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Review article

ROLE OF BACTERIA IN THE TOOTH ABSCESS: A MINI REVIEW

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ABSTRACT: A tooth abscess or root abscess is pus enclosed in the tissues of the jaw bone at the apex of an infected tooth's root(s). Usually the abscess originates from a bacterial infection that has accumulated in the soft, often dead, pulp of the tooth. This can be caused by untreated tooth decay, cracked teeth or extensive periodontal disease. A failed root canal treatment may also create a similar abscess. Recently developed molecular methods have made it possible to characterise mixed micro flora in their entirety, including the substantial numbers of uncultivable bacteria. This paper will review the current literature regarding the molecular techniques used to identify uncultivable bacteria from dental abscess.

Key words: Tooth abscess, Bacterial infections, and Molecular techniques.

INTRODUCTION

An **abscess** (Latin: *abscessus*) is a collection of pus (neutrophils) that has accumulated in a cavity formed within a tissue because of an inflammatory process in response to either an infectious process (usually caused by bacteria or parasites) or other foreign materials (e.g., splinters, bullet wounds, or injecting needles). It is a defensive reaction of the tissue to prevent the spread of infectious materials to other parts of the body. The organisms or foreign materials kill the local cells, resulting in the release of cytokines. The cytokines trigger an inflammatory response, which draws large numbers of white blood cells to the area and increases the regional blood flow. The final structure of the abscess is an abscess wall, or capsule, that is formed by the adjacent healthy cells in an attempt to keep the pus from infecting neighbouring structures. However, such encapsulation tends to prevent immune cells from attacking bacteria in the pus, or from reaching the causative organism or foreign object. Abscesses must be differentiated from emphysemas, which are accumulations of pus in a pre-existing rather than a newly formed anatomical cavity.

The acute dental abscess is frequently underestimated in terms of its morbidity and mortality. There are three types of dental abscess. A gingival abscess (or gumboil) involves only the gum tissue, without affecting either the tooth or the periodontal ligament. A periodical abscess starts at the apex of the root. A periodontal abscess begins in the pocket of gingival over 3 mm. The acute dental abscess usually occurs secondary to dental caries, trauma or failed root treatment. After the intact pulp chamber is breached, colonization of the root canals occurs with a diverse mix of anaerobic bacteria. The walls of the necrotic root canals become colonized by a specialized mixed anaerobic bio film (Chavez de Paz, 2007). While asymptomatic necrosis is common, abscess formation occurs when these bacteria and their toxic products enter the periodical tissues via the apical foramen and induce acute inflammation and pus formation (Nair, 2004). The root canal micro biota is the main stimulus for the development of acute symptoms. The main signs and symptoms of the acute dental abscess (often referred to as a periodical abscess or infection) are pain, swelling, erythematic and suppuration usually localized to the affected tooth, although the abscess can frequently spread causing a spreading odontogenic infection which can be accompanied by sepsis syndrome.

The role of bacteria in the pathogenesis of the lesion is undisputed but modern diagnostic techniques have not identified a single causative pathogen. The dent alveolar abscess is polymicrobial comprising various facultative anaerobes, such as the viridans group streptococci and the *Streptococcus anginosus* group, and strict anaerobes, especially anaerobic cocci, Prevotella and Fusobacterium species. The presence of anaerobes both cultivable and uncultivable tends to predominate. The vast majority of dental abscesses respond to surgical treatment, such as drainage of pus and elimination of the source of infection, with antibiotic use limited to severe spreading infections.

New diagnostic methods for acute dental abscess

Molecular biology approaches used for the identification and differentiation of bacterial species can be categorised into main groups such as (i) hybridization of nucleic acid (DNA/RNA) with DNA probes and genomic fingerprinting, (ii) sequencing of ribonucleic acid (55, 165, 235). (iii) PCR with species-specific primers and PCR fingerprint techniques which can detect DNA polymorph isms in the identification of bacterial species. The application of modern molecular biology enables the identification of microorganisms without the need of bacterial culturing is leading to the identification of an even more diverse oral micro flora, in particular the uncultivable species (Slots, 2000). These molecular techniques based on non-amplification nucleic acid probe procedures are more reliable primary reference standards than the cultural method. Importantly, it provides rapid, accurate and inexpensive microbial identification of pathogens and will probably play increasing importance in future diagnostics. Application of the DNA probe technique in clinical dentistry is useful for identification of the presence of bacterial DNA fragments within any type of sample based on cross-reactivity patterns with known bacterial types. Also, it allows the examination of a large number of samples that are difficult to explore with conventional cultural techniques. Nucleic acid probe assays rely on the unique double stranded structure of the DNA molecule to detect the target microorganisms. Each species contains unique segments of DNA thus, allowing the possibility of constructing nucleic acid probes that will hybridize to these sequences. The detection of target microorganisms is, therefore, based on the binding of the nucleic acid probes to the complementary strands of nucleic acid in these unique regions. This technique requires preparing pieces of single-stranded DNA labelled with an enzyme or radioisotope that can locate and bind. To their complementary nucleic acid sequences with low cross-reactivity to non-target microorganisms. Probes can be of different types; (i) whole genomic, (ii) cloned or (iii) oligonucleotide probes and may target for whole genomic DNA or individual genes. Whole genomic probes are constructed from the entire genome of the target microorganism and are more likely to cross react with non-target organisms due to the presence of homologous sequences between different bacterial species. This type of probe has several advantages over other nucleic acid probes. It is the simplest to construct and the least expensive to produce. Cloned probes are isolated sequences of DNA that do not show cross-reactivity and are produced in quantity by cloning in a plasmid vector within a temporary host microorganism such as Escherichia coli. Oligonucleotide probes are usually 10-50 bp whereas the other probe types are often several kilo base pairs in length. The sequences of oligonucleotide probes are obtained from unique hyper variable regions of ribosomal RNA of the target microorganism. Oligonucleotide probes for oral species are relatively insensitive, detecting only 10^6 cells, compared to cloned and whole genomic probes, which can routinely identify 10^3 target cells (Savitt et al., 1990). This probe based on species-specific sequences may display limited or no cross-reactivity with non-target organisms. Oligonucleotide probes complementary to variable regions of the 165 rRNA genes have been used for the detection of various bacterial species (Chen et al., 1989). Thus, DNA probe technology provides both a sensitive and specific assay and alleviates the concern for transport of fastidious microorganisms. The procedure includes (i) disruption of bacterial cells with denaturation of DNA, (ii) immobilization of DNA onto a nitrocellulose filter, (iii) blocking unbound nitrocellulose with non-specific DNA, (iv) hybridization of the filter with labelled probe, (v) washing and detection of bound probe (Savitt et al., 1990). Chen et at. developed a checkerboard DNA-DNA hybridization assay for the detection of oral bacteria. In this method, the sample DNA is released and immobilized on a nitrocellulose membrane. The membrane-bound DNA is then allowed to hybridize with either digoxigenin-Iabelled whole genomic DNA or 165 rRNA-based oligonucleotide probes. The checkerboard assay is able to simultaneously screen for the presence of up to 43 different bacterial species in 43 specimens using a single nitrocellulose membrane.

