JSTMU Journal of Shifa Tameer-e-Millat University

https://j.stmu.edu.pk

Open Access

REVIEW ARTICLE

Role of defensins in innate immunity

Atteqa Safdar[1], Sara Iftikhar[2], Ghassan Zahid[3]

[1, 2] Research Scholar, Atta-Ur-Rehman School of Applied Bioscience, National University of Science and Technology, Islamabad, Pakistan

[3] Lecturer, Department of Biotechnology, University of Azad Jammu & Kashmir, Muzaffarabad, Pakistan

Author`s Contribution

[1]Major Contribution, conceived the idea and findings

[2] Substantial contribution to the concept

[3] Supervised and final review

Article Info.

Conflict of interest: Nil

Funding Sources: Nil

Correspondence

Atteqa Safdar

Atteqasafdar3@gmail.com

Cite this article as: *Safdar A, Iftikhar S, Zahid G. Role of defensins in innate immunity. JSTMU. 2021; 4(2):117-123.*

A B S T R A C T

Immune system of living organisms ranging from fungi, plants, vertebrates and invertebrates are all aided by polypeptide chains like defensins and cathelicidins. In humans the defensisns are quite fundamental part of innate immune system in combating with day-to-day exposure to unknown pathogens. The defensins are classified as alpha beta and sigma defensins expressed at chromosome 8 at nearly same positions, the sigma defensin is however synthetically developed as reterocyclin, as it has been stopped producing because of evolutionary development of stop codon 7.5 million years ago. The expression of Defensins can be either constitutive or inducible through epithelial cells, Paneth cells or other respective immune cells to regulate the activation of the innate immune responses. These impart their role either by direct microbicidal action, antiviral activity, inactivation or neutralization of microbial products, mobilization or activation of phagocytes and mast cells. Further to this there is lot more to explore about the availability of similar genetic expressions as defensins with unclear functions and *in vivo* experimental models development.

Keywords: *Anti-microbial polypeptides, defensins, Innate immunity, Toll like receptors TLRs activation*

Introduction

The polypeptides chain responsible for supporting immune system is quite old phenomenon, the poly peptides in mammals that take part include defensins, cathelicidins and others.[1] The defensins are quite broad spread in living organisms ranging from insects, fungi, plant, invertebrates and vertebrates. As Humans are vulnerable to many unknown pathogens, the defensins help in an effective immune response against such, disease causing agents. Defensins are classified as positively charged cysteine containing host defense polypeptides originating from either epithelial cells or neutrophils, performing multiple functions along with efficient role in innate and adaptive immune response against foreign microbes and pathogens. Initially defensins were discovery as; alpha defensins (α) from rabbit neutrophils in 1985, subsequently beta defensins β were discovered (1990) in a bovine of mouse and in human intestinal epithelium.[2]

Classification of Defensins:

These were classified as per presence in mammals as α, β and θ defensins. These molecules have amphiphilic structure with much conserved region of six cysteine molecules and di sulfide bonds but still vary in structure, origin and function.

