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Expression of β-defensins in gingival health and in periodontal disease

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BACKGROUND: Human β-defensins (HBDs) are epithelial-derived antimicrobial peptides. The expression of three HBDs (HBD-1, HBD-2, and HBD-3) has been reported in oral mucosa, gingiva, and salivary glands. However, their role in protection against oral infections is not well understood. This study examined the expression of HBD-1, HBD-2, and HBD-3 mRNAs in gingival health and periodontal disease.

METHODS: Gingival tissue discarded from periodontal procedures was obtained from 20 periodontally healthy and 29 periodontally diseased sites. Total RNA was isolated, and expression of β-defensins was analyzed by both 35-cycle and semiquantitative (25-cycle) reverse transcription-polymerase chain reaction (RT-PCR). The level of expression was assigned ordinal scores of no expression, low expression, or high expression. mRNA expression was compared between healthy and diseased groups using exact tests of homogeneity and Cochran–Mantel–Haenszel tests; associations among the variables were assessed using exact tests and Kendall's tau-b statistic.

RESULTS: All 49 samples demonstrated basal mRNA expression of HBD-1, HBD-2, and HBD-3. Significantly higher levels of HBD-3 ($P = 0.012$) expression were found in the healthy tissues as compared to the diseased ones. There was also a suggestion of higher expression of HBD-2 in the healthy tissues ($P = 0.12$). Levels of HBD-1, HBD-2, and HBD-3 mRNA expression were correlated with one another ($P < 0.001$).

CONCLUSIONS: High levels of HBD-3 mRNA expression in healthy tissues suggest a potentially important protective role for defensins in the host immune response to infection by periodontal pathogens.

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Introduction

Periodontitis is primarily a bacterial infection, and as with other infectious processes, bacterial colonization triggers both an innate host response and an acquired immunity. The numerous enzymes and antimicrobial factors present in the saliva or the gingival crevicular fluid aid in the neutralization of bacterial components of supragingival and subgingival plaque. These factors include lysozyme, lactoferrin, peroxidases, bradykinin, thrombin, fibrinogen, complement, antibodies, and neutrophil-derived components and antimicrobial peptides (1–3). This study focused on the periodontal expression of recently discovered components of the innate host response, the β-defensins.

The β-defensins are small, cationic antimicrobial peptides produced by epithelial cells of the skin and many lining mucosa, such as intestinal, airway, and oral tissues (4–12). We previously demonstrated the mRNA expression of three human β-defensins (HBDs): HBD-1, HBD-2, and HBD-3 in gingival tissue (4, 5). Localization studies have shown that mRNA for HBD-1 and HBD-2 is most strongly expressed in the spinous layers of normal gingiva, whereas the peptides are present in more superficial epithelial layers, placing them in optimal position to defend against bacterial infection (13, 14). In fact, Dale & Krisanaprakornkit (14) reported that mRNA expression for HBD-1 and HBD-2 was strongest at the gingival margin, adjacent to plaque formation, and in inflamed sulcular epithelium.

In vitro β-defensins have been reported to exhibit broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, and fungi (15). Their mechanism of action is believed to be related to their ability to bind microbial membranes and form pores that result in killing (16). In addition to their antimicrobial actions, it has recently been recognized that the defensins participate in the awakening of the acquired immune response through chemotaxis of

immature dendritic cells and memory T cells (17). Therefore, the β-defensins possess both anti-microbial activities and actions that provide linkage between the innate and acquired immune responses. They may play a major role in host defenses, which combat microbial colonization of the mucosal and gingival environments.

Studies in gingival keratinocyte models have shown that HBD-1 is constitutively expressed; however, both HBD-2 and HBD-3 are differentially induced by various cytokines and bacterial components (4, 5, 14, 18, 19). Some studies have suggested increased expression of HBD-2 and HBD-3 in tissues with inflammation (10, 19), whereas other investigators have shown decreased expression in inflamed oral tissue samples (20). These reports included a variety of oral mucosal sites and disease conditions. Their expression specifically in periodontal disease has not previously been well defined. The purpose of this study was to evaluate the expression of HBD-1, HBD-2, and HBD-3 in gingival health and in periodontal disease. This information will contribute to our understanding of the role defensins play in the pathogenesis of periodontal disease.

