

Expression of human β -defensin 2 (hBD-2) in *Helicobacter pylori* induced gastritis: antibacterial effect of hBD-2 against *Helicobacter pylori*

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Accepted for publication
26 February 2001

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Abstract

Background—Human β -defensin 2 (hBD-2) plays a role in the innate defence system at mucosal surfaces. Colonisation of *Helicobacter pylori* in the stomach is an important pathological factor in gastrointestinal illnesses, including gastritis, peptic ulcer, and gastric adenocarcinoma. **Aims**—To evaluate the antibacterial role of hBD-2 against *H pylori* infection in the gastric mucosa.

Subjects—Biopsied gastric mucosa specimens from *H pylori* positive (n=6) and *H pylori* negative (n=6) individuals were used. *H pylori* was determined by the presence of urease activity and microscopic examination.

Methods—The specimens were examined for hBD-2 expression by reverse transcription-polymerase chain reaction (RT-PCR), immunohistochemistry, and in situ hybridisation. The antibacterial effect of hBD-2 against *H pylori* was evaluated by the number of colony forming units of *H pylori* after incubation with 0, 10^{-9} , 10^{-8} , 10^{-7} , 10^{-6} , or 10^{-5} M of hBD-2 peptide.

Results—All six *H pylori* positive specimens expressed a high level of hBD-2 mRNA while hBD-2 mRNA was not detected in the *H pylori* negative specimens by RT-PCR. Immunohistochemistry using anti-hBD-2 antiserum revealed that hBD-2 was expressed in the surface epithelium of *H pylori* infected specimens. In gastric specimens obtained after *H pylori* eradication, hBD-2 immunoreactivity had dramatically decreased. In situ hybridisation confirmed that hBD-2 transcripts were localised in the epithelium of *H pylori* infected gastric specimens. Incubation with hBD-2 reduced the growth rate of cultured *H pylori* in a dose dependent manner, and incubation with 10^{-5} M hBD-2 completely inhibited the proliferation of *H pylori*.

Conclusions—*H pylori* infection induces hBD-2 expression in the human gastric epithelium. hBD-2 inhibited the growth of *H pylori* in vitro, suggesting that hBD-2 plays an antibacterial role in *H pylori* induced gastritis.

(Gut 2001;49:481-487)

Keywords: human β -defensin 2; *Helicobacter pylori*; gastritis; antimicrobial peptide

Infection by the Gram negative, spiral shaped bacterium *Helicobacter pylori* has been identified as the main cause of chronic antral gastritis and duodenal ulcer.^{1,2} Colonisation of *H pylori* in the stomach is established by neutralisation of the acidic environment with the ammonia produced by urease from the urea present in mucosal secretions.^{3,4} Although most *H pylori* infections are asymptomatic, *H pylori* type I, which colonises the gastric mucosa of over 50% of the world population, is associated with severe symptomatic gastritis.⁵ On the other hand, it has recently been suggested that *H pylori* has beneficial effects in infected carriers who are exposed to a high level of other gastrointestinal pathogens because *H pylori* infected individuals have a dramatically higher percentage of total IgA secreting cells in the antrum than non-infected subjects.⁶

In most cases, *H pylori* infection is associated with strong systemic antibody responses as well as mucosal antibody responses.⁷ The host defence response against a microorganism depends mainly on the immune system that acts through neutrophils and lymphocytes. Recently, attention has focused on antimicrobial peptides as a component of the innate immune system against microorganisms. Defensins comprise a major family of antimicrobial peptides found in mammals, and can be divided into two classes, the α -defensins and β -defensins, according to their structural features at the genetic and protein levels.⁸ Some α -defensins are stored in the azurophilic granules of phagocytic leucocytes while other α -defensins are constitutively expressed in the epithelial cells of the small intestine.⁹ Two types of human β -defensins were recently identified. Human β -defensin 1 (hBD-1) was isolated as a trace peptide in blood filtrates¹⁰ and is constitutively expressed in various epithelial tissues.¹¹⁻¹³ Human β -defensin 2 (hBD-2) is a cysteine rich, low molecular weight antimicrobial peptide that was originally isolated in 1997 from psoriatic skin.¹⁴ Basal hBD-2 mRNA expression has been detected in the skin, lung,

