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Bacteroides endodontalis and Other Black-Pigmented *Bacteroides* Species in Odontogenic Abscesses

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Twenty-eight odontogenic abscesses were examined for the presence of black-pigmented *Bacteroides* spp. Of the 28 samples, 26 were found to contain one or more species of black-pigmented *Bacteroides*. Abscesses were divided into three categories according to the tissue of origin: endodontal, periodontal, and pericoronal. Four abscesses which developed after extraction were also examined. It was found that *Bacteroides endodontalis*, a newly described species of asaccharolytic black-pigmented *Bacteroides*, was isolated almost exclusively from periapical abscesses of endodontal origin. *B. intermedius* proved to be the most frequently isolated species in all of the samples. *B. gingivalis* was present in all of the periodontal abscesses studied, as well as in two endodontal abscesses. *B. melaninogenicus* was recovered once from a pericoronal abscess. Precautions for the isolation of *B. endodontalis* are discussed.

Abscesses in the maxillofacial region are the most frequently occurring pyogenic infections in oral surgery. These infections are almost always of odontogenic origin. They may be classified according to the primarily infected tissues: endodontal (periapical), periodontal, and pericoronal. Other infections may be postoperative or may be caused by needle-borne infections, jaw fractures, skin lesions, or extractions. Early research on the bacteriology of these kinds of infections suggested a major role for the viridans streptococci (13, 14). However, since the improvement of anaerobic sampling and cultivation techniques it was found that the majority of the microbiota of these abscesses consisted of obligately anaerobic microorganisms (1, 3, 5, 11). Within this group of bacteria gram-negative rods often are encountered. In addition, many other types of bacteria can be isolated, e.g., facultative and obligately anaerobic cocci and rods.

Until now, few attempts have been made to correlate the different forms of dental abscesses with certain bacterial floras or specific combinations of bacterial species. Researchers in bacteriology often did not differentiate among the various forms of odontogenic abscesses, making correlation with the microbial flora involved impossible. Oguntebi and co-workers (12) found *Fusobacterium* spp. and *Streptococcus mitis* in combination in 50% of the examined periapical abscesses. Their observation is suggestive of a specific role for these microorganisms in this type of infection. Only a limited number of species were recovered from the lesions they examined. In a recent study, the bacteriology of periapical abscesses was investigated quantitatively (23). It was confirmed that the majority of the isolates were strict anaerobes or microaerophilic organisms. The bacteria most frequently encountered in this study were gram-negative anaerobic rods (*Bacteroides* and *Fusobacterium* spp.) and anaerobic cocci (*Peptostreptococcus* spp.). In a study of the bacteriology of abscesses of periodontal origin, Newman and Sims (11) also found that the majority of the microbiota consisted of strictly anaerobic bacteria. Among these, black-pigmented *Bacteroides* species were often found.

Bacteroides species, especially the group of black-pigmented species, are involved in both endodontal and periodontal infections (4, 6, 15). The significance of this group of bacteria in human oral diseases has been reviewed in detail by Slots (16). A new asaccharolytic black-pigmented *Bacteroides* species was recently described (19). Being isolated from infected dental root canals, it was named *Bacteroides endodontalis*. So far, three strains of this species have been described (19).

The purpose of this study was to examine the occurrence of black-pigmented *Bacteroides* species in odontogenic abscesses, paying special attention to *B. endodontalis*, and to correlate the different black-pigmented *Bacteroides* species with the different forms of odontogenic abscesses.

MATERIALS AND METHODS

Patients and specimen collection. Twenty-eight patients were involved in this study. They were treated at the Department of Oral and Maxillofacial Surgery, Vrije Universiteit, Amsterdam, The Netherlands. They all suffered from oral pyogenic infections of odontogenic origin. Their ages ranged from 8 to 67 years.

After disinfection of the oral mucosa and incision of the abscess, pus was collected with a sterile cotton swab. All pus samples were obtained through incisions of the oral mucosa, with the exception of patient 1, from whom pus was collected after exposure of the pulpal cavity. The samples were transported in a solid charcoal medium (Microdiagnostics Puurs, Belgium) and processed within 15 min.

