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REGULAR ARTICLE

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Identification of a novel, multifunctional β-defensin (human β-defensin 3) with specific antimicrobial activity

Its interaction with plasma membranes of *Xenopus* oocytes and the induction of macrophage chemoattraction

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Abstract Previous studies have shown the implication of β-defensins in host defense of the human body. The human β-defensins 1 and 2 (hBD-1, hBD-2) have been isolated by biochemical methods. Here we report the identification of a third human β-defensin, called human β-defensin 3 (hBD-3; cDNA sequence, Genbank accession no. AF295370), based on bioinformatics and functional genomic analysis. Expression of hBD-3 is detected throughout epithelia of many organs and in non-epithelial tissues. In contrast to hBD-2, which is upregulated by microorganisms or tumor necrosis factor-α (TNF-α), hBD-3 expression is increased particularly after stimulation by interferon-γ. Synthetic hBD-3 exhibits a strong antimicrobial activity against gram-negative and gram-positive bacteria and fungi, including *Burkholderia cepacia*. In addition, hBD-3 activates monocytes and elicits ion channel activity in biomembranes, specifically in oocytes of *Xenopus laevis*. This paper also shows that screening of genomic sequences is a valuable tool with which to identify novel regulatory peptides. Human β-defensins represent a family of antimicrobial peptides differentially expressed in most tissues, regulated by specific mechanisms, and exerting physiological functions not only related to direct host defense.

Keywords β-Defensin · *Burkholderia cepacia* · Cystic fibrosis · Innate host defense · Antimicrobial peptide · Human

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Introduction

Antimicrobial peptides are effector molecules of the innate immune system (Diamond et al. 2000; Medzhitov and Janeway 2000). Human β-defensin 1 (hBD-1) was originally isolated from hemofiltrate and is expressed in epithelial cells of the urinary and respiratory tract (Bensch et al. 1995; Valore et al. 1998). Human β-defensin 2 (hBD-2) was isolated from psoriatic skin using an affinity chromatography procedure applying columns coated with microbial components (Harder et al. 1997b) and is expressed in skin and epithelia of the respiratory and gastrointestinal tract (Bals et al. 1998a; Singh et al. 1998). Both human β-defensins have been isolated by bioscreening, which applies functional assays to identify candidate substances in biological material. Identification of novel human peptide antibiotics is an important step in the further evaluation of innate immunity. The progress of the Human Genome Project should allow screening of genomic sequences for the presence of structural motifs of defensins and the detection of novel antimicrobial peptides.

The aim of the present study was to identify novel members of the β-defensin family based on functional genomics. We identified a new inducible defensin, called human β-defensin 3 (hBD-3), expressed in epithelial and nonepithelial tissues. Functional studies using synthetic peptide revealed a strong antimicrobial activity, activation of monocytes, and pore formation in cell membranes.

Materials and methods

Identification and characterization of the genomic sequence of hBD-3

The amino acid sequences of hBD-1 (Genbank accession no. NM 005218) and hBD-2 (Genbank accession no. NM 004942) were used to perform a "basic local alignment search tool" (BLAST) search in the High Throughput Genomic (HTG) division of Genbank (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/BLAST) and several overlapping clones were found. Structural motifs of β-defensins (prepropeptide of hBD-1, prepropeptide of hBD-2, and the amino acid sequences CCR, CCK, and FCC) were used to scan the sequences (Genequest software package, version 4.03; DNA Star, Madison, Wis.) and to identify the presence of the β-defensin-typical cysteine pattern. Based on these criteria, we focused on a sequence that we named hBD-3. To search for 5′ sequences of the novel gene, the upstream region of the genomic sequence was used to perform a scan algorithm for the detection of genes (Genscan 1.0, http://bioweb.pasteur.fr/seqanal/interfaces/genscan-simple.html; Burge and Karlin 1997). Sequences 2 kb upstream of the start of translation were used in the screening algorithms Matinspector public domain (www.genomatix.de; Quandt et al. 1995) and TFSEARCH 1.3 (http://www.rwcp.or.jp/papia/; Heinemeyer et al. 1998). To predict putative cleavage sites of the prepropeptide, the software program SignalP V1.1 (www.cbs.dtu.dk/services/SignalP; Nielsen et al. 1997) was used.

