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Human β-defensin (hBD-1, -2) expression in dental pulp

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The purpose of this study was to investigate the expression of human β-defensins (hBD-1, -2) in dental pulps by reverse-transcription polymerase chain reaction (RT-PCR) and immunohistochemistry. The mRNA transcripts of human β-defensin-1 and human β-defensin-2 could be detected by performing RT-PCR. With immunohistochemical staining of pulp tissue using antisera to hBD-1 and -2 it was possible to demonstrate cytoplasmic expression in odontoblasts. The results demonstrate that not only oral keratinocytes at the epithelial surface but also odontoblasts express human β-defensins. Thus odontoblasts take part in the innate immune system and human β-defensins may play an important role in the innate host defense of human dental pulp.

Key words: antimicrobial peptides; dental pulp; human β-defensins; odontoblasts; PCR

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Proteins in saliva with antimicrobial activity, like lysozyme or calprotectin, have been well examined and are known to be major components of the oral defense (8, 21, 26). A more recent observation is that the epithelia at mucosal surfaces secrete antimicrobial peptides (7, 8, 12, 27) and the β-defensins, in particular, have become the focus of research (3, 5, 6, 13). Antimicrobial peptides play a substantial role in oral host defense because the oral mucosa is regularly colonized by different microorganisms, but is not infected. The mechanical barrier, components of the adaptive immune system and the antimicrobial peptides as part of the innate immune system are responsible for the maintenance of the ecologic balance (1, 3, 4, 9, 19, 24). The antimicrobial spectrum of these peptides may also kill oral pathogens when infections take place. The absence of antimicrobial peptides may lead to diseases like periodontitis or opportunistic infections like candidiasis, and perhaps also to caries and pulpitis (3, 6, 14).

Defensins are a group of small (3–5 kDa), cationic, cysteine-rich β-sheet peptides with a broad spectrum of antimicrobial activity. Their function is presumably based on the production of channels or micropores in the membrane of microorganisms (1, 9, 10, 12, 22, 24, 27). Another mode of action is the presentation of a carpet-like structure on the membrane, which activates the adaptive immune system (31). Three subfamilies are distinguished in vertebrates, two of which, the α- and the β-defensins are known to exist in humans (27). Four human β-defensins have been isolated so far, the human β-defensins (hBD)-1, -2 and the more recently discovered hBD-3 and -4 (11, 12, 15, 17, 22, 28). hBD-1 is expressed constitutively in salivary glands and oral epithelial tissues and acts highly effective against gram-negative bacteria (4, 6, 10, 11, 16, 20, 25). hBD-2 was shown to be produced by oral epithelia and by salivary glands, as well. It has a strong bactericidal effect on gram-negative bacteria and has a high antimycotic potency but only a weak bacteriostatic effect on the gram-positive *Staphylococcus aureus* (11).

Recently, we studied the expression of phospholipase A-2 (PLA-2), lysozyme, the human α-defensins and human β-defensins hBD-1 and hBD-2 in oral tissue samples (5, 6).

In the present study we investigated the expression of the β-defensins hBD-1, hBD-2 in dental pulps by reverse-transcription polymerase chain reaction (RT-PCR) and by immunohistochemistry.

Materials and methods

Tissue samples

In the present study, 20 dental pulps of freshly extracted wisdom teeth were obtained from 20 different patients, 10 females and 10 males, during routine surgical procedures with their informed consent. All specimens were completely caries free. The study had been approved by the Institutional Review Board (Ethical Committee). The age of all investigated caucasian patients was in the range of 18–28 years. The pulps were isolated by crushing extracted teeth, immediately frozen and stored at − 80°C. Ten pulps were used for immunohistochemistry and the remaining tissue samples were used for detecting gene expression by RT-PCR.

