Candida Infections and Human Defensins

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Abstract: Background: Candida species infections are an important worldwide health issue since they do not only affect immunocompromised patients but also healthy individuals. The host developed different mechanisms of protection against Candida infections; specifically the immune system and the innate immune response are the first line of defence. Defensins are a group of antimicrobial peptides, components of the innate immunity, produced at mucosal level and known to be active against bacteria, virus but also fungi.

Objectives: The aim of the current work was to review all previous studies in literature that analysed defensins in the context of Candida spp infections, in order to investigate and clarify the exact mechanisms of defensins anti-fungal action.

Methods: Several studies were identified from 1985 to 2017 (9 works form years 1985 to 1999, 44 articles ranging from 2000 to 2009 and 35 from 2010 to 2017) searched in two electronic databases (PubMed and Google Scholar). The main key words used for the research were “Candida”, “Defensins”, “Innate immune system”, “fungi”.

Results and conclusions: The findings of the reviewed studies highlight the pivotal role of defensins antimicrobial peptides in the immune response against Candida infections, since they are able to discriminate host cell from fungi: defensins are able to recognize the pathogens cell wall (different in composition from the human ones), and to disrupt it through membrane permeabilization. However, further research is needed to explain completely defensins’ mechanisms of action to fight C. albicans (and other Candida spp.) infections, being the information fragmentary and only in part elucidated.

Keywords: defensins, Candida, mucosa, innate immunity, infection.

1. INTRODUCTION

Fungal infections are a worldwide health issue whose incidence has dramatically increased over the past three decades, not only affecting immuno-compromised patients (causing gastric or life-threatening systemic candidiasis) but also healthy individuals [1].

More than 200 fungal species have been classified so far, some of which being universally present in human body as commensal resident flora [2]. Among them, Candida strains and particularly Candida albicans, show the strongest clinical relevance being the fourth cause of nosocomial septicemia in many European nations as well as in the USA [3]. These yeasts, normally present in skin and mucosae of human natural cavities (such as oral cavity, gastrointestinal tract, and vagina) [4, 5] can behave as opportunistic pathogens when the host defences are impaired. In fact, more than 17 Candida strains are known as etiological agents for human infections [6] and among them C. albicans is the most prevalent in both mucosal and systemic forms [7], leading to serious morbidity and mortality [3].

Naturally occurring antimicrobial peptides such as defensins are integral part of the innate immune system acting against bacteria, protozoa, fungi, and viruses.

In this review article we will assess the interplay between the most studied human α and β-defensins, and Candida spp., focusing our attention on C. albicans (See Table 1). This work identifies different studies from 1985 to 2017 (9 works from years 1985 to 1999, 44 articles ranging from 2000 to 2009 and 35 from 2010 to 2017) searched in two electronic databases (PubMed and Google Scholar). The main key words used for the research were “Candida”, “defensins”, “innate immune system”, “fungi”.

2. CANDIDA MAJOR TRAITS

The pathogenicity of Candida spp. is related to a complex pattern of attributes, denominated as “putative virulence factors”, that fungi exploit during infection processes; their expression is different among strains and depends upon envi-
Table 1. Summary of studies describing the interplay between *C. albicans* and defensins.

