitis and apparently aggravates the oral condition.<sup>13</sup> Periodontitis is an infection that involves tooth-supporting tissue, including the gingiva, periodontal membrane, and alveolar bone. It affects approximately 30% of the population in Western countries and 70% to 80% of the population in Japan.<sup>13-15</sup> The polymerase chain reaction (PCR) method, which can detect bacteria in tissue samples with high sensitivity,<sup>16-17</sup> was used to determine whether oral bacteria were present in arterial and oral samples from patients with Buerger disease.

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## MATERIALS AND METHODS

Fifteen patients, who had been given a clinical diagnosis of Buerger disease on the basis of Shionoya's criteria<sup>8</sup> and angiographic findings (Fig 1, A) and who had undergone removal of an occluded arterial segment between April 2003 and May 2004 at our institution, were enrolled in this study after they provided informed consent. All the patients had typical characteristics of Buerger disease, including a smoking history, disease onset before the age of 50 years, occlusive lesions in the infrapopliteal artery, either upper limb involvement or phlebitis migrans, and an absence of risk factors, except for smoking, for atherosclerosis. In accordance with Shionoya's criteria, development of additional risk factors for atherosclerosis during the course of Buerger disease did not alter the diagnosis. Pathologic examinations were performed to confirm the diagnosis of Buerger disease (Figs 1, B and 2). One patient, whose disease was finally diagnosed microscopically as fibromuscular dysplasia of the tibial artery, was excluded from this study.

The clinical characteristics of the 14 subjects are shown in Table I. Their mean age was 60 years (range, 39-73 years), and their mean age at the onset of Buerger disease was 37 years. The principal symptoms of Buerger disease include necrosis or ulceration of the toes or feet, rest pain, and claudication. Confirmation of upper extremity involvement was based on the clinical assessment of signs and symptoms (cyanosis or paleness of the fingers), angiographic findings, or Allen's test.<sup>18</sup> The extent of arterial occlusion caused by Buerger disease was classified in four degrees according to the involved arteries, as follows: (1) below the knee joint; (2) 1 plus above the knee joint; (3) 1 plus 2 plus external iliac; and (4) 1 plus 2 plus 3 plus common iliac or aorta. In Japan, symptomatic atherosclerosis is usually observed in persons older than 60 years, so patients older than 60 years were examined angiographically to see whether there were any suggestive changes, such as hard, irregular, or calcified luminal walls or irregularity of the aorta.

The 14 patients with Buerger disease had undergone removal of an occluded segment of the superficial femoral artery (SFA; n = 4), popliteal artery (n = 2), anterior tibial artery (n = 4), or posterior tibial artery (n = 4) under either local or general anesthesia in a sterile operative field to avoid bacterial contamination. In two cases, this procedure was performed concomitantly with other operations (a major above-knee amputation in one case and a colostomy closure in the other).

Segments of arteries were also obtained from seven patients, who underwent surgical treatment for vascular diseases other than Buerger disease, as control specimens after informed consent was obtained. The control specimens were defined as those without atherosclerotic findings in the arterial wall macroscopically and microscopically: three external iliac arteries from three male AAA patients, two splenic arteries from a male AAA patient and a female patient with a renal aneurysm, one common femoral artery

(Fig 3) from a male patient with aortoiliac occlusive disease, and a small artery in the genicular area from a young female patient with popliteal arteriovenous malformations. A questionnaire survey was conducted among the study participants to learn whether they are active smokers at the time of operation.

Fresh and sterile arterial specimens approximately 1.5 cm long were obtained from the patients in the Buerger disease group. Control specimens of various sizes were also obtained during the operations. The specimens were washed thoroughly with normal saline and cut into three sections: one for the pathologic examination, one for the electron microscopic investigation, and one for PCR detection and culture. Specimens for PCR were stored immediately at  $-80^{\circ}\text{C}$  until use. The study was approved by the Ethics Committee of Tokyo Medical and Dental University, and informed consent was obtained from all patients.

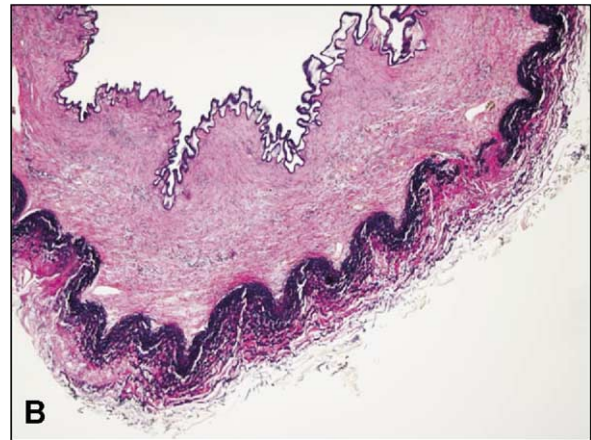
A periodontist performed oral examinations on each patient with Buerger disease at our university dental clinic to check the presence and severity of periodontal disease. On the basis of the probing depth (PD) of the periodontal pockets and attachment loss, patients were classified into four different groups. Grade A was the normal group with a PD of less than 2 mm, and grade B was considered to have moderate periodontitis, with a PD of 2 to 5 mm. Grade C was the group with severe periodontitis, with a PD deeper than 5 mm, and grade D was the edentulous group (assumed to be the consequence of severe periodontitis). Dental plaque and saliva samples were taken at the same time and stored immediately at  $-80^{\circ}\text{C}$ .

