ANTICANCER RESEARCH *24*: 1025-1030 (2004)

# Human Beta-Defensin-2 in Oral Cancer with Opportunistic *Candida* Infection

JENS EDUARD MEYER[1], JÜRGEN HARDER[2], TIBOR GÖRÖGH[1], JAN BERND WEISE[1], SABINE SCHUBERT[3], DIRK JANSSEN[4] and STEFFEN MAUNE[1]

[1]*Department of Otorhinolaryngology, Head and Neck Surgery, Arnold-Heller-Straße 14;*
[2]*Department of Dermatology, Venerology and Allergology, Schittenhelmstraße 7;*
[3]*Department of Medical Microbiology and Virology, Brunswiker Straße 4; and*
[4]*Department of Pathology, Michaelisstr 11, University of Schleswig-Holstein, Campus Kiel, 24105 Kiel, Germany*

**Abstract.** *Candida albicans (CA) is a frequent opportunistic pathogen in cancer patients. Usually, human surfaces are protected, apart from physical barriers, by the production of human β-defensins (hBD). hBD-2 shows a potent antimicrobial activity against CA. We therefore investigated whether CA induces hBD-2 expression in primary oral cells and if immunosuppressive betamethasone alters hBD-2 expression. Additionally, we studied, whether a lack of hBD-2 expression could explain opportunistic infection of tonsillar cancer. Primary oral epithelial cells and fibroblasts were stimulated with Candida albicans in a time- and dose-dependent manner with or without betamethasone preincubation. Total RNA from oral cells and specimens was isolated and hBD-2 expression was analyzed by semiquantitative RT-PCR. Our data demonstrate that opportunistic CA induced hBD-2 expression in a time- and dose-dependent manner, suggesting hBD-2 to be a fast antifungal, epithelia-derived immune response. Treatment with glucocorticoid could lead to diminished innate immunity based on suppression of inducible AP. Malignant transformation induces alteration of hBD-2 expression and leads to a reduced hBD-2 expression and subsequentially to Candida colonization on oral SCCs.*

Fungal infections are frequent and invasive fungal infections have become a major obstacle to the treatment of patients with malignancies (1). *Candida* spp. now rank among the ten most prominent pathogens in these patients (2), of which *Candida albicans* (CA) is the most common species (3).

Patients with malignancies are predisposed to develop fungal infections as the result of impairment of host defense mechanisms from intensive cytotoxic chemotherapy, ablative radiation therapy and corticosteroids, but the exact pathogenesis of fungal infection is still unknown (4).

Recently, Harder *et al.* isolated an antimicrobial peptide from inflamed human skin, which was termed human β-defensin-2 (hBD-2) (5). hBD-2 is an epithelia-derived antimicrobial peptide and is inducible upon contact of epithelia with different microorganisms, including gram-negative bacteria and CA, and by proinflammatory cytokines. hBD-2 follows an organ-specific pattern in the human body and exhibited a strong antimicrobial activity against CA (5-7).

In the present study we addressed the question, as to whether hBD-2 gene expression is inducible by CA in primary oral cells and if this stimulation might be altered through immunosuppression induced by corticosteroids. Finally, we studied *in vivo* hBD-2 gene expression in tonsillar carcinoma with candidiasis compared to control group.

## Materials and Methods

*Patients.* The patients were being treated with tonsillectomy for hyperplasia or for cancer of the tonsils. In each cancer patient, preoperative swabs had been drawn to investigate the surface colonization. The swabs were evaluated by the Department of Microbiology at the University of Schleswig-Holstein, Campus Kiel, Germany. All 8 cancer patients elicited *Candida* colonization on their oral squamous cell carcinomas (SCCs). We extracted specimens of 8 patients in both of the two groups. Specimens were removed without the need of any additional resection and frozen immediately in liquid nitrogen (Ethical Committee approval AZ 310/98).