Abbreviations used in this paper: hBD-2, human β -defensin 2; RT-PCR, reverse transcription-polymerase chain reaction; hBD-1, human β -defensin 1; NSAIDs, non-steroidal anti-inflammatory drugs; Fmoc, fluorenylmethoxycarbonyl; FMP, 2-fluoro-1-methylpyridinium toluene-4-sulphonate; TBS, Tris buffered saline; PBS, phosphate buffered saline; SSC, standard sodium citrate; CFU, colony forming units; NF κ B, nuclear transcription factor κ B; IL, interleukin; PAI, pathogenicity island.

trachea, urogenital tract, and oculus by reverse transcription-polymerase chain reaction (RT-PCR).^{14,15} On proinflammatory induction, hBD-2 expression is strongly upregulated in the epithelium of the respiratory tract and skin.¹⁵⁻¹⁷ Thus hBD-2 is the first human β -defensin whose expression was found to be upregulated at the transcriptional level in response to contact with a microorganism¹⁴ and it plays an important role in host defence against mainly Gram negative bacteria and fungi at the mucosal surface.¹⁶ However, its expression in the epithelia of the gastrointestinal tract, and in particular the stomach, has not yet been fully clarified.

We recently demonstrated that expression of hBD-2 transcripts is induced by *H pylori* in a human gastric cancer cell line.¹⁸ In the present study, to gain an understanding of the innate immune response to *H pylori*, we investigated hBD-2 expression in human gastric mucosa specimens with or without *H pylori* colonisation by RT-PCR, immunohistochemistry, and in situ hybridisation. Finally, the antibacterial effect of hBD-2 against *H pylori* was evaluated by the number of colony forming units (CFU) of *H pylori* after incubation with synthetic hBD-2 peptide in vitro.

Materials and methods

RNA PREPARATION AND RT-PCR

Human gastric tissue specimens were obtained by biopsy from 12 patients who underwent gastroscopic examination. Informed consent was obtained from all patients prior to biopsy. The characteristics of the patients are summarised in table 1. None of the patients had been treated with any non-steroidal anti-inflammatory drugs (NSAIDs) or acid suppressants in the previous year at least. Gastritis activity was classified according to the Sydney system.¹⁹ The presence of *H pylori* colonisation in each specimen was determined by the presence of urease activity, as detected by the PyloriTek test (Serim Research Corp., Elkhart, Indiana, USA) and also by microscopic examination of May-Giemsa stained specimens. RT-PCR for hBD-2 was performed on all 12 specimens. Total RNA was extracted from the tissue with Isogen (Nippon Gene, Tokyo, Japan) according to a modified guanidine-phenol method,²⁰ and quantified by measuring absorbance at 260 nm. cDNA was synthesised from 2 μ g of total RNA with 2.5 μ M of oligo (dT) primer, 1 mM of each deoxynucleotide

triphosphate, and reverse transcriptase (Life Technologies Inc, Rockville, Maryland, USA). Samples of cDNA were used for each PCR with primers for amplification of hBD-2 (5'-ATGAGGGTCTTGATCTCCT-3' (sense), 5'-TATCTTTGGACACCATAGTT-3' (antisense)) or human β -actin (5'-TCCTCCCTGGAGAAGACTA-3' (sense), 5'-AGTACTTGCGCTCAGGAGGA-3' (antisense)) as a control. The primer pairs for both genes were chosen to amplify a region that spanned an intron in the respective genes. The hBD-2 and β -actin primers were predicted to amplify a 296 and 313 bp DNA fragment, respectively. The PCR reaction mixture contained 10 mM Tris HCl (pH 8.3), 50 mM KCl, 2 mM MgCl₂, 1 mM deoxynucleotide triphosphate, 50 pmol of each primer, and 2.5 U of Taq DNA polymerase (Life Technologies Inc.) in a final volume of 50 μ l. The mixture was covered with mineral oil to prevent evaporation. The PCR conditions were 40 cycles of one minute at 94°C, two minutes at 55°C, and two minutes at 72°C. PCR products were visualised on an ethidium bromide stained 1.5% agarose gel. A sample was considered to be positive for hBD-2 or β -actin mRNA if a band at 296 bp or 313 bp, respectively, was visible.

