

## Localization and expression of two human $\beta$ -defensins (HBD-1 and HBD-2) in intestinal biopsies of celiac patients

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Innate immunity is the first line of defense against microorganisms in vertebrates and acts by providing an initial barrier to microorganisms and triggering adaptive immune responses (Ganz and Lehrer 1999; Oppenheim et al., 2002). Peptides such as  $\beta$ -defensins are an important component of this defense, both providing a broad spectrum of antimicrobial activity against bacteria, fungi, mycobacteria and several enveloped viruses (Ganz and Lehrer, 1994) and showing chemotactic activity by linking and activating CCR6 (Hoover DM et al., 2002).  $\beta$ -defensins are small cationic peptides that vary in their expression patterns and spectrum of pathogen specificity (Ganz and Lehrer, 2002).

The expression of the human  $\beta$ -defensins 1 (HBD-1) and  $\beta$ -defensins 2 (HBD-2) in intestinal epithelial cells both *in vitro* and *in vivo* has been studied by O'Neil et al. (1999). Their findings indicated that HBD-1 is expressed by the epithelium of normal human colon and small intestine, with a similar pattern of expression in inflamed colon. In contrast, they evidenced little HBD-2 expression by the epithelium of normal colon, but abundant HBD-2 expression by the epithelium of inflamed colon.

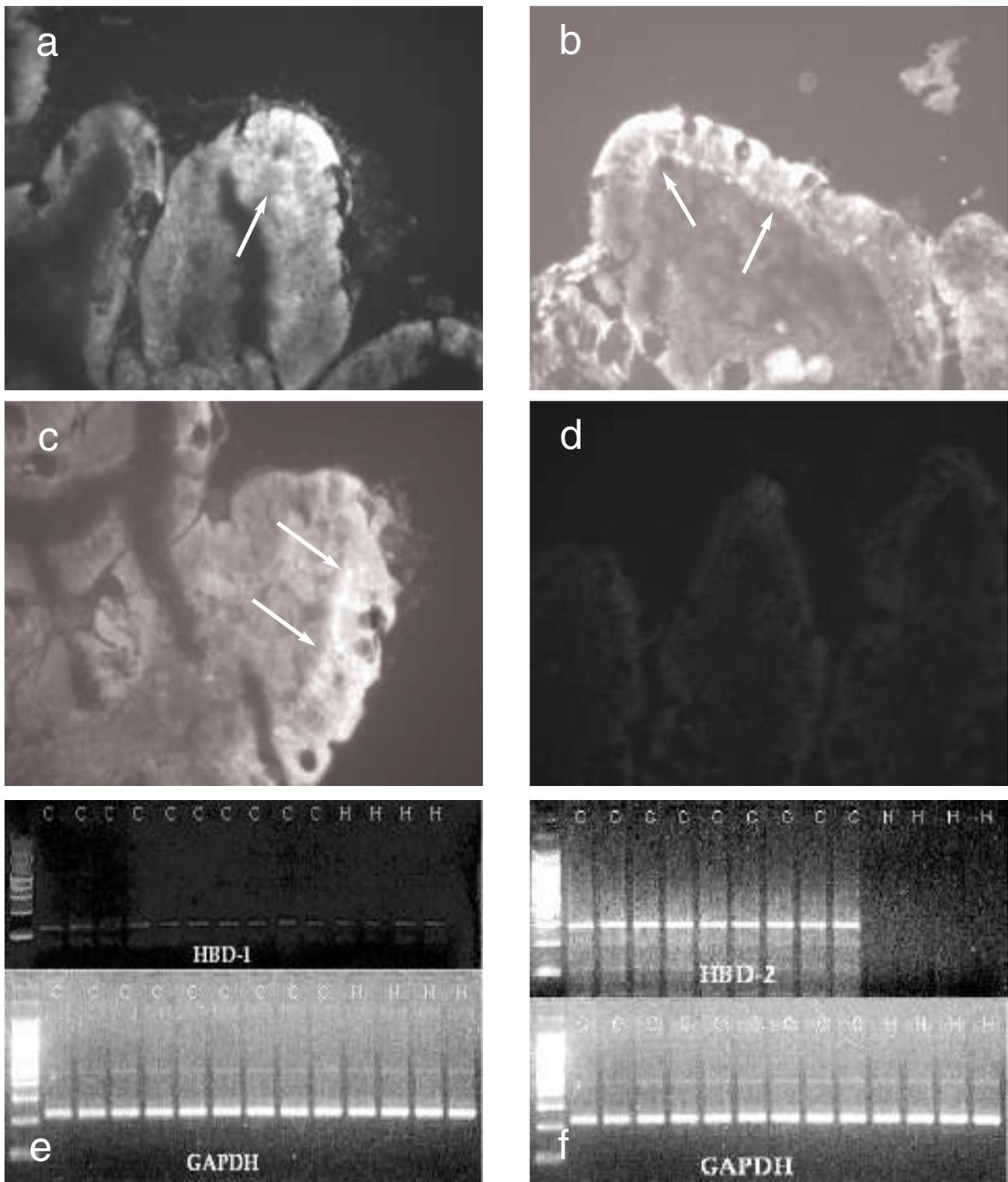
Recently, Wehkamp et al. (2002) investigated

HBD-1 and HBD-2 mRNA and peptide expression in patients with Crohn's disease, ulcerative colitis and unspecified colitis. They demonstrated that HBD-1 is expressed constitutively in colonic tissue irrespective of inflammation, while HBD-2 is barely present in uninflamed colon but it is induced in inflammation.