Cloning of the hBD-3 cDNA

Total RNA was isolated from differentiated respiratory epithelial cells (Trizol reagent; LifeTechnologies, Karlsruhe, Germany) and reverse transcribed (Superscript II reverse transcriptase system; LifeTechnologies). The primers used for the amplification of hBD-1, hBD-2, hBD-3, and glyceraldehyde-phosphate-dehydrogenase (GAPDH) were: (1) GAPDH forward, 5′-TGA AGG TCG GAG TCA ACG GAT TTG GT-3′; GAPDH reverse, 5′-CAT GTG GGC CAT GAG GTC CAC CAC-3′ (predicted size of the PCR product, 900 bp); (2) hBD-1 forward, 5′-CAG TTC CTG AAA TCC TGA GTG-3′; hBD-1 reverse, 5′-AAA GTT CAT TTC ACT TCT GCG-3 (predicted size of the PCR product, 291 bp); (3) hBD-2 forward, 5′-GAA GCT CCC AGC CAT CAG C-3′; hBD-2 reverse, 5′-CAT GTC GCA CGT CTC TGA T-3′ (predicted size of the PCR product, 268 bp); (4) hBD-3 forward, 5′-GAG GAT CCA TTA TCT TCT GTT TG-3′; hBD-3 reverse, 5′-TAT TTC TTT CTT CGG CAG CAT T-3′ (predicted size of the PCR product, 202 bp). PCR conditions were applied as described previously (Bals et al. 1998a) using a Robocycler Gradient 40 with hot top (Stratagene, Heidelberg, Germany). Products were separated by agarose gel electrophoresis, stained with ethidium bromide, and viewed on a UV transilluminator. For quantification of the intensity of PCR products (UV densitometer; BioRad, Munich, Germany), the signals of the specific PCR products were normalized to their GAPDH signal from the same RNA sample. The data of the stimulation experiments are expressed as stimulation index (SI) calculated by dividing the signal intensity of the PCR product of stimulated cells by the intensity of resting cells. For hBD-3, the PCR product was isolated, cloned into pGEM-T (Promega Corporation, Madison, Wis.), and sequenced (Toplab, Martinsried, Germany).

Quantification of hBD-3 expression by real-time RT-PCR

hBD-3 expression was evaluated by real-time RT-PCR (Taqman; Applied Biosystems, Foster City, Calif.; Heid et al. 1996). Normal human total RNA was obtained from placenta, testis, trachea, heart, skeletal muscle, liver, thyroid gland, pancreas, lung, uterus, kidney, salivary gland, prostate, brain, cerebellum, adrenal gland, and mammary gland (Clontech, Heidelberg, Germany) and also isolated from tissues of a previously healthy individual through an organ donor program (esophagus, gastric antrum, gastric fundus, gastric corpus, jejunum, colon, rectum, and urinary bladder) and from a volunteer (neutrophils) and reverse transcribed. The hBD-3 Taqman system consisted of the primers: hBD-3 F, 5′-CCGCC-TCTGACTCTGCAATAAT-3′; hBD-3 R, 5′-TGCTTGCTCTTCC-TGTTTTG-3′; and the dual-labeled fluorescent Taqman probe hBD-3 P, 5′-(FAM) GATTCCTCCATGACCTGGAACAGGCA (TAMRA)-3′. In addition, normalization of the RNA load to the housekeeping gene ribosomic 18S was performed. The 18S Taqman system consisted of the primers: 18S F: 5′-GAAAC-TGCGAATGGCTCATTAAA-3′; 18S R, 5′-CACAGTTATCCAA-GTGGGAGAGG-3′; and the probe 18S P, 5′-(FAM) TCAGT-TATGGTTCCTTTGGTCGCTCGC (TAMRA)-3′. The hBD-2 Taqman system consisted of the primers: hBD-2 F, 5′-GCCATGAGGGTCTTGTATCTCC-3′; hBD-2 R, 5′-GGCTCCA-CTCTTAAGGCAGGTA-3′; and the probe hBD-2 P, 5′-(FAM) TCCTGATGCCTCTTCCAGGTGTTTTTG (TAMRA)-3′. A reference plasmid in pGEM-T containing the same fragment to be amplified was used as a template in triplicate with each PCR. Every PCR experiment included two no-template control wells.

Cell culture experiments and induction of hBD-3 expression

HaCaT cells (keratinocyte cell line; Boukamp et al. 1988) were cultivated by standard procedures in RPMI medium containing 10% fetal bovine serum and 1% L-glutamine. Subconfluent cells in six wells were treated with normal medium (control) or medium containing either 20 ng interleukin-1α (IL-1α)/ml, 100 ng IL-6/ml, 10 ng phorbol-12-myristate 13-acetate (PMA)/ml, 20 ng tumor necrosis factor α (TNF-α)/ml, or 40 ng interferon-γ (IFN-γ)/ml (all reagents from Pepro Tech, Rocky Hill, N.J.). After 24 h, the RNA was extracted. Experiments were repeated three times with identical results. Human respiratory epithelial cells were isolated from large airways resected during surgery and cultivated in air-liquid interface cultures as described previously (Bals et al. 1999). All procedures were approved by the Institutional Review Board (IRB) of the University of Munich. Inocula of *Pseudomonas aeruginosa* PAO1, grown as described previously (Bals et al. 1998b), were added to the cultures. For stimulation with TNF-α (100 ng) or INF-γ (5 ng), the substances were dissolved in 100 µl culture medium and 50 µl volume each was applied to the apical surface and the basolateral buffer. RNA was isolated after 24 h stimulation.

