

A peptide antibiotic from human skin

To avoid opportunistic infections, plants and animals have developed antimicrobial peptides in their epithelia that can form pores in the cytoplasmic membrane of microorganisms¹. After contact with microorganisms, vertebrate skin², trachea and tongue epithelia³ are rich sources of peptide antibiotics¹, which may explain the unexpected resistance of these tissues to infection. Here we report that human skin is protected in a similar way by an inducible, transcriptionally regulated, antibiotic peptide, which resembles those in other mammals.

Patients with psoriasis have fewer skin infections than expected⁴, leading us to speculate that lesional psoriatic skin might produce antimicrobial peptides. Cationic antimicrobial peptides bind strongly to their target bacteria, so we isolated and purified peptide antibiotics from psoriatic scale extracts using a whole *Escherichia coli* affinity column. We subsequently purified bound peptides, which demonstrated antimicrobial activity in a plate assay, to homogeneity using high-performance liquid chromatography. We recovered 200–400 µg of a pure antimicrobial peptide (relative molecular mass 4,000) from 50-g samples of psoriatic scales. Amino-acid sequence analysis of this peptide (Fig. 1) revealed the consensus sequence of a β-defensin with homology to bovine tracheal and lingual antimicrobial β-defensins³, as well as human β-defensin-1 (hBD-1)⁵.

Using degenerate primers based on amino-acid sequence data, we generated the complete complementary DNA sequence from RNA obtained from human foreskin-derived primary keratinocytes. The deduced amino-acid sequence of the precursor peptide (Fig. 1) consisted of 41 residues present in the mature peptide as well as a leader sequence, which may indicate that this is a secreted peptide. The highest homology is to bovine tongue- and trachea-derived antimicrobial peptides³, produced in cow epithelia in response to microorganisms or inflammatory cytokines. We conclude that the peptide, named hBD-2, is the second human β-defensin to be found. The first human β-defensin (hBD-1) is mainly produced by epithelia from the urogenital tract and, to a lesser degree, in trachea and lung⁶, and was originally discovered in human blood filtrates⁵.

We found that hBD-2 was highly effective in killing Gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa*) with LD₉₀ (the dose that achieves 90% reduction of colony-forming units) near 10 µg ml⁻¹. The yeast *Candida albicans* was also effec-

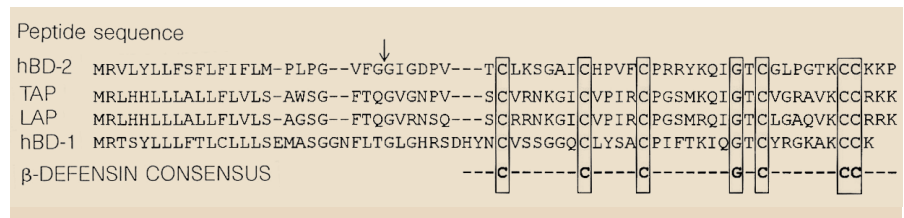


Figure 1 The deduced amino-acid sequence (single-letter code) of the hBD-2 precursor based on the complementary DNA sequence obtained from human keratinocytes, with the sequences of bovine tracheal antimicrobial peptide (TAP), bovine lingual antimicrobial peptide (LAP) and human β-defensin-1 (hBD-1), and the β-defensin consensus sequence. Arrow indicates the amino terminus of hBD-2 obtained from psoriatic scales. Isolation of the peptide was as described in ref. 9. The full-length cDNA structure was derived using an inverse PCR method¹⁰ using specific primers according to the sequence found with degenerate primers. The cDNA sequence is available from the EMBL/Genbank database, accession number Z71389.

tively killed (LD₉₀, 25 µg ml⁻¹), but hBD-2 achieved a bacteriostatic effect on the Gram-positive *Staphylococcus aureus* only at concentrations as high as 100 µg ml⁻¹.

Using reverse transcription and semi-quantitative polymerase chain reaction (RT-PCR) we saw low hBD-2 messenger RNA expression in foreskin-derived keratinocytes. Expression was greatly upregulated with tumour-necrosis factor-α within 1 h of stimulation and persisted for more than 48 h. Gram-negative and Gram-positive bacteria, as well as *C. albicans*, strongly induce hBD-2 (Fig. 2), in contrast

to hBD-1, which is not upregulated by inflammatory stimuli⁶. Thus hBD-2 is the first human β-defensin found to be regulated at a transcriptional level in response to contact with microorganisms.

We detected constitutive hBD-2 expression in freshly isolated foreskin, lung and trachea (Fig. 2). In contrast, considerably less hBD-2 is expressed in kidney, uterus and salivary gland tissue. Both small intestine and liver mRNA preparations failed to demonstrate hBD-2 expression. Therefore, hBD-2 may represent the human counterpart of the bovine lingual and tracheal peptides.

Our observations show that human skin contains a chemical shield of endogenous peptide antibiotics, which are induced by contact with microorganisms and are operative in other human epithelia. Disruption of this shield, as in cystic fibrosis^{7,8}, might be a reason for recurrent infections of skin and other epithelia. It is intriguing to speculate that human peptide antibiotics, like hBD-2, might be ideal therapeutic agents, avoiding the problems of acquired resistance.

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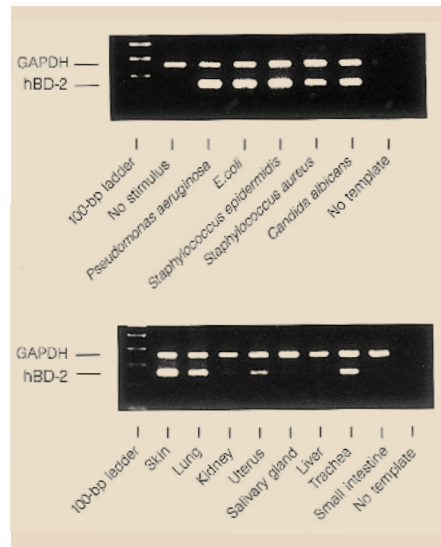


Figure 2 hBD-2 mRNA is upregulated by contact with different microorganisms (upper panel) and constitutively expressed in organs other than skin (lower panel). Foreskin-derived, third-passage keratinocytes were stimulated with different heat-killed microorganisms (10⁷ per ml) for 16 h and analysed for mRNA expression by semi-quantitative RT-PCR using hBD-2 primers (5'-CCAGCCATCAGCCATGAGGGT-3'; 5'-GGAGCCCTTCTGTAATCCGCA-3') and primers for glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) as described¹⁰. Controls: 'no stimulus', buffer without bacteria; 'no template', primer only, no cDNA.

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