Arterial segments were homogenized, and then a High-Pure PCR Template Preparation Kit (Roche, Mannheim, Germany) was used to extract DNA from both the homogenized tissue and oral samples. The PCR method was applied to detect the presence of seven species of putative oral bacteria: *Porphyromonas gingivalis*, *Tannerella forsythensis*, *Treponema denticola*, *Campylobacter rectus*, *Actinobacillus actinomycetemcomitans*, *Prevotella intermedia*, and *Prevotella nigrescens*. Species-specific primers used for PCR were designed according to 16S ribosomal RNA gene sequences as described previously. The sensitivity and specificity of the method have been noted.<sup>17</sup> All samples were processed in duplicate to ensure reproducibility of the results.

For pathologic studies, one third of the arterial specimens were fixed by immersion in 10% neutral buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin, elastic van Gieson stain, or Giemsa stain. Ten fresh arterial specimens from patients with Buerger disease were homogenized and then cultured in aerobic and anaerobic environments immediately after the surgical procedure. The Spearman test was used to check whether the severity of periodontitis correlated with the extent of arterial occlusion in patients with Buerger disease.

## RESULTS

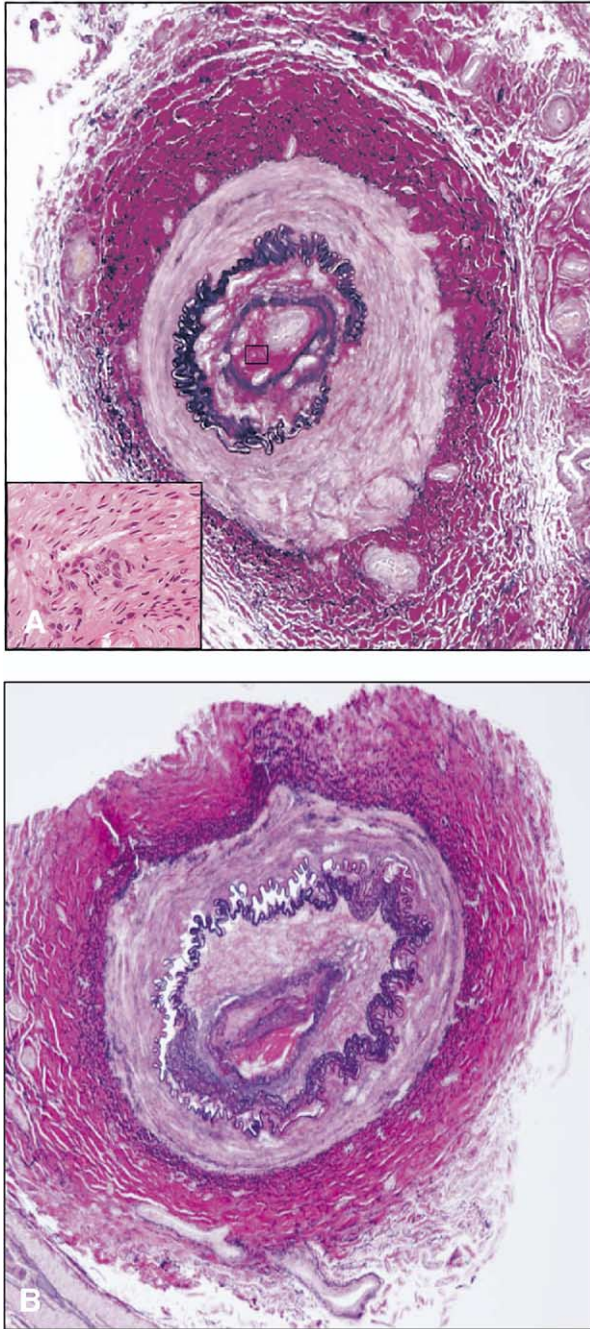
The results of the clinical examination for periodontitis and PCR analysis are shown in Table II. In the PCR



