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Albumin and amino acids upregulate the expression of human beta-defensin 1

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Abstract

Antimicrobial peptides are essential components of the innate immune system and are the first line of defense against invading pathogens. Human beta-defensin 1 (hBD-1) is the most important antimicrobial peptide in human epithelia, its expression is constitutive in most tissues, and it is not induced in instances of infection or inflammation. In addition to its antibacterial activity, hBD-1 has an immunomodulatory activity by recruiting immune cells. Our objective was to determine whether immune-enhancing ingredients, such as arginine, isoleucine, and polyunsaturated fatty acids (PUFA), which are added to immune-enhancing formulas, mediate their beneficial activity by bolstering the immune system through hBD-1 induction. Real-time PCR analysis of hBD-1 in human colon cells, HCT-116, revealed upregulation after treatment with arginine, isoleucine, and bovine serum albumin (BSA). BSA increased transcription as well as secretion of hBD-1. hBD-1 upregulation in response to BSA treatment was concomitant with c-myc over-expression, suggesting that hBD-1 may be regulated via a non-inflammatory pathway involving c-myc. Assuming similar control in vivo, our results may imply that nutrients found in immune-enhancing formulas upregulate hBD-1 expression, which, in turn, recruits the adaptive immune system. This sequence of events bolsters of the immune system, which may lead to fewer infectious complications, less antibiotics usage, and a shorter hospital stay, as indeed has been shown in patients fed immune-enhancing formulas.

Keywords: Defensin; Expression; hBD-1; Immunonutrition; BSA; c-myc

1. Introduction

The innate and adaptive immunity are two essential elements of host defense. Innate immunity is highly conserved from fruit flies to human and is the first line of defense against invading pathogens (Yuan and Walker, 2004). One mechanism of the innate immunity is the secretion of broad-spectrum antimicrobial substances, such as cathelicidins and small cationic polypeptides named defensins (Ganz, 2003; Froy and Gurevitz, 2003). Defensins act as direct antimicrobial effectors by disrupting membrane integrity and function, which ultimately leads to the lysis of the microorganisms (Yang et al., 2002). The two main defensin subfamilies, α- and β-defensins, differ in length and pairing of the six cysteines (Selsted and Ouellette, 2005). α- and β-defensins are salt-sensitive and the direct antimicrobial effect occurs in vacuoles of phagocytes and on mucosal epithelia, where there is low ionic strength (Goldman et al., 1997; Yang et al., 2002). Human alpha-defensins include human neutrophil peptides 1 to 4 (HNP1 to HNP4) and human defensin-5 (HD-5) and HD-6 that are expressed primarily in intestinal Paneth cells (Selsted and Ouellette, 2005).

Human beta-defensins include six members (hBD-1 to hBD-6) that are expressed in a wide variety of tissues (Lehrer and Ganz, 2002; Ganz, 2003). hBD-1, which was originally isolated from the plasma of patients with end-stage renal disease (Bensch et al., 1995), is the most important antimicrobial peptide in human epithelia against infection as its expression is constitutive in most tissues (Lehrer and Ganz, 2002; Ganz, 2003). Human colon epithelial cell lines, such as HT-29 or Caco-2, constitutively express hBD-1 and its expression is not upregulated after stimulation with IL-1α, TNF-α, IFN-γ, LPS, *Salmonella dublin*, or *Escherichia coli*. Similar levels of hBD-1 mRNA were also found in intestinal biopsies taken from healthy and inflamed colonic mucosa of Crohn's disease and ulcerative colitis patients (O'Neil et al., 1999; Wehkamp et al., 2002; Lehrer and Ganz, 2002; Froy, 2005). Although in most cases hBD-1 expression is

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constitutive, there are some instances in which hBD-1 expression has been shown to be upregulated, e.g., in monocytes exposed to LPS, or IFN-γ; in uterine epithelial cells stimulated with Toll-like receptor 3 agonists [poly(I:C) or double-stranded RNA]; and in pulmonary gland epithelial cells exposed to bacilli Calmette–Guerin cell wall components (Duits et al., 2002; Fang et al., 2003; Zhu et al., 2003; Schaefer et al., 2005).

