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Expression and regulation of novel human β-defensins in gingival keratinocytes

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This study evaluated the expression and regulation of β-defensins DEFB-104 and the recently identified DEFB-105–14 in gingival keratinocytes. Keratinocytes from healthy subjects were exposed to cytokines, *Escherichia coli* lipopolysaccharide or *Candida* species. Total RNA was extracted and defensin expression analyzed by reverse transcription-polymerase chain reaction. Three patterns of expression were seen: no expression, constitutive expression and inducible expression. Constitutive mRNA expression was evident for DEFB-104, 107, 109, 111, and 112. DEFB-108 and 114 were induced by interleukin (IL)-1β and *Candida* species. For DEFB-108 expression, synergism was observed when IL-1β was combined with tumor necrosis factor-α or interferon-γ. Downregulation of DEFB-109 occurred following treatment with *Candida albicans*. These findings suggest a role for multiple β-defensins in response to oral infection. Further investigation is needed to better understand their function, both in terms of antimicrobial activities and contributions to innate and acquired immunity.

Key words: antimicrobial peptides; β-defensins; gene expression; gingival keratinocytes; innate immunity

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Mammalian defensins are 3.5–4.5 kDa cationic, antimicrobial peptides of 33–45 amino acids with DEFB-1 (also published as HBD-1) the smallest (33 amino acids) and DEFB-103 (HBD-3) the largest (45 amino acids) in length (2, 8, 44). They are composed of β-sheet structures stabilized by three intramolecular disulfide bonds (29, 54). Human defensins include the α- and β-defensins (12, 33, 41, 44), with the β-defensins differing from α-defensins in the order of the three disulfide bonds between the six cysteine residues of the mature peptides (31, 54, 55). The α-defensins are found in the azurophilic granules of human neutrophils (13) and in the small intestine in the granules of Paneth cells (37). β-defensins are produced by epithelial cells during inflammation and infection (31, 38, 40, 49, 54). In addition to their antimicrobial activity, the β-defensins act as chemokines for immature dendritic cells and memory T cells, therefore, bridging innate and adaptive immunity (52).

To date, mRNA expression of four members of the β-defensin family [DEFB-1, 4 (HBD-2), 103 and 104 (HBD-4)] has been reported at a number of different epithelial sites (2, 14, 15, 19, 21, 24). DEFB-1 is constitutively expressed by the epithelial cells of the respiratory mucosa, urogenital tract, gingiva, parotid glands, buccal mucosa and tongue (17, 26, 33, 34, 36, 46, 51, 53). Recombinant and natural DEFB-1 demonstrate antimicrobial activity against *Escherichia coli* at micromolar concentrations (51).

The cellular sources of DEFB-4 are similar to that of DEFB-1 and include skin, gingival and airway epithelium (1, 19, 22, 31, 33, 36, 46). Unlike DEFB-1, DEFB-4 mRNA is induced in response to bacterial infection or proinflammatory agents. For example, DEFB-4 expression was stimulated when cultured gingival keratinocytes were treated with IL-1β and *E. coli* lipopolysaccharide (33). TNF-α and IL-1β induced DEFB-4 in bronchial, nasal and tracheal epithelial cultures (20, 46). Gram-negative species, in particular, as well as *Staphylococcus aureus* and *Candida albicans* (19, 20), have been shown to upregulate DEFB-4 gene expression. DEFB-4's antimicrobial activity is primarily against gram-negative bacteria such as *E. coli*, *Pseudomonas aeruginosa* and *C. albicans* (19).

In addition to its expression in epithelial cells of skin, esophagus, trachea and gingiva, DEFB-103 mRNA expression has been detected in adult heart, skeletal muscle, placenta, and fetal thymus (9, 14, 24). Like DEFB-4, DEFB-103 is inducible (21, 24), and increased DEFB-103 mRNA has been shown with IL-1β (24), TNF-α (21), interferon-γ (IFN-γ) (14) and various bacteria.

Antimicrobial activity for DEFB-103 has been seen for gram-negative and positive organisms and *Candida* species (21).

DEFB-104 is expressed in testes, gastric antrum, uterus, neutrophils, thyroid gland, lung and kidney (15), but its production has not been investigated in oral tissues. Upregulation of DEFB-104 mRNA was seen in response to both gram-positive and gram-negative bacteria and to phorbol 12-myristate 13-acetate, but not to the proinflammatory cytokines that upregulate DEFB-4 or DEFB-103 (15). This defensin demonstrates antimicrobial activity against gram-negative and positive organisms and the yeast, *Saccharomyces cerevisiae* (15).