**Fig 1.** A, An angiogram of case 6, a 73-year-old man with a smoking history of 15 cigarettes per day for 53 years. Arterial changes typical of Buerger disease can be observed in the left leg and below the right knee. This patient developed gangrene on his left third and fourth toes at age 32 years. A minor amputation was performed at that time. An abdominal aortic aneurysm was diagnosed at age 73 years, and an aneurysmectomy with a bifurcated graft replacement was performed. A segment of his left femoral artery was obtained for this study during the operation. The patient quit smoking after abdominal aortic aneurysm surgery. B, The pathologic examination of the superficial femoral artery (SFA) of case 6. Intimal defects (may be artifacts) and internal elastic lamina are duplicated. Medial muscle fibers were replaced by collagenous fibers and fibroblasts. No inflammatory cells, such as giant cells or neutrophils, are seen. Atherosclerotic changes, such as foam cells, atheroma deposits, or cholesterol crystals, are absent (stain, elastica van Gieson stain; original magnification,  $\times 40$ ).

analysis, all samples from the 14 patients with Buerger disease, except for 1 arterial specimen, were positive for at least 1 species of oral bacteria (Fig 4). *Treponema denticola* was detected in 12 arteries and all oral samples from patients with Buerger disease. *Campylobacter rectus* was found in 6 (43%) of the 14 arterial samples and all oral samples. *Porphyromonas gingivalis* was found in 5 (36%) arterial and 13 (93%) oral samples, *Prevotella intermedia* was found in 3 (21%) arterial and 10 (71%) oral samples, *Tannerella forsythensis* was found in 2 (14%) arterial and 13 (93%) oral samples, and *Prevotella nigrescens* was found in 2 (14%) arterial and 10 (71%) oral samples. *Actinobacillus actinomycetemcomitans* was not detected in any of the arterial or oral samples. As shown in Table II, in most patients the same species of bacteria were detected in both the arterial and oral samples, although more species were detected in the oral samples. None of the control arterial samples was positive for any oral bacteria examined. All the patients with Buerger disease had periodontitis, and 64.3% (9/14) had a severe form.

Concerning the extent of arterial occlusion, there were seven patients with degree 1, four patients with degree 2, two with degree 3, and one with degree 4, as shown in Table I. The extent of occlusion in the patients with



**Fig 2.** Pathologic pictures of different stages of Buerger disease. A, Intermediate-chronic phase of case 12 (aged 39 years) (inset: stain, hematoxylin and eosin; original magnification,  $\times 600$ ). B, Chronic phase of case 9 (aged 65 years). There is more cellular infiltration in the intermediate-chronic phase compared with the chronic phase, from which well-organized thrombi in the lumen can be observed, and cellular infiltration is not as apparent (stain, elastica van Gieson stain; original magnification,  $\times 40$ ).

Buerger disease did not correlate with the severity of the periodontitis ( $P = .475$ ).

All patients with Buerger disease were active smokers or had just stopped smoking for several months at the time of biopsy except for one, who had quit smoking 7 years previously. There were three smokers and four nonsmokers in the control group.

The angiograms of the seven patients with Buerger disease who were older than 60 years were checked for any atherosclerotic changes. One showed iliac arterial stenosis with an irregular arterial wall; one showed a calcification line of the common iliac artery; two showed an irregular abdominal aorta; and the other three had normal angiographic findings in the aortoiliac regions.

Pathologic examinations of the arterial samples from the patients with Buerger disease confirmed intermediate-chronic-stage lesions in four patients (Fig 2, A) and chronic-stage lesions in ten patients (Fig 2, B). The intermediate-chronic-stage samples showed a slight to moderate cellular infiltration around the vasa vasorum or the recanalized small vessels in the thrombus. In the arterial samples we studied, the histologic structure was well preserved as previously described by Buerger,<sup>7</sup> Allen and Brown,<sup>12</sup> and Lie.<sup>9</sup> No atherosclerotic changes or evidence of bacteria on pathologic analysis were observed. Microabscesses and giant cell infiltration associated with the acute phase of bacterial infection were not observed either. There were no atherosclerotic changes in the control specimens, as shown in Fig 3. A routine bacterial culture of arterial specimens ( $n = 10$ ) yielded negative results.

## DISCUSSION

In this study, arterial specimens from patients with a diagnosis of Buerger disease were studied from a clinical, angiographic, pathologic, and microbiologic point of view. Considering the patients' mean age at biopsy, we were extremely careful to differentiate the occlusive lesions from atherosclerosis. As pathologic pictures (Figs 1, B, 2, and 3) show, there were no atherosclerotic changes in the specimens studied. Although some patients developed AAA or atherosclerosis years after they contracted Buerger disease and although both diseases coexisted in the same individual, the two diseases were not observed simultaneously at the same site. In blood vessels already occluded by Buerger disease at a younger age, it seems impossible for atherosclerosis to develop because there is no longer an arterial bloodstream. In the control study, we chose iliac or visceral arteries from four AAA patients that showed, macroscopically or microscopically, no atherosclerotic changes. Usual AAA is now classified as a nonspecific etiology. This means that atherosclerosis is a secondary change in the aneurysmal sac, so we can expect to excise normal or minimally changed arteries. Fig 3 shows that even with aortoiliac occlusive disease present, we obtained an atherosclerosis-free femoral arterial specimen.