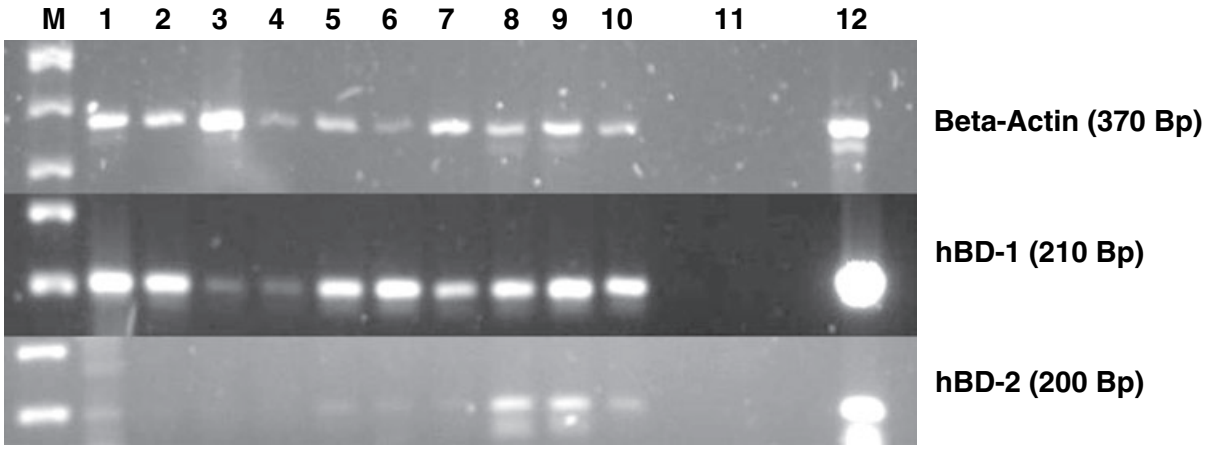

Fig. 1. Expression of β-actin, hBD-1 and hBD-2 transcripts detected by RT-PCR in pulpal tissue samples of 10 different patients. Lanes 1–10 = dental pulp; lane 11 = negative control; lane 12 = positive control. M, Marker.

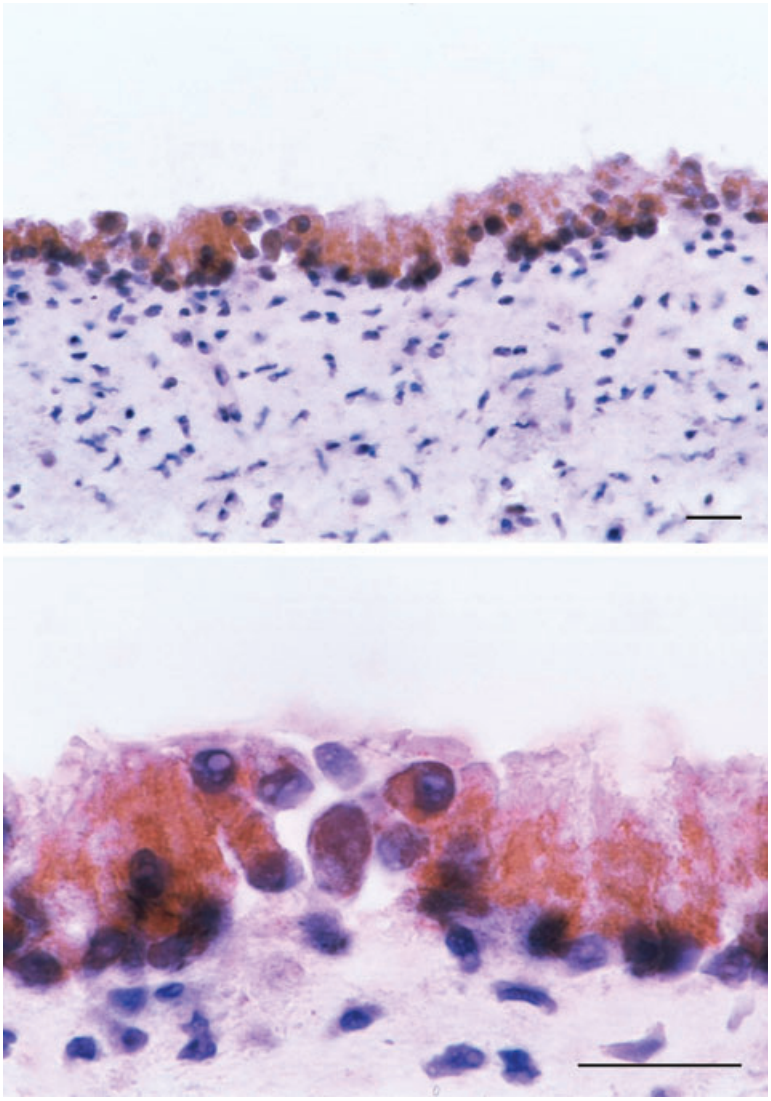

Fig. 2. Representative immunohistological staining with α-hBD-1 from dental pulp: Cytoplasmic expression in odontoblasts. Strept-ABC staining, magnification 450× (above) and 1350× (below). Bar = 20 μm.

RNA-extraction and cDNA-synthesis

Total RNA was extracted from homogenized biopsies according to the RNeasy Mini Kit protocol (Qiagen, Hilden, Germany). Complementary DNA (cDNA) was synthesized from 8 μl of total RNA using the SuperScript II Reverse Transcriptase Kit (Invitrogen, Carlsbad, CA).