Materials and methods

Tissue samples

A total of 49 gingival samples were obtained from patients presenting to the Periodontics Clinic, College of Dentistry, University of Iowa. These tissues were collected in compliance with a protocol approved by the University of Iowa Institutional Review Board for the Use of Human Subjects in Research. Periodontally diseased tissues were obtained from 29 subjects with moderate to advanced chronic periodontitis, who were undergoing periodontal surgery. Periodontally diseased tissues were defined as sites with residual probing depths and attachment loss $\geq$5 mm, and bleeding upon probing after completion of initial therapy. Periodontally healthy tissues were obtained from 20 subjects undergoing crown lengthening in areas without periodontal involvement. Healthy sites had probing depths of $\leq$3 mm, no attachment loss, and no bleeding upon probing, or no clinical signs of inflammation. Other demographic and health informations were recorded, including age, gender, race, and medication and tobacco use. All tissues were rinsed in sterile saline, flash frozen in liquid nitrogen, and stored at -80°C.

Total RNA isolation

Total RNA was isolated from the tissues using the acid guanidinium thiocyanate–phenol–chloroform method (21). The tissue samples were finely minced, then lyzed by incubating with 1000 μl of Tri Reagent (Molecular Research Center, Cincinnati, OH, USA) at room temperature for 5 min. The homogenate was then separated into aqueous and organic phases by the addition of 100 μl bromochloropropane, followed by centrifugation at 11 600 ***g*** for 15 min at 4°C. Total RNA was precipitated by addition of 250 μl isopropanol and 250 μl of a high salt precipitation solution (0.8 M sodium citrate and 1.2 M sodium chloride), and centrifuged at 11 600 ***g*** for 8 min at 4°C. The resulting RNA pellet was washed in 75% ethanol and solubilized in RNase/DNase-free water. Optical density (OD) measurements were taken, and the $OD_{260/280}$ ratio was determined. The RNA was diluted to a concentration of 1 μg/μl using RNase/DNase-free sterile water and stored at -80°C.

Reverse transcription-polymerase chain reaction (*RT-PCR*)

Reverse transcription-polymerase chain reaction was performed using the Superscript system (Superscript^{TM} II RNase H^- reverse transcriptase system; Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Briefly, 1 μl Oligo $(dT)_{12-18}$ (500 μg/ml; Integrated DNA Technology, Coralville, IA, USA), 2 μg total RNA, and 12 μl sterile distilled water were added and incubated at 70°C for 10 min before adding 4 μl 5× First Strand Buffer, 2 μl 0.1 M dithiothreitol (DTT), and 1 μl 10 mM dNTP Mix (10 mM each dATP, dGTP, dCTP, and dTTP; Roche, Indianapolis, IN, USA). The contents of the tube were incubated at 42°C for 2 min prior to adding 1 μl (200 units) of SuperScript II RNase II^- Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA), and were further incubated for 50 min at 42°C followed by 70°C for 15 min. All incubations were carried out in a PTC-200 Peltier Thermal Cycler (MJ Research Inc., San Francisco, CA, USA).