Arterial specimens we studied here included four SFAs: this seems contradictory to the traditional thinking that Buerger disease mainly involves small or medium-sized

**Table I.** Characteristics of 14 male patients with Buerger disease

Patient No./age (y)	Cigarettes per day × No. years	Onset age (y)	PM	ULI	Principal symptoms	Occlusion sites	Sample sites	Other disorders (onset age [y])	Extent of occlusion*
1/47	40 × 27	41	No	Yes	Rt leg ulcer	Lt CIA to PLA; Rt CIA to PLA, PTA, PA, VA	SFA	—	4
2/52	10-22 × 37	32	No	Yes	Lt foot ulcer, necrosis	Lt EIA to PLA, ATA, PEA; Rt ATA	PLA	—	3
3/61	20 × 41	47	Yes	Yes	Lt leg necrosis	Lt SFA, PLA, PTA, ATA; Rt CFA, SFA, ATA	PLA	—	2
4/67	10-30 × 55	50	No	Yes	Claudication	Lt SFA, PLA, ATA; Rt ATA, PTA, PA	SFA	—	2
5/60	10 × 20	38	Yes	No	Claudication, Rt foot ulcer	Lt PLA, PTA, ATA, PEA; Rt ATA, PA	PTA	HT (56)	1
6/73	15 × 53	32	Yes	No	Lt toe necrosis	Lt CIA to PLA, ATA	SFA	HT (41); AAA (73)	3
7/71	20 × 40	36	Yes	No	Rt toe rest pain	Lt ATA, PTA; Rt SFA, PTA, ATA, PEA	SFA	CI (70)	2
8/63	20 × 44	34	No	Yes	Rt leg claudication	Lt PLA, PTA, ATA, PEA; Rt PTA, PEA, VA	PTA	—	1
9/65	20 × 43	25	No	Yes	Claudication, pallor	Lt SFA, PLA, ATA	ATA	—	2
10/54	20 × 31	34	Yes	Yes	Rt foot gangrene	Lt PTA, PEA; Rt ATA, PEA	PTA	—	1
11/59	30 × 23	41	Yes	No	Lt toe gangrene	Lt ATA; Rt ATA	ATA	—	1
12/39	30 × 20	28	No	Yes	Toe gangrene	Lt PTA, PEA; Rt ATA, PTA, PEA	PTA	—	1
13/57	40 × 20	38	Yes	Yes	Rt toe gangrene	Lt PTA, ATA; Rt ATA, PEA	ATA	—	1
14/70	40 × 30	38	Yes	Yes	Rt toe ulcer	Lt ATA, PEA; Rt ATA, PTA	ATA	—	1

PM, Phlebitis migrans; ULI, upper limb involvement; Rt, right; CIA, common iliac artery; PLA, popliteal artery; PTA, posterior tibial artery; PA, pedal artery; VA, visceral artery; SFA, superficial femoral artery; Lt, left; EIA, external iliac artery; ATA, anterior tibial artery; PEA, peroneal artery; CFA, common femoral artery; HT, hypertension; AAA, abdominal aortic aneurysm; CI, cerebral infarction.

\*Extent of the occlusion: (1) below knee joint; (2) 1 plus above knee joint; (3) 1 plus 2 plus external iliac; (4) 1 plus 2 plus 3 plus common iliac or aorta.

arteries. However, in Japan, arterial involvement tends to be more proximal, and data show SFA involvement in 13 (17%) of 75 limbs, femoropopliteal involvement in 41 (30%) of 137 patients, and aortoiliac involvement in 21 (8%) of 259 patients.<sup>8</sup> This indicates that SFA occlusion is not uncommon, and among the four SFAs, two were from distal sites.

The cause of Buerger disease is not yet known, although many etiologic factors have been suggested for years. Habitual use of tobacco is the only indisputable etiologic factor because of its close association with disease activity. However, smoking alone does not seem to be enough to cause Buerger disease. The striking inflamma-

tory component of the acute phase, such as microabscess formation and the existence of multinucleated giant cells, led Buerger<sup>7</sup> to postulate that bacterial infection was a causative agent for the disease. Allen and Brown<sup>12</sup> also suspected that infection foci including oral and throat infection might have served as a contributory etiologic factor. Several pathogens, such as *Treponema pallidum*,<sup>7,8</sup> have been investigated as possible causative agents for Buerger disease and have been rejected.