Detection of transcripts of human β-defensins by PCR

RT-PCR amplifications were performed in a total volume of 50 μl containing 1 μl cDNA, 5 μl 10× buffer, 5 μl 20 mM dNTPs, 2 pmol of each primer, and 1 U of DNA-polymerase.

The following amplification conditions and primers were used:

- For β-actin: 5′-CATGGATGATG-ATATCGCCGCG-3′ (forward), 5′-AC-ATGATCTGGGTCATCTTCTCG-3′ (reverse); 95°C/5 min, 95°C/15 s, 63°C/30 s, 72°C/1 min, using TaqPol (Invitrogen).
- For hBD-1: 5′-CATGAGAACTTCCT-ACCTTCTGC-3′ (forward), 5′-TCACT-TGCAGCACTTGGCCTT-3′ (reverse); 95°C/5 min, 95°C/15 s, 63°C/30 s, 72°C/1 min, using Herculase Hotstart DNA Polymerase (Stratagene, La Jolla, CA).
- For hBD-2: 5′-CATGAGGGTCTTGTA-TCTCCTCT-3′ (forward), 5′-CCTCCT-CATGGCTTTTTGCAGC-3′ (reverse); 95°C/5 min, 95°C/15 s, 63°C/30 s, 72°C/1 min, using Pfu Turbo Hotstart DNA Polymerase (Stratagene).

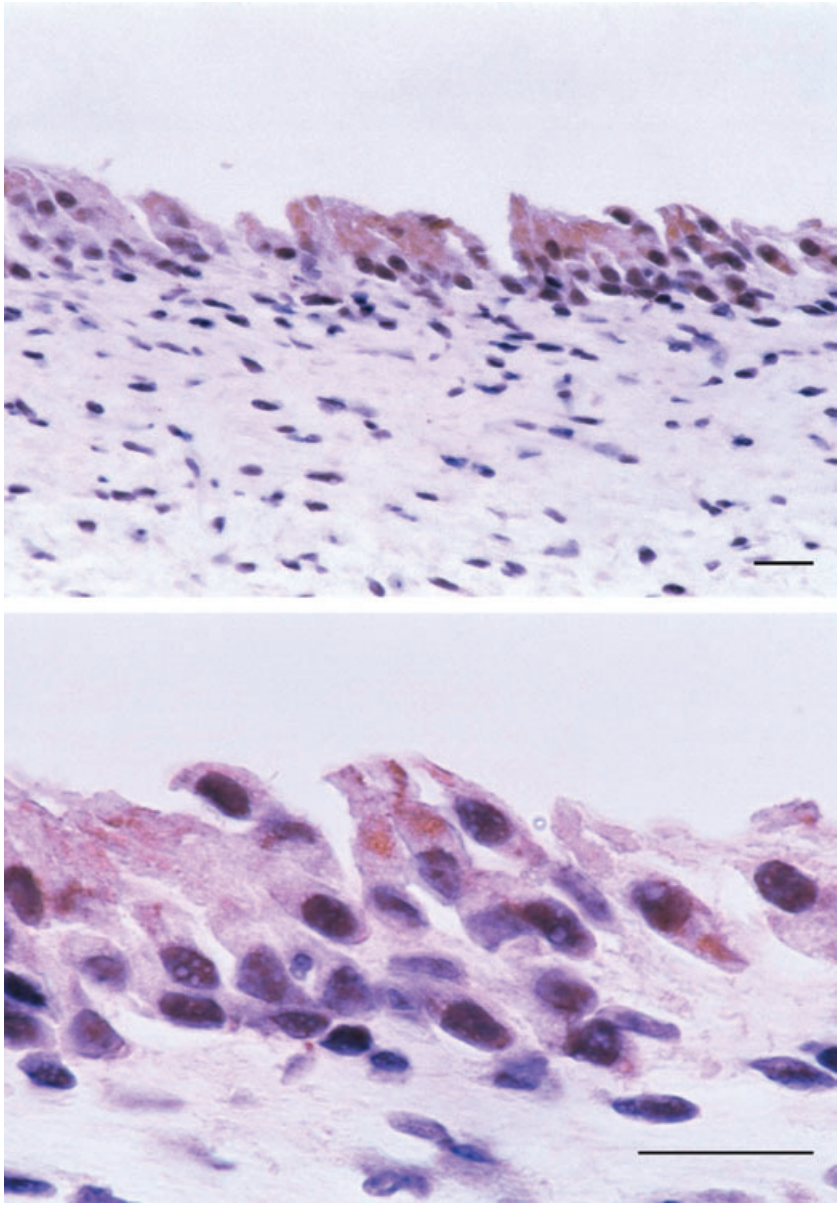

Fig. 3. Representative immunohistological staining with α-hBD-2 from dental pulp: Cytoplasmic expression in odontoblasts. Strept-ABC staining, magnification 450× (above) and 1350× (below). Bar = 20 μm.

