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Cell adhesion properties of human defensins

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ABSTRACT

Effector peptides of innate immunity play an important role in host defense. They act directly by inactivating microbes but also link innate to adaptive immunity. A variety of innate immune functions has been described for these peptides, including chemoattraction and cytokine release. In this study, we describe the effect on cell morphology and cell adhesion of human defensins. We find that Human Defensin 5, the major product of specialized gut epithelial cells, causes changes in cell morphology. HD-5 induces cell adhesion, binds to fibronectin and facilitates binding of T cells to intestinal epithelial cells. These effects were found also for a second prominent defensing, termed Human Neutrophil peptide-1, but not for other human defensins.

1. Introduction

Defensins constitute a major family of antimicrobial peptides that plays a protective role against microbial invasion of various epithelial surfaces, including the skin, respiratory tract and gastrointestinal tract. Primarily, these small cationic peptides act as effectors of the innate immune system with the ability to kill a variety of microbial pathogens, including bacteria, fungi and viruses [1,2]. Based on a difference in disulfide connectivity of six conserved cysteine residues, defensins have been divided in two families, termed α and β. Both families are believed to have evolved from a common ancestral β-defensin gene [3,4], and share similar tertiary structures despite low amino acid sequence identity [5–7]. In humans, β-defensins are widely expressed in epithelial cells. Defensins of the α-family are expressed predominantly in neutrophils (termed human neutrophil peptides or HNP) or in specialized intestinal epithelial cells called Paneth cells in the case of Human Defensin 5 and 6 (HD-5 and HD-6) [8,9].

In addition to their antimicrobial activities, increasing evidence suggests that defensins play a significant role in innate and adaptive immunity and aberrant expression of defensins has been linked to infectious diseases [10,11]. Defensins have been shown to chemoattract a variety of host cells [12–14] and have shown the ability to modulate host cell cytokine responses [15,16]. Recently, a specific deficiency of HD-5 was observed in patients with ileal Crohn's disease, a chronic inflammatory disease [17,18]. These findings have led to the notion that HD-5 as an innate immune effector molecule may play an important role in the maintenance of mucosal balance and that deficiency of HD-5, resulting in modulation of mucosal antibacterial capacity may contribute to pathogenesis [19]. Here, we examine the interaction of this enteric α-defensin and other members of the defensing family with host cells and its role in innate and adaptive immunity.

2. Materials and methods

2.1. Materials

Chemicals used for solid phase peptide synthesis were obtained as described [20]. The Caco-2 and Jurkat cell lines were obtained from the American Type Culture Collection (Manassas, Va).

2.2. Solid phase peptide synthesis

Chemical synthesis and folding of peptides in this study was carried out as described [20,21]. The molecular mass of the peptides was verified by electrospray ionization mass spectrometry (ESI-MS) as described [20].

2.3. Cell culture

Subconfluent monolayers of Caco-2 cells and Jurkat cells in suspension were maintained in RPMI 1640 medium (Gibco),

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supplemented with 10% FBS (Valley Biomedical, Winchester, Va), 2 mM L-glutamine (Quality Biological, Gaithersburg, MD), 20 mM HEPES, 1 x nonessential amino acids, 1 mM sodium pyruvate and 5% Penicillin/Streptomycin (Sigma) in a humidified incubator at 37 °C with 5% CO_2. Caco-2 cells were used between passages 18–35 and were plated at a density of 4×10^4 cells per cm^2. Cell numbers of Jurkat T cells were determined by haemocytometer counts and cell suspensions were adjusted to 1×10^5 cells/ml.

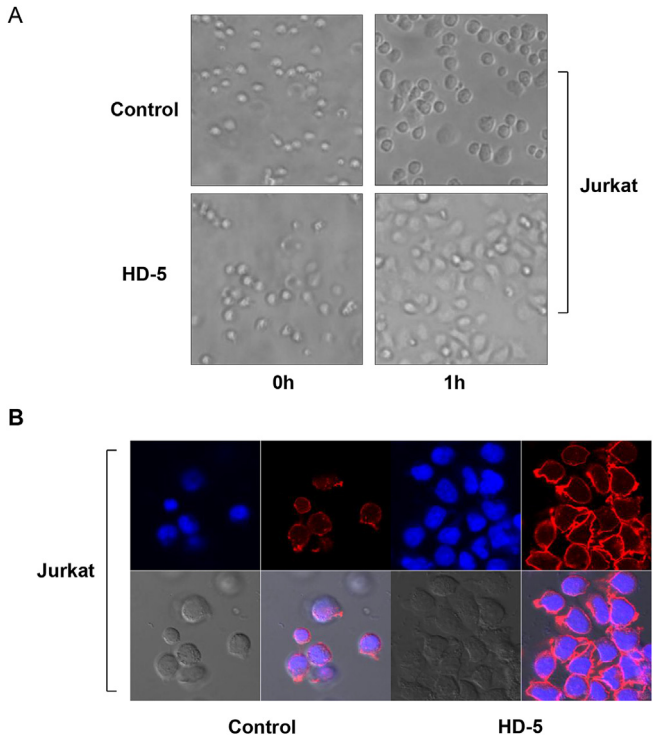

Fig. 1. Cytoskeletal reorganization induced by HD-5. Jurkat T cells were exposed to HD-5 at a concentration of 50 μg/ml for 1 h (**A**) and visualized by phase contrast microscopy; or 4 h (**B**) in serum-free medium. Reorganization of the actin cytoskeleton was visualized by phalloidin staining using Hoechst counterstaining to visualize cellular nuclei.

