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Human β-Defensin-2 Induction in Nasal Mucosa after Administration of Bacterial Lysates

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Background and Aims. The airway epithelium produces antimicrobial peptides (AMPs) that prevent colonization of host tissues by a wide range of pathogens. Human β-defensin 2 (hBD-2) is one of the most well-documented AMPs in humans. Several bacterial products can induce production of this peptide. Bacterial immunostimulants containing bacterial lysates have long been used in the treatment of respiratory infections, but their effects on hBD-2 release have not been investigated. We undertook this study to induce production of hBD-2 after stimulation of the nasal mucosa with bacterial lysates.

Methods. A nasal lavage (NL) was performed in 12 healthy volunteers under basal conditions and after a nasal challenge with separate and subsequent stimuli with either bacterial lysates (20 million), cholecalciferol (400 IU), or sham-challenge with glycerol plus isotonic saline solution. Immunohistochemistry was performed in nasal biopsies 48 h after stimulation with bacterial lysates to identify the presence of hBD-2.

Results. Increased levels of hBD-2 (4668.99 ± 2829.33 pg/mL) were measured with ELISA in NL fluids following bacterial challenge. However, hBD-2 concentrations were below the limit of detection in NL fluids at baseline and after the administration of cholecalciferol or the sham-challenge. Through immunohistochemistry, hBD-2 was predominantly localized to the epithelium.

Conclusions. hBD-2 can be induced in the nasal mucosa after administration of bacterial lysates. Stimulation of the innate immune system to produce hBD-2 could be used to prevent or even treat infections caused by respiratory pathogens.

Key Words: Antimicrobial peptides, Defensins, Host defense, Nasal mucosa, Immunostimulants, Bacterial lysates.

Introduction

Respiratory tract infections (RTIs) represent one of the most common and important causes of human disease in terms of morbidity, mortality, and economic cost to society. In fact, RTIs are the leading reasons for absences at work and school. Despite their structural vulnerability, the respiratory airways successfully resist infection through a variety of mechanical, humoral, and cellular mechanisms (1). First, airway epithelia have the ability to clear and eliminate inhaled particles using mechanical clearance of mucus, involving ciliary activity and regulation of the proper amount of salt and water on airway surfaces via transepithelial ion transport (1,2). Second, airway epithelium secretes antibodies, mostly A isotype (secretory IgA − sIgA), which exert an important protective activity against RTI (3). Finally, the airway epithelium produces antimicrobial peptides (AMPs), which prevent colonization of host tissues by a range of pathogens at nanomolar concentrations (4). Defensins are perhaps the most well-documented AMPs in

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humans. To date, six human β-defensins (hBD) are known: hBD-1 through hBD-6 (although genomic analyses suggest there may be more) (5–7). Among these, hBD-2 has been investigated extensively in several diseases (8). hBD-2 was first isolated from psoriatic scales as an inducible peptide (9). Subsequently, it was shown that the cytokines interleukin-(IL)-1, tumor necrosis factor (TNF), and bacterial products such as lipopolysaccharides (LPS) stimulate the production of hBD-2 from airway epithelial cells (10–11). More recently, it has been reported that other bacterial products such as peptidoglycans and flagellin also induce production of hBD-2 (12).

Bacterial immunostimulants containing bacterial lysates (BL) have long been used in the treatment of respiratory infections, in particular in children suffering recurrent respiratory tract infections (13,14). Currently there are large numbers of these in the market: bacterial components mimic or, to a certain extent, repeat the immune response evoked by the intrusion of a pathogen into the human body and, although the mechanism of action is not fully understood, BL induce a range of effects on the immune system. These effects include enhanced expression of adhesion molecules on monocytes and neutrophils, upregulation of the respiratory burst-superoxide and nitrite production by alveolar macrophages, production of key proinflammatory cytokines (tumor necrosis factor α [TNF-α], IL-8, IL-6, monocyte chemotactic protein-1, interferon gamma) (15–19). However, the effect of bacterial immunostimulants on hBD-2 release has not yet been investigated. In the present study we administered intranasal BL to normal subjects and showed hBD-2 release. Through immunohistochemistry, the airway epithelium was identified as the major cell source of hBD-2.

Materials and Methods

Study Population

Twelve healthy volunteers aged 18–25 years participated in the study (none was atopic as assessed by skin prick test to common allergens). All subjects were nonsmokers without any pulmonary diseases and were not taking any medication either at the time of the study or during the preceding 3 months. The study was approved by the institutional ethics committee and complied with good clinical practices (GCP). Informed written consent was obtained from all participants.

Study Design and Nasal Challenge

A nasal lavage (NL) under basal conditions was performed in each subject using the modified Greiff and Grünberg method (20,21). Then, nasal challenges with separate and subsequent stimuli with either BL (20 million), cholecalciferol (VD3, 400IU), or sham-challenge with glycerol plus isotonic saline solution (1 g glycerol and sodium chloride at 0.65 g/100 ml of solution) were performed. The total dose was divided into four and administered every 12 h, for 36 h for every challenge. Finally, NL were performed 12 h after the last dose of each stimulus. A 15-day washout period was given after each nasal challenge. All volunteers answered a standard questionnaire following nasal challenges to evaluate the presence of secondary effects such as nasal obstruction, itching, burning, rhinorrhea, sneezing, or fever.

Sample Processing

NL were centrifuged at 1500 rpm for 10 min to precipitate mucus and detritus. Bacterial cultures were done to discard any ongoing infection. Once the mucus was eliminated, the supernatant was concentrated 10X by lyophilization. The concentrated supernatant was then stored at −70°C until the ELISA test was performed (see below).

Immunostimulant Agents

BL and VD3 were used to perform nasal challenges. BL were obtained from IPI ASAC (International Pharmaceutical Immunology, ASAC S.A., Mexico; distributor of ASAC Pharmaceutical Immunology, Alicante, Spain). This formula has a BL concentration of 2600 million/mL and includes the following bacteria: *Streptococcus pneumoniae, Brahmanela catarrahalis, Staphylococcus aureus, Haemophilus influenzae, α-hemolytic Streptococcus*, β-*hemolytic Streptococcus, Streptococcus fecalis, Staphylococcus epidermidis, Bordetella pertussis, Proteus* sp, *Pseudomonas* sp, *Escherichia coli,* and *Corynebacterium diphteriae*. VD3 was obteined from Sigma (Deisenhofen, Germany). Both agents were diluted with glycerol and isotonic saline solution and were then instilled into the nasal mucosa (42 μl per dose).

ELISA

Concentrations of hBD-2 in NL were measured using specific sandwich ELISA using the Human BD-2 ELISA Development Kit from PeproTech (Rocky Hill, CT) following manufacturer's recommendations. Briefly, ELISA plate (Maxisorb Immunoplate, Nunc, Wiesbaden, Germany) was covered with the capture antibody (0.25 μg/mL) overnight at room temperature, then washed three times with 0.05% Tween-20 in PBS, and blocked with 1% bovine serum albumin (Sigma) in PBS (overnight/4°C). Samples (100 μl each) were added in duplicate and incubated 2 h at room temperature. After three washes, biotinylated antigen-affinity purified goat anti-hBD-2 (0.5 μg/mL) was added and incubated 2 h at room temperature. The reaction was amplified adding avidin peroxidase diluted at 1:2000 in PBS containing 0.05% Tween-20 and 0.1% BSA for 30 min at room temperature. Azino′bis