Black-pigmented Gram-negative anaerobes in endodontic infections

M. Haapasalo

Department of Cariology, Institute of Dentistry, University of Helsinki, Helsinki, Finland

Abstract: Necrotic dental root canal infections are polymicrobial infections dominated by anaerobic bacteria. The number of different species in one canal is usually low, approx. 4–7 species. The species isolated most frequently belong to the genera Prevotella, Porphyromonas, Fusobacterium, Peptostreptococcus, Eubacterium and Streptococcus. The frequency of isolation of black-pigmented Gram-negative anaerobes in endodontic infections varies from 25% to > 50%. Pr. intermedia is the most commonly found pigmented species, followed by Pr. denticola and two Porphyromonas species, P. gingivalis and P. endodontalis. Several studies have shown that P. gingivalis and P. endodontalis are closely related to the presence of acute symptoms in endodontic infections, whereas other black-pigmented Gram-negative anaerobes are not. However, several other species may also be involved in acute infections. Moreover, Porphyromonas species have occasionally been isolated from cases with no symptoms. Although Porphyromonas spp. are clearly related to symptoms at the beginning of therapy, they are not important for the prognosis of the treatment.

Key words: Porphyromonas gingivalis; Porphyromonas endodontalis; Black-pigmented anaerobic rods; Endodontics

Introduction

Dental root canal infections are caused by oral bacteria that invade the pulp. The required role of bacteria for the development of apical periodontitis has been shown in the studies by Kakehashi et al. [1] and Sundqvist [2]. Earlier studies of the microbiology of endodontic infections indicated that aerobic and facultative bacteria are dominant isolates [3]. Moreover, it was long assumed that often no bacteria at all can be isolated from the infected root canal, and that periapical tissue destruction may be caused by tissue degradation products from the necrotic pulp. The development of anaerobic techniques during the last 20 years has considerably changed our understanding of the microbiology of endodontic infections. It has been shown clearly in many studies that necrotic dental root canal infections are polymicrobial infections dominated by strictly anaerobic species [2,4–9]. It is also known that the presence of bacteria in the canal is a prerequisite for periapical bone loss [1,2], excluding some specific conditions such as occlusal trauma and cemental dysplasia, where the pulp usually is vital.

Bacteriology of endodontic infections

Factors influencing the composition of the flora

The composition of the bacterial flora in endodontic infections is influenced by three major
factors: the origin of the infection, the ecological conditions in the infected root canal, and the host defence mechanisms. Convincing data have accumulated that the bacteria in root canal infections originate from the oral cavity. Species most often isolated are similar to those found in deep periodontal pockets [4–10]. After the infection reaches the pulp through the dentinal tubules, the pulp gradually becomes necrotic (if vital before infection). The oxygen tension in the necrotic pulp goes down, and the low redox potential favours the survival and growth of anaerobes over facultative and aerobic species. Kobayashi et al. compared the bacterial flora of the root canal and the periodontal pocket in teeth with both endodontic and periodontal infections [11]. The aerobe : anaerobe ratio was 0.23 in the periodontal pocket, but it was as low as 0.0022 in the necrotic root canal. In other infections of the body, host defence is usually a major factor influencing the type and composition of the infective flora. However, in the necrotic root canal the host’s defence is limited to a very small area in the most apical part of the canal. The necrotic canal may be regarded as a closed environment in some aspects. The bacteria are supplied with nutrients, but they are not challenged much by specific or non-specific defence mechanisms. Attachment to surfaces or to other bacteria is not supposed to play a role in root canal ecology as it is in the periodontal pocket [12,13].

Bacterial flora in apical periodontitis

No bacteria can be found in a healthy pulp. The number of different bacteria isolated from the necrotic root canal in apical periodontitis is usually quite low, averaging approx. 4–7 species per tooth [4–9]. Anaerobes constitute about 70–95% of the isolates, and based on the cell counts, anaerobic dominance is even more pronounced [11]. The most frequent isolates belong to the genera Prevotella, Fusobacterium, Wolinella, Porphyromonas, Peptostreptococcus, Eubacterium and Streptococcus [4–9]. Table 1 lists the genera most regularly isolated from endodontic infections. It must be emphasized that Table 1 is based on studies where sampling has been done prior to any root canal therapy. Normal chemo-mechani-

| Table 1 |
| Bacterial genera often isolated in endodontic infections |
| Gram-negative anaerobes | Gram-negative facultatives |
| Prevotella | Eikenella |
| Porphyromonas | Capnocytophaga |
| Fusobacterium | Haemophilus |
| Wolinella | |
| Veillonella | |
| Gram-positive anaerobes | Gram-positive facultatives |
| Peptostreptococcus | Streptococcus |
| Eubacterium | Lactobacillus |
| Lactobacillus | Actinomyces |
| Actinomyces | Propionibacterium |
| Propionibacterium | |

cal root canal treatment dramatically changes the ecological conditions in the canal, and in most cases the flora is removed and killed rapidly [14].

Bone destruction in the periapical area is a result of antigenic stimulation from the canal. Bacterial antigens give a start to a highly complex cascade of immunological reactions which are only partially understood at present [15]. Various bacteria possess antigens capable of producing periapical pathology [15,16]. Thus a large number of species may be called endodontic pathogens, if the ability to induce apical periodontitis is used as a criterion. However, this kind of ‘non-specific flora’ hypothesis is only one aspect of endodontic microbiology. There is strong evidence suggesting that the severity of endodontic infections as well as persistent infections are related to certain types of bacterial flora. Much attention has been focused on the role of black-pigmented Gram-negative anaerobes at the beginning of the therapy.

