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B-defensins – Underestimated peptides in influenza combat

Barbara Małgorzata Kalenik, Anna Góra-Sochacka, Agnieszka Sirko*

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5A, 02-106 Warsaw, Poland

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ABSTRACT

Defensins are a family of host defense peptides present in vertebrates, invertebrates and plants. They display broad antimicrobial activity and immunomodulatory functions. Herein, the natural anti-influenzal role of β-defensins, as well as their potential usage as anti-influenza vaccine adjuvants and therapeutic agents, is reviewed. This article summarizes previously published information on β-defensin modes of action, expression changes after influenza infection and vaccination, biotechnological usage and possible boosting of their production by dietary supplementation.

1. Introduction

Antimicrobial peptides are conserved in evolution and produced by many species, including humans. These incredible proteins are not only able to directly inhibit many bacteria, viruses and fungi but also show important roles in the modulation of the immunological response. Moreover, they pose a low risk of triggering microbial resistance (Zhang and Sunkara, 2014). The natural occurrence of these proteins in mucosal liquid has a strong influence on host protection, as they are an essential component of the primary immune defense in the lungs (LeMessurier et al., 2016). These features make them extremely interesting candidates for therapeutic agents and vaccine adjuvants. Although reviews on the antimicrobial potential of host defense peptides have previously been published (Hsieh and Hartshorn, 2016; Mattar et al., 2016; Zhang and Sunkara, 2014), their significance as immunomodulating agents and enhancers of vaccine efficacy remains underestimated. Herein, we focus on reports related to influenza infection and vaccination and discuss the potential role of defensins in defense against influenza virus and their prospective application as vaccine adjuvants.

2. Defensins

Defensins are short, cationic peptides produced by vertebrates, invertebrates and plants. They contain 6–8 (in vertebrates) highly conserved cysteine residues that form intramolecular disulphide bonds and are assumed to possess a conserved structural fold (Mattar et al., 2016). Vertebrate defensins are classified as α-, β- and θ-defensins. The β-defensins consist of 38–42 amino acids, contain a conserved pattern of 6 cysteins with pairing 1–5, 2–4 and 3–6 (Sugiarto and Yu, 2004). The α-defensins are shorter than the β-defensins and have different Cys pairings; however, their folding remains similar (Ganz, 2003). The α-defensins are found exclusively in mammals. The θ-defensins probably evolved in primates, although in humans they acquired mutations resulting in a premature stop codon. Avian β-defensins were initially named gallinacins; however, independent reports by Lynn et al. (2004) and Xiao et al. (2004) demonstrated inconsistent numeration resulting in the termination of that moniker (Lynn et al., 2007). Additionally, β-defensin-related molecules called ovo defensins were found in birds (Zhang and Sunkara, 2014). Even though 6 cysteines in the C-terminal mature region form a disulfide bonding pattern identical to β-defensins, the cysteine spacing patterns in the primary structures of ovo defensins are different.

3. Functions of β-defensins

Depending on the cell type and the modulated process, β-defensins display many functions (Fig. 1). They overlap functionally with chemokines and bind to their receptors (Yang et al., 2002), thus causing chemoattraction of various cells such as macrophages, monocytes, T cells, mast cells, neutrophils, immature dendritic cells and fibroblasts (Sugiarto and Yu, 2004; Kohlgraf et al., 2010). In addition, these types of cells are influenced by β-defensins in numerous ways. For example, they can activate macrophages (Barabas et al., 2013), enhance

Abbreviations: DC, dendritic cells; HA, hemagglutinin; HBD-1, Human β-defensin 1; HBD-2, Human β-defensin 2; HBD-3, Human β-defensin 3; hpi, hours post infection; IAV, influenza A virus; MBD-1, Mouse β-defensin 1; MBD-2, Mouse β-defensin 2; MBD-3, Mouse β-defensin 3; MBD-4, Mouse β-defensin 4; pDC, plasmacytoid dendritic cells; PR8, A/H1N1/PR/08/1934 influenza strain; rMBD-2, recombinant mouse β-defensin 2; rMBD-3, recombinant mouse β-defensin 3; rMBD-4, recombinant mouse β-defensin 4

* Corresponding author.
E-mail address: asirko@ibb.waw.pl (A. Sirko).