*Primary cell culture of human oral epithelial cells.* Human epithelial cells were cultured from healthy oral mucosa obtained from the edge of resected hyperplastic tonsils. The submucous layer and

*Correspondence to:* Jens E. Meyer, MD, Dept. of Otorhinolaryngology, Head and Neck Surgery, University of Schleswig-Holstein, Campus Kiel, Arnold-Heller-Str. 14, 24105 Kiel, Germany. Tel: ++49-431-597-2241, Fax: ++49-431-597-22, e-mail: jmeyer@hno.uni-kiel.de

*Key Words:* hBD-2, antimicrobial peptides, innate immunity, cancer, *Candida*, defensin.

5'-GTGAAGCTC***CCAGCCATCAGCCATGAGGGT****CTTGTATCTCCTCTTCTCGTTC*
*CTCTTCATATTCCTGATGCCTCTTCCAGGTGTTTTTGGTGGTATAGGCGATCCTG*
*TTACCTGCCTTAAGAGTGGAGCCATATGTCATCCAGTCTTTTGCCCTAGAAGGTA*
*TAAACAAATTGGCACCTGTGGTCTCCCTGGAACAAAATGCTGCAAAAAGCCATG*
*AGGAGGCCAAGAAGCTGCTGTGGCTGA****TGCGGATTCAGAAAGGGCTCC***CCTCA
TCAGAGACGTGCGACATGTAAACCAAATTAAACTATGGTGTCCAAAGATAC-3'

Figure 1. *The nucleotide sequence of the coding region of the human beta-defensin-2 (hBD-2) gene consisting of 319bp is shown. The amplified RT-PCR product is indicated in italics and the forward and reverse primers are highlighted by boldface.*

vessels of the specimens were removed. After an incubation in 0.5% trypsin-PBS mixture for 1 h at 37˚C, trypsination was stopped with DMEM (Dulbecco's Minimal Essential Medium) containing 10% FCS (Fetal Calf Serum). Epithelial cells were centrifuged at 1,000 rpm for 5 min at room temperature, resuspended and cultured in cell culture flasks at 37˚C with 5% $CO_2$ in KGM (Keratinocytes Growth Medium) supplemented with bovine pituitary extract (BPE) and gentamycin. For stimulation cell cultures were transferred to 6-well tissue culture plates.

*Primary cell culture of human oral fibroblasts.* Human fibroblasts were cultured from healthy oral connective tissue obtained from the residues of the above mentioned oral tissue. The tissue was cut into small pieces, placed on the floor of cell culture flasks and cultured at 37˚C with 5% $CO_2$ in DMEM supplemented with 10% FCS, glutamine, penicillin and streptomycin. After 14 days, fibroblasts had sprouted from the connective tissue pieces which were then gently removed. Cell cultures were grown to confluence and split on 6-well tissue culture plates for stimulation after another passage.

*Stimulation of primary cell cultures.* Two wells of a 6-well tissue culture plate were used for each concentration and time-point of the stimulation. After the cells had reached 70-80% confluence, the medium was removed, cells were washed twice with PBS and cultured in KGM lacking bovine pituitary extract (BPE) or, respectively, DMEM lacking FCS for 24 h. Cultivated preconfluent primary oral cells were incubated for 12 h with various densities of heat-inactivated CA (0, $10^2$, $10^3$, $10^4$, $10^5$, $10^6$, $10^7$ CA per ml stimulation medium) and with $10^7$ CA per ml over various periods of time (0, 1, 2, 4, 6, 12 h). For the corticosteroid inhibition, cells were pretreated with 0-1,000 ng per ml betamethasone for 8 h, washed twice with PBS and stimulated with $10^7$ CA for 6 h. Overnight cultures of CA (clinical isolate) were grown at 37˚C in Trypticase Soy Broth (TSB), washed with PBS, diluted in BPE/FCS free cell culture medium, heat-inactivated (60 min incubation at 70˚C) and then added at the indicated concentrations to the cells. Subsequently, cells were lysed by the following procedures in order to isolate RNA. All procedures were performed at least twice.