PCR amplification of cDNA

Standard PCR was performed to amplify the cDNA fragment using specific primers designed from previously published sequences of HBD-1, HBD-2, and HBD-3 (5, 8, 12). The primer sequences and their predicted product sizes are shown in Table 1. Each reaction contained 25 pM of the primers, except for HBD-2, where 12.5 pM was used. In addition, 3 mM Mg^{2+} and 1 μl of the RT reaction product were added for a total reaction volume of 20 μl. At least two separate reactions were performed and analyzed for each tissue sample. An initial denaturing step (95°C for 3 min) was followed by either 25 or 35 cycles of denaturing (94°C for 30 s), annealing (60°C for 30 s), and extending (72°C for 30 s), followed by 5 min at 72°C for elongation. Recombinant cDNA for HBD-1, HBD-2, and HBD-3 was amplified by PCR as positive controls. Oligonucleotide primers that hybridize to the glyceraldehyde 3-phosphate dehydrogenase gene, or GAPDH 'housekeeping' gene, were used to normalize loading of the PCR products. Non-quantitative (35 cycles) PCR was performed to screen for expression of defensin mRNA, and semiquantitative (25 cycles) PCR

Table 1 Primer sequences and predicted product sizes

Primers	*Forward (5′–3′)*	*Reverse (5′–3′)*	*Predicted product size (bp)*
HBD-1	GATCATTACAATTGCGTCAGCAGTGG	CTCACTTGCAGCACTTGGCCTTC	111
HBD-2	GGTATAGGCGATCCTGTTACCTGC	TCATTGGCTTTTTTGCAGCATTTTGTTC	126
HBD-3	TGTTTGCTTTGCTCTTCCTG	CTTTCTTCGGCAGCATTTTC	191
GAPDH	GTCAGTGGTGGACCTGACCT	AGGGGTCTACATGGCAACTG	421

was performed on the samples to assess for differential expression of mRNA in healthy and diseased gingival tissue groups.

Analysis of PCR products

Amplified products were analyzed by electrophoresis on a 2% agarose gel in 1× TAE buffer (0.8 mM Tris-acetate, 0.04 mM $Na_2EDTA\text{-}2H_2O$ (pH 8.5)) and visualized by UV illumination (Foto UV21®; Fotodyne, Hartland, WI, USA) after staining with ethidium bromide. A 100-bp ladder (Gibco, BRL® Life Technologies, Grand Island, NY, USA) was also run to assess the molecular sizes. Relative amounts of defensin expression were scored as ordinal grades of no expression (−), low-level expression (+), or high-level expression (++) from the 25-cycle PCR gels by two independent and blinded examiners. At least two separate PCR reactions from each RT-PCR sample were completed and scored. RT-PCR was repeated when ambiguous results were obtained between examiners, or between PCR reactions.

Statistical analysis

Levels of mRNA expression for HBD-1, HBD-2, and HBD-3, based upon semiquantitative PCR evaluations, were compared for the healthy and diseased tissue samples. Because of the modest sample size, exact tests of homogeneity were performed comparing frequency of no, low, and high mRNA expression. These tests do not take into account the ordinal nature of the data and do not rely upon asymptotic results to obtain the *P*-values. This procedure is an extension of the Fisher's exact test for fourfold tables (22).

In addition, the Cochran–Mantel–Haenszel Chi-square test (23) was used to consider whether the distribution of PCR results for mRNA of each peptide differed for healthy and diseased groups, using ratings measured on an ordinal scale. An alternative approach to scoring the responses, Ridit analysis (24, 25), was also applied; results obtained were identical in interpretation to those obtained using integer scoring, and are not presented here.

Exact tests (22) were also used to test the hypothesis of independence for each pair of defensin mRNA levels, but did not take the ordinal nature of the data into account. Kendall's tau-b statistic (23) was used to measure the strength of the associations between the pairs of semiquantitative evaluations of PCR results for HBD-1, HBD-2, and HBD-3, as measured on an ordinal scale (none, low, or high mRNA expression levels). However, because of the small sample sizes, the results of this test should be interpreted descriptively, with reliance upon the exact tests for assessment of significance of independence among the measures.