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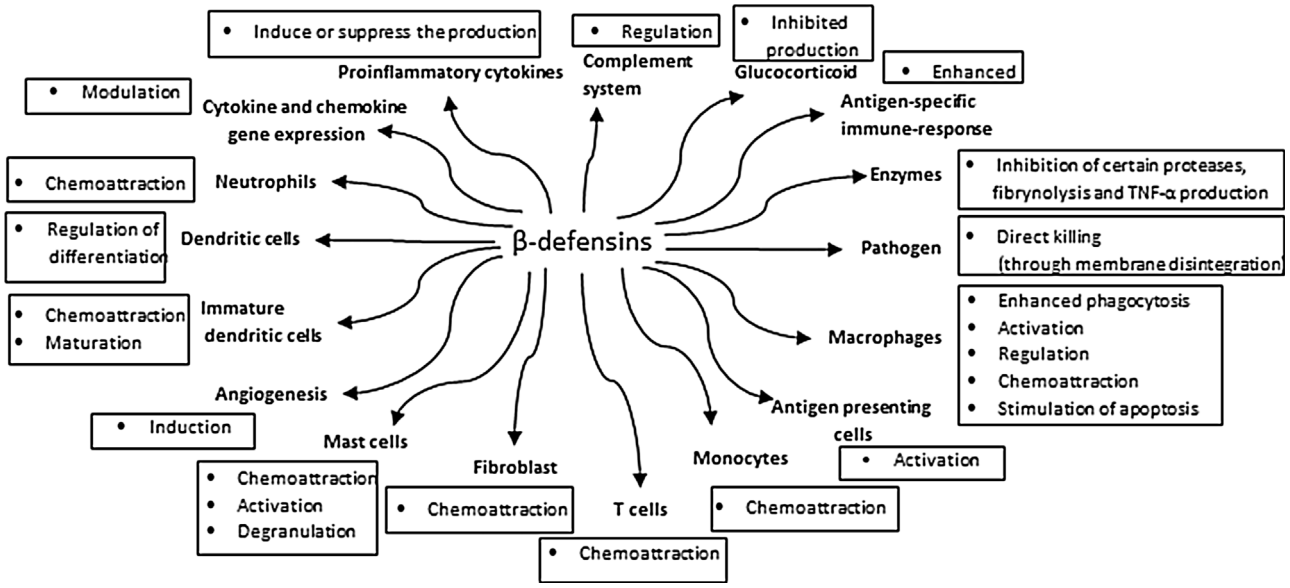

Fig. 1. Impact of β-defensins on different targets. TNF-α: tumor necrosis factor-α.

phagocytosis of macrophages (Kohlgraf et al., 2010) and increase neutrophil uptake (Tecle et al., 2007), regulate macrophages during chronic inflammation (Sugiarto and Yu, 2004), provoke mast cell degranulation (Sugiarto and Yu, 2004; Kohlgraf et al., 2010) and regulate maturation (Biragyn et al., 2002) and differentiation (Zhang and Sunkara, 2014) of dendritic cells. Moreover, one of the β-defensins was proven to activate professional antigen presenting cells via toll-like receptors (TLR-1 and TLR-2) (Funderburg et al., 2007). They also induce or suppress the production of cytokines and chemokines depending on the antigen (Kohlgraf et al., 2010). Furthermore, they regulate the complement system; inhibit production of glucocorticoids, certain proteases, fibrinolyses and TNF-α production to inhibit tissue injuries, and prevent bacteria spreading, inflammation and septic shock (Sugiarto and Yu, 2004; Kohlgraf et al., 2010). In addition, they can also cause induction of angiogenesis (Zhang and Sunkara, 2014).