SYNTHESIS OF hBD-2 PEPTIDE

The hBD-2 peptide was synthesised using the solid phase method.²¹ Starting with 9-fluorenylmethoxycarbonyl (Fmoc)-Pro-HMP resin, the peptide chain was elongated using the FastMoc™ Fmoc strategy on an Applied Biosystems Model 433A peptide synthesiser (PerSeptive Biosystems, Foster City, California, USA) according to the single coupling protocol.^{22,23} The protected peptide resin obtained was treated with Reagent K for two hours at room temperature. The crude peptide was subjected to the disulphide bond formation reaction according to a previously described method.¹⁷ The crude hBD-2 was loaded on a high performance liquid chromatography column (Cosmosil 5C18AR, 20 \times 250 mm; MetaChem Technologies Inc., Torrance, California, USA) using aqueous acetonitrile containing 0.1% TFA as the eluent. The peak eluted at the same retention time as standard hBD-2 (Peptide Institute, Osaka, Japan), and was collected and lyophilised to give purified hBD-2. Purity was assessed by matrix assisted laser desorption ionisation-time of flight mass spectrometry (PerSeptive Biosystems) and amino acid analysis.

PREPARATION OF ANTI-hBD-2 ANTIBODY

The synthetic hBD-2 peptide was coupled to keyhole limpet haemocyanin.²⁴ Rabbits were immunised subcutaneously with the antigen. Titration of anti-hBD-2 antibody was carried out according to the Ouchterlony double gel diffusion test.²⁵ Antibodies were purified from antisera by affinity chromatography on immobilised synthetic peptides.²⁶ Purified synthetic peptides (1.0 mg) were coupled to the column packed with 2-fluoro-1-methylpyridinium toluene-4-sulphonate (FMP)-cellulofine (Seikagaku Kougyo Co., Tokyo, Japan). After

Table 1 Patient characteristics

Case	Sex	Age (y)	Biopsy site	Urease activity	Severity of gastritis*	Symptom or diagnosis
1	M	71	Antrum	Negative	Mild	Epigastric discomfort
2	F	81	Antrum	Negative	Normal	Epigastric discomfort
3	F	54	Antrum	Negative	Normal	No symptom
4	M	61	Antrum	Negative	Normal	Epigastric discomfort
5	M	73	Antrum	Negative	Mild	Gastric cancer
6	M	87	Body	Negative	Mild	Gastric ulcer
7	F	62	Antrum	Positive	Severe	Chronic active gastritis
8	M	68	Antrum	Positive	Moderate	Chronic active gastritis
9	M	58	Antrum	Positive	Severe	Chronic active gastritis
10	M	67	Body	Positive	Severe	Gastric cancer
11	F	72	Body	Positive	Moderate	Chronic active gastritis
12	M	74	Antrum	Positive	Severe	Chronic active gastritis

*According to the updated Sydney system.¹⁸

washing the column with Tris buffered saline (TBS), the antiserum in an appropriate volume (10 ml) was added to the peptide conjugated FMP-cellulofine column and incubated for one hour at room temperature. After washing the column with TBS supplemented by 1% Triton X-100, the antibodies were eluted with 0.1 M glycine HCl, pH 2.5, and collected in a tube containing 0.05 volume of 1 M Tris solution. Eluted fractions were dialysed against phosphate buffered saline (PBS) and used as anti-hBD-2 antibody in the immunohistochemical analysis. The hBD-2 specific antibody did not cross react with hBD-1 when tested against the recombinant peptides by western blotting.

IMMUNOHISTOCHEMISTRY

To demonstrate the distribution of hBD-2 expression in gastric tissue, immunohistochemical analysis was performed on 10% buffered, formalin fixed, paraffin embedded samples of gastric specimens from the 12 patients described above, using the ABC-Peroxidase kit (Vector Laboratories, Inc., Burlingame, California, USA). Three patients with *H pylori* positive gastritis (case Nos 7, 8, and 9 in table 1) were treated with antibiotics (20 mg/day sodium rabeprazole, 600 mg/day clarithromycin, and 1500 mg/day amoxicillin) for five days perorally to eradicate *H pylori*, and then re-biopsied on the 28th day after completion of treatment. The presence of *H pylori* after antibiotic treatment was evaluated by both microscopic examination and the urease test. Samples for immunohistochemical analysis were cut into 4 µm sections, deparaffinised in xylene, and rehydrated in PBS. The deparaffinised sections were immersed in 0.3% hydrogen peroxide in methanol to block the internal peroxidase, preincubated with 1% normal bovine serum to prevent non-specific binding, and then incubated overnight at 4°C with 100-fold diluted anti-hBD-2 antibody. After washing in PBS, the slides were incubated for 30 minutes with biotinylated anti-mouse IgG and then with peroxidase conjugated avidin. The reaction products were resolved using diaminobenzidine. The specimens were counterstained with haematoxylin. Negative controls were prepared in each case by replacing the primary antibody with non-immunised rabbit serum, and did not show any staining (data not shown).