<table>
<thead>
<tr>
<th>Interaction with <em>C. albicans</em></th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td><strong>α-Defensins</strong></td>
<td></td>
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<tr>
<td>HNP-1 Cause depletion of intracellular ATP in <em>C. albicans</em> inducing loss of cell viability</td>
<td>[55]</td>
</tr>
<tr>
<td>Isolated in neonatal faeces showing low activity against <em>C. albicans</em></td>
<td>[67]</td>
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<tr>
<td>Augmented expression in whole blood cells infected with <em>C. albicans</em></td>
<td>[56]</td>
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<tr>
<td>Augmented expression during <em>Candida</em> esophagitis</td>
<td>[58]</td>
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<tr>
<td>DEFB1 and DEFB103A polymorphisms increase susceptibility to recurrent vulvovaginal candidiasis</td>
<td>[61]</td>
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<tr>
<td>HNP-3 Augmented expression during <em>Candida</em> esophagitis</td>
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<tr>
<td>DEFB1 and DEFB103A polymorphisms increase susceptibility to recurrent vulvovaginal candidiasis</td>
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<tr>
<td>HNP-2 Isolated in neonatal faeces showing low activity against <em>C. albicans</em></td>
<td>[67]</td>
</tr>
<tr>
<td>Augmented expression during <em>Candida</em> esophagitis</td>
<td>[58]</td>
</tr>
<tr>
<td>HNP-4 Kill <em>C. albicans</em> yeast form (not pathological) <em>in vitro</em></td>
<td>[64]</td>
</tr>
<tr>
<td><strong>HD-5</strong> Concentration-dependent microbicidal activity</td>
<td>[66]</td>
</tr>
<tr>
<td>Isolated in neonatal faeces showing low activity against <em>C. albicans</em></td>
<td>[67]</td>
</tr>
<tr>
<td><strong>HD-6</strong> Permeabilize <em>C. albicans</em> membrane, probably through “nanonets”</td>
<td>[70]</td>
</tr>
<tr>
<td><strong>β-Defensins</strong></td>
<td></td>
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<tr>
<td>hBD-1 Together with hBD-2 and hBD-3 cause membrane permeabilization and cell death</td>
<td>[36]</td>
</tr>
<tr>
<td>A polymorphisms in DEFB1 gene (-44C/G) possibly associates with type I diabetes and oral candidiasis</td>
<td>[86]</td>
</tr>
<tr>
<td>hBD-2 hBD-2 mRNA is upregulated after <em>C. albicans</em> contact in human keratinocytes</td>
<td>[79, 81]</td>
</tr>
<tr>
<td>Upregulated in oesophagus during <em>C. albicans</em> infection</td>
<td>[80]</td>
</tr>
<tr>
<td>hBD-3 Upregulated in oesophagus during <em>C. albicans</em> infection</td>
<td>[79, 80]</td>
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</table>

Environmental conditions (reviewed in [8]), infection sites (systemic or mucosal) and immune host responses ([9]). The most important putative virulence factors are yeast-to-hypae transition (dimorphic transition), phenotypic switching (e.g. ability to switch between different cell phenotypes), adherence, biofilm formation (on human tissue and surfaces of medical devices), cell-wall associated adhesins, invasion, hydrolytic enzymes secretion and host immune defence elusion ([9, 10]). The dimorphic transition is one of the most interesting features of *C. albicans*: through morphogenic changes in its membrane with the constitution of β glucan cyclic form structure, known as hyphal formation ([10], the harmless commensal *C. albicans* yeast converts in a fungal pathogen capable of invading and affecting the host cells ([11]). The fungal membrane structure is crucial for the recognition of the pathogen by host and it has been supposed that innate immune system can discriminate between yeast and hyphal form of *C. albicans* ([12], even if the mechanisms are still debated. Moreover, the hyphal features seem to increase proinflammatory cytokine production in human peripheral blood mononuclear cells (PBMCs) and to enhance inflammosome activation in macrophages ([13]).

Considering *C. albicans* localization, the commensal form can be found in the intestine and vagina of more than 50% of healthy asymptomatic subjects, whereas the hyphal form is exclusive of subjects with active infection ([14]). In most cases, *C. albicans* infections are localized at mucosal level such as in vulvovaginal diseases or oral thrush, rarely at
skin and nails. Instead, when patients are immune-compromised due, for example to systemic e.g. antibiotic exposure, prolonged hospitalization, neutropenia (caused by multiple cycle of chemotherapy) or infectious diseases (i.e. AIDS), C. albicans could colonize lungs or oesophagus leading to visceral infections that could be fatal [4, 7].

INTERPLAY BETWEEN CANDIDA AND HOST IMMUNE CELLS

Interaction between C. albicans cells and the host is a complex molecular process that involves both adaptive and innate immune responses. During infection, the first host defence response is leaded by Pattern Recognition Receptors (PRRs) that can identify Pathogen Associated Molecular Patterns (PAMPs, pathogen signatures present on cell wall) [15]. Different types of PRRs have been identified, such as Toll Like Receptors (TLRs), C-type Lectin Receptors (CLR), NOD-Like Receptors (NLRs), mannose receptors (MR), dectin-1, dectin-2, dendritic cell-specific ICAM-grabbing non-integrin (DC-SIGN), complement and immunoglobulin Fc receptors (Crs, FeRs) [16-21].