Results

There was basal mRNA expression of HBD-1, HBD-2, and HBD-3 in each of the 49 samples tested, assessed by 35-cycle PCR, suggesting that mRNA expression for each defensin is present regardless of the tissue status, or inducibility state of the defensin itself. Figure 1 presents, on selected samples, the differences observed between semiquantitative conditions (25 cycles) (panel A) and 35 cycles (panel B) in which all samples demonstrated a basal level of HBD-2 expression. Utilizing semiquantitative PCR (25-cycle PCR), differential expression patterns were observed for all three defensins in the healthy and diseased gingival tissue groups (Fig. 2; Table 2). The majority of samples demonstrated low level expression (+) of HBD-1 in both

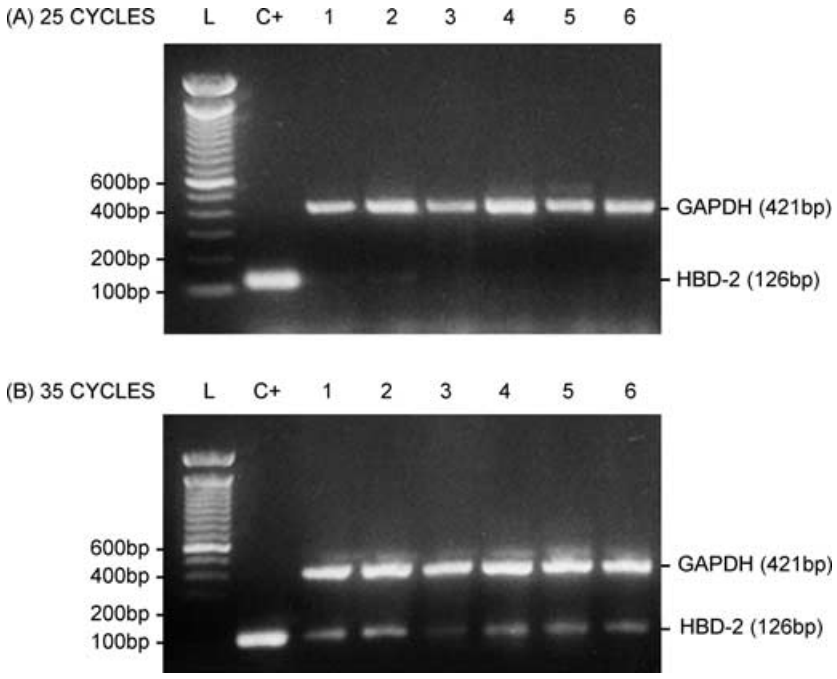

Figure 1 Basal mRNA expression of HBD-2 in semiquantitative (25-cycle) RT-PCR (A) and non-quantitative (35-cycle) RT-PCR (B) conditions for six different samples (lanes 1–6). L, 100-bp ladder; C+, positive control. Molecular sizes of the genes of interest are presented to the right of the panels.