IN SITU HYBRIDISATION

Biopsied 4% paraformaldehyde fixed, frozen samples were sectioned at 10 µm. Digoxigenin labelled hBD-2 RNA probes were prepared by subcloning the full length hBD-2 cDNA into the SmaI site of pBluescript KS (+) (Promega, Madison, Wisconsin, USA), and the resultant vector was used as a template for the riboprobe. The open reading frame of hBD-2 cDNA shows 39% identity with hBD-1. The antisense probe was generated using the EcoRI digested linearised template and T7 RNA polymerase. Similarly, the sense probe was prepared for negative control experiments using the BamHI digested template and T3 RNA polymerase.

The fixed sections were digested by placing them in 10 µg/ml proteinase K in TE (pH 8.0) at 37°C for 30 minutes, followed by postfixation in 4% paraformaldehyde. The slides were immersed in 0.2 N HCl for 10 minutes and were subsequently acetylated for 10 minutes in freshly prepared 0.25% acetic anhydride in 0.1 M triethanolamine (pH 8.0). After prehybridisation in 4× standard sodium citrate (SSC)/50% deionised formamide for one hour at 37°C, the slides were hybridised in a solution of 200 ng/ml antisense or sense probe in 50% deionised formamide, 10 mM Tris HCl (pH 7.6), 1 mM EDTA (pH 8.0), 300 mM NaCl, 0.25% sodium dodecyl sulphate, 1× Denhardt's solution, 10% dextran sulphate, and 200 µm/ml yeast tRNA at 42°C for 16 hours. After hybridisation, the specimens were rinsed in 2× SSC for 30 minutes at 37°C and digested with 20 µg/ml of RNaseA at 37°C for 30 minutes. After washing once with 1× SSC at 37°C for one hour and twice with 0.1× SSC at 37°C for 30 minutes each, the sections were subjected to immunohistochemical analysis for detection of the hybridised probes using an alkaline phosphatase conjugated antidigoxigenin antibody (Boehringer Mannheim, Indianapolis, Indiana, USA). The alkaline phosphatase reaction was visualised with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium chloride.

ANTIMICROBIAL ASSAY

The toxin producing strain of *H pylori*, ATCC49503 (tox⁺ strain 60190), was used in this assay. *H pylori* was recovered from frozen stocks by seeding on a blood agar plate (Mueller Hinton II agar with 7% horse blood) at 37°C for three days under microaerophilic conditions (10% O₂ and 10% CO₂) generated with Anaeropack Campylo (Mitsubishi Gas Chemicals Corp., Tokyo, Japan). Bacteria harvested from the plates using cotton swabs were suspended in 200 ml of brain heart infusion broth containing 10% fetal calf serum and then grown in liquid culture at 37°C for three days with vigorous shaking in a controlled microaerophilic atmosphere. Mixtures containing 2.4×10⁴ CFU of *H pylori* and synthetic hBD-2 peptide at concentrations of 0, 10⁻⁹, 10⁻⁸, 10⁻⁷, 10⁻⁶, or 10⁻⁵ M in 100 µl of 10 mM phosphate buffer (pH 7.4) with 50 mM NaCl were incubated at 37°C for 20 minutes. The *H pylori* cells from each preparation were plated on a blood agar plate and incubated at 37°C for three days under microaerophilic conditions. The number of CFU was counted. This antimicrobial assay is performed under hypotonic (hypo-osmolal) conditions because the antimicrobial function of hBD-2 is highly salt sensitive and almost completely inhibited by more than 150 mM of NaCl.^{27, 28}

Results

RT-PCR

To evaluate the effect of *H pylori* colonisation in gastric tissue on hBD-2 mRNA expression, gastric specimens from six *H pylori* positive and six *H pylori* negative patients, as determined by the urease test and microscopic examination,