*Semiquantitative reverse transcription polymerase chain reaction (SQRT-PCR).* Oral cells were washed twice with PBS and total RNA of two wells of a 6-well tissue culture plate was isolated using the TRIzol reagent according to the manufacturer's instructions (Gibco-BRL, Eggenstein, Germany). Total RNA was quantified by measuring the optical density at 260 nm and the sample was stored in 50% formamide at -80˚C after the concentration had been adjusted to 1 μg/μl. SQRT-PCR was carried out as previously described (8). Briefly, 1 μg total RNA was reverse transcribed (Gibco BRL) and cDNA

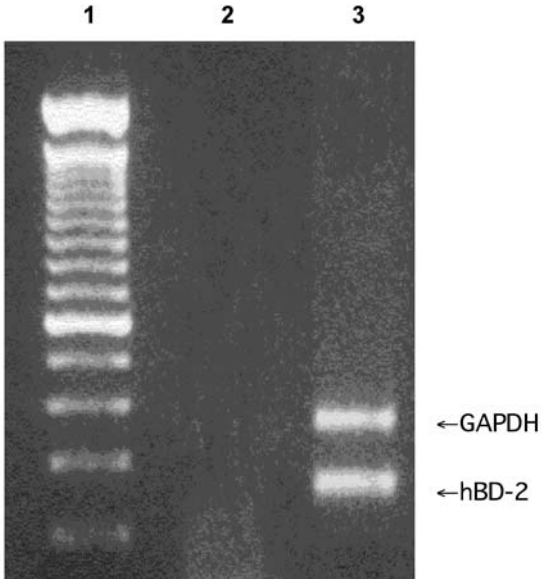


Figure 2. *Ethidium bromide agarose gel electrophoresis showing distinct fragments of GAPDH and hBD-2 gene fragments generated by RT-PCR. The 100bp molecular weight ladder (lane 1) is indicated on the left. Lane 2 is the negative control. The expression of the hBD-2 gene was measured densitometrically and related to the constitutive expression level of the GAPDH gene (lane 3), allowing a semi-quantitative determination of gene expression.*

equivalent to 50 ng RNA used as a template in a duplex-PCR reaction containing 0.5 μM of hBD-2-specific intron spanning primers (5'-CCAGCCATCAGCCATGAGGGT-3'; 5'-GGAGGCCTTTCTG AATCCGCA-3'; Figure 1) and 0.05 μM of intron spanning sets of primers specific for glycerin-3-phosphatdehydrogenase (GAPDH; 5'-ATGAGCCCCAGCCTTCTCCAT-3', 5'-CCAGCCGAGCCACA TCGCTC-3'). The GAPDH primer sets were designed to differentiate between genomic and cDNA-templates and served as internal control for equal amounts of cDNA. Amplification was done using 32 cycles with denaturation at 94˚C for 1 min, primer annealing at 60˚C for 30 sec and extension at 72˚C for 2 min (T3 PCR-thermocycler, Biometra, Göttingen, Germany). PCR products were subjected to electrophoresis on a 2 % agarose gel and visualized by ethidium bromide staining. PCR signals were quantified by densitometric analysis (Video BioDoc, Scan Pack 3.0).