Cultivation and isolation. The swabs were streaked directly on four 5% horse blood agar plates (no. 2; Oxoid Ltd., London, England) supplemented with 0.05% hemin and 0.01% menadione. All inoculations were done aerobically. Pus from the swabs was then suspended in 1.0 ml of BM medium containing 1% Trypticase soy (BBL Microbiology Systems, Cockeysville, Md.), 1% proteose peptone (Difco Laboratories, Detroit, Mich.), 0.5% glucose (Merck & Co., Inc., Rahway, N.J.), 0.5% NaCl (Merck), 0.5% yeast extract (Difco), and 0.075% cysteine-hydrochloride (Sigma Chemical C., St. Louis, Mo.) in distilled water. The pH was adjusted to 7.4, and the medium was autoclaved for 20 min at 121°C. After sterilization, the medium was supplemented with filter-sterilized hemin (0.05%) and menadione (0.01%). This pus suspension was homogenized with a tuberculin

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TABLE 1. Black-pigmented *Bacteroides* species and motile organisms associated with odontogenic abscesses

Subject	Sex[a] and age	Clinical remarks	Black-pigmented *Bacteroides* species	Spirochetes[b]	Motile organisms[b]	Origin[c]
1	M, 32	Radicular cyst	*B. endodontalis*	−	−	ED
2	M, 22	Trauma	*B. endodontalis*	ND	ND	ED
3	M, 40	Radicular cyst	*B. endodontalis*	−	−	ED
4	M, 22	Trauma	*B. endodontalis*	−	−	ED
5	M, 34	Carious exposure	*B. endodontalis, B. intermedius*	+	+	ED
6	M, 52	Radicular cyst	*B. endodontalis, B. intermedius*	−	−	ED
7	M, 23	Carious exposure	*B. endodontalis, B. intermedius*	+	+	ED
8	M, 35	Radicular cyst	*B. endodontalis, B. intermedius*	−	−	ED
9	F, 21	Carious exposure	*B. endodontalis, B. gingivalis*	−	−	ED
10	F, 15	Apical lesions	*B. intermedius*	ND	ND	ED
11	M, 16	Disclosed earlier	*B. intermedius*	−	+	ED
12	M, 18	Restoration including pulp chamber	*B. intermedius*	−	−	ED
13	M, 23	History of surgical treatment	*B. intermedius*	−	−	ED
14	F, 35	Apical lesions	*B. intermedius*	−	−	ED
15	M, 20	Carious exposure	*B. intermedius*	++	+	ED
16	M, 30	History of apex resection	*B. gingivalis*	ND	ND	ED
17	M, 8	History of apex resection	*B. intermedius*	−	+	ED
18	F, 57	Generalized periodontitis	*B. gingivalis, B. intermedius*	ND	ND	PD
19	F, 66	Generalized periodontitis	*B. gingivalis, B. intermedius*	−	+	PD
20	F, 71	Generalized periodontitis	*B. gingivalis, B. intermedius*	−	+	PD
21	M, 19	Third lower molar	*B. intermedius, B. melaninogenicus*	++	+	PC
22	F, 21	Third lower molar	*B. intermedius*	+	+	PC
23	F, 24	Extraction of third lower molar	*B. endodontalis, B. gingivalis*	++	+	EX
24	F, 59	Including maxillary sinusitis	*B. intermedius*	−	−	EX
25	M, 40	Total extraction	*B. intermedius*	−	−	EX
26	M, 21	Third lower molar	*B. intermedius*	ND	ND	EX

[a] M, Male; F, female.
[b] −, Not detected; +, present; ++, present in high numbers; ND, not determined.
[c] ED, Endodontal; PD, periodontal; PC, pericoronal; EX, extraction.

syringe and used for dark-field examination to assess the presence of spirochetes and other motile microorganisms (9). Plates were incubated in jars under 80% N_2–10% CO_2–10% H_2 at 37°C for up to 21 days. The cultures were first examined after 7 days. Two morphologically different black- or brown-pigmented colonies were subcultured on 5% horse blood agar plates supplemented with 0.05% hemin and 0.01% menadione and were incubated for 5 days.