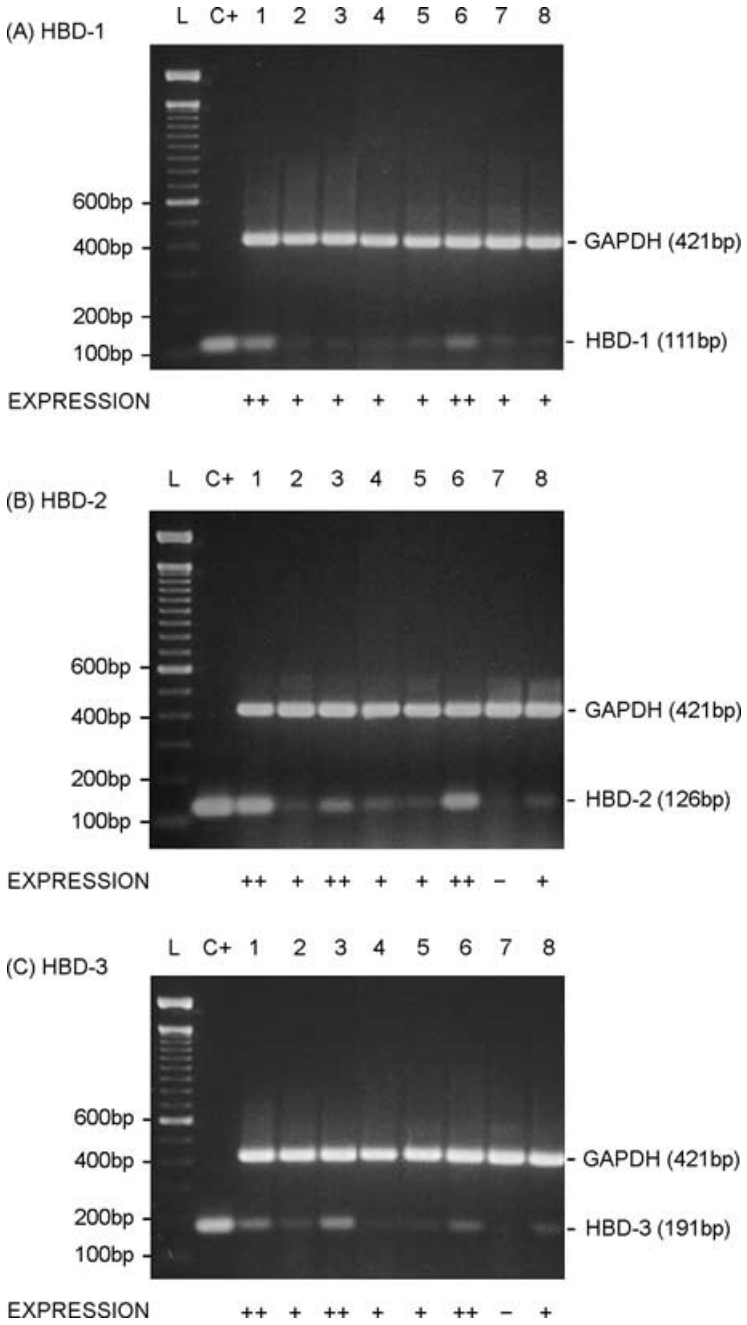

Figure 2 Differential expression of HBD-1 (A), HBD-2 (B), and HBD-3 (C) mRNA among healthy gingival tissue samples (lanes 1–4) and diseased gingival tissue samples (lanes 5–8) in relation to housekeeping gene (GAPDH) using semiquantitative (25-cycle) RT-PCR. L, 100-bp ladder; C+, positive control; (++), high-level expression; (+), low-level expression; (−), no expression. Molecular sizes of the genes of interest are presented to the right of the panels.

the healthy and diseased groups (65 and 75.9%, respectively; Table 2). Comparison of HBD-1 mRNA expression for the healthy and diseased tissue samples showed no statistical difference between the two groups (Table 2). This was true whether the analytic approach used was the exact test of homogeneity, the Cochran–Mantel–Haenszel test, or the Ridit analysis ($P > 0.5$ in all cases).

HBD-2 demonstrated more variability in expression patterns. Among the healthy samples, 35% displayed high-level expression (++), 35% low-level expression (+), and 30% did not express HBD-2 (−). For the diseased samples, 13.8% exhibited high-level expression (++), 31% had low-level expression (+), and 55.2% did not express HBD-2 (−) (Table 2). Statistical analysis suggested an association between HBD-2 mRNA expression levels and tissue status ($P = 0.120$, by exact homogeneity test). When the ordinal nature of the PCR scorings was taken into account, there was a stronger indication that healthy tissues tended to have higher levels of HBD-2 mRNA ($P = 0.045$, by the Cochran–Mantel–Haenszel test; data not shown).