Synthesis of hBD-3 peptide

Synthetic hBD-3 corresponding to the amino acid sequence LQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK was assembled using standard Fmoc (fluorenylmethoxycarbonyl) chemistry on a preloaded Tentagel S PHB resin (Rapp Polymere, Tübingen, Germany) using a 433A peptide synthesizer (Applied Biosystems, Weiterstadt, Germany). Cysteine residues were trityl-protected. For cleavage and deprotection, the dry peptidyl resin was treated with trifluoroacetic acid, ethanedithiol, and water in a ratio of 94:3:3 (vol) for 4 h at room temperature. The product was then precipitated by addition of cold tert-butylmethylether. The crude material was dried, dissolved in water, filtered, and prepurified by preparative Vydac C18 HPLC (The Separations Group, Hesperia, Calif., USA). The combined fractions containing the reduced product were subjected to oxidative folding at a peptide concentration of 0.6 mg/ml in 5% acetic acid/dimethylsulfoxide

5:1 (vol). pH was adjusted to 6.0 by the addition of ammonium carbonate. The chromatographically homogeneous hBD-3 obtained from this mixture after purification by C18 HPLC was lyophilized and analyzed by electrospray mass spectrometry (molecular weight 4,656.9 Da; calculated 4,656.6 Da) and sequencing by automated Edman degradation on a 494-protein sequencer (Applied Biosystems).

Antimicrobial activity assays

For antimicrobial, salt sensitivity, and synergy testing, we used the conventional inhibition zone assay on agar dishes and micro-broth dilution assays as described earlier (Bals et al. 1999). As test organisms we used *Saccharomyces cerevisiae* ATCC 9763, *Staphylococcus aureus* ATCC 25923, *Streptococcus pneumoniae* ATCC 33400, and *Burkholderia cepacia* ATCC 17770 purchased from the Deutsche Sammlung für Zellkulturen und Mikroorganismen (DSZM, Braunschweig, Germany). In addition, *Pseudomonas aeruginosa* PAO 1, *Staphylococcus carnosus* TM 300 (provided by F. Goetz, University of Munich), and *Escherichia coli* BL 21 (obtained from J. Alves, Hannover Medical School) and clinical isolates of *B. cepacia* (provided by B. Grabein, University of Munich) were included. As positive control substances, the synthetic alpha-helical peptide MBI 28 and the human cathelicidin LL-37 (Bals et al. 1998b) were used.

In vitro chemotaxis

Donor-blood buffy coats were supplied by the blood bank of the Annastift (Hannover, Germany). Monocytes and neutrophils (purity 90–95%) were isolated and chemotaxis was assessed in 48-well chambers (Neuroprobe, Cabin John, Md., USA) using polyvinyl-pyrrolidone-free polycarbonate membranes with 5-μm pores (Nucleopore, Neuroprobe) for monocytes or neutrophils (Pardigol et al. 1998). Changes in the cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) were measured in monocytes and neutrophils. The cells were loaded with 2 μm Fluo-4 (Molecular Probes, Eugene, Ore., USA) by incubation for 30 min at 37°C in HBSS supplemented with 20 mM Hepes and 0.1% BSA. Afterwards, they were washed twice and resuspended (2×10^6 cells/0.5 ml for analysis in the flow cytometer, and 0.5×10^6 cells/150 μl in the fluorometric image plate reader, FLIPR; Molecular Devices, Sunnyvale, Calif., USA) in the same medium. $[Ca^{2+}]_i$ changes were recorded on the FACSCalibur system (Becton Dickinson, Heidelberg, Germany), by analyzing FL1 (linear scale) versus time, and on the FLIPR by analyzing fluorescence changes after stimulation of the cells.

Biological assay for oocyte membrane interaction

Two-electrode voltage-clamp experiments were performed to assess oocyte membrane interaction as described previously (Becker et al. 1996).