Recently, more than 28 new putative human β-defensin genes were identified using a computational genomics strategy. Preliminary evidence indicates that at least 12 are transcribed (42). The purpose of this study was to assess the expression and inducibility of 10 of these new defensins, as well as DEFB-104, in gingival keratinocytes.

Material and methods

Keratinocyte cultures

Gingival keratinocyte cultures were established as previously described (25) from healthy gingival tissues obtained from 12 healthy individuals undergoing crown-lengthening procedures. Tissues were procured in accordance with a protocol approved by the University of Iowa's Institutional Review Board for the Use of Human Subjects in Research. Keratinocytes in passage two or three were seeded (2.5×10^5 cells/well) into six-well tissue culture plates (Corning, Acton, MA) and incubated at 37°C in serum-free medium (KGM, Clonetics, Biowhittaker Inc, Walkersville, MD). When cells reached approximately 80–90% confluency, the cultures were induced for 24 h with one of the following treatments: 100 or 200 ng/ml of IL-1β, TNF-α or IFN-γ; 100 ng/ml of IL-2, IL-6, IL-8 or IL-12; 10 μg/ml of *E. coli* lipopolysaccharide or 5×10^4 cell/ml of five different live *Candida* species (*Candida tropicalis* I11C1E (18), *Candida parapsilosis* ATCC 22019, *Candida krusei* ATCC 6258, *Candida glabrata* 35B11 (32), and *C. albicans* ATCC820). All cytokines and lipopolysaccharide used in this study were obtained from Sigma-Aldrich Corp. (St Louis, MO). The cytokines IL-1β, TNF-α, IFN-γ, IL-6, IL-8 and IL-12 were included because of their proinflammatory functions, and the fact that DEFB-4 and 103 are induced by proinflammatory cytokines (14, 19, 21, 24, 33, 46). Based on evidence that β-defensins are chemotactic for T cells (52), cytokines involved in T-cell regulation were selected, including IL-2 and IL-12 (16, 48). Total RNA was isolated after the inductions were terminated.

Total RNA isolation

Total RNA was isolated from the cell cultures using the acid guanidium thiocyanate-phenol-chloroform method (4). Tri Reagent (Molecular Research Center, Cincinnati, OH) was used to collect and lyse the cells by incubation for 5 min at room temperature. The homogenate was then separated into aqueous and organic phases by the addition of 100 μl bromochloropropane (Molecular Research Center) followed by centrifugation at 12,000 rpm for 15 min at 4°C. Total RNA was precipitated by addition of 500 μl isopropanol and centrifuged at 12,000 rpm for 8 min at 4°C. The resulting RNA pellet was washed twice 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 –20°C.

Reverse transcription polymerase chain reaction (RT-PCR)

Reverse transcription polymerase chain reaction was performed in a final volume of 20 μl using the Superscript transcription system (Gibco BRL, Grand Island, NY). In brief, 1 μl Oligo (dT) (500 μg/ml) and 9 μl distilled water were added to 2 μg of total RNA and incubated at 70°C for 15 min. A mixture of 4 μl 5× Superscript Buffer (250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM $MgCl_2$ (Gibco BRL), 0.01 M DTT (Gibco BRL) and 500 μM dNTP Mix (Roche, Indianapolis, IN) was added and incubated at 42°C for 2 min; 200 U of SuperScript II (Gibco BRL) was then added, and the incubation continued at 42°C for 50 min, 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).

PCR amplification of cDNA

The PCR reaction was performed to amplify the cDNA fragments using specific primers for DEFB-1–114. DEFB-105–114 primer sets were designed to the predicted sequences of the exon 2 genes. Primer sequences and their predicted product sizes are shown in Table 1. Each reaction contained 1× PCR buffer (Bioline, Randolph, MA), 1.5 mM $MgCl_2$, 200 μM each dNTP (Roche), 0.5 U of *Taq* Polymerase (Bioline), 500 μM of each forward and reverse primer and 1 μl of the RT reaction product for a total volume of 20 μl. β-actin was used as a housekeeper gene at a concentration of 500 μM of each forward and reverse primer (Table 1). An initial denaturation step (94°C for 3 min) was followed by either 25 or 35 cycles of denaturation (94°C for 30 s), annealing (60°C for 30 s), and extension (72°C for 30 s), followed by 5 min at 72°C for elongation. Amplification of the housekeeping gene, β-actin, was carried out in combination with or separately from the defensin genes and performed at 25 cycles.