TLRs are a family of transmembrane glicoproteins that recognize specific PAMPs and play a crucial role in detecting fungal infection; recognition is mediated through a leucine rich-repeat standing in outer domain that activate a downstream signalling through its cytoplasmatic domain (Toll-Interleukin Receptor, TIR domain) and a series of adaptor molecules such as Myeloid Differentiation factor (MyD-88), TIR domain-containing Adaptor Protein / MyD88-adaptor-like (TIRAP/Mal), TIR-domain containing adapter-inducing interferon-β (TRIF), and TRIF-related adaptor molecule (TRAM), that are necessary for inducing inflammatory responses [22]. MyD-88, the most well characterized adapter, form the “myddosome” with the IL-1 receptor associated kinase (IRAK), thus activating Nuclear Factor-kb (NF-kb) and Mitogen-activated protein kinase (MAPK) signaling pathways that comprehend three different pathways: p38, c-Jun N-terminal kinase (JNK), and extracellular signal-regulated kinase1/2 (ERK1/2) [22]. All TLRs, with the exception of TLR3, express MyD-88, and it has been demonstrated that mice MyD-88/- are more susceptible to C. albicans infections, even if the strain is less virulent, highlighting the importance of TLRs in the protection process during candidiasis [23, 24]. Specifically, only C. albicans hyphae pathogen form promotes a strong activation of the p38 pathway, inducing the transcription factor c-Fos, and of the ERK1/2 pathway giving raise to MAPK phosphatase 1 (MKP1) that in turns regulate p38 and JNK signalling. C-Fos together with NF-kB pathway activation lead to the production of cytokines and inflammatory mediators that stimulate innate and adaptive immune response against C. albicans [25].

Different studies conducted in mice suggest that the TLRs most involved in C. albicans infections are TLR2 [23, 26], TLR4 [27] and TLR9 [28]; interestingly, TLRs are able to trigger an antimicrobial peptides response (reviewed by [25]) rather than activating proinflammatory cytokines. TLR2 and TLR4 pathways have been shown to increase hBD-2 expression following pathogen stimulation [29] by lipopolysaccharide (LPS) in intestinal cells [30] and by Entamoeba histolytica in Caco2 cell line [31]. On the other hand the same hBD-3 could activate TLRs 1 and 2 responses in antigen presenting cells (such as monocytes and dendritic cells) thereby stimulating adaptive immune responses [32].

DEFENSINS

At the mucosal level, the innate immune system, possibly through NLRP3 inflammasome, is one of the first lines of defence that can discriminate between the two different forms of C. albicans [10]. The immune system can activate the production of soluble effectors such as antimicrobial peptides (AMPs) or cytokines, or activate the complement system with the aim of damaging the pathogen and protecting the host organism [7]. AMPs are defined as host defense peptides since they are able to kill microorganisms, but also have multiple functions in modulating the host immune system, such as stimulation of chemotaxis and wound healing [33]. Defensins are an evolutionary ancient group of AMPs that can intervene in infections caused by bacteria, virus and fungi [34].

Defensins are able to discriminate between host cells and pathogens due to differences in their membrane composition, being the latter characterised by negatively charged phospholipids and lack of cholesterol [35], and after recognition, these peptides, due to their cationic characteristic, can disrupt micro-organism through permeabilization of its membrane [34, 36] (Figure 1).

Human defensins have been divided in two main groups, α- and β-defensins according to their cysteine residues localization and disulphide bonds formation. Several α- and β-defensins have been widely described in humans: the better characterized are α-defensins 1-4, produced by neutrophils [37]; the α-defensins 5 and 6 expressed by intestinal epithelial Paneth cells [38]; the β-defensins 1, 2, 3 and 4 expressed by epithelial cells [39, 40] (Table 2 reports the tissue specific production of the defensins [41]).

So, defensins are abundant in immune cells and tissues involved in defence and are present at highest concentration in leucocyte granules (>10 mg/ml) (reviewed in [42]). When a neutrophil ingest a pathogen, granules empty their content in vacuoles promoting the contact with antimicrobial agents. Paneth cells (small intestinal defence cells) are other sites where defensins are rich in concentration, particularly in secretory granules that release their content in intestinal crypts (about 10 mg/ml in the crypts) (reviewed in [42]). Finally, epithelial cells produce defensins constitutively or in an inducible manner with an average concentration of 10-100 μg/ml (reviewed in [42]). It is important to note that defensins antimicrobial action should be more efficient in granules where the peptides are more concentrated respect to epithelial barriers where they are released in the extracellular region and so they are more diluted. Additionally, the granules containing defensins are produced constitutively by neutrophils being ready to be released in the site of infection; instead, inducible defensins are produced by epithelial cells after an infectious stimulus and only one member of the family (the human β defensin 1) is produced constitutively. Nevertheless, defensins exhibit a strong activity in saliva samples, where both neutrophils secretion and mucosal production are present. Different studies have been conducted on
salivary defensins, since in this location defensins are particularly expressed and active [43-47]. Indeed, the oral cavity is highly exposed to external stimuli and microorganisms, so the mucosal protection is dramatically important in preserving oral health by rinsing and delivering antimicrobial agents such as defensins that could have a critical role for oral thrush sustained by Candida.