*Cloning and sequencing procedure of PCR-generated DNA fragments.* Twenty microliter RT-PCR product was loaded onto agarose gel

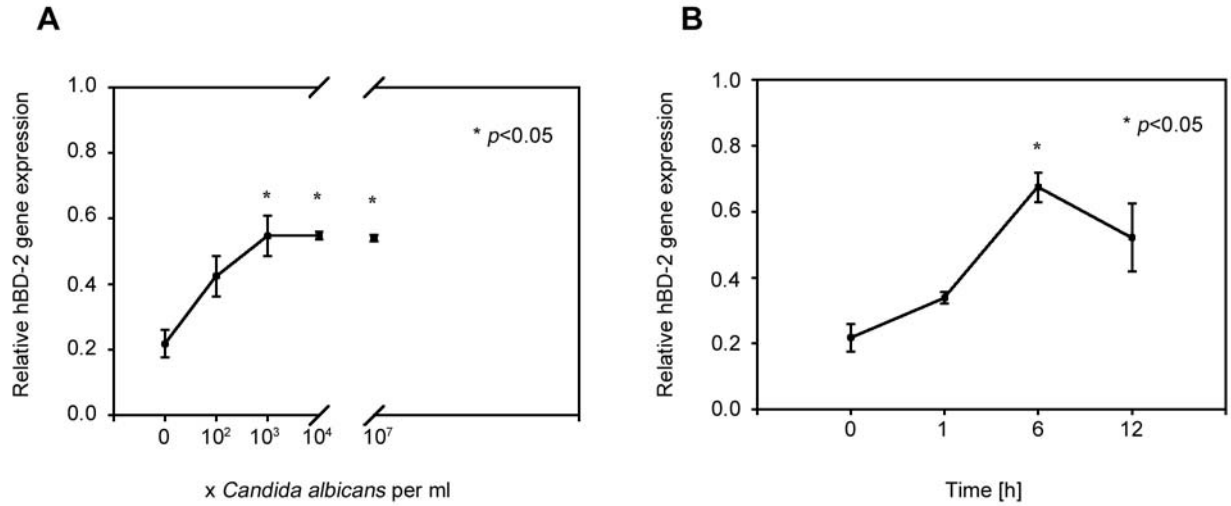


Figure 3. *Total mRNA was amplified in a semiquantitative RT-PCR with specific hBD-2 primers after a dose- (a) and time-dependent (b) stimulation with Candida albicans. Relative hBD-2 gene expression was calculated to GAPDH gene expression and is depicted as arbitrary units.*

containing 0.01% ethidium bromide and separated at 80 V for 2 h. After electrophoresis the expected 250 bp cDNA fragment was visualized by UV monitoring (Biometra) and subsequently cut out from the gel (Figure 2). The DNA was isolated from the gel slice using the GFX-PCR Kit according the manufacturer's instructions (Amersham, Braunschweig, Germany). This purified double-stranded cDNA fragment was ligated into pGEM-T cloning vector and cloned into JM-105 high efficiency competent cells (Promega, Heidelberg, Germany). Using both forward and reverse M13 oligonucleotides, five clones were sequenced on both strands using the DNA-sequencing apparatus Abi Prism 310 (Applied Biosystems, Weiterstadt, Germany) and examined for potential overlaps.

*Statistical analysis.* hBD-2 gene expression was specified as the ratio of hBD-2 over GAPDH. Mean and standard deviations were calculated for each trial. The significance of results was compared with control group and, respectively, measurement without stimulation or inhibition was evaluated by Student's *t*-test. If the probability was less than 5%, the results were marked by a star. All calculations were performed with Sigma Plot and Sigma Stat software from Jandel Scientific.

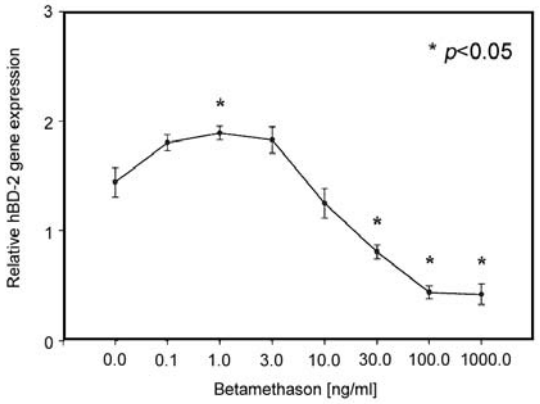


Figure 4. *Primary oral epithelial cells were preincubated with betamethasone in different concentrations and stimulated with $10^7$ Candida albicans per ml over 6 h. Total mRNA was amplified in a semiquantitative RT-PCR with specific hBD-2 primers. Relative hBD-2 gene expression was calculated to GAPDH gene expression and is depicted as arbitrary units.*