Analysis of PCR products

PCR products were analyzed by electrophoresis on a 2% agarose gel in 1× TAE buffer (0.8 mM Tris-acetate (Fisher Scientific, Fairlawn, NJ); 0.04 mM $Na_2EDTA\text{-}2H_2O$ (Fisher Scientific; pH 8.5), stained with ethidium bromide and visualized by UV illumination (Foto UV21®; Fotodyne, Hartland, WI). A 100-bp ladder (Gibco, BRL) was used as a standard to assess the molecular sizes. The intensity level of each reaction was scored as no expression (–), expression (+), inducible expression (++) and synergistic induction (S) and compared to expression of the β-actin.

DNA sequencing

Sequencing of DEFB-108 (exon 2) and DEFB-114 (exon 2) fragments were performed for two different subjects using the primer sets presented in Table 1. DEFB-109 partial exon 1 and 2 (198 bp) was amplified and sequenced for three subjects using the following primers: Forward 5′-TCTCTTATCCCCAGTAAGAGGT and Reverse 5′-GGTTCTTGATAATCTGACATG. The PCR fragments were ligated into a plasmid using the pGEM®-T easy vector system (Promega Corp., Madison, WI). The ligated plasmid was used to transform, *E. coli* JM109 (Promega Corp). Plasmid DNA was extracted using QIAprep® Spin Miniprep Kit (QIAprep Inc., Valencia, CA). The nucleotide sequence of the plasmid insert was determined in both directions with an ABI model 3700 auto sequencing system (Perkin-Elmer/Applied Biosystems, Foster City, CA) using the PCR cycle sequencing protocol and fluorescent dye terminator dideoxynucleotides (Perkin-Elmer/Applied Biosystems).

Table 1. Primer sequences and their predicted product sizes

Primers	Genomic sequences		Product size (bp)
	Forward (5′ to 3′)	Reverse (5′ to 3′)	
DEFB-1	GATCATTACAATGCGTCAGCAGTGG	CTCACTTGCAGCACTTGGCCTTC	111
DEFB-4	GGTATAGGCGATCCTGTTACCTGC	TCATTGGCTTTTTTGCAGCATTTTGTTC	126
DEFB-103	TGTTTGCTTTGCTCTTCCTG	CTTTCTTCGGCAGCATTTTC	179
DEFB-104	ATGCAGAGACTTGTGCTGCT	GCTCTCATCCCATTTTCTCAA	194
DEFB-105	TGTCTGTGAGTCGTGCAAGC	GCAGCAGAGAAAGTTCAGCC	100
DEFB-106	CTCTTTCTCTTTGCCGTGCT	TCTATAATGCTCCCACATGGC	182
DEFB-107	GTCTTTATTTTGGCTGCTCTCA	TGCAGCAAAATGGTGCTAAT	169
DEFB-108	GCAAATTCAAGGAGATCTGT	TGGGTGTAGTGCTCTCAATT	147
DEFB-109	CGGAAGGTCATTGTCTCAATT	GGTTCTTGATAATCTGACATG	163
DEFB-110	TTTGAAAGATGCGAAAAAGTG	TACGCAGCACTGACTTCTCC	102
DEFB-111	ACCTGGAACTCATTGCTGCT	GAAGGATGTGCTGGGAAAAC	105
DEFB-112	TCATGTACAGCGATTGGAGG	CTGTCACGCAGCAATGAGTT	100
DEFB-113	ATGTCAGCTTGTTCGTGGTG	GGTATTCCCATACCGCACAG	101
DEFB-114	CCAAACGTTACGGTCGTTGTA	TCAAAACATATCATCTTCTTCATA	122
β-actin	CGGAACCGCTCATTGCC	ACCCACACTGTGCCCATCTA	300

Nomenclature

Nomenclature of defensins is presented in this manuscript as described by the HUGO Gene Nomenclature Committee: www.gene.ucl.ac.uk/cgi-bin/nomenclature/searchgenes.pl.

