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Mini review

Periodontal bacteremia and various vascular diseases

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Background and Objective: Weak oral bacteria, such as periodontal bacteria, have been found in various vascular lesions, including atheroma, the thrombus of the occluded artery of Buerger's disease, the abdominal aortic aneurismal wall and varicose veins. Serum titer levels of each bacterium are now available and have shown a significant relationship between severity and individual differences.

Material and Methods: The Medline and Tokyo Medical and Dental University databases were searched to identify the literature currently available on oral bacteria and vascular diseases.

Results: It is estimated that lymph vessel openings trap bacteria en route from the mouth to the bloodstream and then carry them to the vein of the venous angle near the supraclavicular area. In the case of periodontal bacteria, a typical bacterium, such as *Porphyromonas gingivalis,* strongly activates platelets and makes them mass, including the bacteria without phagocytosis. Simultaneously, cytokines and serotonin are discharged. Platelet engulfment was clearly demonstrated by electron microscopy analysis. An animal study using rats showed the formation of a small arterial thrombus after continuous intravenous infusion of *P. gingivalis* for 2–4 wk.

Conclusion: Weak oral bacteria, such as periodontal bacteria, may play an important role in the development of various vascular diseases, such as Buerger's disease, atherosclerosis and varicose veins, through bacteremia.

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Since 1999, various weak oral bacteria have been identified in atherosclerotic lesions (1). Of these bacteria, *Chlamydia pneumoniae*, which resides in the mouth, pharynx, or bronchus, has been investigated in detail and was confirmed to be transported to blood vessel walls by monocytes (2). This invasion mechanism appears to be a factor in the development of atherosclerosis. Additionally, cytomegalovirus can be absorbed from the oral cavity, resulting in opportunistic infections; and, recently, cytomegalovirus was found to be present in the so-called inflammatory abdominal aortic aneurismal walls (3). *Helicobacter pylori* is a well-known bacterium that resides in the stomach and may also be present in the oral cavity. Moreover, *H. pylori* has been identified in blood vessel walls. However, our studies have shown that its frequency in vessel walls may not be significant. Since 2000, periodontal bacteria (several species in particular) have been shown to be present within blood vessel walls (4) (Fig. 1).

Weak bacteria and strong bacteria

Weak bacteria usually present normally and cause opportunistic infections. Even when such microbes invade the body, the resulting infectious conditions are usually not serious and recovery occurs without complications, except in susceptible individuals, such as those who are immunosuppressed. By contrast, strong bacteria can destroy arterial walls or heart valves after bacterial adhesion, as described by Virchow in 1847 and confirmed by Oslor (5). In severe cases, this destruction of the blood vessels can even result in patient mortality. These strong bacteria include those that cause syphilis and tuberculosis, and certain strains of *Staphylococcus*, *Salmonella*, *Klebsiella* and *Streptococcus*. Strong bacteria, such as *Streptococcus* spp.,

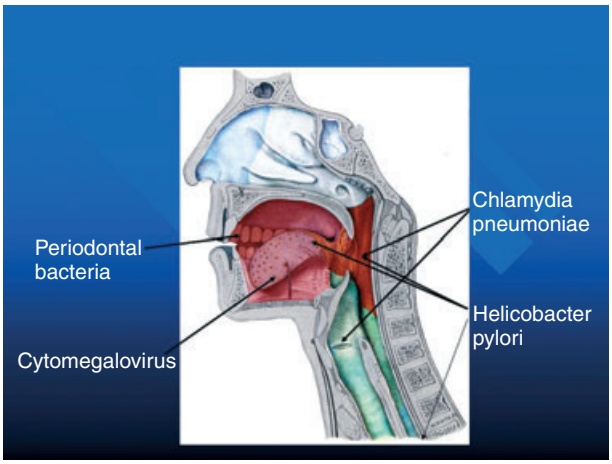

Fig. 1. Weak oral bacteria.

are present in the oral cavity from where they can easily infect and destroy heart valves and blood vessels after tooth extraction. In general, doctors control these potentially lethal conditions through the administration of antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA), which exists in the upper pharynx, does not directly invade the bloodstream but can invade after the development of MRSA pneumonia, resulting in fatal complications. Our recent experiences include a case of infection at a site of total parenteral nutrition, leading to infectious aneurysm rupture and death of the patient, as well as a case of infection at the site of percutaneus transluminal angioplasty, followed by the formation of a large iliac artery aneurysm and high fever after major surgery.