## Results

*Candida albicans stimulates hBD-2 mRNA expression in oral epithelial cells, but not in oral fibroblasts in a time- and dose-dependent manner.* Analyses of hBD-2 mRNA expression in oral epithelial cells by RT-PCR revealed that unstimulated oral epithelial cells showed a constitutive hBD-2 mRNA expression. A dose-dependent induction with significant effects at *Candida albicans* densities higher than $10^2$ per ml were seen (Figure 3). Above $10^3$ *Candida albicans* per ml, hBD-2 mRNA expression turned into a plateau phase. Time-course studies in oral epithelial cells revealed a maximum induction within 6 h of stimulation, starting early at 1 h after stimulation (Figure 3b). Twelve hours after stimulation, hBD-2 mRNA expression decreased again up to two-thirds of maximum expression (Figure 3a). We found that oral fibroblasts did not express hBD-2-mRNA (data not shown).

*Corticosteroids inhibit hBD-2 mRNA expression in oral epithelial cells in a dose-dependent manner.* Pretreatment of the oral epithelial cells with betamethasone showed a dose-

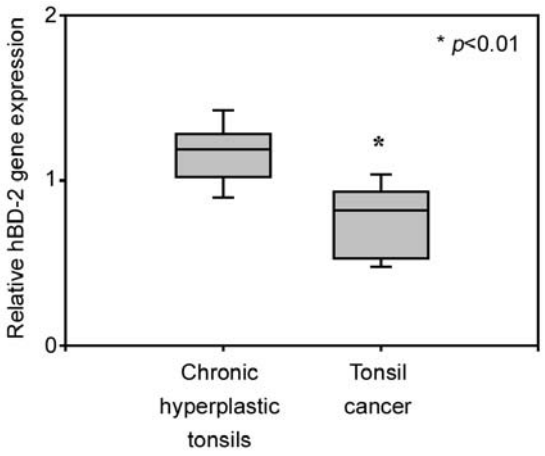


Figure 5. *hBD-2 gene expression of normal hyperplastic tonsils was compared to hBD-2 gene expression of tonsil cancer specimens after a semiquantitative RT-PCR using specific hBD-2 primers. Relative hBD-2 gene expression was calculated to GAPDH gene expression and is depicted as arbitrary units.*

dependent inhibition of hBD-2 mRNA expression (Figure 4). Interestingly, constitutive hBD-2 mRNA expression was also inhibited.

*Hyperplastic tonsils showed a higher hBD-2 mRNA expression than tonsillar carcinoma.* hBD-2 mRNA was expressed in all tissue samples. In oropharyngeal cancer patients hBD-2 mRNA expression was significantly less than in hyperplastic and otherwise healthy tonsils (Figure 5).

## Discussion

Harder *et al.* were able to show that hBD-2 has a potent antimicrobial activity against CA and the highest hBD-2 gene expression was detected in lung, trachea and tonsils, indicating a prominent role of hBD-2 in the respiratory tract host immune response (7). In our study, hBD-2 mRNA was expressed *in vivo* in tonsils, tonsillar cancer and in oral epithelial cells, while control experiments with oral fibroblasts confirmed the fact that the main source of hBD production seems to be the epithelium (6,8-11).

Several studies supported the idea of a constitutive role of hBD-1 in maintaining normal gingival health and an inducible part for hBD-2 in oral inflammation (9,12-16). Interestingly, we also found a constitutive hBD-2 mRNA expression in unstimulated primary oral epithelial cells. Krisanaprakornkit *et al.* and Mathews *et al.* also independently described a constitutive hBD-2 gene expression, suggesting that the oral cavity is in a steady state with invading germs and emphasizing the important role of hBD-2 in this complex defense system (10,12).