α-DEFSINS

In Humans six α-defensins have been described [39]; the first four are called Human Neutrophil Peptides (HNPs-1 to -4) because they are stored in Neutrophil Azurophilic Granules [37] before being released in the tissue, where they take part to non-oxidative killing of phagocytized pathogens [39]. Human α-defensin-5 (HD-5) and Human α-defensin-6 (HD-6) are often referred as intestinal defensins since produced in Paneth intestinal cells [38]; furthermore, HD-5 is located in the genital female tract, too [48].

HNP-1 and HNP-3 are encoded by DEFA1 and DEFA3 genes respectively; the HNP-2 peptide seems to be codified by DEFA1 and DEFA3 genes and obtained by post-translational proteolytic cleavage, since no separate gene has been identified. Finally, DEFA4, DEFA5 and DEFA6 genes encode for HNP-4, HD-5 and HD-6 respectively. These genes are located at 8p22-23, together with genes encoding β-defensins [49]. DEFA1 and DEFA3 genes are characterized by copy number polymorphisms and unequal number of these genes can be inherited [50]. The α-defensins have 29-35 aminoacidic residues and HNP-1, HNP-2 and HNP-3 have the same amino acidic structure, except the N-terminus: HNP-1 presents an Alanine while HNP-3 an Aspartic acid and these residues precede a Cysteine. This Cysteine residue corresponds to the N-terminus of HNP-2 [51]. The tertiary structure of the α-defensins is quite similar, the difference resides in the position of the six cysteine residues forming the three intra-molecular disulfide bonds: Cys1–Cys6, Cys2–Cys4, and Cys3–Cys5 [52]. HNP-3, HNP-4, HD-5 and HD-6 form characteristic dimers as observed using crystallography methods [52, 53]. HD-5 tertiary structure presents high positively charged residues that confer to HD-5 the highest antimicrobial activity among human α-defensins [52].

α-defensins have multiple functions in the immune system: they stimulate mast cell degranulation, regulate complement activation, enhance migration of T cells and increase macrophage phagocytosis [54].

Antibacterial activity of HNP-1 against C. albicans has been investigated by Edgerton et al. [55]: the antimicrobial mechanism is very similar to Histatin 5, causing an efflux of ATP and a depletion of intracellular stocks of the pathogen [55].