Details of Buerger's disease infection theory

Many investigators, including Leo Buerger, believed that Buerger's disease is an infectious disease. They had studied patients and conducted animal experiments, but were unable to identify the pathogen. Reports were published by many doctors, such as Edgar Allen, Lauderdale, Rabinowitz, Goodman, Horton & Dorsey, Schmidt-Weyland, Barotolo, Roncon, Winternitz and Haga *et al.* (6). Buerger stated, in his paper published in 1914, that, 'Thrombo-angiitis obliterans is an infectious disease in which a specific type of organism is at work; and although it has not yet been possible to demonstrate either bacteriologically or morphologically the presence of the offending agent, the pathological findings clearly indicate whether future studies should be directed in order that the causative factor may be discovered.' (7). In 1928, Professor Allen of the Mayo Clinic also had suspicions about oral bacteria as a cause and mentioned that 75% of 87 Buerger's disease sufferers had periodontal infection and 80% showed tonsil enlargement or pus attachment (8). Allen believed that Buerger's disease was an infectious disease up until his death in 1967. After his death, molecular biological approaches and immunological techniques became sufficiently popular to dismiss the infection theory and no papers were written that discussed the infection theory. Buerger's disease was classified as an inflammatory autoimmune disease.

Periodontal bacterial invasion of the arterial wall

Epidemiological evidence connecting periodontitis and vascular diseases with atherosclerotic changes were not widely reported prior to 2000. Periodontal bacteria are not detectable using standard culture techniques and they are extremely difficult to identify. Periodontal microbes consist of anaerobic bacilli or spirochetes and include more than 300 species overall. Usually six or seven species are examined as representative bacteria. In general, a fresh oral specimen is examined using dark-field microscopy from which the shape or movement of the bacteria can be confirmed, but not the species. When the bacteria are not expected to live in the vessel thrombi or plaques, methods for identification are extremely limited. Therefore, the established polymerase chain reaction (PCR) method was used for detecting DNA of oral bacteria and became popular (9). After the presence of bacteria is confirmed, immunofluorescence methods help to locate the bacteria in the vessel walls. PCR methods for oral bacteria are available. Dr Buerger and Professor Allen were unable to locate these bacteria even though they both strongly suspected bacterial infection in Buerger's disease.

To date, periodontal bacterial DNA has been detected in carotid arterial plaques, coronary arterial plaques, abdominal aortic aneurysmal walls (86% of patients) and intraluminal thrombi (88%), atherosclerotic vessel plaques (52%), occluded arteries of Buerger's disease patients (93%),

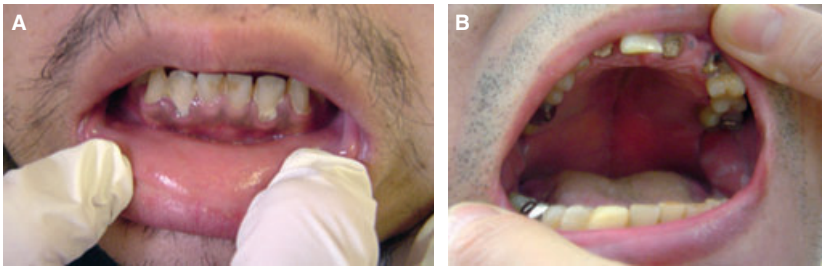

Fig. 2. Oral conditions in patients with Buerger's disease (two recent cases; left (A), 34-year-old man; right (B), 55-year-old man, with no fingers and below-knee amputation of the right leg).

migrating phlebitis samples (two cases, 100%) and primary varicose veins (48%) (10–12; Fig. 2).