Cloned β-actin, hBD-1 and hBD-2 served as positive control, while water used instead of pulpal cDNA served as negative control.

PCR products were separated on 2.0% agarose gels. If the specific PCR-product was detectable after ethidium bromide staining, a sample was evaluated as 'positive' for the expression of the respective transcript.

Immunohistochemical preparation of the paraffin-embedded tissues

Immunohistochemical staining was performed using the streptavidin-biotin-complex (Strept-ABC) technique. Formalin-fixed tissue samples were paraffin-embedded according to routine procedures (23). Four-μm-thick sections were cut with a standard microtome (Reichert-Jung, Heidelberg, Germany) and afterwards treated with 1.5% H_2O_2 in ice-cold methanol for 10 min to block endogenous peroxidase. The slides were subsequently immersed in 0.01 M citrate buffer, pH 6.0, and cooked for 5 min at 90°C in a pressure cooker to unmask the relevant antigens. Slides were washed in phosphate-buffered saline (PBS) and incubated with 2% goat nonimmune serum for 20 min to block nonspecific binding. Indirect immunohistochemistry was performed with rabbit-antisera to hBD-1 and hBD-2 (Bio-Logo, Kiel, Germany) as well as preimmune serum (Bio-Logo) as control. Subsequent sections of each tissue were stained in parallel using the Strept-ABC method according to the instructions of the manufacturer (DAKO, Hamburg, Germany). Antibodies were used at a final concentration of 10 μg/ml in 4% normal human serum. The primary antibody was detected with rabbit anti-mouse peroxidase (DAKO) using DAB as chromogene, counterstained with hemalum. Stained slides were dehydrated and mounted with Eukitt (Merck Eurolab, Darmstadt, Germany).

Results

The expression of transcripts for the human β-defensins hBD-1 and -2 was investigated by RT-PCR in a series of 10 samples from dental pulps (Fig. 1). A strong expression of hBD-1 transcripts was detected in eight samples. In contrast, a strong expression of hBD-2 mRNA could be detected in only two samples. Immunohistochemical staining of pulps using antisera to hBD-1 and -2, demonstrated consistent expression in the cytoplasm of odontoblasts (Fig. 2 and 3). hBD-1 was distinctly expressed in the cytoplasm of odontoblasts (Fig. 2), while hBD-2 showed a weaker cytoplasmic expression in the odontoblast cell-layer (Fig. 3).

In this study, we could demonstrate with two independent methods a different expression of β-defensin-1 and -2 on the transcript and protein level in pulpal cells.

Discussion

Odontoblasts are the first pulpal cells in contact with dental pathogens due to their peripheral localization in the dental pulp and their extension into dentin. The contact of odontoblasts with immunoglobulins during microbial invasion of dentin and the juxtaposing of dendritic cells to odontoblast cell bodies implies that odontoblasts may play a role in adaptive pulpal immune responses. Recently, it was shown that odontoblasts take an active part in the recruitment of neutrophils in response to bacterial by-products (18). Our observation of an expression of β-defensins indicates that the nonspecific, natural and rapidly acting defense may also be an important function of odontoblasts. In addition to their antimicrobial activity, defensins seem to have important signaling potential in the interaction between innate

and acquired immune responses (2, 9, 30, 32, 33).

Our data suggest minor gene activation of hBD-2 in odontoblasts from dental pulps in complete caries-free and uninfected wisdom teeth. In agreement with recently published results, we could demonstrate a consistently high expression of hBD-1 (11, 16). In contrast, hBD-2 expression seems at least to be weaker in pulpal cells. An inducible effect on hBD-2 expression remains to be proven by further experiments. The data derived from RT-PCR experiments support the results from immunohistological staining.

In addition to antimicrobial activity, a further role of human β-defensins may be an involvement in odontoblast development. Certain fractions of pulp cells have the ability to differentiate into odontoblasts, thus forming dentin. Dentin sialophosphoprotein is a marker protein of odontoblasts. Macrophage inflammatory protein-3α and hBD-2 have been described to stimulate dentin sialophosphoprotein gene expression in pulp cells (29).

In summary, the results of immunohistochemistry confirm for the first time – to our knowledge – that not only oral keratinocytes at the epithelial surface, but also odontoblasts, mesenchymal in origin, express human β-defensins. In addition to the formation, maintenance, and repair of dentin, cytoskeletal functions like collagen formation and control of the passage of extracellular material, this may reflect an important function of odontoblasts which was unknown up to now, as β-defensins seem to play a role in the innate host defense of human dental pulp.

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