In experimental conditions, neutrophils have been reported to secrete HNP-1 and HNP-3 at high concentration when exposed to C. albicans [56]. Moreover, patients af-
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Table 2. Most studied defensins tissue distribution.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>RNA expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEF1</td>
<td>α-Defensin 1 (HNP-1)</td>
<td>spleen, lung and liver; heart muscle; endometrium; thyroid gland; adipose tissue; cerebral cortex; kidney; breast; caudate; pituitary gland; kidney; breast; caudate; skeletal muscle; hypothalamus.</td>
</tr>
<tr>
<td>DEF3</td>
<td>α-Defensin 3 (HNP-3)</td>
<td>spleen; lung; liver; heart muscle; thyroid gland; breast; pituitary gland; kidney; cerebral cortex; caudate; skeletal muscle; hippocampus; hypothalamus; pancreas; cerebellum; vagina; salivary gland; skin; stomach; adrenal gland; prostate; ovary; colon; fallopian tube; small intestine; oesophagus; testis; urinary bladder.</td>
</tr>
<tr>
<td>DEF4</td>
<td>α-Defensin 4 (HNP-4)</td>
<td>spleen and lung; liver; heart muscle; adipose tissue; endometrium; breast; pituitary gland; thyroid gland; cerebral cortex; kidney; testis; skeletal muscle; caudate; hypothalamus.</td>
</tr>
<tr>
<td>DEF5</td>
<td>Human Defensin-5 (HD-5)</td>
<td>small intestine; colon; breast; stomach; testis; oesophagus; pancreas; pituitary gland; liver; spleen; adrenal gland; ovary; vagina; cerebellum; salivary gland; hypothalamus; caudate; adipose tissue; heart muscle, lung, hippocampus; thyroid gland; prostate; cerebral cortex; skin.</td>
</tr>
<tr>
<td>DEF6</td>
<td>Human Defensin-6 (HD-6)</td>
<td>intestine; colon; oesophagus; testis; skin; breast; stomach; adrenal gland; pituitary gland; adipose tissue; vagina; ovary; liver; pancreas; salivary gland; spleen.</td>
</tr>
<tr>
<td>DEF1</td>
<td>β Defensin-1 (hBD-1)</td>
<td>salivary gland, kidney and pancreas; skin, oesophagus; vagina; liver; uterine cervix; prostate; breast; stomach; urinary bladder; colon; pituitary gland; small intestine; adipose tissue; fallopian tube; thyroid gland; skeletal muscle; testis; heart muscle; endometrium; hypothalamus; adrenal gland; spleen; ovary; cerebral cortex; caudate.</td>
</tr>
<tr>
<td>DEF4A</td>
<td>β Defensin-2 (hBD-2)</td>
<td>oesophagus and salivary gland; lung; stomach; vagina; skin; small intestine.</td>
</tr>
<tr>
<td>DEF103A*</td>
<td>β Defensin-3 (hBD-3)</td>
<td>tonsil; skin; oesophagus; breast; epididymis; adipose tissue.</td>
</tr>
<tr>
<td>DEF104A*</td>
<td>β Defensin-4 (hBD-4)</td>
<td>epididymis; oesophagus; small vesicle.</td>
</tr>
</tbody>
</table>

*The Human Protein Atlas” data [41] have been reported, choosing GTEx dataset for RNA expression, except for DEF103A and DEF104A, reporting * HPA dataset. Distribution data have been reported in order from the higher to lesser RNA and protein expression.

Affected by oral candidiasis with possible precancerous lesions, the areas of epithelia infected by C. albicans showed higher presence of inflammatory cells and increased concentration of HNPs, particularly in the extracellular matrix around neutrophils, rather than in healthy regions [57] (Figure 2).

C. albicans takes part to the commensal gastrointestinal flora and in some cases (patients affected by diabetes or malignancy) could colonize oesophagus leading to Candida esophagitis, characterized by inflammation with neutrophils infiltration and subsequently strong secretion of HNP-1 and -3 [58].

HNP-1 and HNP-3 also take part in the innate defence of vagina [59] and DEF1 and DEF3 genes polymorphisms could influence predisposition to develop vulvovaginal candidiasis (VVC), a fungal infection caused by C. albicans [60]. VVC can evolve in recurrent VVC (RVVC) when four or more episodes of VVC occur during a year, affecting 7-8% of women that experienced a first episode in their childbearing age and resolving after menopause [14]. Boatto and co-workers observed that genetic variations within DEF1 and DEF3 genes could be related with RVVC and its severity [61].

Several works measured the fungicidal activities of HNP-1, -2 and -3 against Candida spp.. Raj and colleagues found that the candidacidal activity (tested through the loss of C. albicans viability) of synthetic HNP-1 is about 5-fold higher than synthetic HNP-2, while HNP-3 seems to have no effect on C. albicans [62]. The difference between HNP-1 and HNP-3 is an aminoacidic residue at N-terminus, Ala (not polar) and Asp (negatively charged) respectively; these differences could be crucial for their fungicidal effect [62, 63].
To the best of our knowledge, only one study has been conducted on HNP-4 and C. albicans in its yeast, not pathologic form, reporting that HNP-4 has also microbicidal activity [64]. Furthermore, HD-5 recombinant analogue is able to kill C. albicans in a concentration dependent manner [65] and it has been shown that vaginal epithelial cells infected with C. albicans respond better to the infection if transfected with HD-5 recombinant plasmids (associated with LL-37 recombinant plasmids), inhibiting the fungus growth [66]. Antimicrobial components, in particular HNP-1, HNP-2 and HD-5, have been found in meconium and neonatal faecal extracts, reflecting their presence in foetal and neonatal gut; however extract of neonatal faeces (but not of meconium) have shown low antimicrobial activity against C. albicans [67].

Concluding, Mathews and Nagaraj observed that synthetic analogues of HD-6 could interact with C. albicans in vitro and kill it, speculating that HD-6 can damage C. albicans by permeabilizing its membrane [68] maybe through the formation of “nanonets”, little structure formed by HDs-6 self-assembly that entrap pathogen microorganism, [69], although this mechanism has been reported in bacteria and not yet in fungi [70].