Differences between oral bacteria and intestinal bacteria

The oral cavity and the intestinal cavity both contain significant numbers of bacteria. In particular in the oral cavity, periodontal plaques, gingival pockets and pharyngeal-tonsil pus permit bacteria to live and multiply without moving down the alimentary tract. In the intestine, the bacteria move as a result of bowel peristalsis. Periodontal pockets resemble ulcers and the total ulcer area could be as large as the size of a hand in severe cases. The gingival area of the periodontal region is surrounded by a multitude of lymph vessels with an open junction that can capture and trap bacteria. Then, from the tooth root, lymph vessels directly connect to the venous angle of the neck, where the lymph fluid mixes with venous blood (13). It is well known that for several minutes after dental care or brushing, we can identify the oral bacteria because of the above-mentioned system. Without transportation via lymph vessels, direct invasion of periodontal bacteria into arteries or veins cannot occur in a short period of time.

By contrast, owing to the preventive mechanism of the mucosal barrier, intestinal bacteria cannot invade the portal system as easily as periodontal bacteria appear to be able to invade the vascular system. Even when bacteria are able to invade the portal vein, the liver can prevent bacteria from entering the systemic circulation as a result of the presence of strongly phagocytic cells such as Kupffer cells. At present, weak intestinal bacteria do not appear in the systemic circulation vessels. The above-mentioned mechanism solves the question about weak oral bacteria being the only bacteria that are present in the systemic circulation vessels.

How are bacteria transported to the blood vessel wall?

The results obtained following continuous intravenous oral bacterial infusion in rats showed newly formed thrombi in the small arteries of the extremities with 50% of the specimens showing the presence of bacterial DNA (14). Other findings demonstrated a reduced inflammatory response surrounding the occluded lesions, which was explained by the fact that the weak bacteremia caused thrombus formation, and the occlusions came from the embolic episodes as a result of the bacteria-included thrombus. Unexpectedly, the bacteria appeared on the arterial side in the absence of killing events carried out by white blood cells or organ phagocytic cells in the venous circulation.

In 2004, we began using plateletrich plasma to stimulate effective wound healing. When we accidentally added periodontal bacteria (*Porphyromonas gingivalis*) to the sample and studied the mixed fluid through a stereoscopic microscope, we observed active movement. Upon examining the sample by electron microscopy, we saw that periodontal bacteria (*P. gingivalis*) were engulfed by platelets and morphologically no changes were observed in the bacteria for 1 h. Platelet aggregation was also observed. By contrast, the bacteria captured by granulocytes were killed within 1 h. These observations confirm that *P. gingivalis* aggregate strongly with platelets, enter the platelets and survive (15) (Figs 3 and 4). *Treponema denticola*, another periodontal bacterium, showed relatively weak reactions with platelets.

P. gingivalis-induced platelet aggregation became maximal in a few minutes and the mass became more than 20 microns over the size of the small artery. This suggested that the healthy small arteries of the rats could become embolized by the platelet aggregation, as shown in animal experiments (14). Moreover, clinically, in Buerger's disease, the healthy but spastic arterial lumen of the fingers or toes may be occluded with sudden onset.

In addition, the aggregative reactions of periodontal bacteria with platelets are strong enough to stimulate the secretion of various cytokines and of products such as serotonin, E-selectin, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1. As periodontitis itself results in the expression of inflammatory molecules such as interleukin-6 and tumor necrosis factor-alfa, it should be considered a systemic disorder (16).

