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The concentration-dependent chemokine release and pro-apoptotic effects of neutrophil-derived α-defensin-1 on human bronchial and alveolar epithelial cells

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Abstract

Defensins play a pivotal role in antimicrobial reactions, inflammatory responses, wound repair, and specific immunity. In inflammatory and infectious lung diseases, α-defensins are released from recruited neutrophils, and modulate a variety of lung cell functions. We found that human bronchial and alveolar epithelial cells treated with low and moderate concentrations (5 and 10 μg/ml) of purified neutrophil-derived α-defensin-1 secreted more interleukin (IL)-8 and monocyte chemoattractant protein (MCP)-1 in a dose- and time-dependent manner. Under moderate and high concentrations (10 and 20 μg/ml) of α-defensin-1, we observed typical apoptotic changes in the lung epithelial cells after stimulation for 24 h. Furthermore, α-defensin-1 triggered lung cell detachment in a time- and dose-dependent manner at moderate and high concentrations. Prior to the detachment, caspase-3 activity significantly increased. On confocal laser microscopy, rapid translocation of α-defensin-1 to the endoplasmic reticulum (ER) was noted. These findings suggest that neutrophil-derived α-defensin-1 has pro-inflammatory and apoptotic effects in human bronchial and alveolar epithelial cells, which are concentration-dependent and may be associated with ER activity.

Keywords: Defensin; Interleukin (IL)-8; Monocyte chemoattractant protein (MCP)-1; Apoptosis; Caspase; Endoplasmic reticulum (ER)

Introduction

The defensins are part of the antimicrobial peptide families that comprise the host innate defensive systems, which permit multicellular organisms to live in harmony with microbes (Zasloff, 2002). In humans, there are two main defensin subfamilies, α- and β-defensins (Ganz et al., 1990; Goldman et al., 1997), which are widely distributed in phagocytes, epithelial cells, and tissues that are involved in human host defense against microbial infections (Lehrer et al., 1993; Ganz, 2003). The α-defensins are expressed in neutrophils and Paneth's cells of the intestinal tract, whereas the β-defensins are mainly produced by epithelial cells of various origins (Van Wetering et al., 1999). Human neutrophil defensins, also called human neutrophil peptides (HNP), are small (3.5–4 kDa), cationic polypeptides (Ganz et al., 1990). They are constitutively synthesized by the neutrophil precursors during specific differentiation stages of neutrophil development (Cowland and Borregaard, 1999; Harwig et al., 1994) and are packaged in the primary (azurophil) granules (Rice et al., 1987).

In addition to their antimicrobial activity (Selsted et al., 1984; Lehrer and Ganz, 2002), α-defensins have the capacity to be chemotactic for monocytes (Territo et al., 1989), naïve T cells (Chertov et al., 1996) and dendritic cells (Yang et al., 2000) by inducing chemokine/cytokine synthesis (Van Wetering et al., 1997; Zhang et al., 2004; Sakamoto et al., 2005). α-Defensins also have the capacity to enhance bacterial adherence to airway epithelial cells (Rice et al., 1987; Gorter et al., 1998), induce LTB4 and IL-8 release by alveolar macrophages (Van Wetering et al., 1997; Paone et al., 1999), and induce histamine release by mast cells (Befus et al., 1999). High α-defensin concentrations

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are present in inflammatory tissues (Van Wetering et al., 1997), as well as in the airway secretions of patients with chronic inflammatory lung disorders (Ashitani et al., 1998). Furthermore, high α-defensin concentrations have been reported to be cytotoxic to airway epithelial cells (Aarbiou et al., 2002; Sakamoto et al., 2005). However, most of the intracellular molecular mechanisms that are related to α-defensin-associated biological phenomena have not been fully elucidated.

In this study, we calibrated the cell survival to study the chemokine release from human lung bronchial and alveolar epithelial cells treated with neutrophil-derived α-defensin-1. We found that α-defensin-1 had diverse concentration-dependent biological effects on the cells, which appear to be associated with endoplasmic reticulum (ER) activity.

Materials and methods

Reagents

α-Defensin-1 was purchased from Peptide Institute, Inc. (Osaka, Japan). The broad-spectrum caspase inhibitor (zVAD-FMK) and fluorogenic caspase-3 substrate (Ac-DEVD-AMC; *N*-acetyl-Asp-Glu-Val-Asp-7-amino-4-methylcoumarin) were purchased from Calbiochem (La Jolla, CA, USA). Propidium iodide (PI) and *n*-octyl-*b*-D-glucopyranoside (OG), and Hoechst nuclear dye were obtained from Sigma–Aldrich (St. Louis, MO, USA). Goat anti-human α-defensin-1 antibody, rabbit anti-mouse IgG antibody conjugated with FITC, and swine anti-goat IgG antibody conjugated with Cy3 were obtained from Chemicon International (Temecula, CA, USA). Mouse anti-human ER-specific glycoprotein antibody was obtained from Molecular Probes (Eugene, OR, USA). Anti-fade mounting medium was purchased from Vector Laboratories (Burlingame, CA, USA). HRP-conjugated rabbit anti-goat IgG antibody and the SuperSignal substrate were obtained from Pierce (Rockford, IL, USA). CytoBusterProtein Extraction Reagent was obtained from Novagen (Madison, WI, USA). Bovine serum albumin, cell culture media, and supplements were purchased from Life Technologies (Grand Island, NY, USA). Chemokine ELISA kits for IL-8 and MCP-1 were obtained from R and D Systems Inc (Minneapolis, MN, USA). Reverse transcriptase and *Taq* DNA polymerase were purchased from Invitrogen (San Diego, CA, USA) and Promega (Madison, WI, USA), respectively.