Identification of black-pigmented *Bacteroides* species. All pure cultures were examined for the following characteristics: Gram staining, cell morphology, aerotolerance, production of catalase, and fermentation of 1% glucose in BM medium. Fermentation of other carbohydrates as well as the production of indole from tryptophan and the reduction of nitrate was carried out with the Minitek system (BBL Microbiology Systems). Asaccharolytic black-pigmented isolates were tested for the ability to agglutinate 3% sheep erythrocytes (17). Asaccharolytic hemagglutination-negative isolates were further examined for the guanine-plus-cytosine contents of their DNAs and for DNA homology with DNA of a reference strain of *B. endodontalis* as described previously (20, 22). Final identification of black-pigmented isolates was carried out by the method of Johnson and Holdeman (7).

Classification of abscesses. Abscesses were classified by the following criteria. Infections were described as periapical abscesses of endodontal origin if there was clear radiographic evidence of a periapical lesion and a lack of periodontal breakdown along the root of the infected tooth. Infections were classified as periodontal abscesses when a clinical examination revealed periodontal pockets 6 mm or more in depth which bled upon being probed, in combination with radiographic evidence of alveolar bone loss and an absence of periapical lesions. Infections were classified as pericoronal abscesses if there was a clear inflammation of the operculum and if no periapical lesions were present. Abscesses developing within 7 weeks after extraction and with no radiographic evidence of alveolar bone loss and no periapical lesions before the extractions were classified as extraction abscesses.

RESULTS

Black-pigmented *Bacteroides* species. Of 28 odontogenic abscesses, 26 proved to contain one or more species of black-pigmented *Bacteroides*. The concentration of these bacteria in the mixed infections varied from 10 to 50% of the cultivable microflora, with a mean of ca. 30%. The occurrence of black-pigmented *Bacteroides* species and motile organisms and the types of abscesses from which they were isolated are summarized in Table 1.

The majority of the odontogenic abscesses (17 [65%]) were of endodontal origin. In addition, there were three periodontal abscesses, two pericoronal abscesses, and four extraction abscesses.

The black-pigmented *Bacteroides* species recovered from the 26 odontogenic abscesses were *B. intermedius*, *B. gingivalis*, *B. melaninogenicus*, and *B. endodontalis*. *B. intermedius* was isolated from 73% of the samples, followed by *B. endodontalis* (38%), *B. gingivalis* (23%), and *B. melaninogenicus* (4%). In periapical abscesses of endodontal origin, three of these species, *B. endodontalis* (53%), *B. intermedius* (63%), and *B. gingivalis* (12%), were found.

Spirochetes and other motile organisms. The presence of spirochetes and other motile organisms was examined in 21 samples. Two samples from periodontal abscesses contained motile rods; no spirochetes could be found. In one sample from an extraction abscess, relatively high numbers of medium-sized and small-sized spirochetes were present. In two samples from pericoronal abscesses, large-sized, medium-sized, and small-sized spirochetes were found. Three samples from periapical abscesses of endodontal origin contained both spirochetes and other motile organisms. In these abscesses, all three types of spirochetes were detected. In two other samples from endodontal abscesses, only other motile organisms were found.

DISCUSSION

All samples contained leukocytes, many cocci and coccobacillary forms, quite a few fusiforms, and some straight rods and filamentous forms.

More than 90% of the abscesses examined harbored one or more species of black-pigmented *Bacteroides*. In comparison with other studies, this incidence is rather high. Oguntebi and co-workers (12) found *B. intermedius* in 2 of 10 periapical abscesses. Williams and co-workers (23) recovered black-pigmented *Bacteroides* species from 30% of samples from abscesses of endodontal origin. They did not isolate *B. intermedius*, but they did isolate *B. melaninogenicus* and *B. asaccharolyticus*. Labriola and coworkers (8) reported that 24% of samples from 50 orofacial abscesses contained black-pigmented *Bacteroides* species. Periodontal abscesses were investigated by Newman and Sims (11). They cultured black-pigmented *Bacteroides* species from all nine patients examined and recovered *B. gingivalis*, *B. intermedius*, and *B. melaninogenicus* from seven, five, and two of the nine samples, respectively. The incidence of *B. gingivalis* and *B. intermedius* in periodontal abscesses was confirmed in our examination, although the number of these infections in our study was small.