In our study we investigated the localization and the expression of HBD-1 and HBD-2 in intestinal biopsies of celiac patients, by using both solution and *in situ* RT-PCR.

Frozen intestinal biopsies from 9 celiac patients were collected from the gastroenterology unit of IRCCS Burlo Garofolo in Trieste (Italy). The diagnosis of celiac disease was performed following the ESPGHAN indications (Walker-Smith et al., 1990). Four uninflamed intestinal biopsies from healthy individuals were used as controls. The study was approved by the Ethical Committee of the Burlo Garofolo Children's Hospital and verbal consent was obtained from all the patients. Total RNA was extracted from intestinal biopsies of celiac patients and controls using TRIzol reagent (Life Technologies). RNA (1  $\mu$ g) was reverse transcribed and HBD-1 and HBD-2 cDNA amplified as described by O'Neil et al. (1999). Primers for HBD-1 were: 5'-CCAGTTCCTGAAATCCTGAGTGT-3' (forward) and 5'-CTGTGAGAAAGTTACCACCTGAGG-3' (reverse) and for HBD-2, 5'-GCTCCAGCCATCAGCC-3' (forward) and 5'-CATGTCGCACGTCTCTGATGA-3' (reverse). GAPDH amplification kit (Applied Biosystems) was used as *housekeeping* control. The amplification profile was: 1 hold at 95°C for 10-min followed by 35 cycles of 1-min denaturation at 95°C, 1-min annealing at 60°C, and a 1.5-min extension at 72°C. The same amplification profile was used for GAPDH.

RT *in situ* PCR was performed as described by Boniotto et al. (2003) using Cy3 fluorescent nucleotides (Amersham Pharmacia) and the primers and annealing temperature described



**Figure 1:** a) HBD-1 gene expression revealed by *in situ* RT-PCR: HBD-1 cDNA amplification signal is localized at the level of the surface epithelium (arrows) in the intestinal biopsy of an uninfamed healthy control Magnification is 400X. b) HBD-1 gene expression in intestinal biopsy of a celiac patients: a positive signal is detectable over the surface epithelium (arrows). Magnification is 400X. c) HBD-2 expression in duodenal mucosa of celiac patients: positive signals are localized over the epithelium (arrows). Magnification is 400X. d) HBD-2 expression in duodenal mucosa of healthy control: no positive signal of amplification is visible. Magnification is 400X. e) Solution RT-PCR amplification of HBD-1 in 9 celiac patients and 4 healthy controls: all subjects were characterized by an HBD-1 amplicon of 117bp. GAPDH amplification gave an amplicon of 230bp detectable in all the samples. f) Solution RT-PCR amplification of HBD-2 generated products of 280 bp only in the 9 celiac subjects. No HBD-2 amplicon was detected in healthy controls. GAPDH amplicon (230bp) was detected in all the samples.

above. Negative controls were used for RT and IS-PCR, without either RT or primers.

To determine the expression and the localization of HBD-1 and HBD-2 in intestinal biopsies of celiac patients an RT *in situ* PCR was performed. As shown in Figure 1, HBD-1 was equally expressed by surface epithelium of uninflamed duodenal biopsies from healthy controls (Figure 1a) and by the epithelium of the inflamed duodenal mucosa of celiac patients (Figure 1b). Conversely there was little to no expression of HBD-2 by the epithelium of uninflamed biopsies (Figure 1d), but HBD-2 was expressed by the epithelium of inflamed duodenal mucosa of celiac patients (Figure 1c). No HBD-1 and HBD-2 amplification signal was detected in both no RT and no primer controls (data not shown).

Mucosal  $\beta$ -defensins mRNA expression was also measured by solution RT-PCR: HBD-1 cDNA was detectable in 9 celiac patients as well as in uninflamed duodenal epithelium of healthy controls (Figure 1e); HBD-2 transcript was expressed differentially, being present in celiac patients only, but not in healthy controls (Figure 1f).

The results obtained by solution and *in situ* RT-PCR indicate that HBD-2 mRNA but not HBD-1 mRNA is expressed preferentially in inflamed areas. The expression and distribution of the HBD-1 and HBD-2 in our celiac patients resulted similar to the other gastrointestinal pathologies such as Crohn disease and ulcerative rectocolitis (Wehkamp et al., 2002). Our data confirm the hypothesis (O'Neil et al., 1999) that HBD-1 and HBD-2 occupy distinct functional niches distinguishing an inducible (HBD-2) from a constitutive (HBD-1) form of intestinal epithelial peptide antibiotic. Constitutively expressed HBD-1 could mediate epithelial interactions with the commensal flora,

whereas HBD-2 may participate in the host defense response to enteric microbes that can break the epithelial barrier. Furthermore, as  $\beta$ -defensins show a chemotactic activity, the increased expression of HBD-2 could account for the infiltration of T lymphocytes observed in intestinal biopsies taken from celiac patients.

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