Results

Expression profile of new β-defensins in the oral cavity

Initial screening of the expression and stimulation of DEFB-104 and the new β-defensins was performed utilizing a keratinocyte culture from a single healthy subject (subject 299) in both non-stimulated conditions (control medium) and medium supplemented with lipopolysaccharide or various cytokines (IL-1β, IL-2, IL-6, IL-8, IL-12, TNF-α or IFN-γ). Tables 2 and 3 show the expression and induction patterns of the defensins tested at both 25 and 35 PCR cycles, respectively. Three patterns of expression were observed: no expression, constitutive expression and inducible expression. As previously described (26, 33, 36), DEFB-1 was constitutively expressed in oral epithelium, while DEFB-4 and DEFB-103 showed inducible expression, also consistent with previous reports (14, 19, 33). The initial screening of DEFB-104 and the new defensins (DEFB 105-114) utilizing 25-cycle PCR demonstrated lack of expression for DEFB-104, 105, 106, 110, 111, 112 and 113; constitutive expression for DEFB-107 and DEFB-109; and inducible expression for DEFB-108 and DEFB-114 with IL-1β. When 35-cycle PCR was used, DEFB-104, 111 and 112 demonstrated constitutive expression, suggesting the low level transcripts of these defensins which were only detectable at the higher cycle PCR. IL-2, IL-6, IL-8, IL-12 and lipopolysaccharide did not regulate the expression of the defensins tested. Sequence verifications of the PCR products obtained confirmed the gene products for DEFB-108 (exon 2), DEFB-109 (exon 1 and 2) and DEFB-114 (exon 2) for two different subjects.

Table 2. Screening of new β-defensins (25-cycle PCR)

No expression	Constitutive expression	Inducible expression
DEFB-104	DEFB-1	DEFB-4 (IL-1β, TNF-α, *E. coli*)*
DEFB-105	DEFB-107 weak	DEFB-103 (IL-1β, TNF-α, IFN-γ)*
DEFB-106	DEFB-109 weak	DEFB-108 weak (IL-1β)*
DEFB-110		DEFB-114 weak (IL-1β)*
DEFB-111		
DEFB-112		
DEFB-113		

*Indicates inducers of expression.

Table 3. Screening of new β-defensins (35-cycle PCR)

No expression	Constitutive expression	Inducible expression
DEFB-105	DEFB-1	DEFB-4 (IL-1β, TNF-α, *E. coli*)*
DEFB-106	DEFB-104	DEFB-103 (IL-1β, TNF-α, IFN-γ)*
DEFB-110	DEFB-107 weak	DEFB-108 weak (IL-1β)*
DEFB-113	DEFB-109	DEFB-114 weak (IL-1β)*
	DEFB-111	
	DEFB-112	

*Indicates inducers of expression.

Impact of combination of various cytokines on induction of selected β-defensins

Of the 10 new β-defensins, additional analyses were performed for DEFB-108, 109, 111, 112 and 114, which represented both constitutive and inducible defensins, utilizing a keratinocyte culture from subject 310. As reported above, IL-1β induced DEFB-108 mRNA expression. In addition, synergistic induction of DEFB-108 expression was noted when IL-1β was combined with TNF-β or IFN-γ (Fig. 1, Table 4); however, TNF-α or IFN-γ alone did not induce DEFB-108. DEFB-114 demonstrated inducible expression with the following individual cytokines and cytokine combinations: IL-1β, IL-1β + TNF-α and IL-1β + IFN-γ (Table 4).

Selective response of DEFB-108, 109 and 114 to various *Candida* species

When keratinocyte cultures from four different individuals (subjects 277, 236, 221, 212) were challenged with five species of *Candida* [*C. tropicalis (Ct), C. parapsilosis (Cp), C. krusei (Ck), C. glabrata* (*Cg*) and *C. albicans* (*Ca*)], DEFB-108 was upregulated by *Cp, Ck* and *Ca,* but not *Cg* and *Ct* in select subjects, while DEFB-114 was induced only by *Ck* in subjects 277 and 221 (Table 5). The induction was both subject and species specific. DEFB-109 demonstrated constitutive expression for all species except *C. albicans* (*Ca*), where downregulation of expression was