The checkerboard method is particularly suitable for large-scale microbiological studies of the sub gingival micro biota. This method overcomes many of the limitations of culture and is more rapid, cost-effective than antibody-based techniques and able to identify uncultivable or difficult to grow species. However the disadvantages of this method include the fact that detection is limited to species for which probes are available, the need for precise quality control and the possibility of cross reactions (Haffajee et al., 1999). Close attention to specimen collection and processing on selective and non-selective agars under appropriate atmospheric conditions has improved the routine diagnostic yield from acute dental abscesses. However, despite meticulous attention to detail, it is apparent that many genera of bacteria have yet to be cultured from many infectious diseases including the acute dental abscess (Siqueira & Rocas, 2005). The use of culture-independent or molecular diagnostic techniques has expanded our insight into the microbial ecology of the dental abscess. There is an increasing reliance on genetic methods of identification with 16S rRNA gene sequencing frequently being used for research purposes. Broadly speaking, the molecular analysis may take one of two approaches. Firstly, the use of molecular cloning and sequencing techniques to identify uncultivable micro-organisms using 16s rRNA or rDNA has led to the identification of several novel species (Dymock et al., 1996). The second approach utilizes PCR or DNA–DNA hybridization chequerboard techniques (Siqueira et al., 2001d, 2002a) and more recently 16S rRNA gene sequencing and species-specific primers searching for the presence of specific microbes (Dymock et al., 1996; Riggio et al., 2006; Rocas & Siqueira, 2005; Sakamoto et al., 2006; Siqueira et al., 2001b, c, 2002b, 2003). This approach has demonstrated the higher prevalence of more fastidious organisms such as Treponema species in the acute dental abscess. Treponema species are strictly anaerobic, motile, helically shaped bacteria. Within the oral cavity they are more usually associated with diseases of the periodontium. There are a number of different species described from the oral cavity including *Treponema amylovorum, Treponema denticola, Treponema maltophilum, Treponema medium, Treponema pectinovorum, Treponema socranskii* and *'Treponema vincentii'* (Chan & McLaughlin, 2000). The treponemes are difficult to cultivate and differentiate and only *T. denticola, T. pectinovorum, T. socranskii* and *'T. vincentii'* have been readily cultivated. Recent work using PCR detection has indicated a surprisingly high prevalence of Treponema species within the acute dental abscess. Siqueira & Rocas (2004c) found that *T. denticola* was present in up to 79 % of dental abscesses, with lower detection rates reported by other workers (Baumgartner et al., 2003; Siqueira et al., 2001a, c; Gomes et al., 2006; Cavrini et al., 2008). Other Treponema species were found in lower numbers, including *T. Socranskii* (in 26 % of aspirates), *T. pectinovorum* (14–21 % of aspirates), *T. amylovorum* (16 % of aspirates) and *T. medium* (5 % of the microbiology of the acute dental abscess aspirates). Other species such as *Treponema lecithinolyticum*,*'T. vincentii'* and *T. maltophilum* were not detected. Despite the possession of a number of potential virulence factors, the precise role of these poorly understood and under-reported organisms in the pathogenesis of the acute dental abscess is unclear.

CONCLUSION

With the advent of molecular diagnostic techniques, our insight into the diversity of the polymicrobial collection that comprises the dental abscess is expanding. Factors the microbiology of the acute dental abscess influencing the process of bacterial succession from the saccharolytic cariogenic flora to the more anaerobic and proteolysis flora of the dental abscess remain unknown. Determination of factors that influence the spread of infection from a localized collection at the apex of a tooth to a cellulites and life-threatening sepsis would aid treatment decisions. There are surprisingly few well-controlled studies into the most appropriate treatment regimen for the acute dental abscess, with most of the evidence pointing towards a key role for prompt surgical intervention and timely review. In conclusion, molecular techniques such as those described in this review are a new, culture independent era of medical microbiology and there is great hope that the approach will assist in elucidating the role of microorganisms in the pathogenesis of oral infections. Clearly, further work is required to characterize the microbial populations associated with oral health and disease. Thus, molecular methods provide the tools for the detection of uncultivable microorganisms and hopefully will allow a far better understanding of dental abscess.

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