To study the influence of CA on hBD-2 gene expression, we stimulated oral epithelial cells with CA. hBD-2 mRNA was induced in a time- and dose-dependent manner, which elicits oral epithelial cells to be the source for an antimicrobial activity towards CA. Interestingly, hBD-2 mRNA expression was up-regulated as early as 1 h after stimulation. In contrast to the adaptive immune response, this result emphasizes hBD-2 mRNA expression vicariously for other AP as a fast immune response to fungal infections.

In addition, hBD-2 mRNA expression was early up-regulated at $10^2$ CA per ml. This is a minor density of CA, pointing to a possible role for hBD-2 in the control of opportunistic *Candida* albicans on human body surfaces and contributing to a natural balance between infections and immune system. Additionally, Abiko *et al.* also accredited hBD-2 *in vivo* as playing a role in the defense of oral candidiasis (17).

To investigate the effect of immunosuppression on hBD-2 gene expression, we preincubated oral epithelial cells with different concentrations of betamethasone. We found a down-regulation of hBD-2 gene expression after pretreatment with betamethasone below constitutive hBD-2 mRNA levels, suggesting that *in vitro* immunosuppression *via* glucocorticoids leads to a decreased hBD-2 mRNA expression. In contrast, Duits *et al.* found that dexamethasone did not reduce hBD-1 and -2 mRNA expression in Primary Bronchial Epithelial Cells (PBEC), whereas hBD-3 mRNA expression was inhibited (18). Duits *et al.* speculated that the induction of hBD-3 might be linked to NF-kappa B and AP-1 consensus binding sites in the promoter region, although it has recently been published that the hBD-2 promoter region also contains both consensus binding sites (6). Furthermore, Duits *et al.* stimulated hBD-2 gene expression with *Pseudomonas aeruginosa*, whereas we stimulated hBD-2 expression with CA. As fungi are more lipophilic and have a completely different surface pattern, it is intriguing to speculate that different aspects of innate immune responses are differentially regulated. Therefore, it could well be that *Candida albicans* induces hBD-2 through NF-kappa B and AP-1 consensus binding sites.

Simmaco *et al.* described in frogs that glucocorticoid treatment inhibits the transcription of all genes encoding antibacterial peptides by inducing the synthesis of I kappa B alpha (19,20). I kappa B is an inhibitor of NF kappa B *via* cytoplasmatic inactivation through dimer constitution, supporting the hypothesis that glucocorticoids directly interact with NF-kappa B and AP-1 (21). Therefore, a possible mechanism of glucocorticoids down-regulating hBD-2 mRNA expression could be through I kappa B and consecutively lower concentrations of NF-kappa B leading to lower hBD-2 mRNA expression.

Abiko *et al.* studied several tumor cell lines and four SCC samples for hBD-1 and –2 expression. The results indicated that SCCs in which hBD expression is down-regulated may

be susceptible to infection (22). As *Candida* is associated with oral carcinomas (23) and CA especially is often a permanent resident on oral cancers, it is interesting to investigate the role of the epithelia-derived hBD-2 in oral SCCs. Therefore, we investigated hBD-2 mRNA expression in normal tonsils and in CA-positive tonsillar carcinomas. In specimens of tonsillar cancer we found comparably lower hBD-2 mRNA expression than in control specimens, suggesting that locally limited, opportunistic candidiasis on oral cancers could interfere with locally diminished hBD-2 gene expression. Interestingly, Sawaki *et al.* showed that more intense HNPs and hBD-2 expression signals were restricted in the candidiasis area of normal buccal epithelia, whereas hBD-2 was also found in the non-infected buccal epithelia, suggesting that malignant transformation induces alteration of hBD-2 expression (24).