Human beta-defensin 2 (hBD-2) and hBD-3 are induced by bacterial patterns or pro-inflammatory cytokines. hBD-2 and hBD-3 expression is upregulated in several diseases, such as inflammatory lung, bowel, and skin diseases (Harder et al., 1997; Fellermann et al., 2003; Claeys et al., 2003). While hBD-2 is mainly present in skin, respiratory, and gastrointestinal tracts, hBD-3 is expressed in epithelial and non-epithelial tissues, such as heart, liver, and skeletal muscle (Harder et al., 1997, 2001; Schroder and Harder, 1999; Garcia et al., 2001a). hBD-4 is expressed in the testis, gastric antrum, uterus, neutrophils, thyroid gland, lung, and kidney (Garcia et al., 2001b). Its expression can be upregulated by bacterial infection but not by inflammatory factors that upregulate hBD-2 and hBD-3 (Garcia et al., 2001b). Recently, hBD-5 and hBD-6 have been identified and localized to the epididymis (Yamaguchi et al., 2002). However, their antibacterial role in the testis is not yet understood. In addition to their direct antimicrobial activities, hBD-1 and hBD-2 possess chemotactic activities for immature dendritic cells and memory T-cells by binding to the chemokine receptor CCR6, while hBD-3 and hBD-4 elicit monocyte chemotaxis (Yang et al., 1999; Garcia et al., 2001a, 2001b; Harder et al., 2001; Jia et al., 2001; Raj and Dentino, 2002). Moreover, hBD-2 activates mast cells to induce chemotaxis, histamine release, and prostaglandin (PGD_2) production, suggesting the involvement of hBD-2 in allergic reactions (Niyonsaba et al., 2001, 2002). Thus, β-defensins contribute to adaptive immunity through the induction of immune cell migration and maturation. Altogether, defensins play a crucial and indispensable role in both the innate and adaptive immune responses.

Chronic disease, major surgery, trauma, or malnutrition results in immune compromise and can increase the risk of infection and sepsis, which may lead to organ failure and death (Bone et al., 1992). The three most commonly infected sites are the respiratory tract, the urinary tract, and the bloodstream (Richards et al., 2000). There is increasing evidence that early feeding of patients reduces the occurrence of infectious complications (Bengmark and Gianotti, 1996; Vincent and Preiser, 1999). Several key nutrients (arginine, glutamine, dietary nucleotides, and fish oil) have been shown to enhance immune function in patients (Daly et al., 1988; Kinsella et al., 1990; Kirk et al., 1993; Grimble, 2001). Several large clinical studies have demonstrated that patients fed immune-enhancing formulas had significantly fewer infectious complications, needed antibiotics for a shorter period of time, and had a shorter hospital stay than did patients fed standard, high-nitrogen formulas (Daly et al., 1992; Kemen et al., 1995; Beale et al., 1999). However, the mechanism by which nutrients bolster the immune system is unclear. Therefore, we examined the hypothesis that hBD-1 upregulation is a possible mechanism by which immune-enhancing ingredients bolster the immune system. We found that arginine, isoleucine, and albumin are specific hBD-1 upregulators in human colon tumor cells, HCT-116. In addition, we found that hBD-1 upregulation coincided with c-myc over-expression, suggesting the involvement of c-myc in hBD-1 induction.

2. Materials and methods

2.1. Cell culture and treatments

Human colon carcinoma cells (HCT-116) were maintained at 37 °C in a humidified incubator with 95% air and 5% CO_2 in Dulbecco's modified eagle medium (DMEM) (Sigma, Israel), 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin (Biological Industries, Israel). Cells were grown to 80% confluence and the medium was then replaced by DMEM as control or with DMEM supplemented with different concentrations of L-arginine, L-isoleucine, linolenic acid-conjugated Fraction V bovine serum albumin (BSA), linoleic acid-conjugated Fraction V BSA, Fraction V BSA, or fatty acid free Fraction V BSA (Sigma, Israel) for 6 h. After 6 h, media were collected and cell total RNA was extracted.

2.2. Fatty acid conjugation with Fraction V bovine serum albumin

Fatty acids were conjugated to BSA, as was previously described (Svedberg et al., 1990). Briefly, 20% (w/v) BSA was heated to 37 °C and 200 mM fatty acids in ethanol were added to yield 8 mM fatty acid-conjugated BSA. The conjugate was further diluted with DMEM to a final concentration of 0.8 mM. Control cells stimulated with only BSA were treated with medium prepared as above except that only the carrier ethanol was added to the albumin.

2.3. RNA extraction and quantitative real-time polymerase chain reaction

For hBD-1 expression analyses, total RNA was extracted from HCT-116 cells using TRI Reagent (Sigma, Israel). Total RNA was DNase I-treated using RQ1 DNase (Promega, USA) for 2 h at 37 °C, as was previously described (Froy et al., 2003). 2 μg of DNase I-treated RNA were reverse transcribed using MMuLV reverse transcriptase (Promega, USA) and random hexamers. 1/20 of the reaction was then subjected to quantitative real-time PCR using the Sybr Green Master kit (Applied Biosystems, USA) and the ABI Prism 7300 Sequence Detection System. Primers for hBD-1, hBD-1-F 5′-ttacttttgtctgagatggcctcag-3′, hBD-1-R 5′-gccaaggcctgtgagaaagtta-3′ and hBD-2, hBD-2-F 5′-tgatgcctcttccaggtgttt-3′, hBD-2-R 5′-ggcaggtaacaggatcgcc-3′ were tested alongside the normalizing gene Glyceraldehyde 3 phosphate dehydrogenase (*Gapdh*), GAPDH-F 5′-catgttcgtcatgggtgtgaa-3′, GAPDH-R 5′-tgcaggaggcattgctgat-3′.

2.4. Western blot analysis

After stimulation of HCT-116 with BSA for 6 h, the supernatants were removed, and the cells were harvested, centrifuged