The Table 1 reports the principal interaction between α-defensins and C. albicans

**Figure 2.** *Candida* infection through endocytosis and subsequent phagocytosis: after phagocytosis IL1β, TNF and IL6 are produced. These cytokines promote hBD2 and overall hBD3 production as well as stimulate neutrophils to produce reactive oxygen species (ROS) and Neutrophil Extracellular Traps (NETs), thus triggering the formation of Candida iphae. hBD3 is able to activate antigen presenting cells (APC) such as monocytes and dendritic cells, thus prompting the immune response.

**β-DEFENSINS**

β-defensins are small cationic peptides produced by all epithelial tissues [39] with antimicrobial activity against gram-negative bacteria, enveloped virus and fungi [34], including Candida spp. [71]. β-defensins do not only damage infective micro-organisms, but also exhibit chemo-attractant functions for immature dendritic cells, monocytes/macrophages, and mast cells [35]. Differently respect to α-defensins they possess a shorter and less anionic pro-peptide, have up to 45 aminoacidic residues, contain relatively more lysines than arginines and present a Cys1–Cys5, Cys2–Cys4, Cys3–Cys6 cysteine pairing for the formation of the three intra-molecular disulfide bonds.

The tertiary structure of β-defensins, consist in three β-sheet arranged in an antiparallel sheet linked by three disulphide bridges and an alpha helix of variable length, stabilized by a disulphide bridge [72].

Eleven Human β-Defensins (hBDs) have been identified and functionally characterized so far: hBD-1 to -8 and hBDs 18, 29, 31 [73]. The genes encoding β-defensins map on chromosome 8p22 (where all known α and β-defensins are clustered) and present inheritable copy number polymorphisms [74]. When comparing to α-defensins, β-defensins are encoded by genes with two instead of three exons [74].
The most studied and well-characterized β-defensins are hBD-1, hBD-2, hBD-3, and hBD-4 encoded by DEFB1, DEFB4, DEFB103A and DEFB104 genes respectively [40, 49, 74].

HBD-1 is constitutively produced (except for inflammation state or microbe stimuli) while the other β-defensins are inducible [75]. HBD-1 is present in the urogenital tract, trachea and respiratory tract; hBD-2 and hBD-3 are expressed mostly in the respiratory tract; hBD-4 mRNA has been found in human testis, stomach, lung and neutrophils [76].

Many studies have been conducted about different expression levels of β-defensins associated with Candida spp. and C. albicans infection, the latter being the most virulent and the earlier recognized by epithelium among Candida spp. [77].

Recombinant hBD-2 and hBD-3 have similar targets, presenting specific strain activity against Candida spp. in vitro, specifically against C. albicans [71, 78], C. tropicalis and C. parapsilosis [71] but not against C. glabrata [71, 78] and C. krusei, [71] possibly due to their synergistically action [71]. Nevertheless, recombinant hBD-2 and hBD-3 can inhibit adherence of C. glabrata to human oral epithelial cells [78]. Moreover, it has been demonstrated that only hyphal growth and not the yeast form of C. albicans stimulates recombinant hBD-2 and hBD-3 mRNA expression in vitro [78].

A study conducted in Caco-2, an intestinal epithelial cell line, showed that hBD-2 is overexpressed after stimulation with different Candida spp. - albicans, krusei, tropicalis and parapsilosis [56]. In an another experimental study, a model of reconstituted human oral epithelia (RHOE) has been infected in vitro with different strains of Candida spp.: C. albicans wild-type, C. glabrata, C. tropicalis, C. krusei and C. parapsilosis infection induced β-defensins expression; instead C. albicans mutants and C. dubliniensis had no effect. After the infection, C. albicans seems to inhibit innate immune system control and impairs β-defensins expression with the aim of invading host epithelia [77]. However it has not been clarified if β-defensins expression is modulated directly by Candida recognition or by the damage caused by the fungus [25] (Figure 3).