The present paper describes the results of transcription level experiments. In contrast to our results, Sawaki *et al.* quantified hBD-2 peptide concentration in ten oral SCCs, showing significantly higher hBD-2 than in normal oral epithelia (25). In this study it was not specified what kind of SCCs were extracted, since Mizukawa *et al.* demonstrated that hBD-2 production is linked to cancer cell differentiation (26). So the results of Sawaki and coworkers would not be surprising, if almost only well-differentiated SCCs were investigated.

Takes together, our data demonstrate that opportunistic *Candida albicans* infections induced hBD-2 expression in a time- and dose-dependent manner, suggesting hBD-2 to be a fast antifungal, epithelia-derived immune response. Treatment with glucocorticoids could lead to diminished innate immunity based on suppression of inducible AP, as shown here exemplarily for hBD-2. Malignant transformation induces alteration of hBD-2 expression and leads to a reduced hBD-2 expression and subsequently to *Candida* colonization on oral SCCs. Furthermore, because of the small molecular weight, a compact structure and the killing activity of AP, it seems to be difficult for microorganisms to acquire resistance, making these peptides very attractive for therapeutic use as antifungal agents (8).

## Acknowledgements

This study was supported by the Deutsche Forschungsgemeinschaft Grant Ma 2071/1 and the Bonner CF-Studie, Germany.

## References

1 Wall SD and Jones B: Gastrointestinal tract in the immunocompromised host: opportunistic infections and other complications. Radiology *185(2)*: 327-35, 1992.

2 De Pauw BE: Practical modalities for prevention of fungal infections in cancer patients. Eur J Clin Microbiol Infect Dis *16(1)*: 32-41, 1997.

3 Wingard JR: Importance of *Candida* species other than *C. albicans* as pathogens in oncology patients. Clin Infect Dis *20(1)*: 115-25, 1995.

4 Walsh TJ, Lee JW, Roilides E and Pizzo PA: Recent progress and current problems in management of invasive fungal infections in patients with neoplastic diseases. Curr Opin Oncol *4(4)*: 647-55, 1992.

5 Harder J, Bartels J, Christophers E and Schröder JM: A peptide antibiotic from human skin. Nature *387*: 861, 1997.

6 Harder J, Siebert R, Zhang Y, Matthiesen P, Christophers E, Schlegelberger B and Schroder JM: Mapping of the gene encoding human beta-defensin-2 (DEFB2) to chromosome region 8p22-p23.1. Genomics *46(3)*: 472-5, 1997.

7 Harder J, Meyer-Hoffert U, Teran LM, Bartels J, Maune S and Schröder JM: Mucoid *Pseudomonas aeruginosa*, TNF-alpha and IL-1beta, but not IL-6, induces human beta-defensin-2 in respiratory epithelia. Am J Respir Cell Mol Biol *22(6)*: 714-721, 2000.

8 Schröder JM: Epithelial peptide antibiotics. Biochem Pharmacol *57(2)*: 121-34, 1999.

9 Krisanaprakornkit S, Weinberg A, Perez CN and Dale BA: Expression of the peptide antibiotic human beta-defensin 1 in cultured gingival epithelial cells and gingival tissue. Infect Immun *66(9)*: 4222-8, 1998.

10 Krisanaprakornkit S, Kimball JR, Weinberg A, Darveau RP, Bainbridge BW and Dale BA: Inducible expression of human beta-defensin 2 by *Fusobacterium nucleatum* in oral epithelial cells: multiple signaling pathways and role of commensal bacteria in innate immunity and the epithelial barrier. Infect Immun *68(5)*: 2907-15, 2000.

11 Dunsche A, Acil Y, Dommisch H, Siebert R, Schröder JM and Jepsen S: The novel human beta-defensin-3 is widely expressed in oral tissues. Eur J Oral Sci *110(2)*: 121-4, 2002.