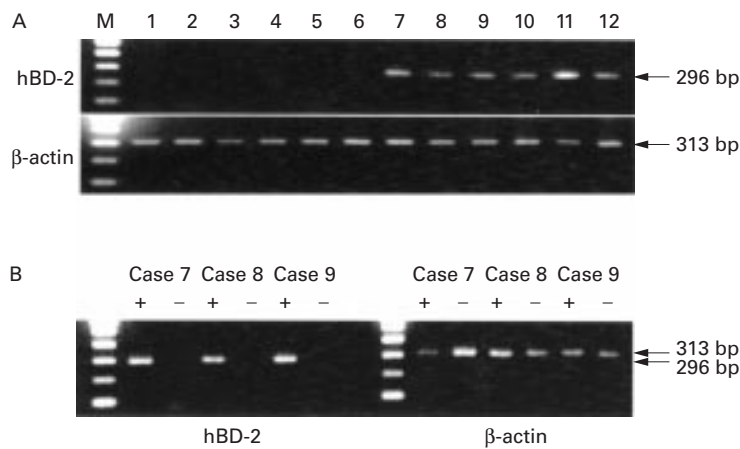


Figure 1 Reverse transcription-polymerase chain reaction (RT-PCR) analysis of human β -defensin 2 (hBD-2) expression in gastric mucosa specimens. (A) Lanes 1–12 correspond to gastric specimens obtained from cases 1–12, respectively, in table 1. Cases 1–6 were not infected with *Helicobacter pylori* and cases 7–12 were infected with *H pylori*. (B) Expression of hBD-2 mRNA in gastric mucosa specimens obtained from cases 7–9 before and after *H pylori* eradication. Cases 7–9 in table 1 were applicable in this experiment. +, before *H pylori* eradication; –, after *H pylori* eradication; M, 100 bp DNA ladder as a marker.

were used for RT-PCR analysis. A high level of hBD-2 mRNA was detected in all six *H pylori* positive cases while there was no visible band for hBD-2 mRNA in the six *H pylori* negative cases (fig 1A). On the other hand, a similar level of β -actin mRNA expression as a positive control was detected in all 12 samples. *H pylori* was successfully eradicated in case Nos 7, 8, and 9 by antibiotic treatment according to the urease test and microscopic examination. After *H pylori* eradication, the level of hBD-2 transcripts had decreased to an undetectable level by RT-PCR (fig 1B).

IMMUNOHISTOCHEMISTRY

We performed immunostaining for hBD-2 with a newly developed polyclonal antibody. hBD-2 expression was detected in the gastric specimens from the six *H pylori* positive patients but not in the specimens from the six *H pylori* negative patients (fig 2). In the *H pylori* positive gastric mucosa specimens, hBD-2 immunoreactivity was restricted to the surface epithelium and gastric pits and was not detected in the lamina propria, inflammatory cells, or other gastric glands. The gastric mucosa specimens obtained after treatment with antibiotics for eradication of *H pylori* (n=3) were also examined. *H pylori* were not detected in the gastric mucosa specimens obtained after antibiotic treatment, by both the urease test and microscopic examination. hBD-2 immunoreactivity had dramatically decreased in the gastric mucosa specimens obtained after antibiotic treatment (fig 3).

IN SITU HYBRIDISATION

To confirm localisation of hBD-2 expression in *H pylori* infected gastric mucosae at the transcriptional level, we performed in situ hybridisation with hBD-2 riboprobes on frozen sections of the gastric mucosa specimens from cases 1 and 2 in the *H pylori* negative group and cases 11 and 12 in the *H pylori* positive group. Using the hBD-2 antisense probe, a positive signal for hBD-2 mRNA was detected in the surface epithelium of the gastric mucosa specimens obtained from the *H pylori* positive cases. No signal was evident in any of the specimens using the sense probe as a control study (fig 4). As another control experiment, RNaseA treatment of the gastric mucosa specimens from *H*