In our study, patients were categorized into four groups according to several clinical parameters. The bacteriological results from our study on odontogenic abscesses suggest a correlation between the type of infection and the occurrence of certain species of black-pigmented *Bacteroides*.

The presence of *B. gingivalis* in periodontal abscesses was expected, as these microorganisms are frequently involved in advanced adult periodontitis. *B. gingivalis* was recovered twice from endodontal abscesses. Although this species is not commonly isolated from this type of disease, in these cases it may have been present in the dental plaque and from this reservoir may subsequently have infected the dental pulp chambers. *B. intermedius* was found to be involved in all four types of odontogenic abscesses, the overall incidence in the samples being 73%. It appears that this species is not distinctly correlated with any one of the four types of infections and may therefore be less specific. *B. endodontalis*, however, was found almost exclusively to be involved in dental abscesses of endodontal origin. *B. endodontalis* was found in 53% of samples from this type of abscess. Earlier, this asaccharolytic black-pigmented *Bacteroides* species was isolated from infected dental root canals (18, 19). This suggests a specific association of *B. endodontalis* with endodontal infections. This species was isolated only once from another abscess, an extraction abscess from which *B. endodontalis* was isolated in combination with *B. gingivalis*.

As with periodontal abscesses (11), endodontal abscesses were, with no exception, mixed infections with *Bacteroides* species among the infecting types. In experimental anaerobic mixed infections in guinea pigs, black-pigmented *Bacteroides* species have been found to play a key role (10, 18). It may be possible that *B. endodontalis* plays a similar role in anaerobic mixed infections.

It is not clear which properties of *B. endodontalis* are responsible for its association with endodontal infections. Isolation of this species as a member of the human oral microflora has not been reported previously, but we recently recovered *B. endodontalis* from supragingival plaque and the tongues of patients with gingivitis. This observation suggests that the reservoir for *B. endodontalis* may be dental plaque.

Asaccharolytic black-pigmented *Bacteroides* species are not commonly isolated from endodontal infections and periapical abscesses. In one report, a sample was found to harbor an asaccharolytic black-pigmented *Bacteroides* strain (23). This strain was identified as *B. asaccharolyticus* but may in fact be a *B. endodontalis* strain, as may the two *B. asaccharolyticus* strains isolated by Brook et al. (2) from periapical lesions from children. There are several possible explanations for the rare isolation of asaccharolytic black-pigmented *Bacteroides* species from endodontal infections and periapical abscesses of endodontal origin. *B. endodontalis* grows slowly, even on enriched media. An incubation time of 5 days is often too short for the recognition of *B. endodontalis* as a black-pigmented species. Isolation may require an incubation time of 7 days or more before pigmentation is visible. Furthermore, *B. endodontalis* is probably very sensitive to oxygen. In a study on the oxygen tolerance of oral anaerobic bacteria, two black-pigmented *Bacteroides* strains, now identified as *B. endodontalis*, proved to be the most oxygen sensitive of all the black-pigmented *Bacteroides* strains examined (3). In our study, all media had to be freshly prepared to obtain satisfactory growth. In addition, media used for the isolation of *Bacteroides* species frequently contain vancomycin in combination with kanamycin. Recently, it was found that asaccharolytic black-pigmented *Bacteroides* species are very sensitive to vancomycin (21). Within this group of bacteria, *B. endodontalis* was found to be one of the most sensitive species.

In three cases of endodontal abscesses, both spirochetes and other motile organisms were found. These observations are in disagreement with those of other studies (12, 23). We noticed that in these cases, pulpal involvement by caries was present. An explanation for the presence of spirochetes and other motile organisms in these three abscesses may be their migration from the oral cavity to the pulpal tissue because of the severe caries. From there they may have become involved in the subsequent periapical abscesses. To our knowledge, this is the first observation of the presence of spirochetes and other motile organisms in abscesses of endodontal origin. Further study will be necessary to elucidate the role of these bacteria in these diseases.

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