Results

Identification of hBD-3 by screening genomic sequences

We used the peptide sequences of hBD-1 and hBD-2 to perform BLAST searches in the HTG division of Genbank and identified several overlapping clones (Genbank accession nos: AF252830, AF189745, AF202031, AF57570, AF59450, AF200455, and AF205406). A putative novel defensin was identified from clone AF252830 (chromosomal region 8p23). Using the Genscan 1.0 software, the exon 1 of the novel defensin was identified from the upstream region of the genomic sequence found identically in several HTG clones. A map of the hBD-3 gene together with putative functional sequence elements is displayed in Fig. 1a. The full-length cDNA for hBD-3 was determined by RT-PCR using RNA isolated from cultivated lung epithelia. The cDNA sequence (Genbank accession no. AF295370) was identical to the predicted sequence and consisted of a 204-bp open reading frame encoding a peptide of 67 amino acids(Fig. 1b). The deduced peptide revealed the β-defensin-specific structural hallmarks (Fig. 1c).

hBD-3 is expressed in epithelial and nonepithelial tissues

The tissue distribution of hBD-3 expression was evaluated by real-time quantitative RT-PCR and detected in epithelial and nonepithelial cells and tissues (Fig. 2a). Specific transcripts were detectable in organs of the gastrointestinal and respiratory tract. Interestingly, we found substantial baseline expression of hBD-3 in leukocytes, placenta, and testis, as well as in the heart and skeletal muscle.

hBD-3 expression is regulated by mechanisms different from hBD-2

Since β-defensins have been found to be constitutively expressed or induced by infection and inflammation, we analyzed whether expression of hBD-3 is regulated. We found low expression of hBD-3 in the keratinocyte cell line HaCaT under basal conditions. Expression was unchanged following 24-h exposure to either IL-1α, IL-6, PMA, or TNF-α. However, IFN-γ treatment gave rise to a 136-fold increase in hBD-3 transcripts, as measured by real-time quantitative RT-PCR (Fig. 2b). Further, we inoculated cultures of differentiated airway epithelia with *P. aeruginosa* and inflammatory mediators. RT-PCR of the cultures revealed the constitutive expression of hBD-1 and hBD-3 and the induction of hBD-2 expression after bacterial infection (Fig. 2c). In contrast to hBD-2, which was upregulated by TNF-α, hBD-3 expression was increased by IFN-γ (Fig. 2d).

hBD-3 is an endogenous antibiotic

In order to evaluate the biological functions of hBD-3, a predictably antimicrobially active peptide was synthesized by solid-phase chemistry. In conventional inhibition zone assays, hBD-3 inhibited the growth of *Staphylococcus carnosus* TM300, *Micrococcus luteus* ATCC 19212, *E. coli* BL21, and *Saccharomyces cerevisiae* ATCC 9763 as effectively as the reference peptide MBI 28 (data not shown). The minimal inhibitory concentrations (MIC) of hBD-3 against various microorganisms were tested by conventional micro-broth dilution assays. Synthetic hBD-3 showed a strong antimicrobial activity against