Several studies have suggested that Buerger disease is an immunologic disorder or is caused by cellular sensitivity to collagen, and the susceptibility to Buerger disease may be genetic.<sup>19-23</sup> However, these theories cannot account



**Fig 3.** A pathologic picture of a control arterial specimen. This is a common femoral arterial specimen from control patient 6 (aged 73 years; male), who underwent abdominal aortic reconstruction surgery. Macroscopically, the inner surface of the arterial specimen was smooth and shiny, and the arterial wall was soft and elastic. The microscopic view shows mild fibrous thickening in the intima. The media is almost intact, with a little disorder of the muscle fibers. No calcification or atheromatous degeneration was found. There was also no infiltration of any inflammatory cells in the adventitia (stain, elastica van Gieson stain; original magnification,  $\times 40$ ).

for the formation of phlebitis migrans, a principal characteristic of Buerger disease, or explain why Buerger disease affects both arteries and veins simultaneously and, occasionally, nerves. The prevalence of Buerger disease in India and Japan was similar before, but it has recently decreased in Japan.<sup>18,24</sup> These hypotheses cannot explain this decrease either. The various features of Buerger disease might be better explained by considering the disease as a systemic reaction to bacterial infection or to an antigen originating from bacteria rather than as an immunologic disorder, although no previous study has successfully identified any specific pathogen in the vessel lesion.

Although Buerger disease is seen worldwide, it is more prevalent in the Middle and Far East than in developed areas such as North America and Western Europe. Socioeconomic conditions seem to influence this distribution.<sup>18</sup> Control of chronic infection, including oral infection, may contribute partly. Many studies that have investigated the association between oral health and peripheral arterial diseases have implied that oral infection, especially periodontal disease, may be involved in atherosclerosis through a potential oral infection-inflammatory pathway.<sup>2,3,25</sup>

As we mentioned previously, smoking has been regarded as a causative factor for Buerger disease.<sup>7,8,18</sup> A

strong relationship between smoking and oral disease has also been described.<sup>13,14</sup> Although periodontitis generally affects older people, young people who smoke are at an increased risk of a characteristic pattern of this disease.<sup>13</sup> Ex-smokers respond better than active smokers to dental treatment.<sup>26</sup> In our study, all the patients with Buerger disease had been heavy smokers since youth, and all had moderate to severe periodontitis. Besides directly damaging blood vessels, cigarette smoking exacerbates patients' periodontal disease and stimulates more extensive bacterial invasion to blood vessels. Thereby, the microorganisms (especially *Treponema denticola* and *Porphyromonas gingivalis*) may be transported by monocytes, lodge in peripheral arteries, and induce thrombosis formation in small to medium-sized vessels (including veins) in the hand or leg that have already been affected by smoking.

Oral bacteria have been found in arterial lesions caused by atherosclerosis and Buerger disease. If endothelial changes that could permit easy invasion of the bacteria to the subendothelial space occur at approximately the age of 50 years, it may be speculated that before 50 years, oral bacteria cannot invade through the healthy endothelial cell layer. Therefore, they make thrombi by using their strong thrombogenic activity in the vessel lumen, which shows infectious findings such as microabscess or giant cells, with an intact arterial wall structure. After the age of 50 years, the endothelial layer changes with aging to permit bacterial invasion in some cases, and bacteria easily enter the intimal layer to encourage atherosclerotic changes. This may explain the detection of oral bacteria in the two different categories. However, susceptibility to Buerger disease may be genetic, and more investigation is required. Different combinations of the oral bacteria may contribute to the two distinctive diseases by different mechanisms. Our results showed that *Treponema denticola* was the most frequently detected bacteria in Buerger disease, but in atherosclerotic lesions, *Porphyromonas gingivalis* was found more often.<sup>5</sup>

More than 500 species of bacteria are found in the mouth, and people with poor dental hygiene may have an oral bacterial count of more than 20 billion. Most of these bacteria remain in the gingival crevice or periodontal pocket, an anaerobic environment. The bacteria also form a biofilm; this makes it difficult to eradicate them with antibiotics and hampers the host's production of effective antibodies because of the symbiotic state of the chronic biofilm infection. Oral bacteria may be involved in transient bacteremia and enter the bloodstream frequently through dental treatment, tooth brushing, or even chewing.<sup>27,28</sup> Many studies have shown the existence of oral bacteria in atherosclerotic coronary disease, internal carotid plaque, or peripheral atherosclerotic vascular lesions.<sup>1-5,29-32</sup>

Oral pathogens have been observed adhering to and invading the buccal epithelium<sup>33</sup> and arterial endothelium,<sup>34,35</sup> and they play a role in thrombus formation despite the host's protective mechanisms, such as phagocytosis.<sup>36</sup> The activities of *Porphyromonas gingivalis* have been especially well documented in this regard. Infectious microorganisms may also contribute to the progression of