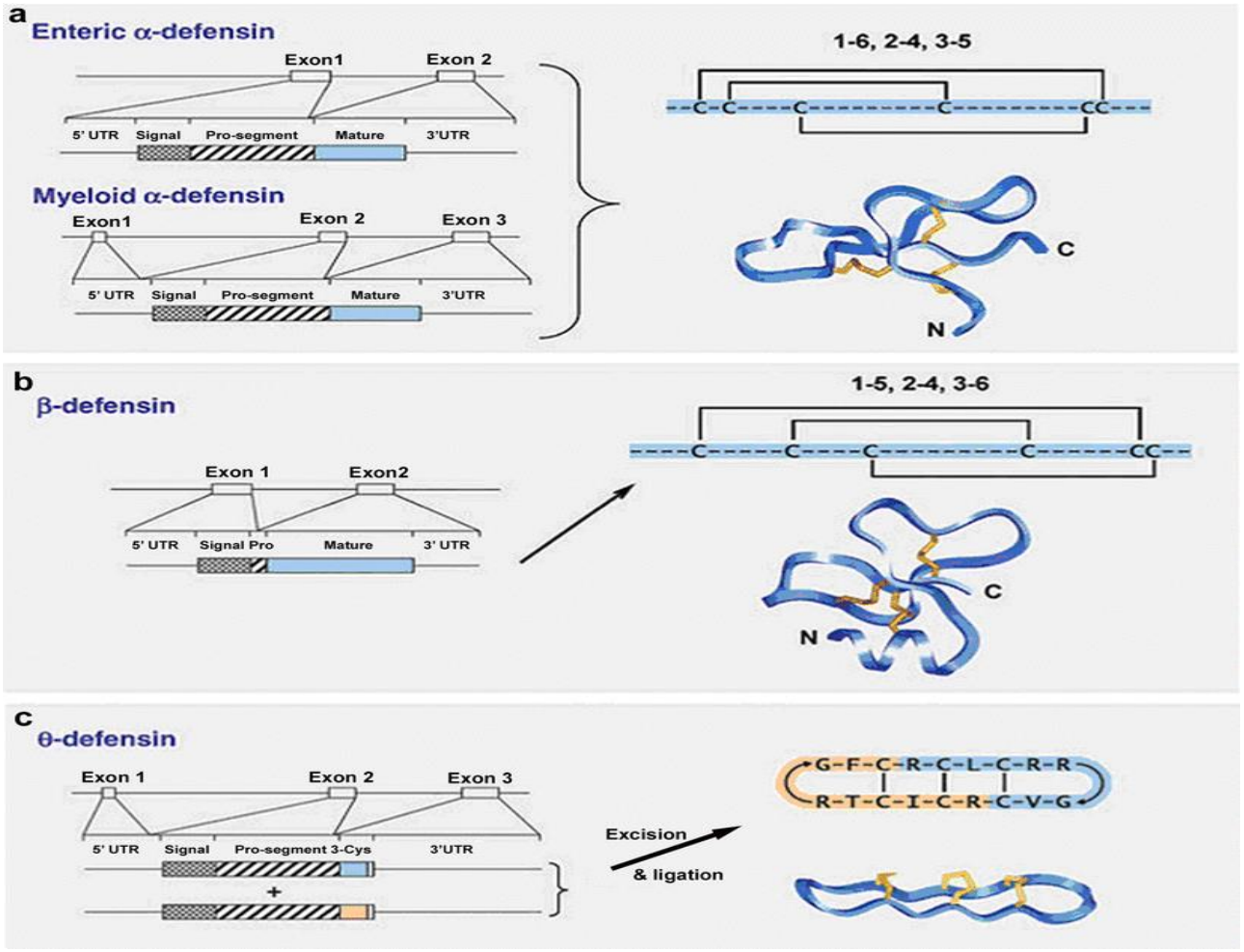

Figure 1: **Defensin genes and peptides** [22]

Alpha defensins:

In humans these defensins originate from neutrophils and epithelial cells. The first four have been studied and extracted from neutrophils in humans are (HNP1~4) as shown in Figure 1, while the other two were found in Paneth cells present in intestinal epithelial called as human defensins HD5 and HD6 or enteric defensins.[3,4] The cysteine disulphide bonds are between the 1-6, 2-4, 3-5 cysteine residues [5,6] and have 29 to 35 amino acid sequence.[7]

Beta defensins:

In humans these are composed of more than 36 amino acid residues and are found in epithelial tissues mostly, so far 28 genes for beta defensins have been discovered but only Human Beta defensin (HBD1~6) and HBD 2, 3 are studied extensively.[8] While the cysteine links are at the connections are 1-5, 2-4, 3-6 forming disulphide bridges, these bonds not only add positive charge but also add stability to structure.[9]

Sigma defensins:

In humans the development of sigma defensin have silenced because of an evolutionary development of a stop codon 7.5 million year ago [10] however, this have been developed synthetically as reterocyclin.[9] It has a circular hair pin structure with cysteine di sulfide bonds between 1-6, 2-5, 3-4 residues.

Genetic Expression of Defensins:

The human defensins alpha and beta specifically are expressed at nearly same location at Chromosome 8, there loci and genetic expression along with their cellular expression, role in immunity and concentration in diseased condition is mentioned in Table 1. In this no genetic expression is mentioned for HNP (Natural human

neutrophil defensin) HNP2 it is thought that HNP1 and /or HNP3 genetic expressions might have gone by a proteolytic activity to express HNP2. [11]

Defensins Synthesis and regulation & release:

Defensins are synthesized and released when the host body is exposed to a pathogen, the immune system in response to that produce these polypeptides either from epithelial cells or from neutrophils. Their expression could be constitutive or inducible i.e., in response to chemical mediators or other cells or in response to pathogenic exposure respectively. The cytokines or the mediators for development and release of the defensins are mentioned in Table 1. Defensins are expressed whenever the host immune cells get exposed to pathogenic content, as in case of toll like receptors (TLRs) activation. TLRs 1,2,4,5.6 and 8 are present on the surface of immune cells and some of the epithelial cells while TLR 3, 7 and 9 lies on the endosomes of the immune cells.