HBD-3 displayed similar patterns of expression as HBD-2. Forty per cent of the healthy samples were high-level

Table 2 Comparison of healthy and diseased samples using semiquantitative PCR scores for HBD-1, HBD-2, and HBD-3 mRNA expression

	HBD-1 (%)			*HBD-2 (%)*			*HBD-3 (%)*		
	None (−)	*Low (+)*	*High (++)*	*None (−)*	*Low (+)*	*High (++)*	*None (−)*	*Low (+)*	*High (++)*
Disease ($N=29$)	3.4 (1)[b]	75.9 (22)	20.7 (6)	55.2 (16)	31.0 (9)	13.8 (4)	41.4 (12)	51.7 (15)	6.9 (2)
Health ($N=20$)	5.0 (1)	65.0 (13)	30.0 (6)	30.0 (6)	35.0 (7)	35.0 (7)	15.0 (3)	45.0 (9)	40.0 (8)
P-value[a]		>0.50			0.12			0.012	

[a]Significance probability associated with exact test of homogeneity comparing the frequency of no, low, and high mRNA expression in healthy tissue samples vs. diseased tissue samples.
[b]Numbers in parentheses reflect numbers of tissue samples in the respective categories.

expressors (++), and 45% were low-level expressors (+). Only 15% of the healthy samples did not express HBD-3 (−). In contrast, only 6.9% of the diseased samples demonstrated high-level expression of HBD-3 (++), with 51.7% displaying low-level expression (+) and 41.4% displaying no expression at all (−) (Table 2). Significantly higher HBD-3 mRNA levels were reported for healthy samples, regardless of the analytic approach. The exact significance procedure yielded a *P*-value of 0.012, while the Cochran–Mantel–Haenszel test using integer scoring was associated with an approximate *P*-value of 0.0042. For HBD-3, the numbers appear to be sufficient for validity of the Cochran–Mantel–Haenszel tests, and the smaller *P*-value is consistent with the greater power associated with tests utilizing the ordinal nature of the data (Table 2).

Strong evidence was found for an association of expression among the three defensins; these results are summarized in Table 3. The strongest associations were seen between HBD-2 and HBD-3, for the combined samples, and for the healthy and diseased samples (Kendall's tau-b of 0.800, 0.844, and 0.762, respectively). These results were confirmed by exact tests of independence ($P < 0.0001$ in all instances). In the combined samples, we also found strong evidence that HBD-1 expression was correlated with both HBD-2 and HBD-3 expression (Kendall's tau-b of 0.444 and 0.491, respectively); these results were also confirmed by the exact procedures ($P < 0.01$ in all instances). The same relationships were strongly suggested within the healthy and diseased samples, but these results should be interpreted with some caution because of the limited sample sizes and the fact that the less powerful exact procedures did not confirm the Kendall's tau-b results, except for the positive association between HBD-1 and HBD-3 in the healthy samples ($P = 0.029$ by exact test).

Discussion

The aim of this study was to determine if there were differences in levels of mRNA expression of HBD-1, HBD-2, and HBD-3 in healthy gingival tissue as opposed to periodontally diseased gingival tissue. Based on 35-cycle PCR, a basal level of expression existed for all three defensins in the healthy and diseased tissues in all patients. Semiquantitative analyses revealed differential expression of HBD-1, HBD-2, and HBD-3 among patients. These findings suggest that although mRNA for these defensins may be present in all individuals, tissue status affects levels. Significantly higher levels of HBD-3 mRNA expression correlated with healthy tissue status. There was also a trend for increased HBD-2 expression in healthy tissue. Finally, associations in the level of expression for HBD-1, HBD-2, and HBD-3 were positively correlated for both healthy and diseased gingival samples.

In this and other studies, little difference was noted between levels of HBD-1 expression in healthy and diseased samples (26). In addition, HBD-1 mRNA is constitutively expressed in keratinocyte cell cultures and is not up-regulated when exposed to inflammatory mediators (4, 9). Our data from clinical samples confirm the constitutive or basal nature of HBD-1 mRNA expression (4, 9, 26), as the majority of samples in both the healthy and diseased categories demonstrated a low level of expression with semiquantitative PCR.