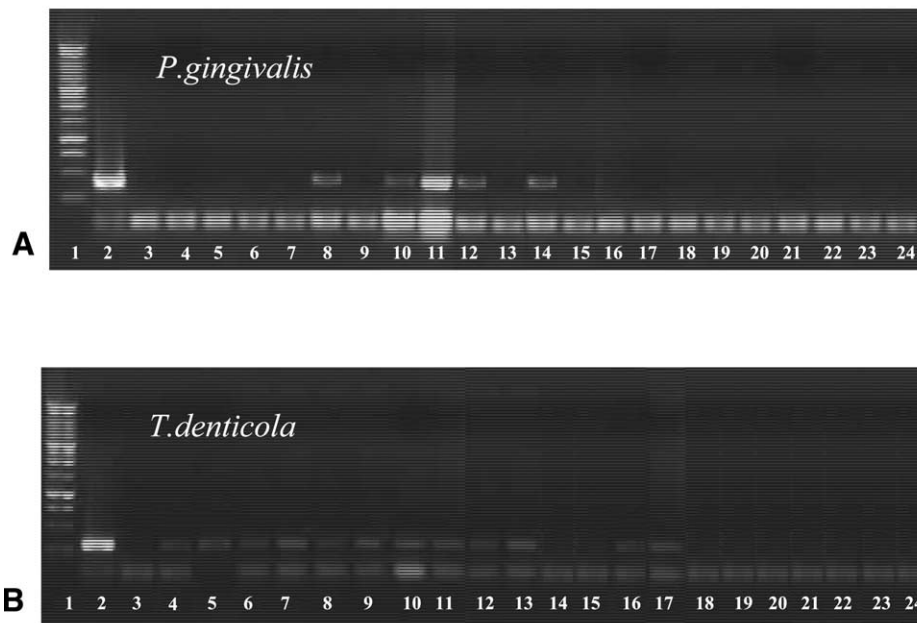
**Table II.** Results of periodontal examination and PCR detection of patients with Buerger disease and of controls

Subject No.*	Periodontitis grade†	Artery	Oral cavity
Patient 1	C	Td	Tf, Td, Cr, Pn
Patient 2	B	Td, Cr	Pg, Tf, Td, Cr, Pi, Pn
Patient 3	C	Tf, Td, Cr, Pn	Pg, Tf, Td, Cr, Pi, Pn
Patient 4	C	Td, Cr, Pi	Pg, Tf, Td, Cr, Pi
Patient 5	C	Pg, Td, Cr, Pn	Pg, Tf, Td, Cr, Pi, Pn
Patient 6	B	Tf, Td, Pi	Pg, Tf, Td, Cr, Pi, Pn
Patient 7	C	Pg, Td, Cr, Pi	Pg, Tf, Td, Cr, Pi, Pn
Patient 8	D	Pg, Td, Cr	Pg, Td, Cr
Patient 9	C	Pg, Td	Pg, Tf, Td, Cr, Pi, Pn
Patient 10	B	Td	Pg, Tf, Td, Cr, Pn
Patient 11	C	Pg	Pg, Tf, Td, Cr, Pi
Patient 12	C	None	Pg, Tf, Td, Cr, Pi, Pn
Patient 13	B	Td	Pg, Tf, Td, Cr, Pi, Pn
Patient 14	C	Td	Pg, Tf, Td, Cr
Control 1	—	None	—
Control 2	—	None	—
Control 3	—	None	—
Control 4	—	None	—
Control 5	—	None	—
Control 6	—	None	—
Control 7	—	None	—

PCR, Polymerase chain reaction; *Pg*, *Porphyromonas gingivalis*; *Tf*, *Tannerella forsythensis*; *Td*, *Treponema denticola*; *Cr*, *Campylobacter rectus*; *Pi*, *Prevotella intermedia*; *Pn*, *Prevotella nigrescens*.

\*All patients with Buerger disease were male, and their ages are shown in Table I. The ages of the control patients were 69, 62, 25, 72, 77, 73, and 65 years. The resected sites were iliac, splenic, arteriovenous malformation, iliac, iliac, femoral, and splenic arteries, respectively.

†Grade A was normal (pocket depth on probing, <2 mm); grade B, moderate periodontitis (pocket depth, 2-5 mm); grade C, severe periodontitis (pocket depth, >5 mm); and grade D, edentulous.