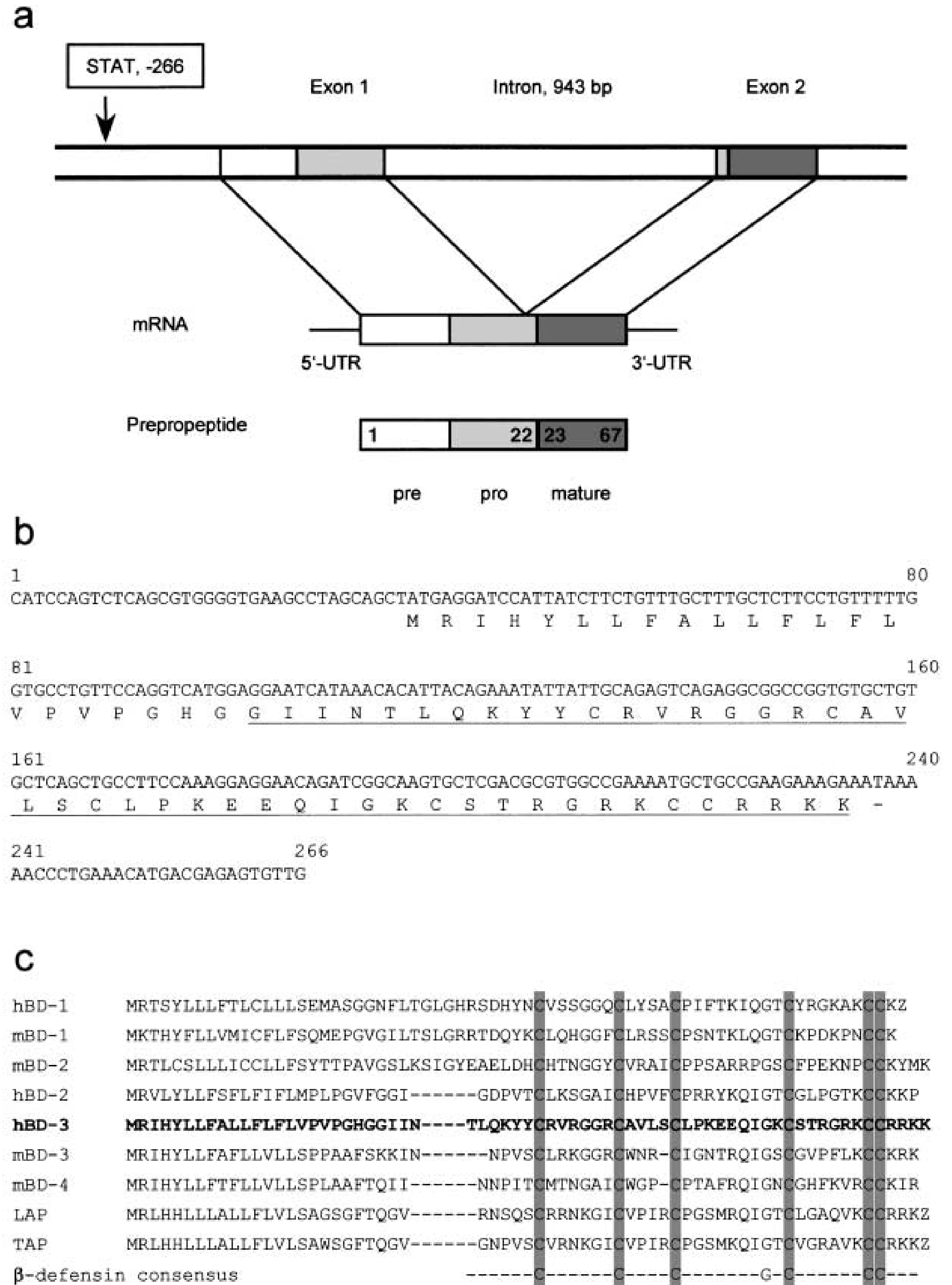

Fig. 1a–c Gene structure, cDNA, and amino acid sequence of human β-defensin 3 (hBD-3). **a** Structure of the human β-defensin 3 gene together with a schematic drawing of the gene, the cDNA, and the predicted structure of the prepropeptide. The 5′ and 3′ untranslated regions (*5′-UTR*, *3′-UTR*) flank the sequence coding for the signal peptide, propeptide, and mature peptide. **b** Complementary DNA and deduced amino acid sequence of hBD-3. The *underlining* indicates the putative mature peptide; the *dash* represents the stop codon. **c** Comparison of the putative prepropeptide sequences of hBD-1, hBD-2, hBD-3, mBDs 1–4, tracheal antimicrobial peptide (*TAP*), and lingual antimicrobial peptide (*LAP*)

Streptococcus pneumoniae ATCC 33400 (6.6 μg/ml), *P. aeruginosa* PAO 1 (26.5 μg/ml), *Staphylococcus carnosus* (2.6 μg/ml), *E. coli* (6.6 μg/ml), *Saccharomyces cerevisiae* ATCC 9763 (13.2 μg/ml), and *Staphylococcus aureus* ATCC 25923 (more than 26.5 μg). Interestingly, *B. cepacia* was killed at low concentrations of the peptide (6.6 μg/ml for ATCC 17770, 6.6 μg/ml against a clinical isolate). MICs of LL-37 were in the same range as published previously (Bals et al. 1998b). The antimicrobial activity of hBD-3 against *Staphylococcus carnosus* decreased with elevated concentrations of NaCl and was synergistic with lysozyme (data not shown).

Chemotactic activity of hBD-3 on monocytes

Defensins have been described to attract different types of human leukocytes (Yang et al. 1999). hBD-3 was tested as a potential chemoattractant for monocytes and neutrophils, which are strongly involved in the innate immune response. hBD-3 induced migration of monocytes with a maximal response at 50 nM (Fig. 3a). It had a moderate efficacy compared with formyl-methionyl-leucyl-phenylalanine (fMLP). No activity was found on neutrophils (data not shown). However, hBD-3 did not induce Ca^{2+} mobilization, while fMLP or certain chemokines elicited