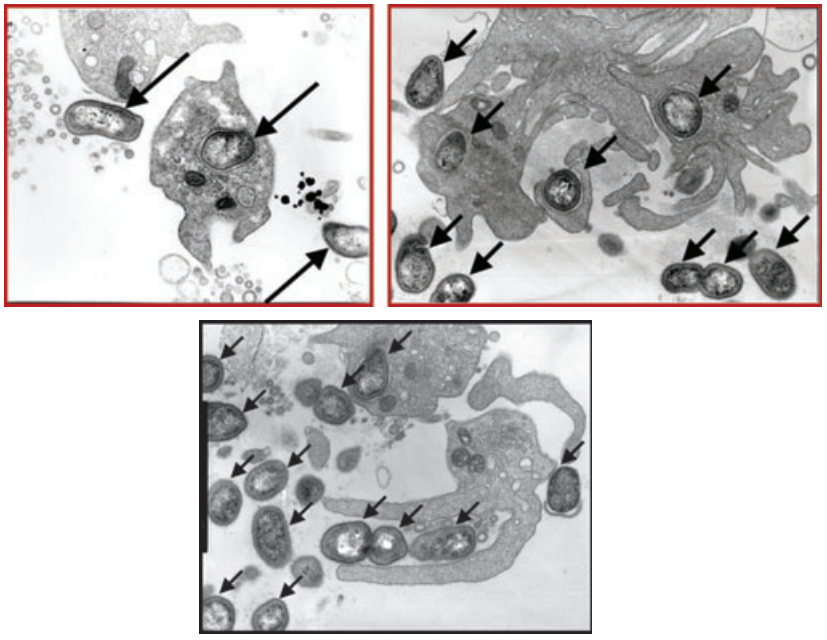

Fig. 3. Transportation of the oral bacteria to vessels? Platelets may play a vital role in the transportation of the oral bacteria. *Porphyromonas gingivalis* is a round-shaped mass (→).

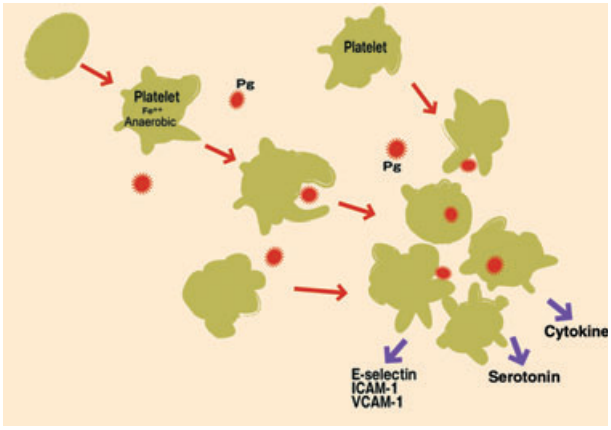

Fig. 4. A schema of the reactions between oral bacteria and platelets. ICAM-1, intercellular adhesion molecule-1; Pg, *Porphyromonas gingivalis* (a red notched round body); VCAM-1, vascular cell adhesion molecule-1.

Serum bacterial antibody titer changes in periodontal disease

The human body reacts to all bacteria with the production of antibody. The antibodies thus produced resist infection and should act to prevent pending infection. This immune function can prevent the development and progression of periodontal disease. However, this theory remains controversial. It can be difficult for antibodies in the saliva and blood to protect from clinical infection, and the treatment of periodontal disease can also be challenging owing to variations in the number and types of bacteria present and to the pathogenesis of the disease. Chen *et al*. reported that the antibody titer for periodontal bacteria is significantly elevated in patients with Buerger's disease (17), reconfirming that individuals with Buerger's disease have very poor periodontal conditions (8). In Buerger's disease, the antibody titers may actually vary relative to the severity of periodontitis. As the bacterium is weak and can be treated easily with antibiotics, the titer level decreases rapidly. However, this evidence should be discussed in more detail from the dental point of view.

The direct link between *C. pneumoniae* and atherosclerosis has been previously studied and the effects of antibiotic treatment were confirmed in animal experiments (18). However, large clinical trials failed to show any significant differences with antibiotic regimens (19). The fact that *C. pneumoniae* is opportunistic, normally present and weak, results in further problems in determining antibody-titer changes, immune products or the advantages of intermittent treatment with antibiotics. Further experiments are needed to resolve these issues.

Do all individuals with serious periodontitis develop vascular disease?