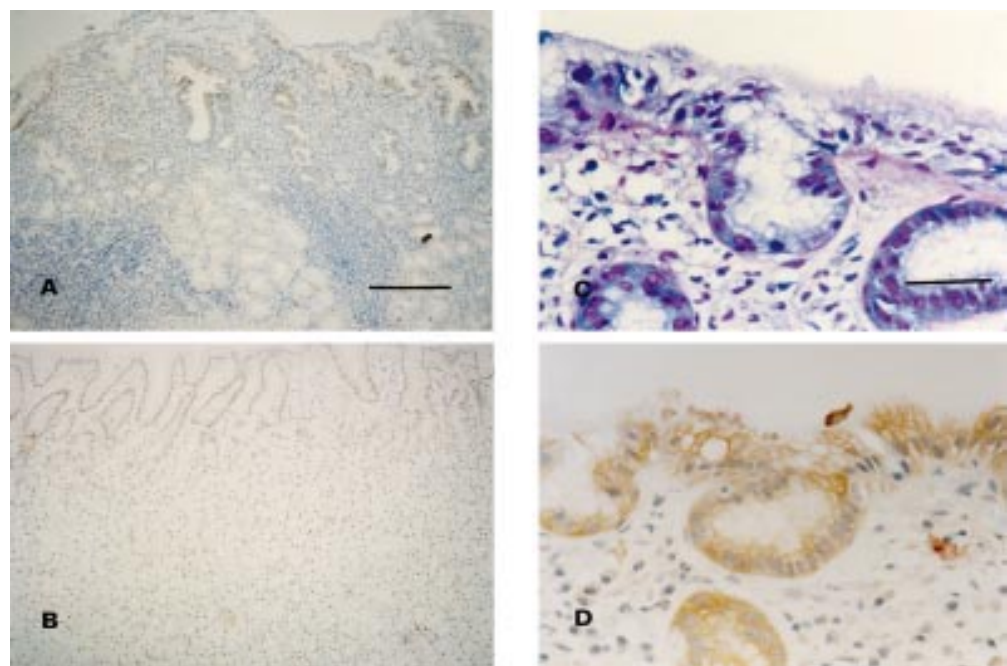


Figure 2 Immunohistochemical analysis of human β -defensin 2 (hBD-2) expression in gastric mucosa specimens with (case 9) (A) or without (case 3) (B) *Helicobacter pylori* colonisation. Both (A) and (B) have the same magnification. The scale bar in (A) represents 200 μ m. (C) May-Giemsa stained section adjacent to (D) and demonstrating colonisation of *H pylori* at the mucosal surface (case 9). (D) Immunoreactivity for hBD-2 in gastric epithelium at a high magnification can be seen. The scale bar in (C) represents 800 μ m.

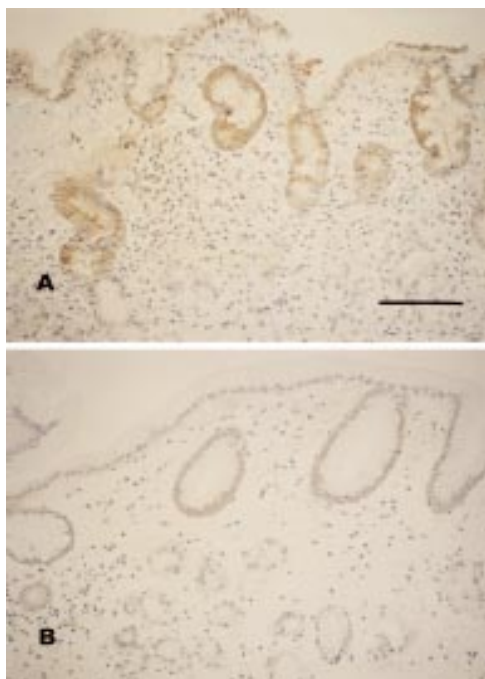


Figure 3 Immunohistochemical analysis of human β -defensin 2 (hBD-2) expression in gastric mucosa specimens obtained before (A) and after (B) *Helicobacter pylori* eradication in case No 7. (A) and (B) have the same magnification. The scale bar in (A) represents 200 μ m.

pylori infected individuals completely abolished the positive signal for hBD-2 mRNA (data not shown).