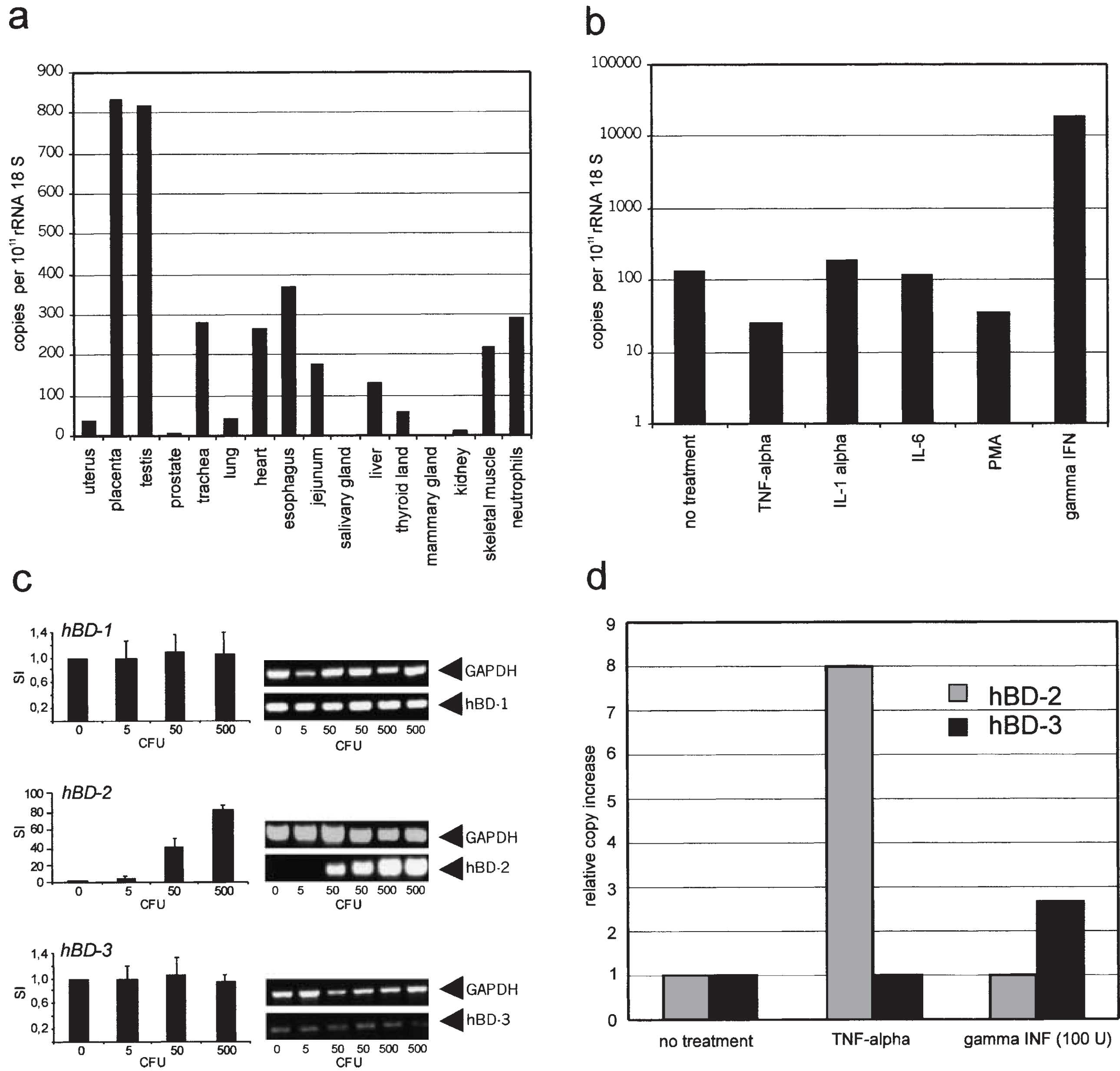

Fig. 2a–d Expression profile and regulation of hBD-3. **a** Tissue distribution of hBD-3 measured by real-time quantitative RT-PCR is displayed as copy number of hBD-3 per 10^{11} copies of ribosomal 18S RNA. **b** Real-time quantitative RT-PCR analysis of hBD-3 expression in HaCaT cells in response to inflammatory mediators. **c** RT-PCR analysis of hBD-3 expression in respiratory epithelia in response to infection with different numbers of colony-forming units (*CFU*) of *P. aeruginosa* PAO1. Glyceraldehyde-phosphate-dehydrogenase (*GAPDH*) was used as positive control. Induction is expressed in the charts as stimulation index (*SI*); see Methods. **d** Real-time quantitative RT-PCR analysis of hBD-3 expression in differential respiratory epithelia in response to inflammatory mediators. Relative numbers of copies are normalized to the group that received no treatment. Data are expressed as mean ± SEM where applicable. Experiments were performed in triplicate. (*TNF* Tumor necrosis factor, *IL* interleukin, *PMA* phorbol-12-myristate 13-acetate)

a transient rise in $[Ca^{2+}]_i$ in monocytes and neutrophils (data not shown).

hBD-3 elicits ion channel activity in oocyte cell membrane

It has been demonstrated that the capability of antimicrobial peptides to form channels in biomembranes extends the functional spectrum of these molecules (Lencer et al. 1997). When oocytes from *Xenopus laevis* were challenged with hBD-3 in two-electrode voltage clamp recordings, the induction of ionic currents was observed (Fig. 3b). Detailed tail current analyses in the presence of KCl, NaCl, and $CaCl_2$, as well as salts of permeable cations and impermeable anions and vice versa (not shown) identified the peptide-induced transporter as