When we hypothesize the possibility that all vascular lesions may be affected with oral bacterial infections, we can propose three factors that are strongly related. First, endothelial cells undergo age-dependent changes. The endothelial cell activity in a 20–30-year-old person and a 50–60-year-old person is quite different regarding adhesive factors, action against bacteria and platelets or monocyte reactions. Second, oral bacteria are affected by many internal and external factors, such as smoking, diabetes and pregnancy. The presence of these effects is important when considering the infection progression. Third, lesions may be influenced by genetic conditions. The influence seems to be reasonable, not dominant, as in cancer. Atherosclerosis runs in families, but Buerger's disease does not. In over 200 Buerger's disease patients studied, no parent–child connections were observed, and Buerger's disease was present in only one set of identical twins.

However, recent studies on Buerger's disease have shown a specific HLA locus and infection susceptibility for basilar bacteria, such as periodontal bacteria (20). Varicose veins seem to occur in mothers and daughters. Interestingly, approximately 50% of varicose veins contain periodontal bacterial DNA, suggesting that pregnancy may be linked to varicosity development when the woman suffers from periodontitis during pregnancy. Varicose veins become evident after delivery in many women and weak bacteria may be involved in the destruction of venous valves.

Hypothesis and future views of vascular lesion development

We believe that *C. pneumoniae* can be transported to vessels containing monocytes, adhere to the damaged vascular regions and then act as one of the factors in atherosclerosis. On the other hand, it is also possible that cytomegalovirus will appear in the aneurismal wall along the same periodontal bacterial routes. *H. pylori* is not commonly associated with vascular lesions, but serum titers of *H. pylori* were high in our results of Buerger's disease research. However, at the present time the role of *H. pylori* is not well understood. Nevertheless, using recently reported evidence, we have developed a hypothesis for Buerger's disease, atherosclerotic occlusion, secondary atherosclerotic changes in aneurismal disease and primary varicose veins.

Buerger's disease

Pathogens in Buerger's disease are probably periodontal bacteria but pyloric bacteria may also be involved. Among periodontal bacteria, *T. denticola* and *P. gingivalis* are well known and may associate to form dental plaques. *P. gingivalis* may therefore be an initiator of platelet aggregation, and from the venous angle of the neck, the clump of bacteria can enter the bloodstream and stimulate platelet aggregation after uptake into platelets.

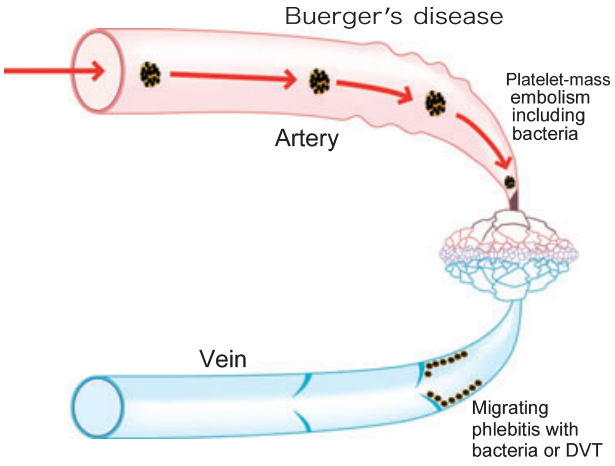

Fig. 5. Hypothesis of Buerger's disease development. DVT, deep-vein thrombosis.

It is suggested that aggregation reaches a maximum level when the platelet thrombi pass through the lung, after which the thrombi start to move in the arterial bloodstream. When the arterial wall is young but spastic from cigarette smoking, the platelet thrombi containing oral bacteria do not adhere to the arterial wall but make a small arterial embolism. It is suggested that the digital arterial obstruction observed in angiography of Buerger's disease patients may represent initial findings. This change will grow to the proximal arterial regions as a result of packing mechanism. Microorganisms that pass through capillaries can be caught at the venous valves, resulting in phlebitis migrens or deep-vein thrombosis formation in the extremities. In Buerger's disease, spastic changes of the small end arteries are key findings. The literature shows that small arterial changes are very common all over the body, but are symptomatic only in the extremities (Fig. 5).