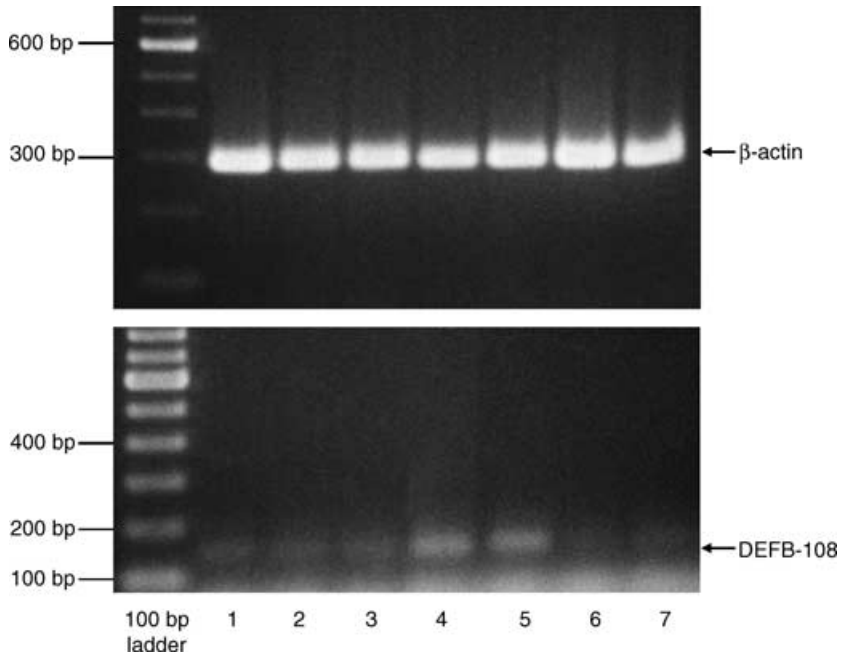

Fig. 1. Expression patterns of DEFB-108 mRNA when exposed to different cytokines in subject 310 utilizing 35-cycle PCR. Synergistic expression was seen when IL-1β was combined with TNF-α or IFN-γ (lanes 4 and 5). β-actin (300 bp) was used as a control housekeeping gene. Molecular sizes in base pairs are presented on the left of the panel. 1: IL-1β; 2: TNF-α; 3: IFN-γ; 4: IL-1β + TNF-α; 5: IL-1β + IFN-γ; 6: TNF-α + IFN-γ; 7: Serum-Free Media.

seen in all four individuals (Fig. 2, Table 5).

Variability of defensin expression among individuals

In order to determine if basal level of expression varied among subjects, non-stimulated keratinocyte cultures from 12 different persons were examined for expression of DEFB-108, 109 and 114. Analysis of expression utilizing 35-cycle PCR showed variation of DEFB-108, 109 and 114 mRNA expressions among subjects as shown for DEFB-108 in Fig. 3. No association was seen among defensin expression for individual subjects. In other words, individuals with strong expression of DEFB-108 did not necessarily demonstrate strong expression of DEFB-109 or DEFB-114.

Discussion

Previous studies demonstrated the expression of the human β-defensins, DEFB-1, 4 and 103 in human gingival keratinocytes (6, 7, 9, 10, 28, 33, 39). This study evaluated the mRNA expression and regulation of DEFB-104 and 10 novel β-defensins (DEFB-105–114) (42) in human gingival keratinocytes. Seven of these were expressed in gingival keratinocytes, two of which were inducible. Although no expression was detected for four of the new defensins (DEFB-105, 106, 110 and 113) utilizing 35-cycle PCR, their role as innate defenders in the oral cavity should not be ruled out, pending evaluation of a larger sample size, other oral tissues and other induction conditions. The initial screening of the new defensins was conducted utilizing keratinocytes from a single individual, and the PCR conditions for these particular defensins may not have been optimal to detect expression. Further analysis of these defensins is warranted in a greater number of subjects.

Cytokines were investigated in this study with the intent of identifying pathways that may be involved in the regulation of these new defensins. Several other *in vitro* studies have focused on the effects of proinflammatory cytokines and various bacterial components on defensin production. These studies have repeatedly reported the induction potential of DEFB-4 and DEFB-103 by IL-1β, and TNF-α (14, 21, 24, 27, 28, 33). Preliminary data in our laboratory and others has also shown an induction potential for DEFB-103 with IFN-γ (14).