When the TLR 1 /2 complex gets activated by exposure to microbes with the help of its adaptor proteins MYD88/ TRAF6 it activates the kinases pathways ultimately activating NFkB that enters into the nucleus of cell and generate the transcription of required proteins and this case defensins and cytokines production that ultimately leads to the pro or anti-inflammatory responses. Similarly, the activation of TLR5 and 4 the transmembrane receptors activate MAPK pathways along with JNK and p38 pathways that ultimately end up in generation of cytokines and chemokines that act as precursors for defensin release and immune response generation. The anti and the pro inflammatory processes are well summarized in above schematic Figure 2.

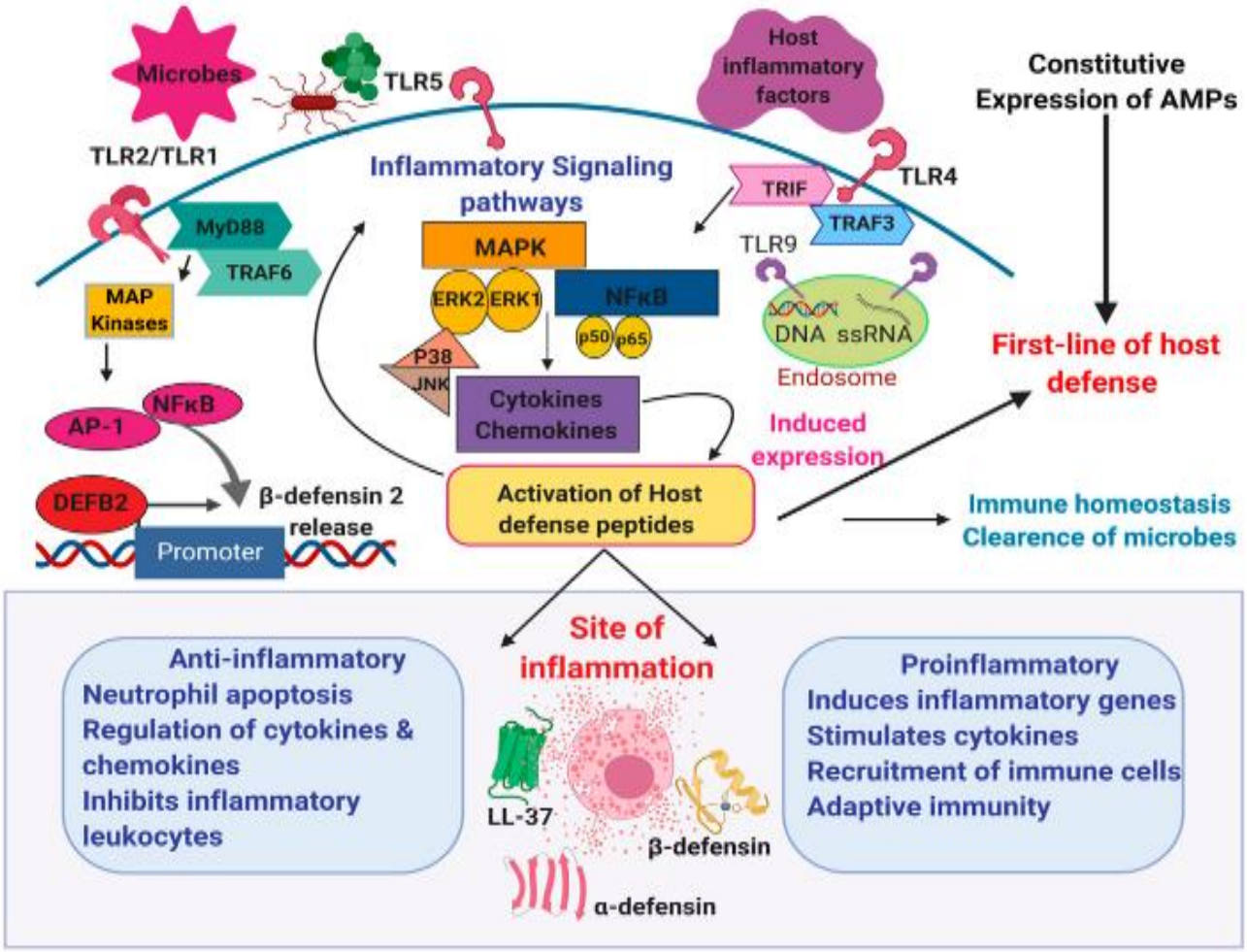

Figure 2: Expression of defensins in immunity [12]