**Fig 4.** Polymerase chain reaction results of artery samples from patients with Buerger disease. Lane 1, 1-Kilobase DNA ladder; lane 2, positive control; lane 3, negative control; lanes 4-17, arterial samples from patients with Buerger disease; lanes 18-24, arterial samples from control patients.

atherosclerosis by regulating inflammation inside arterial walls locally or systemically.<sup>28,37,38</sup> In this study, one oral bacterium, *Treponema denticola*, was detected in most of the arterial specimens and in all of the oral samples from patients with Buerger disease. As a mobile spirochete, it is capable of invading periodontal tissue and has been found in gingival ulcerations and gangrene associated with acute necrotizing periodontitis. The invasion of endothelial cells by *Treponema denticola* has also been reported.<sup>39</sup>

With respect to the role of these bacteria in the pathogenesis of Buerger disease, it is not certain whether these bacteria work as a causative factor, whether they aggravate the disease, or whether they are accidentally entrapped in the lesions. The PCR method was used to detect oral bacteria in this study. Although recent advances have made this technique highly accurate and specific, it cannot distinguish whether the bacteria detected were alive or dead or how long these microorganisms have existed in the lesions, because PCR detects only the genetic material DNA and can reveal the presence of very old DNA.<sup>40</sup> Generally, oral bacteria are not virulent enough to cause febrile bacteremia or sepsis. Furthermore, because most of them are strictly anaerobic, it is difficult for them to survive in the artery in the presence of oxygen, which might explain why we failed to culture any oral bacteria from artery specimens *in vitro*. It is more likely that virulent components of oral bacteria, acting as an inflammatory core, were continuously involved with Buerger disease, rather than live microorganisms. Another reason for our failure to culture may be that no patient with acute-phase disease was included in the study. As Kurihara et al<sup>5</sup> studied occluded arteries by thromboendarterectomy and showed that oral bacteria existed only in the internal layers of the vessel wall of atherosclerotic lesions, research using methods that render bacteria visible is necessary to clarify whether bacteria are in the thrombus, arterial layer, or both.

Although the prevalence of Buerger disease will continue to decrease in Japan and other developed countries with the decline in smoking and the improvements in oral hygiene and dental care, investigations for methods to prevent and treat the disease must continue because of its high prevalence in South and East Asia.

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## REFERENCES

- Chiu B. Multiple infections in carotid atherosclerotic plaques. *Am Heart J* 1999;138:S534-6.
- Mattila KJ, Valle MS, Nieminen MS, Valtonen VV, Hietaniemi KL. Dental infections and coronary atherosclerosis. *Atherosclerosis* 1993; 103:205-11.
- Herzberg MC, Meyer MW. Effects of oral flora on platelets: possible consequences in cardiovascular disease. *J Periodontol* 1996;67:1138-42.
- Okuda K, Ishihara K, Nakagawa T, Hirayama A, Inayama Y, Okuda K. Detection of *Treponema denticola* in atherosclerotic lesions. *J Clin Microbiol* 2001;39:1114-7.
- Kurihara N, Inoue Y, Iwai T, Umeda M, Huang Y, Ishikawa I. Detection and localization of periodontopathic bacteria in abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* 2004;28:553-8.
- Winiwater F. Über eine eigenthümliche Form von Enderarteriitis und Endophlebitis mit Gangrän des Fusses. *Arch Klin Chir* 1878;23:202-26.
- Buerger L. Thromboangiitis obliterans: a study of the vascular lesions leading to pre-senile spontaneous gangrene. *Am J Med Sci* 1908;136: 567-80.
- Shionoya S. Etiology, pathology, pathophysiology, clinical manifestation, diagnosis. In: Shionoya S, editor. *Buerger's disease. Pathology, diagnosis and treatment*. Nagoya (Japan): University of Nagoya Press; 1990. p. 38-198.
- Lie JT. Thromboangiitis obliterans (Buerger's disease) in women. *Medicine* 1987;66:65-72.
- Mills JL, Porter JM. Buerger's disease: a review and update. *Semin Vasc Surg* 1993;6:14-23.
- Olin JW, Young JR, Graor RA, Ruschhaupt WF, Bartholomew JR. The changing clinical spectrum of thromboangiitis obliterans (Buerger's disease). *Circulation* 1990;82(5 Suppl):IV3-8.
- Allen EV, Brown GE. Thromboangiitis obliterans. A clinical study of 200 cases. *Ann Intern Med* 1928;1:535-49.
- Haber J, Wattles J, Crowley M, Mandell R, Joshipura K, Kent RL. Evidence for cigarette smoking as a major risk factor for periodontitis. *J Periodontol* 1993;64:16-23.
- Preber H, Linder L, Bergström J. Periodontal healing and periopathogenic microflora in smokers and non-smokers. *J Clin Periodontol* 1995;22:946-52.
- Dental Health Division of Health Policy Bureau, Ministry of Health, Labor and Welfare, Japan. Report on the survey of dental diseases. Tokyo: Oral Health Association of Japan; 1999. p. 149-56.
- Stelzel M, Conrads G, Pankuweit S, Maisch B, Vogt S, Moosdorf R, et al. Detection of *Porphyromonas gingivalis* DNA in aortic tissue by PCR. *J Periodontol* 2002;73:868-70.
- Ashimoto A, Chen C, Bakker I, Slots J. Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis lesions. *Oral Microbiol Immunol* 1996;11:266-73.
- Olin JW. Thromboangiitis obliterans (Buerger's disease). *N Engl J Med* 2000;343:864-9.
- Kobayashi M, Ito M, Nakagawa A, Nishikimi N, Nimura Y. Immunohistochemical analysis of arterial wall cellular infiltration in Buerger's disease (endarteritis obliterans). *J Vasc Surg* 1999;29:451-8.
- Lee T, Seo JW, Sumpio BE, Kim SJ. Immunobiologic analysis of arterial tissue in Buerger's disease. *Eur J Vasc Endovasc Surg* 2003;25:451-7.
- Adar R, Papa MZ, Halpern Z, Mozes M, Shoshan S, Sofer B, et al. Cellular sensitivity to collagen in thromboangiitis obliterans. *N Engl J Med* 1983;308:1113-6.
- Eichhorn J, Sima D, Lindschau C, Turowski A, Schmidt H, Schneider W, et al. Antiendothelial cell antibodies in thromboangiitis obliterans. *Am J Med Sci* 1998;315:17-23.
- Ohtawa T. HLA antigens in arterial occlusive diseases in Japan. *Jpn J Surg* 1976;6:1-8.
- Matsushita M, Nishikimi N, Sakurai T, Nimura Y. Decrease in prevalence of Buerger's disease in Japan. *Surgery* 1998;124:498-502.
- Hung HC, Willett W, Merchant A, Rosner BA, Ascherio A, Joshipura KJ. Oral health and peripheral arterial disease. *Circulation* 2003;107: 1152-7.
- Grossi SG, Skrepinski FB, Decaro T, Zambon JJ, Cummins D, Genco RJ. Response to periodontal therapy in diabetes and smokers. *J Periodontol* 1996;67:1094-102.
- Carroll GC, Sebor RJ. Dental flossing and its relationship to transient bacteremia. *J Periodontol* 1980;51:691-2.
- Daly CG, Mitchell DH, Highfield JE, Grossberg DE, Stewart D. Bacteremia due to periodontal probing: a clinical and microbiological investigation. *J Periodontol* 2001;72:210-4.
- Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999; 340:115-26.



30. Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ. Identification of periodontal pathogens in atheromatous plaques. *J Periodontol* 2000;71:1554-60.
31. Coles KA, Plant AJ, Riley TV, Smith DW. Cardiovascular disease: an infectious aetiology? *Rev Med Microbiol* 1998;9:17-27.
32. Mendez MV, Scott T, LaMorte W, Vokonas P, Menzoian JO, Garcia R. An association between periodontal disease and peripheral vascular disease. *Am J Surg* 1998;176:153-7.
33. Rudney JD, Chen R, Sedgewick GJ. Intracellular *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in buccal epithelial cells collected from human subjects. *Infect Immun* 2001;69:2700-7.
34. Dorn BR, Dunn EA Jr, Progulsk-Fox A. Invasion of human coronary artery cells by periodontal pathogens. *Infect Immun* 1999;67:5792-8.
35. Deshpande RG, Khan MB, Genco CA. Invasion of aortic and heart endothelial cells by *Porphyromonas gingivalis*. *Infect Immun* 1998;66:5337-43.
36. Lourbakos A, Yuan YP, Jenkins AL, Travis J, Andrade-Gordon P, Santulli R, et al. Activation of protease-activated receptors by gingipains from *Porphyromonas gingivalis* leads to platelet aggregation: a new trait in microbial pathogenicity. *Blood* 2001;97:3790-7.
37. Li L, Messas E, Batista EL Jr, Levine RA, Amar S. *Porphyromonas gingivalis* infection accelerates the progression of atherosclerosis in a heterozygous apolipoprotein E-deficient murine model. *Circulation* 2002;105:861-7.
38. Sessa R, Di Pietro M, Schiavoni G, Santino I, Benedetti-Valentini F, Perna R, et al. *Chlamydia pneumoniae* DNA in patients with symptomatic carotid atherosclerotic disease. *J Vasc Surg* 2003;37:1027-31.
39. Peters SR, Valdez M, Riviere G, Thomas DD. Adherence to and penetration through endothelial cells by oral treponemes. *Oral Microbiol Immunol* 1999;14:379-83.
40. Fish SA, Shepherd TJ, McGenity TJ, Grant WD. Recovery of 16S ribosomal RNA gene fragments from ancient halite. *Nature* 2002;417:432-6.

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