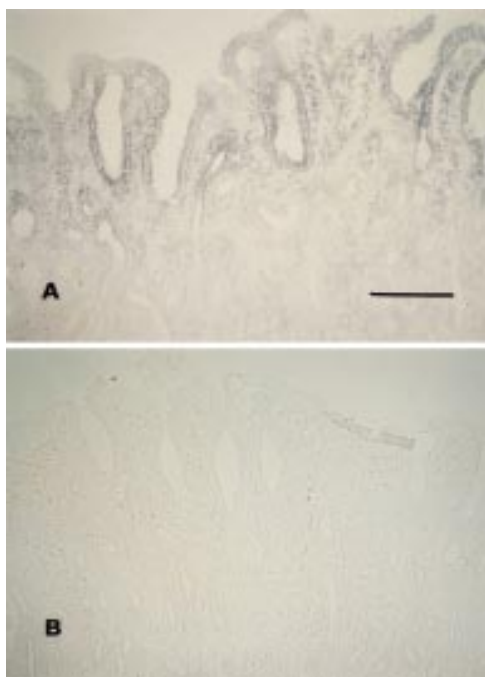


Figure 4 In situ hybridisation of human β -defensin 2 (hBD-2) mRNA in *Helicobacter pylori* infected gastric mucosa specimens. (A) A hybridisation signal with the hBD-2 antisense probe was predominantly observed in the surface epithelium and pits, and also in the inflammatory cells scattered in the interstitium (case 12). The full length hBD-2 antisense probe may cross react with hBD-1 as a leucocytic defensin because the open frame of hBD-2 cDNA shows 39% identity with hBD-1.³⁴ (B) No signals were detected on hybridisation with the hBD-2 sense probe as a negative control (case 12). (A) and (B) have the same magnification. The scale bar in (A) represents 100 μ m.

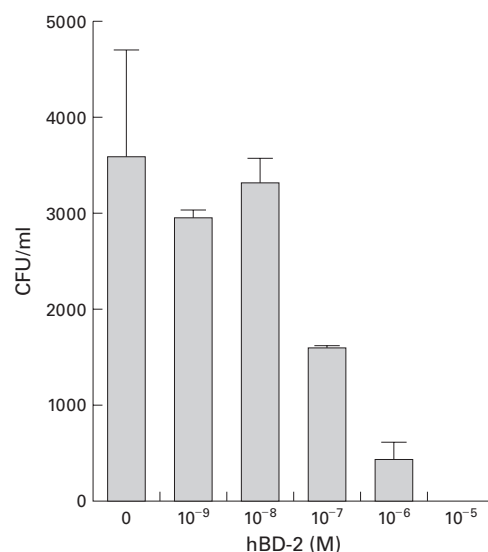


Figure 5 Antimicrobial effect of synthetic human β -defensin 2 (hBD-2) peptides on *Helicobacter pylori* colony formation in an *in vitro* assay. Each bar represents mean (SEM). CFU, colony forming units.

ANTIMICROBIAL ASSAY

To determine whether hBD-2 has an antibacterial effect on *H pylori*, *H pylori* were incubated in solutions containing various concentrations of synthetic hBD-2 peptide for 20 minutes and then plated for three days. The number of CFU of *H pylori* was counted. As shown in fig 5, in *H pylori* that had been pretreated with higher concentrations of synthetic hBD-2, the number of CFU decreased in a dose dependent manner. The number of CFU of *H pylori* on preincubation with hBD-2 at concentrations of 0, 10^{-9} , 10^{-8} , 10^{-7} , 10^{-6} , or 10^{-5} M was 3567 (1129), 2933 (88), 3300 (265), 1567 (33), 433 (167), and 0 (0), respectively. Thus the growth rate of *H pylori* was suppressed by 50% on preincubation with 10^{-7} M (0.3 μ g/ml) hBD-2 peptide, and was completely inhibited on preincubation with 10^{-5} M (30 μ g/ml) of hBD-2 peptide.

Discussion

Although a low level of hBD-2 mRNA is present in healthy epithelia, its expression is greatly upregulated by inflammatory stimuli.¹⁴ The present study revealed that the gastric tissue of patients with *H pylori* infected gastritis overexpressed hBD-2. The gastric specimens from all six *H pylori* positive cases overexpressed hBD-2 mRNA, as assessed by RT-PCR, while those from six *H pylori* negative cases showed only faint hBD-2 expression, suggesting that *H pylori* colonisation induces hBD-2 expression in the gastric mucosa. In our recent report, we demonstrated that the cag pathogenicity island (PAI) of *H pylori* is important for inducing hBD-2 mRNA expression in the MKN45 gastric carcinoma cell line.¹⁸ The 5' flanking region of the hBD-2 gene contains consensus binding sequences for nuclear transcription factor κ B (NF κ B).²⁹ Since cag PAI is essential for *H pylori* mediated interleukin (IL)-8^{30 31} and intercellular adhesion molecule 1³² secretion and these require activation of