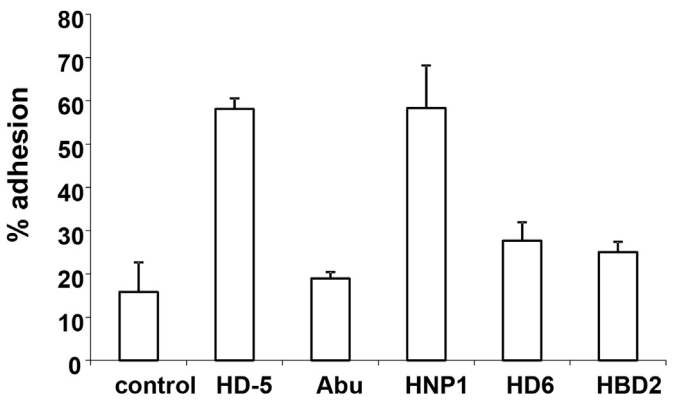

Fig. 2. Cell adhesion by human defensins. Adhesion of Caco-2 cells to fibronectin-coated plates in the presence of 20 μg/ml of each defensin peptide as indicated. For all experiments, adhesion was performed in serum-free medium for 1 h in the presence of defensins. Cells were washed three times with PBS, fixed and stained with crystal violet. Adhesion was measured by determining the absorption at 540 nm and was corrected for adhesion to plates coated with bovine serum albumine. Data represent one experiment carried out in triplicate of three experiments.

2.4. Cell-to-cell adhesion

Jurkat T cells (2×10^6 cells/ml) cells were labeled for 30 min with 5 μM calcein-AM (Sigma Aldrich). Cells were gently washed in serum-free RPMI 1640 medium (Gibco) containing 2 mM L-glutamine (Quality Biological, Gaithersburg, MD), 20 mM HEPES, 1 x nonessential amino acids, 1 mM sodium pyruvate and 5% Penicillin/Streptomycin (Sigma) and resuspended in the same medium at 1×10^5 cells/ml. 250 μl of the cell suspension was subsequently incubated with confluent Caco-2 cells pre-treated with defensins at 20 μg/ml for 2 h and washed twice with serum-free RPMI medium. Following incubation, cells were washed with phosphate-buffered saline twice, fixed in cold phosphate-buffered saline containing 4% paraformaldehyde. Adherent cells were subsequently visualized by fluorescence microscopy.

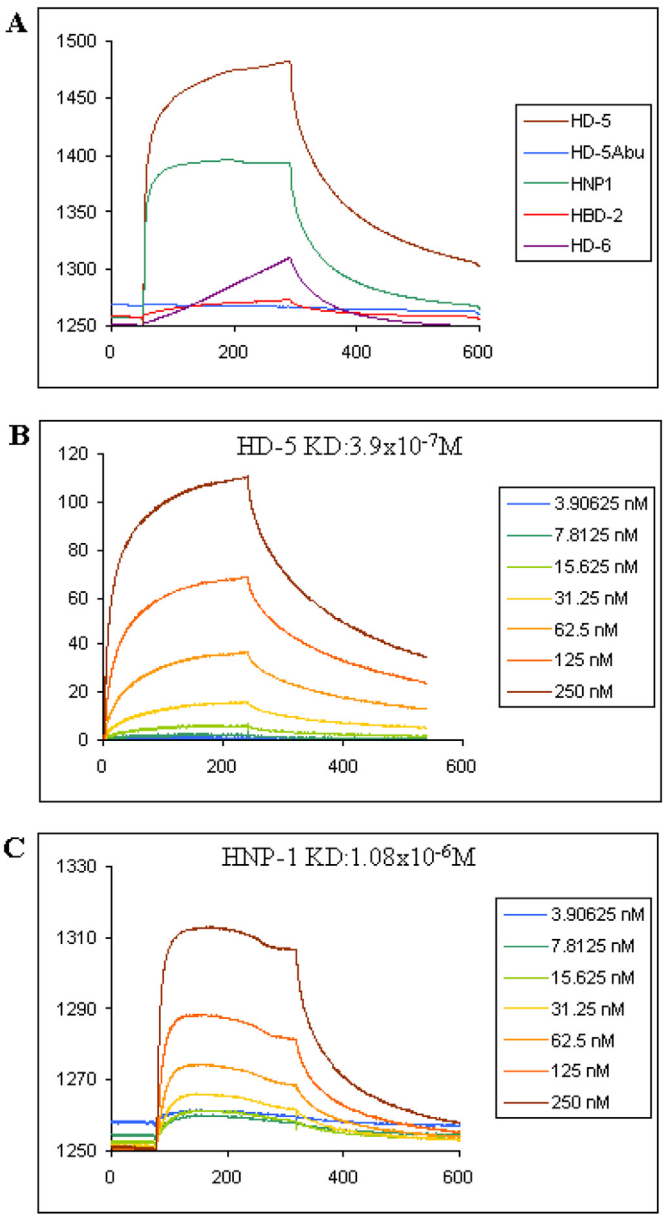

Fig. 3. Binding of human defensins to fibronectin. (**A**) Representative sensorgrams of defensins at 1 μM concentration. Representative sensorgrams of HD-5 (**B**) or HNP1 (**C**) (from 250 nM to 3.90625 nM) A sensor chip with 500 RUs of fibronectin was used.

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