Our data also demonstrated basal levels of expression for HBD-2 and HBD-3 mRNAs in gingival tissue. Many reports have shown an induction of these defensins in cell culture models with various inflammatory mediators (i.e. IL-1β, TNF-α, IFN-γ) and LPS, as well as increased expression in inflamed tissues (4, 10). Therefore, our finding of

Table 3 Summary of associations utilizing semiquantitative PCR scores of the three defensins (HBD-1, HBD-2, and HBD-3) using Kendall's tau-b coefficient[a]

	Healthy samples ($N=20$)		*Diseased samples ($N=29$)*		*All samples ($N=49$)*	
Association considered	*Kendall's tau-b*	*P-value*[b]	*Kendall's tau-b*	*P-value*[b]	*Kendall's tau-b*	*P-value*[b]
HBD-1 vs. HBD-2	0.511	<0.01	0.354	0.043	0.444	<0.001[c]
HBD-1 vs. HBD-3	0.604	<0.01[c]	0.408	<0.01	0.491	<0.001[c]
HBD-2 vs. HBD-3	0.844	<0.001[c]	0.762	<0.001[c]	0.800	<0.001[c]

[a]Kendall's tau-b coefficients are given to describe the strength of association between pairs of semiquantitative PCR results for the three defensins.
[b]Approximate significance probability associated with the test of the null hypothesis that tau-b equals zero (i.e. that there is no association between the indicated pair of semiquantitative mRNA ratings).
[c]Confirmed by exact test of independence; this procedure is less powerful than the Kendall's tau-b, but addresses concerns about small sample size.

diminished expression of both HBD-2 and HBD-3 for the diseased samples and higher HBD-2 and HBD-3 expression scores in the healthy samples was somewhat unexpected. However, while pro-inflammatory mediators are known to induce the expression of defensins, and are most often associated with diseased tissues, they are also present in healthy tissues, as are growth factors which have also been shown to induce expression (27), and can contribute to the patterns of expression seen. Additional factors that may impact the expression of β-defensins in healthy gingival tissue samples (and could also explain the variable basal expression seen among subjects) include the possibility of variable gene copy numbers as recently reported for specific defensins (28). Third, degradation of the defensins in disease is possible. For example, HBD-2 and HBD-3 were recently shown to be degraded and inactivated by the cysteine proteases cathepsins B, L, and S (29). This study suggested that during infection, enhanced expression of cathepsins may increase degradation of HBD-2 and HBD-3, with resultant bacterial colonization and infection (29). This would support the finding of diminished expression in periodontally diseased tissues. Alternately, the expression profiles in healthy and diseased tissues may actually reflect down-regulation of the defensins during disease. For example, down-regulation of HBD-1 and another innately produced antibacterial peptide, LL-37, was reported for patients with bacillary dysenteries and in *Shigella*-infected cell cultures (30). Thus, down-regulation of the host defense may be another bacteria-mediated virulence mechanism. Finally, concerns with previous studies demonstrating up-regulation of the defensins in inflammatory states include limited numbers of clinical samples evaluated and definitions of disease status (4, 19, 31, 32).

The increased expression of β-defensins in healthy tissues in this study is consistent with another recent study in which HBD-1, HBD-2, and HBD-3 were widely expressed in non-inflamed oral tissues and less so in inflamed tissues (20). Therefore, their expression in healthy tissues may assist in preventing the onset and/or progression of disease. While the results of our study were similar to those reported by Dunsche et al. (20), there were major differences in the study designs. For example, the Dunsche et al. (20) study utilized only 35-cycle PCR and not 25-cycle or semiquantitative analysis. Second, their study included a variety of tissues in both the healthy and inflamed categories. Healthy samples were obtained from gingiva, mucosa, tongue, dental pulp, dental follicle, and salivary gland. Inflamed samples included gingivitis sites, periodontal sites with bleeding and attachment loss and erythematous lesions associated with candidal infections, as well as lesions from apical periodontitis (20). These entities represent a variety of diseased states in various tissues, which may be characterized by different host immune responses and divergent expression patterns for the β-defensins.