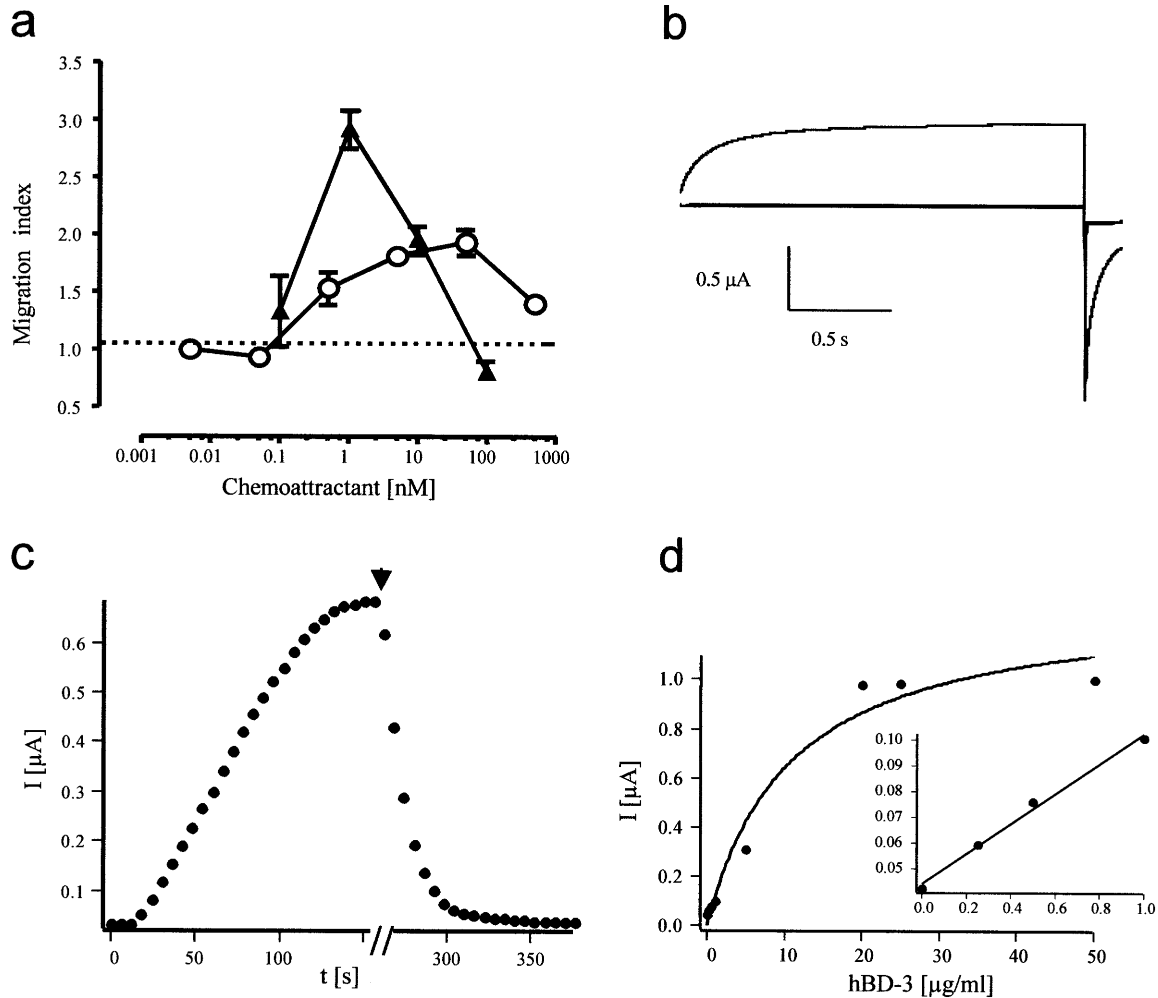

Fig. 3a–d hBD-3 is a monocyte chemoattractant and forms ion channels in biomembranes. **a** Chemotactic responses of human monocytes to hBD-3 (*open circle*) and formyl-methionyl-leucyl-phenylalanine (*filled triangle*). Migration is expressed as migration index defined as *n*-fold increase in cell migration in the presence of chemoattractant over cell migration in the presence of medium alone (mean ± SEM per five high-power fields in triplicate wells). One out of four similar experiments performed with cells from different donors is shown. **b** hBD-3 exposure of *Xenopus laevis* oocytes elicits outward currents. Rapidly decaying tail currents during repolarization indicate the deactivation of hBD-3-induced ion channels. **c** Time dependence of the hBD-3 response. **d** Concentration dependence of the hBD-3 response

a nonselective ion channel. After stimulus onset, the outward currents gradually increased, reaching a steady state within 1–2 min. Removal of the peptide by bath perfusion resulted in a complete deactivation of the ionic current (Fig. 3c). The reversible nature of the peptide-evoked electrical signals allowed dose-response curves to be obtained on individual oocytes (Fig. 3d). When 5 µg/ml of hBD-3 or control peptides (e.g., other defensins and chemokines) were added, channel activation was only obtained with hBD-3, indicating that the channel formation was hBD-3-specific.