B-defensins are able to bind microorganisms and their components, which attenuates the proinflammatory cytokine response and simultaneously enhances antigen-specific antibody responses (Kohlgraf et al., 2010; Zhang and Sunkara, 2014). Moreover, they are able to directly kill microorganisms through disintegration of their membrane (Sugiarto and Yu, 2004; Yang et al., 2002). Particular defensins differ in their abilities to bind various antigens (Doss et al., 2009) and cause an immune system response; however, their function overlaps with some chemokines and other antimicrobial peptides such as cathelicidins (Niyonsaba et al., 2002; Yang et al., 1999). For that reason, knockouts of any single defensin gene may not result in a clear-cut phenotype. The avian β-defensin 13 and the mouse β-defensin 2 serve as endogenous TLR4 ligands but the former upregulates co-stimulatory molecules and the latter downregulates TLR4 expression (Yang et al., 2010; Biragyn et al., 2002). Interestingly, improper expression of β-defensins is associated with colonic Crohn's disease (Gersemann et al., 2012).

4. Antiviral potential of β-defensins

Antiviral potential of β-defensins differ from one peptide to another (Mattar et al., 2016). Perhaps it is the reflection of their unique features or different mechanisms of antiviral action. Sequences of the β-defensins described below are shown in Fig. 2. The available crystal structures of various β-defensins indicate the tendency to form monodimers (eg HBD-1, HBD-2 and HBD-3) and higher-order oligomers like octamers (Suresh and Verma, 2006). Dimerization is not energetically favored for a select groups (e.g. sheep, goat and mouse defensins), suggesting that they function efficiently as monomers (Suresh and Verma, 2006).

4.1. Anti-influenza activity of human β-defensins

The anti-influenza activity has been reported for three human β-defensins. The neutralizing activity of HBD-1 and HBD-2 against A/H3N2/1982 was demonstrated in Madin-Darby Canine Kidney (MDCK) cells (Doss et al., 2009). Both defensins significantly reduced the infectivity of the influenza virus compared with the control ($p < 0.05$) at all tested concentrations. Human β-defensins do not bind significantly to the surfactant protein D (thus they do not reduce its antiviral activity), which is the most potent collectin for inhibiting influenza A viruses (IAV), and it opsonizes viral hemagglutinin (HA) and neuraminidase in a calcium-dependent manner (Doss et al., 2009). HBD-2 slightly (but statistically significantly) increased IAV uptake by neutrophils (Tecle et al., 2007). This feature might be advantageous when considering use of these peptides for the anti-viral therapy in lungs. In turn, the human β-defensin 3 (HBD-3) has blocked viral fusion by creating a protective barricade of immobilized surface proteins (Leikina et al., 2005).

Human β-defensins are less potent than α-defensins in direct inhibition of influenza viruses; however, it is possible that β-defensins play an important immunomodulatory role (Hsieh and Hartshorn, 2016). Human β-defensin 1 (HBD-1) is constitutively expressed in low amounts by peripheral blood mononuclear cells (PBMC). Its expression rate in PBMC increases after stimulation with A/H1N1/PR/08/1934 (PR8) influenza virus; however the fold-change depends on the individual (Ryan et al., 2011). Stimulation of plasmacytoid dendritic cells (pDC) and monocytes with influenza virus also increases the expression rate of HBD-1; however, such stimulation of normal human bronchial epithelial cells results in decreased expression of this antimicrobial peptide (Ryan et al., 2011). The expression of human β-defensin 2 (HBD-2) increases upon stimulation of numerous cell types with proinflammatory cytokines (Krisanaprakornkit et al., 2000; Lehmann et al., 2002; McDermott et al., 2003).

4.2. Anti-influenzal activity of mice β-defensins

The anti-influenza activity of mice β-defensins was investigated by several research groups (Table 1). The mouse β-defensin 1 (MBD-1) has been proven to be a functional homolog of HBD-1 (Morrison et al.,