12 Mathews M, Jia HP, Guthmiller JM, Losh G, Graham S, Johnson GK, Tack BF and McCray PB Jr.: Production of beta-defensin antimicrobial peptides by the oral mucosa and salivary glands. Infect Immun *67(6)*: 2740-5, 1999.

13 Bonass WA, High AS, Owen PJ and Devine DA: Expression of beta-defensin genes by human salivary glands. Oral Microbiol Immunol *14(6)*: 371-4, 1999.

14 Chae SW, Lee SH, Cho JH, Lee HM, Choi G and Hwang SJ: Expression of human beta-defensin 1 mRNA in human palatine tonsil. Acta Otolaryngol *121(3)*: 414-8, 2001.

15 Weise JB, Meyer JE, Helmer H, Wittrock H and Maune S: A newly discovered function of palatine tonsils in immune defence: the expression of defensins. Otolaryngol Pol *56(4)*: 409-13, 2002.

16 Dale BA, Kimball JR, Krisanaprakornkit S, Roberts F, Robinovitch M, O'Neal R, Valore EV, Ganz T, Anderson GM and Weinberg A: Localized antimicrobial peptide expression in human gingiva. J Periodontal Res *36(5)*: 285-94, 2001.

17 Abiko Y, Jinbu Y, Noguchi T, Nishimura M, Kusano K, Amaratunga P, Shibata T and Kaku T: Upregulation of human beta-defensin 2 peptide expression in oral lichen planus, leukoplakia and candidiasis. An immunohistochemical study. Pathol Res Pract *198(8)*: 537-42, 2002.

18 Duits LA, Rademaker M, Ravensbergen B, van Sterkenburg MA, van Strijen E, Hiemstra PS and Nibbering PH: Inhibition of hBD-3, but not hBD-1 and hBD-2, mRNA expression by corticosteroids. Biochem Biophys Res Commun *280(2)*: 522-5, 2001.

19 Simmaco M, Boman A, Mangoni ML, Mignogna G, Miele R, Barra D and Boman HG: Effect of glucocorticoids on the synthesis of antimicrobial peptides in amphibian skin. FEBS Lett *416(3)*: 273-5, 1997.

20 Simmaco M, Mangoni ML, Boman A, Barra D and Boman HG: Experimental infections of *Rana esculenta* with *Aeromonas hydrophila*: a molecular mechanism for the control of the normal flora. Scand J Immunol *48(4)*: 357-63, 1998.

21 Barnes PJ: Anti-inflammatory actions of glucocorticoids: molecular mechanisms. Clin Sci (Lond) *94(6)*: 557-72, 1998.

22 Abiko Y, Mitamura J, Nishimura M, Muramatsu T, Inoue T, Shimono M and Kaku T: Pattern of expression of beta-defensins in oral squamous cell carcinoma. Cancer Lett *143*: 37-43, 1999.

23 Krogh P, Hald B and Holmstrup P: Possible mycological etiology of oral mucosal cancer: catalytic potential of infecting *Candida albicans* and other yeasts in production of N-nitrosobenzylmethylamine. Carcinogenesis *8(10)*: 1543-8, 1987.

24 Sawaki K, Mizukawa N, Yamaai T, Fukunaga J and Sugahara T: Immunohistochemical study on expression of alpha-defensin and beta-defensin-2 in human buccal epithelia with candidiasis. Oral Dis *8(1)*: 37-41, 2002.

25 Sawaki K, Mizukawa N, Yamaai T, Yoshimoto T, Nakano M and Sugahara T: High concentration of beta-defensin-2 in oral squamous cell carcinoma. Anticancer Res *22(4)*: 2103-7, 2002.

26 Mizukawa N, Sawaki K, Yamachika E, Fukunaga J, Ueno T, Takagi S and Sugahara T: Presence of human beta-defensin-2 in oral squamous cell carcinoma. Anticancer Res *20(3B)*: 2005-7, 2000.

*Received October 1, 2003*
*Accepted February 25, 2004*