In the present study, expression of two of the new defensins, DEFB-108 and DEFB-114, was induced with IL-1β, suggesting a common regulatory pathway as that previously described for DEFB-4. Thus it is likely that DEFB-4, 108 and 114 work cooperatively in response to a single cytokine, IL-1β. They may, therefore, display synergistic or additive antimicrobial activity as seen for DEFB-4 or DEFB-104 and other innate molecules (14, 47). This further demonstrates that the defensin family acts as a multifactorial component in innate defense. When a combination of cytokines was utilized (IL-1β with TNF-α or IFN-γ), synergism was seen for DEFB-108 mRNA production. This is in agreement with the synergistic activity

Table 4. Expression of DEFB-108, 109, 111, 112 and 114 with various cytokine treatments

DEFB	IL-1β	TNF-α	IFN-γ	IL-1β + TNF-α	IL-1β + IFN-γ	TNF-α + IFN-γ	SFM
108	++	+	+	S	S	+	+
109	+	+	+	+	+	+	+
111	+	+	+	+	+	+	+
112	+	+	+	+	+	+	+
114	++	+	+	++	++	+	+

+: basal expression; ++: inducible expression; S: synergistic induction; SFM: Serum-Free Media.

Table 5. Regulation of DEFB-108, 109 and 114 when exposed to different *Candida* species

	DEFB-108						DEFB-109						DEFB-114					
Subject	Ct	Cp	Ck	Cg	Ca	SFM	Ct	Cp	Ck	Cg	Ca	SFM	Ct	Cp	Ck	Cg	Ca	SFM
277	+	-	+	+	++	+	+	+	+	+	−	+	+	+	++	+	+	+
236	+	+	++	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
221	+	++	++	+	++	+	+	+	+	+	−	+	+	+	++	+	+	+
212	+	+	+	+	−	+	+	+	+	+	−	+	+	−	+	+	+	+

Ct : *C. tropicalis*; Cp : *C. parapsilosis*; Ck : *C. krusei*; Cg : *C. glabrata*; Ca : *C. albicans;* SFM: Serum-Free Media; −: no expression, +: basal expression, ++: inducible expression.

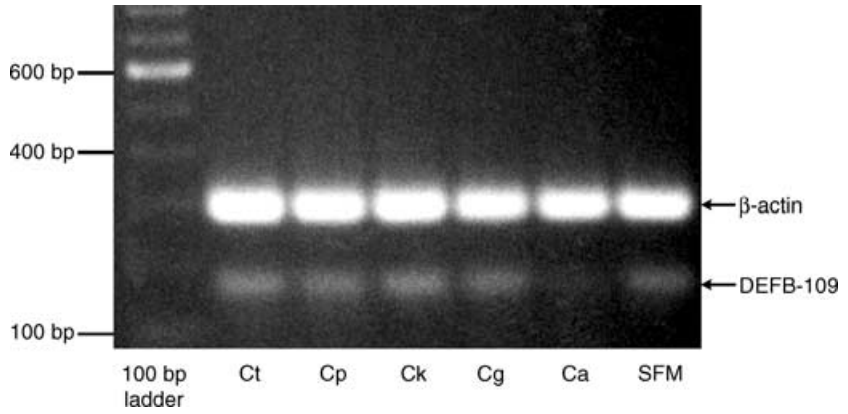

Fig. 2. Expression of DEFB-109 subjected to 5×10^4 cell/ml of five live *Candida* species in subject 277 utilizing 35-cycle PCR. β-actin (300 bp) was used as a control housekeeping gene. Molecular sizes in base pairs are presented on the left of the panel. Ct: *C. tropicalis*; Cp: *C. parapsilosis*; Ck: *C. krusei*; Cg: *C. glabrata*; Ca: *C. albicans*; SFM: Serum-Free Media.