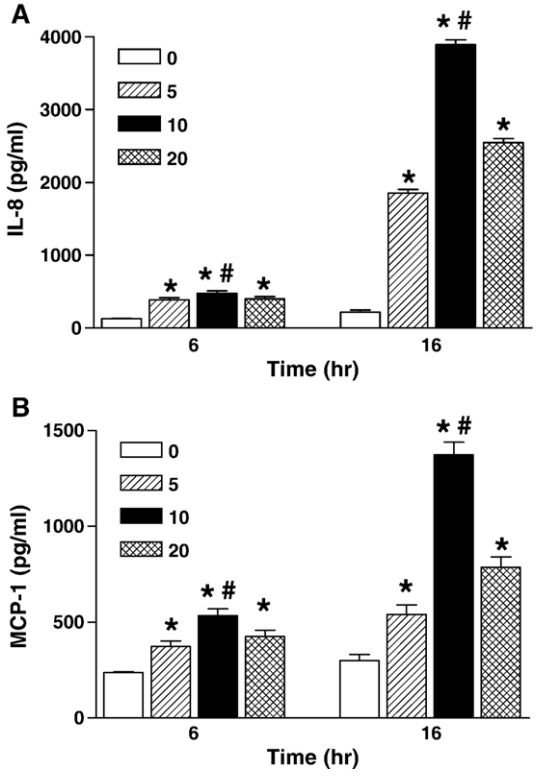

Fig. 1. Induction of IL-8 and MCP-1 secretion in lung A549 cells stimulated with neutrophil-derived α-defensin-1 at concentrations of 5, 10 and 20 μg/ml. (A) IL-8 and (B) MCP-1 protein secretion determined by ELISA; means±SEM of three independent assays are shown. *$p<0.01$, compared with the cells in the absence of defensin; #$p<0.01$, compared with the cells treated with α-defensin-1 at concentrations of 5 and 20 μg/ml.

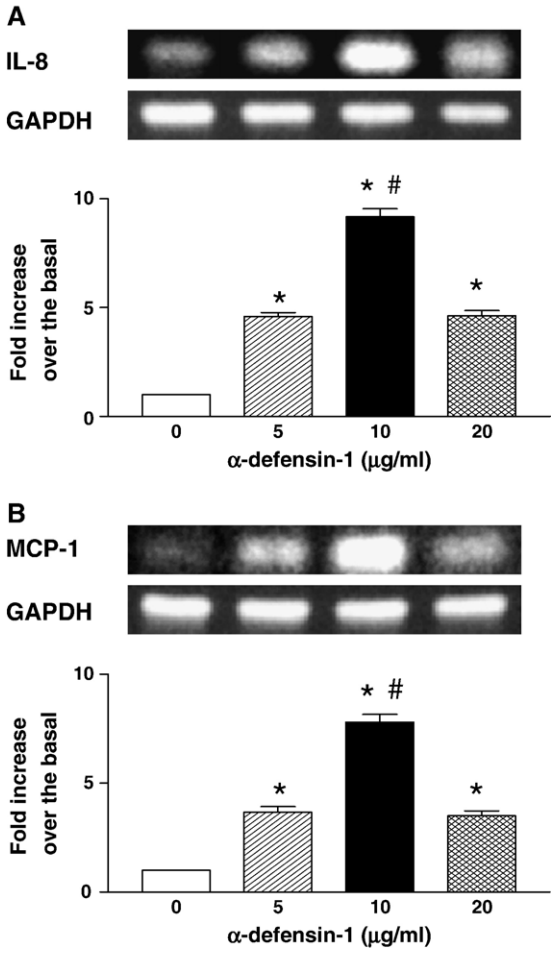

Fig. 2. Relative expression of (A) IL-8 and (B) MCP-1 genes determined by RT-PCR corrected for RNA density using GAPDH expression levels. Cells were treated with α-defensin-1 (5, 10 and 20 μg/ml) for 4 h. Fold change over expression in untreated control cells is demonstrated and represents the mean± SEM of three independent assays. *$p<0.01$, compared with the cells in the absence of defensin; #$p<0.01$, compared with the cells treated with α-defensin-1 at concentrations of 5 and 20 μg/ml.