Other works have shown that hBD-2 and hBD-3 are upregulated in oesophagus during Candida infection [79, 80]. In esophagitis caused by C. albicans infections, the cytokine Interleukin-1β (IL-1β) is able to enhancing the expression of hBD-2, while hBD-3 increased as the final step of “A Disintegrin And Metalloprotease” Metallopeptidase Domain 17” (ADAM17) / TNF-α / Epidermal Growth Factor Receptor / MAPK / Activator Protein-1 pathway [80]. HBD-2 could be also induced in human keratinocytes by the interaction between two cytokines, IL-22 and TNF-α, that act in synergy [81] to keep epidermal barrier integrity during C. albicans infections, suggesting that hBD-2 could be directly involved in C. albicans safeguard [82].

Moreover, rectal mucus contains antimicrobial peptides, possibly indicating that they are present in the digestive system. Particularly, hBD-1 and hBD-3 have been detected in rectal mucus (together with cathelicidin LL-37, lysozyme and other proteins) showing strong antimicrobial activity against the yeast form of C. albicans and different Gram-positive and Gram-negative bacteria [83].

Female hormonal imbalance could also change the expression of hBD-2. In fact, in vaginal epithelial cells exposed to LPS, hBD-2 production is stimulated by oestrogens and inhibited by progesterone [84], so, women taking oral contraceptives may have a decreased expression of hBD-2 and could have higher tendency to develop genital infections [85].

On other hand, host genetic background can influence β-defensins production; for example, hBD-1 could be modulated by the Single Nucleotide Polymorphism (SNP) -44

**Figure 3.** Neutrophils produce higher quantities of HNP1-3 in the presence of C. albicans infection when compared to neutrophils of an uninfected individual. In the case of oral mucosa C. albicans infection HNP1-3 are localized in the extracellular matrix in the proximity of the areas of mucosal lesions.
C/G, at the 5’ UTR of the DEFBI gene in patients affected by type-1 diabetes with oral candidiasis [86].

Interestingly, a recent study conducted by Rasching and coworkers [87] investigated the redox behaviour of hBD-1, demonstrating that reduction of its disulfide bonds causes an increased antimicrobial activity; the reduction is carried out by thioredoxin system that colocalizes with reduced hBD-1 in intestine, where mucosae protect the host both from pathogen and from microbiota overgrowth [87]. Further-more, Rascing et al. demonstrated that hBD-1 oxidized is active only against some Gram-negative bacteria, such as Escherichia coli and Salmonella enteritidis. This important study shows that an antimicrobial peptide can act with dif-ferent defence strategies depending on environmental condi-tions: reduced but not oxidized hBD-1 can form neutrophils extracellular traps (NETs) structures to entrap the pathogen preventing bacterial translocation. These types of actions have been studied in bacteria but it is not excluded that simi-lar mechanisms can be implemented after fungal challenges.

Table 1 reports the principal interaction between β-defensins and C. albicans

CONCLUSIONS

Defensins’ mechanisms of action to fight C. albicans (and other Candida spp.) infection have been only in part eluci-dated, and the information is fragmentary. A lot of investiga-tions are necessary to draw a complete picture of defensins’ functional activity with the aim of understanding how these peptides interact with C. albicans, a microorganism that, from harmless commensal, could become a pathogen. Never-theless, the studies here reviewed clearly indicate the direct role of defensins on the safeguard of the human body against C. albicans infection. Both α and β-defensins in fact are ac-tively expressed during the hyphal transition of C. albicans and concur with other immune components in the fighting against this pathogen mostly at mucosal and epithelial barri-ers sites.

Nowadays, it is also important to consider the widespread use of antibiotics that has led to the development of bacteria resistance and fungal super-infections. This is a urgent emergence for worldwide health, especially for immuno-compromised and hospitalized people; the investigation of novel therapeutic strategies to fight pathogen are rapidly ongoing and in this context defensins could be considered as potential adjuvant at least at topic mucosal level, being able to discriminate mammalian cells from pathogens. Further-more defensins non-specific antimicrobial action should be considered favourably since they could overcome the medi-cal problem of microorganism antibiotic resistance.

Due to their role of immuno-modulators defensins could be used as vaccine adjuvants or as antigens carriers to en-hance antigen presentation to antigen presenting cells [88]. However, Shuyi et al. [89] reported hBD-3 expression as increased by LPS through the epidermal growth factor path-way activation, leading to more aggressive lymphatic metas-tasis of oral squamous cell carcinoma. So, the use of defenses should be considered cautiously since they might accel-erate growth of existing cancer or badly induce malignant transformation of normal epithelia. A topic use of defensins at the mucosal surfaces might be the better way of employing them in the fight against Candida and pathogens affect-ing human mucosal surfaces.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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