Differential expression of β-defensin mRNA among patients has not been previously reported for gingival tissue and is supported by the strong associations of expression among the three defensins. These associations were statistically significant whether all samples were considered, or the diseased and healthy samples were examined separately. In other words, if one β-defensin was expressed at high level, another β-defensin tended to be expressed at high level also. This finding may reflect variation in regulation of the expression of β-defensins from patient to patient, or genotypic diversity including differences in genomic copy number recently described for specific β-defensins, whereby levels of mRNA expression were correlated with genomic copies of defensin genes (28).

Factors such as medication use or smoking may have influenced the results and should be examined in future studies. While the number of subjects in the present study was small, there was no evidence that smoking status was related to expression of the defensins. Furthermore, there was no evidence for any relationship of defensin expression with other demographic characteristics, including age and gender. Future studies should include increased numbers of subjects, so such factors can be better assessed. In addition, future analysis of mRNA expression should rely upon real-time PCR, a method involving fluorescence-based kinetic RT-PCR, which would significantly improve quantification of expression and reproducibility as the standard measure, especially as the cost of the technology decreases.

The findings of increased (or decreased) levels of mRNA expression in the present study do not necessarily correlate with increased (or decreased) amounts or functional activity of the peptide. There may be post-transcriptional regulation occurring, which prevents translation of the defensins. Furthermore, post-translational regulation may prevent the peptide from being functional. Previous studies have demonstrated that expression of HBD-2 mRNA is paralleled by the levels of peptide expression (10, 33). Whether the levels and activities of the defensin peptides *in vivo* correspond with mRNA expression and what effect this has on the immune response remain to be elucidated.

The most well-known effects of β-defensins are their antimicrobial properties; however, their exact function in gingival tissue is not known. HBD-1 and HBD-2 are reportedly active against Gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aerugenosa* (33). Interestingly, studies in our laboratory indicate that HBD-1, HBD-2, and HBD-3 demonstrate poor antimicrobial activity against common anerobic periodontopathic organisms (34). Their primary activity may be directed against the earlier colonizers or commensal flora, as has been suggested for the constitutively expressed HBD-1 (35).

Recent data have shown that β-defensins have other important functions, one of which is their association with epithelial differentiation. HBD-1 and HBD-2 peptides have been found exclusively in cultured keratinocytes expressing involucrin, which is an early marker for differentiation (14). Furthermore, they are not expressed in the relatively undifferentiated junctional epithelium in gingival tissue biopsies (14). *In vitro*, HBD-1 promotes the cell differentiation processes of keratinocytes by inducing the differentiation marker keratin 10 (36). Another important role is their link between the innate and acquired arms of the host response. HBD-1 and HBD-2 can stimulate immature dendritic cells via the CCR6 receptor, thereby enhancing antigen uptake, processing, presentation, and ultimately induction of antigen-specific immunity (17). Therefore, a primary role for the β-defensins in the pathogenesis of periodontal disease may be one of triggering the adaptive immune response.

The present study was focused on gingival expression of β-defensins in healthy and diseased gingival tissue samples. Our findings suggest that β-defensins may be protective in periodontal disease. In order to better understand their role in periodontal diseases, examination of their expression should be evaluated in experimentally induced gingivitis, or in sites showing progressive attachment loss.

As our understanding of their role in periodontal disease and other common oral infections unfolds, clinical applications of the β-defensins will undoubtedly be explored. The potential ability of the β-defensins to influence epithelial differentiation makes them candidates for management of oral ulcerations. Their ability to enhance antigen-specific immune responses, when given with antigens, makes them attractive as useful immunoadjuvants (37, 38).

Ultimately, the knowledge gained from this and future studies will aid in our understanding of the role β-defensins play in the oral cavity, specifically in periodontal disease and other common oral infections, and will potentially lead to the development of novel preventive and therapeutic regimens for the management of these diseases.

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