NFκB,^{33,34} *H pylori* mediated hBD-2 mRNA induction should also involve direct or indirect activation of NFκB. Further studies are needed to evaluate this hypothesis.

hBD-2 is specifically produced by human epithelial cells^{14,27} but not fibroblasts,¹⁶ and the presence of hBD-2 may have an antimicrobial effect on organ surfaces that are continuously exposed to various microorganisms. The defence mechanism via hBD-2 is completely independent of the leucocyte dependent immune defence mechanisms.¹⁶ Immunohistochemistry revealed that hBD-2 is localised in the *H pylori* infected gastric surface epithelium and gastric pits but not in other gastric glands, inflammatory cells, nor in the lamina propria. That hBD-2 is expressed in the epithelium of the stomach with *H pylori* colonisation was confirmed by in situ hybridisation at the transcriptional level. These findings suggest that exposure to *H pylori* upregulates hBD-2 expression in the gastric surface epithelium. We have recently demonstrated that hBD-2 mRNA is inducibly expressed in MKN45 cells after incubation with *H pylori* while hBD-2 expression is not detected in cells exposed to a culture medium after filtering off *H pylori*.¹⁸ Interestingly, hBD-2 immunoreactivity had dramatically decreased in gastric mucosa specimens obtained 28 days after completion of antibiotic treatment. Furthermore, the level of hBD-2 transcripts in the *H pylori* eradicated gastric mucosa specimens had decreased to an undetectable level by RT-PCR. These findings support the importance of direct contact of *H pylori* with the surface epithelium for inducing hBD-2 expression in the host tissue. Furthermore, it is established that proinflammatory cytokines such as tumour necrosis factor α and IL-1β, which are known to be involved in *H pylori* induced gastritis,³⁵ have been found to upregulate hBD-2 mRNA expression in a dose dependent manner.³⁶ Thus hBD-2 may be an inducible antimicrobial factor produced by gastric mucosa either on contact with *H pylori* or by endogenously released proinflammatory cytokines.

The presence of hBD-2 elicits antimicrobial activity on host organ surfaces.¹⁴ To evaluate the effect of gastric hBD-2 on the growth of *H pylori*, we performed an in vitro antimicrobial assay with synthetic hBD-2. It is well known that hBD-2 activity is highly salt sensitive. Indeed, the ability of hBD-2 to inhibit growth of *Escherichia coli* diminished eightfold when the salt concentration was increased from 20 to 150 mM.²⁸ Therefore, this assay was performed under a hypotonic environment containing 50 mM NaCl. In our experiment, the growth rate of cultured *H pylori* decreased on synthetic hBD-2 pretreatment in a dose dependent manner, and growth was completely inhibited on preincubation with 10⁻⁵M (30 μg/ml) synthetic hBD-2. It has been reported that the LD90 values of natural hBD-2 preparations against *E coli*, *Pseudomonas aeruginosa*, and *Candida albicans* are 10, 10, and 25 μg/ml, respectively.¹⁶ Thus hBD-2 seems to have antimicrobial activity against *H pylori* as well as other Gram negative bacteria and fungi,

suggesting that loss of hBD-2 induction in the stomach may exacerbate *H pylori* induced gastritis. Although colonisation of the human stomach by *H pylori* is a predisposing factor for gastrointestinal illnesses,³⁷ it has recently been demonstrated that *H pylori* may actually have beneficial effects on infected carriers who are heavily exposed to other gastrointestinal pathogens.³⁸ The gastric hBD-2 induced by *H pylori* infection may also play a role in protection from other pathogens.

In conclusion, we demonstrated overexpression of hBD-2 mRNA in all of the biopsied gastric mucosa specimens with *H pylori* colonisation. Immunohistochemistry and in situ hybridisation revealed that in *H pylori* infected gastric mucosa specimens, hBD-2 is specifically expressed in the gastric surface epithelium and pits. The synthetic hBD-2 peptide showed an antimicrobial effect against *H pylori* in vitro. Since a high level of hBD-2 is expressed in the gastric mucosa of patients with severe active gastritis, hBD-2 may be involved in the pathophysiology of *H pylori* induced gastritis.

The authors are grateful to Mr Kawata for his technical assistance in preparing the figures in this paper.

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