Atherosclerotic lesions

Atherosclerotic lesions develop mainly in aged patients. Atherosclerotic changes can also be seen within aneurysms. A common problem is that the endothelial layer itself differs between young and aged or old luminal surfaces. Platelet thrombi containing oral bacteria will adhere to the walls near the outlet of the heart. The coronary arteries, aorta and large arteries will be adhered to first. While several weak oral bacteria may contribute to this adhesion mechanism, monocytes carrying *C. pneumoniae*, and platelets carrying periodontal bacteria, probably make the most significant contributions (21).

A recent study has shown that oral bacteria easily adhere to aged endothelial cells and that oral bacteria such as *P. gingivalis* can be easily cultured from samples of the aged endothelial surface compared with normal endothelial cell layers (16). In diabetic patients, peripheral arterial occlusion in arteries occurs below the knee, similarly to what is found in Buerger's disease. In this case, peripheral arterial occlusion appears to proceed by the same mechanism as Buerger's disease, namely via an embolic mechanism (Fig. 6).

Primary varicose veins

It is possible that the venous valve incompetency may come from periodontitis and periodontal bacteremia during pregnancy. Pregnant women usually have normal endothelial function (i.e. that does not allow bacterial adhesion to the arterial luminal surface), as most pregnant women are 20–30 years of age and nonsmokers. However, periodontitis seems to be common during pregnancy, and postdelivery varicose veins have been confirmed as evidence of this periodontitis. In addition to the occurrence of varicose veins, some effects on fetal growth have been reported. The hypothesis suggests that platelet thrombi containing oral bacteria pass through the arteries and capillaries, and thus contact and destroy the superficial venous valves. The inflammatory changes of the valve areas have been observed and oral bacteria were confirmed to be present in varicose veins in 50% of the patients.

Summary

Weak oral bacteria, such as periodontal bacteria or *C. pneumoniae*, have been observed in various arterial and venous lesions from epidemiological data reported prior to the discovery of bacterial invasion into vessels. Rich lymph vessels easily bring the bacteria from the mouth to the neck and the venous angle, which is directly open to

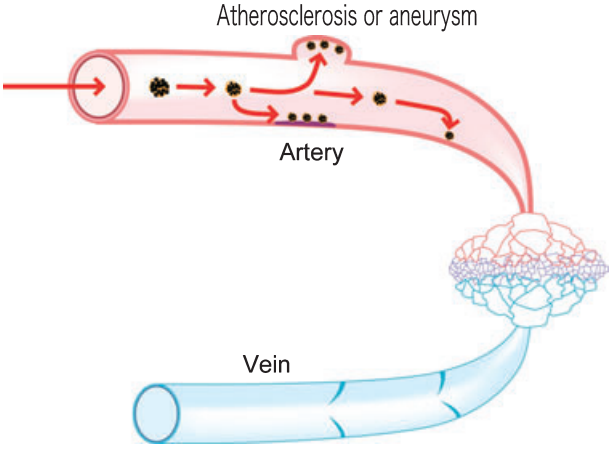

Fig. 6. A hypothesis of bacteria residing in atherosclerotic or aneurysmal lesions.

the blood vessels. Periodontal bacteria travel within platelets and *C. pneumoniae* can be carried by monocytes. The transportation system of other weak oral bacteria has not yet been clarified. Periodontal bacteria, especially *P. gingivalis*, aggregate platelets and form thrombi. At the same time, secretions of, for example, serotonin, various cytokines and adhesion factors also appear in the blood. The characteristic of the arterial lesions is dependent on the age of the patient and on the condition of the endothelial cells. In young patients, infectious incidents occur as a result of embolic mechanisms in Buerger's disease or adhesion to the valves of superficial veins in varicose veins. In aged patients, incidents result in adhesion in the proximal aorta, coronary arteries or large arteries. The hypothesis here unifies the evidence of vessel lesion development and explains possible discrepancies between vascular diseases.

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