Isolation of Propionibacterium acnes from sclerosing osteomyelitis of mandibles

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Introduction

Unusual bone proliferation instead of bone destruction characterizes sclerosing osteomyelitis1-2). The etiology of this disease is unknown2-4), and specific or distinctive microorganisms have not been isolated2,3). However, it has been suggested that a low-grade infection is involved2,4), possibly with anaerobes.

Propionibacterium spp. have been isolated sometimes from osteomyelitis5,6), diffuse sclerosing osteomyelitis7), and from normal bone marrow8,9). Because P. acnes is the most common inhabitant of skin10), specimens are often contaminated with this microorganism7,9). Therefore, isolation of P. acnes from lesions is often ignored. Special precautions must be used to decrease the possibility of contamination and to isolate distinctive strains of P. acnes from the lesion of sclerosing osteomyelitis. With these limitations in mind, we attempted to isolate distinctive microorganisms, especially obligate anaerobes including P. acnes with adoption of anaerobic glove box system11,12).

Materials and Methods

Samples.—Samples (5 mg-0.17 g as wet weight) were obtained from three patients with chronic sclerosing osteomyelitis of the mandible. Diagnosis was made by pathological observation and roentgenological examination. 99mTc-scintigraphy13) was also performed to aid the diagnosis. The samples were taken with special precautions against the contamination of microorganisms from the skin or the mouth. Surgical instruments for skin or oral mucous membrane were kept separate from those used for the lesion and the adjacent tissue. Exposure of the lesion to blood or tissue fluids was carefully avoided. Precautions were also made not to expose the lesion to the air. Pieces of intact tissue adjacent to the lesion were also obtained for controls.

Isolation of microorganisms.—The samples were transported in gas-tight bottles filled with N2 or CO2 gas and transferred to an anaerobic glove box (Model AZ-Hard, Hira-sawa Works, Tokyo, Japan) within a few minutes. After dispersion of the samples in 1 ml of 40 mM potassium phosphate buffer, pH 7.0, with a glass homogenizer, aliquots of 0.1 ml of the samples were spread over the surface of triplicate BH1-blood agar plates14) and incubated under aerobic (in air) or anaerobic conditions (10% H2 and 10% CO2 in N2 in the anaerobic box). The buffer solution and the agar plates had been spread over the surface of triplicate BH1-blood agar plates14) and incubated under aerobic (in air) or anaerobic conditions (10% H2 and 10% CO2 in N2 in the anaerobic box). The buffer solution and the agar plates had been kept in the anaerobic box for at least 24 hrs prior to use. All the colonies on the plates were isolated and identified.

Identification.—The isolates were identified according to the VPI manual14) and to the Bergey’s manual15). For identification of the isolates, following characteristics were examined: Gram staining; acid production from adonitol, arabinose, cellobiose, erythritol, esculin, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, melezitose, melibiose, raffinose,
rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose and xylose; hydrolysis of esculin and starch; liquefaction of gelatin; production of indole; reduction of nitrate; presence of catalase; growth in the presence of bile; motility; ammonia liberation from arginine; heat test for spore inspection; and spore location. Fatty acids produced in PYG broth were assayed with a gas-chromatographic procedure as described previously\(^{12}\).

For determination of lactic, succinic and formic acids by the gas-chromatography, methyl derivatives of these acids were prepared by the methods described by Holdeman et al.\(^{14}\)

### Results

Microorganisms were isolated from all the samples from the lesions. Colony forming units (CFU) were \(8 \times 10^8 \) to \(6.3 \times 10^2\) per sample (\(3.7 \times 10^3\) to \(1.6 \times 10^4\) per g as wet weight).

In two cases, only obligate anaerobes were isolated. They were non-spore-forming Gram-positive rods that produced propionate and acetate in peptone-yeast extract-glucose broth\(^{14}\), and they were classified as \(P.\ acnes\) because of their production of indole. They fermented fructose, galactose and glucose but no acid was produced from adonitol, arabinose, cellobiose, inositol, lactose, melezitose, melibiose, raffinose, salicin, starch, sucrose, trehalose and xylose. They reduced nitrate but they did not hydrolyze esculin nor starch. Additional characteristics of these isolates are shown in Table 1.

In the third case, both facultative bacteria and obligate anaerobes were found. The predominant isolates (52 of 63) were Gram-positive facultative cocci which were identified as \textit{Staphylococcus} species. Eleven of 63 isolates were obligate anaerobes. Five were \(P.\ acnes\), and two were classified as \textit{Arachnia propionica} according to the following characteristics: Non-spore-forming Gram-positive rods that produced propionic acid and fermented fructose, galactose, glucose, lactose, sucrose and trehalose; no acid was produced from adonitol, arabinose, cellobiose, erythritol, esculin, inositol, melezitose, starch and xylose; acid production from maltose, mannitol, mannotose, melibiose, rhamnose, salicin and sorbitol varied with strains; esculin and starch were not hydrolyzed; one isolate liquefied gelatin but the others did not; indole was not produced; nitrate was reduced; no catalase activity was found. Another isolate could not be classified as the species listed in VPI manual\(^{14}\). Its characteristics were as follows: acid production from cellobiose, fructose, galactose, glucose, lactose, mannotose, melibiose and sorbitol; weak acid formation (pH 5.5–6.0) from maltose and melezitose; no acid production from adonitol, arabinose, erythritol, esculin, inositol, mannotol, raffinose, rhamnose, ribose, salicin, starch, sucrose, trehalose or xylose; no hydrolysis of esculin or starch; no production of indole; no reduction of nitrate; no catalase activity.

Control specimens of unaffected tissue adjacent to the lesions were free from microorganisms.

### Discussion

Distinctive bacteria, except pyrogenic cocci which just appear in acute stage of sclerosing osteomyelitis...
osteomyelitis\(^3\), are not isolated from the lesions with a conventional culture technique\(^1-4\). However, obligately anaerobic rods, especially \(P. acnes\), were isolated in relatively low concentrations from 3 lesions of the disease with adoption of the anaerobic glove box system. \(P. acnes\) is a common skin contaminant of clinical specimens or of subsequent cultures\(^16\). In the present study, 2 patients were operated via skin. However, control specimens from intact tissue adjacent to the lesions were free from microorganisms, suggesting that the lesions were not contaminated during the operations. \(P. acnes\) was also isolated from the lesion of the third case where the specimen was taken through oral mucous membrane. No bacterial contamination was found when the sterilized buffer solution, instead of sample, was spread over the blood agar plate and cultured after homogenization and dilution, indicating that homogenization, dilution, inoculation and incubation were carried out aseptically.

Two invasive routes of \(P. acnes\) may be possible. The one is hematogenous infection with \(P. acnes\) delivered from skin\(^8\). The other is infection with \(P. acnes\) of oral origin. \(P. acnes\) is one of the predominant bacteria in deep areas of human carious dentine of teeth\(^11,17\), and isolated from fluid of periodontal pocket\(^18\). All the patients in this study suffered from dental caries with apical periodontitis or from pericoronitis which were located over the lesions that were sampled. This suggests that the route of infection was apical (via carious teeth) or marginal (via lesion of periodontitis) infections\(^4\) in which \(P. acnes\) occurs.

It is suggested that this disease is also associated with high host resistance\(^2,4\). The vital resistance of host\(^19,28\), which causes irregular osteogenic histological reaction of bone\(^2,4\).

The etiological significance of \(P. acnes\) in this disease is not clarified enough, but the lesions appear to be infected with \(P. acnes\) of oral origin.

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References