Discussion

In the present study we describe the discovery of a novel human β-defensin by screening genomic sequences. The novel defensin, called human β-defensin 3 (hBD-3), exhibits the characteristic properties of the β-defensin family with regard to its genomic, cDNA, and peptide sequence, as well as function. Importantly, the regulation, expression pattern, antimicrobial spectrum, and other functions of hBD-3 differ significantly from other β-defensins.

The strategy used to identify the novel human defensin was based on the fact that α- and β-defensins are located in a cluster at chromosome 8p23 (Linzmeier et al. 1999). After screening several candidate clones, we discovered a novel β-defensin named hBD-3, closely related to the β-defensin family. The potent antimicrobial activity and the capability to activate monocytes are consistent with the role of hBD-3 as host defense substance involved in the direct killing of microorganisms and in mediation of inflammation. It has been shown that defensins also serve as mediators of inflammation (Yang et al. 1999) and it is speculated that they fulfill various other functions, including regulation of cell turnover (Zucht et al. 1998). The antimicrobial activity of hBD-3 is in the

range of other antimicrobial peptides. However, it shows a different profile, including strong activity against *B. cepacia*. This bacteria, usually considered to be resistant to cationic peptides (Hancock 1997), is associated with severe pulmonary infection in cystic fibrosis. The pore formation in biomembranes by hBD-3 demonstrated in our study indicates that hBD-3 has an impact on the function of multiple cell types by influencing their electrophysiology. It has been demonstrated previously that mammalian antimicrobial peptides form pores in host cells and may influence cellular functions (Lencer et al. 1997).

Studies of expression of hBD-3 show a low baseline level not only in epithelial but also in nonepithelial tissues. In contrast to hBD-1 and hBD-2 (Bensch et al. 1995; Bals et al. 1998a; Goldman et al. 1997; O'Neil et al. 1999; Valore et al. 1998; Zhao et al. 1996), a significant expression was detected in leukocytes, mammary gland, heart, and skeletal muscle. The regulation of hBD-1 and hBD-2 has been described in several reports (Harder et al. 1997a; Hiratsuka et al. 1998; O'Neil et al. 1999; Singh et al. 1998). In contrast to hBD-2 that is induced by *P. aeruginosa* and TNF-α, no regulation of hBD-3 was found after application of these stimulants. Surprisingly, we found a significant upregulation of hBD-3 in response to IFN-γ. Consistent with this result, the 5′ genomic sequence of the hBD-3 gene contains a signal transducer and activator of transcription (STAT) binding site. It is an intriguing hypothesis that this regulatory pathway could permit an activated macrophage, secreting IFN-γ, to stimulate hBD-3 secretion, inducing local antimicrobial activity and augmenting recruitment of other macrophages. The concept of a large family of antimicrobial peptides with differential functions against diverse classes of microorganisms has been demonstrated for the host defense system of *Drosophila* (Lemaitre et al. 1997) and may also be applicable to the human innate immune system. Functional genomics offers opportunities to further evaluate this hypothesis and to apply this knowledge for diagnostic and therapeutic measures.

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Appendix

While this manuscript was submitted for publication and was under review, a paper by Harder et al. was published, also describing hBD-3. The data of both articles are in full agreement; however, our paper contains substantial additional results:

1. We describe that hBD-3 is specifically upregulated by IFN-γ, unlike hBD-1, which is constitutively expressed, and unlike hBD-2, which is upregulated by TNF-α and IL-1. We also show that hBD-3 is a defensin expressed in muscular tissue and heart in contrast to earlier statements that β-defensins are characteristically expressed in epithelial tissues.
2. hBD-3 attracts monocytes, showing that a human β-defensin activates cells of the innate immune system in contrast to other studies that show attraction of T cells and dendritic cells.
3. We show that a human defensin induces ion current across specific biological membranes in oocytes. This finding demonstrates an important novel biological function of defensins which is beyond their normal role in host defense; it is evident that hBD-3 plays a role in fertilization.
4. We describe a specific and relevant antimicrobial activity of hBD-3 in detail. hBD-3 is a potent antibiotic peptide against *B. cepacia*. This bacterium is resistant to antimicrobial peptides and conventional therapeutic antibiotics, which implies that human β-defensin 3 might be a useful compound in the development of an innovative drug against multi-resistant bacteria, playing a special role in the treatment of patients with cystic fibrosis and chronic bronchitis.

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