Black-pigmented Gram-negative anaerobes in endodontic infections

Black-pigmented Gram-negative anaerobic rods of the genera Porphyromonas and Prevotella are routine isolates in endodontic infections. The frequency of isolation varies between studies, most probably because of differences in the selection of cases and the methods used in sampling, sample transport, culturing, and identification. When routine cases of apical periodontitis, including both acute and asymptomatic infections,
have been studied, black-pigmented Gram-negative anaerobes have been reported in 5–70% of cases [5,7–9,17,18]. Often more than one species of this group is found in the same canal. However, many studies have attempted to isolate and identify only some species of this heterogenous group, usually those presently classified as Porphyromonas spp. and Pr. intermedia. Much less attention has been given to other Prevotella spp. from clinical samples; so far, only a few laboratories have reported the isolation of P. endodontalis.

P. gingivalis has been isolated regularly both from the root canal and from the periodontal pocket. However, P. endodontalis is only exceptionally found in other than endodontic infections. The reason for the site specificity of P. endodontalis is not clearly understood, but it is possible that, unlike P. gingivalis, P. endodontalis lacks adhesins on the cell surface that are needed for establishment in the pocket flora [12,13,19].

**Black-pigmented Gram-negative anaerobes and symptoms**

Black-pigmented Gram-negative anaerobes have been studied intensively for their virulence properties in vitro. In vivo, they have been related to the presence of acute symptoms in endodontic infection [2,6–9,20]. We studied 62 teeth with one root canal and apical periodontitis [6]. Half of the teeth (31) had acute symptoms at the beginning of the therapy. The occurrence of individual species of this group in symptomatic and asymptomatic teeth is shown in Table 2. Van Winkelhoff et al. studied the occurrence of Porphyromonas spp. and Pr. intermedia in abscesses of endodontic origin. One or more of these species were present in all 17 cases: Pr. intermedia in 11, P. endodontalis in nine, and P. gingivalis in two [21]. However, the presence of Porphyromonas spp. in the infective flora does not guarantee the occurrence of acute symptoms. Sundqvist et al. studied 72 cases of apical periodontitis and found black-pigmented Gram-negative anaerobes in 22 teeth; 16 of these had symptoms and six were asymptomatic. Two of the six asymptomatic teeth had a Porphyromonas spp. in the flora [9]. The absence of symptoms in these two teeth could not be explained by the total viable count either. In conclusion, it is obvious that Porphyromonas spp. increase the probability of acute symptoms in endodontic infections, but these species may be isolated also from asymptomatic teeth, though this seems to be relatively rare. However, Porphyromonas spp. are not required for symptoms in root canal infections, several other species may be involved as well [4,5,9,12].

The exact mechanism by which the Porphyromonas spp. cause symptoms in endodontic infections are not known. P. gingivalis possesses a range of virulence factors such as hydrolytic enzymes, capsule and outer membrane vesicle formation, and it has been suggested to be more resistant to phagocytosis than many other species isolated from oral infections [10,13,22]. However, very little is known about the interaction of P. endodontalis with the host’s defence system. P. endodontalis also has a thick capsule and produces outer membrane vesicles [23]. It often happens that bacteria from the root canal are introduced into the periapical area during instrumentation of the canal. In such situations the balance between the bacteria and host defence is broken and a new balance is reached only after an acute stage. In this kind of flare-up, Porphyromonas spp. may play a key role.

**Table 2**

Isolation of black-pigmented Porphyromonas and Prevotella spp. from acute and asymptomatic teeth with apical periodontitis [6]

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of isolates from patients with symptoms</th>
<th>no symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. gingivalis</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>P. endodontalis</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Pr. intermedia *</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Pr. denticola</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>Pr. loescheii</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* Three Pr. intermedia strains were isolated together with P. gingivalis.

**Black-pigmented Gram-negative anaerobes and prognosis of endodontic therapy**

Black-pigmented Gram-negative anaerobes are closely related to ‘short-term prognosis’ in en-
dodontics. Their presence is often accompanied by symptoms such as pain, swelling, tenderness to percussion, and an open sinus tract [2,6–9,20]. The ultimate goal in endodontics is complete healing of the periapical lesion and regeneration of bone structure. Data on the relationship between the original canal flora and long-term prognosis is very limited [12,24]. However, the information available strongly indicates that black-pigmented Gram-negative anaerobes do not have a negative influence on the prognosis of the endodontic treatment. A case of apical periodontitis with *P. gingivalis* in the flora has been described which did not respond to conventional therapy, and an adjacent vital molar had to be extracted because of the massive bone loss, which reduced also the periodontal bone support of the affected tooth [25]. We have also reported an extraradicular infection with *Pr. intermedia* originating from the root canal that was resistant to conventional therapy [26]. These kind of cases are, however, rare. When the prognosis of the treatment is influenced by the bacteria, Gram-positive species are usually involved [27]. These include, e.g. *Actinomyces* spp. and *Arachnia propionica*, or *Enterococcus faecalis*. The latter and Gram-negative, facultative enteric rods are typical of the flora isolated from teeth where the chemo-mechanical therapy has not been adequate, and often they enter the canal after the beginning of the treatment.

In conclusion, although *Porphyromonas* spp. are important pathogens in untreated endodontic infections, they are not important for the prognosis of the therapy, where the quality of the chemo-mechanical treatment, tooth anatomy, and certain Gram-positive bacteria seem to be more important.

References


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