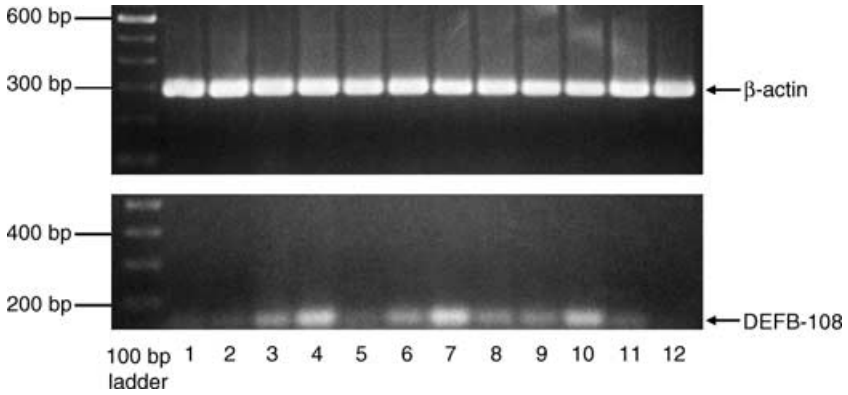

Fig. 3. Expression of DEFB-108 mRNA in non-stimulated keratinocyte cultures from 12 different individuals utilizing 35-cycle PCR. Variability of basal level expression is noted among culture samples. β-actin (300 bp) was used as a housekeeping gene. Molecular sizes in base pairs are presented on the left of the panel. 1: subject #212; 2: #221, 3: #236; 4: #277; 5: #291; 6: #294; 7: #296; 8: #298; 9: #299; 10: #303; 11: #310; 12: #312.

resulting from interactions between NF-κB, MAP-kinase or JAK-STAT pathways reported for numerous inducible genes (5, 11, 35). The data in the present study do not support a role for other cytokines, such as IL-6, IL-8 and IL-12, in the regulation of the expression of the new defensins analyzed. Future analysis of potential inducers should include real-time PCR in order to quantitatively assess their impact, alone and in combination, on individual defensin induction.

Interestingly, a new finding was the downregulation of DEFB-109 by *C. albicans*. A similar phenomenon has been described for the human cathelicidin LL37 and DEFB-1 expression as part of an immune escape strategy in the invasion of the lower gut epithelial cells by *Shigella* bacteria (23). Downregulation of DEFB-109 by *C. albicans* could therefore be part of the host–pathogen interaction promoting *C. albicans* as a commensal. This hypothesis would, however, require further investigation at the protein level. Recently, Semple et al. (45) suggested that DEFB-109 is a pseudogene based on the presence of a stop codon at base pair 40 in exon 1. This report was however based on a single clone. A search in the GeneBank database using DEFB109 as a Blast query, demonstrated that this gene may possess different alleles that could include functional or null alleles which would, in turn, contradict its pseudogene status. Our data showing the regulation of DEFB109 by *Candida* supports the fact that this gene may be functional in the innate immune response to infection. Future investigation should analyze the sequences of multiple clones to determine if DEFB-109 encodes for a translated product.

Differential basal expression was noted among non-stimulated keratinocyte cultures from 12 different individuals. This is consistent with our previous work demonstrating the differential expression of DEFB-1, 4 and 103 in gingival tissue samples from 19 different subjects (3). Future studies should evaluate whether or not any association exists for expression of the novel defensins with each other as well as with healthy or diseased tissues in order to better understand their role in disease pathogenesis.

The β-defensins are produced by epithelial cells, placing them in a strategic position to fend off infectious agents, such as bacterial, yeast or viral infections, in the oral cavity. However, their antibacterial, antifungal and antiviral spectrums have not been extensively characterized against oral pathogens. In addition to their antimicrobial actions, defensins may enhance wound healing to infection or trauma-induced injury. The defensins induce chemotaxis of dendritic and memory T cells, and therefore may also provide an important link between the innate and adaptive arms of the host response to microbial challenges at skin and mucosal surfaces (52). β-defensins also enhance antigen-specific immune responses, making them potentially useful immunoadjuvants (50). DEFB-4 has also been shown to affect the ability of lipopolysaccharide to stimulate production of TNF-α from macrophages (43) therefore mitigating the toxic effects of endotoxin. This is important in life-threatening sepsis, but also has implications for oral infections such as periodontal disease, where proinflammatory cytokines play a key role in tissue destruction. Taken together, these host response functions make β-defensins an important player in the body's defense against microbial invasion.

In conclusion, the role of cationic peptides in innate antimicrobial defenses and stimulation of the adaptive immune response has become increasingly apparent. The knowledge of a large family of antimicrobial peptides serving in different capacities against various microorganisms has previously been shown for *Drosophila* (30). Because gingival keratinocytes are ideally positioned as the first line of defense in the oral cavity, the repertoire of β-defensins expressed in gingiva warrants further study to better understand their role in oral infections as part of the innate and acquired immunity. Finally, future clinical applications may maximize the utility of naturally occurring antimicrobial peptides by combining peptides with complementary antimicrobial and host response modulatory factors for therapeutic applications.

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