Table 1: Cell sources and regulation of mammalian defensins [12]

Defensin				
Conventional name	Type	Cell source	Synthesis	Release
HNP1 ~ 4	α	Neutrophils CD8 T cells	Constitutive Inducible	Degranulation Secretion?
HD5 ~ 6	α	Paneth cells	Constitutive	Degranulation
Mouse cryptidin	α	Paneth cells	Constitutive	Degranulation
HBD1	β	Keratinocytes and epithelial cells	Constitutive and inducible	Secretion
HBD2 ~ 4	β	Keratinocytes and epithelial cells	Inducible	Secretion
BNDBs	β	Neutrophils	Constitutive	Degranulation
Bovine TAP	β	Epithelial cells	Inducible	Secretion
RTD-1	θ	Neutrophils, monocytes	Constitutive	Degranulation
Cathelicidins		Neutrophils, keratinocytes, epithelial cells, mast cells, and monocytes/macrophages	Constitutive and inducible	Degranulation or secretion
EDN		Eosinophils, neutrophils, macrophages, and placental epithelial cells	Constitutive and inducible	Degranulation or secretion

*Abbreviations: EDN, eosinophil-derived neurotoxin; HNP, human neutrophil peptide; HD, human defensin; HBD, human β-defensin; BNDB, bovine neutrophil-derived β-defensin; TAP, tracheal antimicrobial peptide; RTD-1, rhesus theta defensin-1.

Role in Innate Immunity:

- **Direct microbicidal action:**

The direct microbicidal action against bacteria is documented by alpha defensins and they perform it by detecting a more negatively charged cell membrane and forming a pore in it with a dimeric alignment of alpha defensins. This pore makes the internal constituents exposed to external environment and cell death. HNP1, 2, 3 and 4 along with HBD1 and HBD2 are found efficient in killing most of the gram positive, gram negative, fungi and few of parasites.[9] However, sigma defensin have shown this property in limited in vitro where salt and protein concentrations were found to be hindering their action in schematic Figure 3.

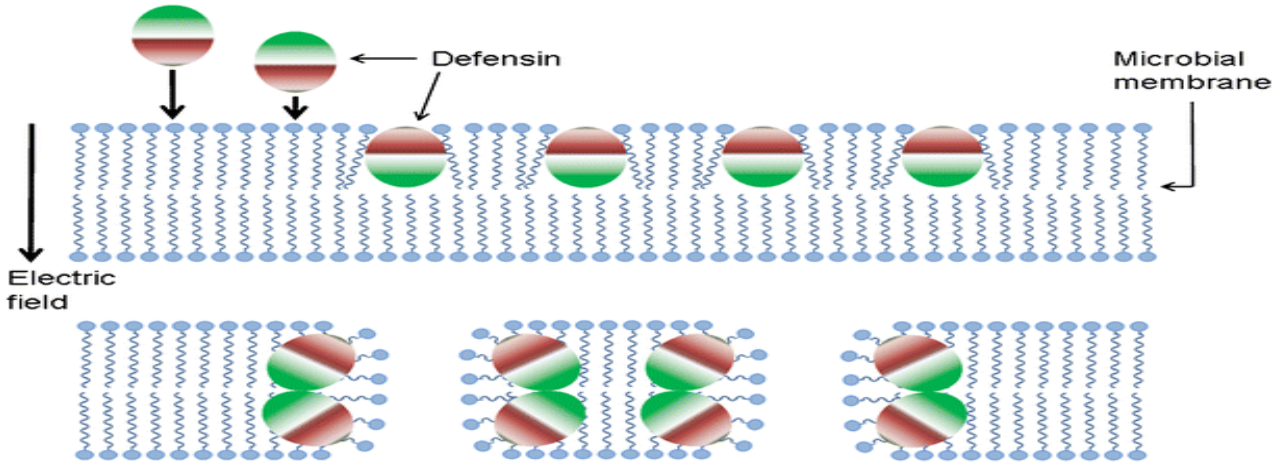

Figure 3: Direct microbicidal action against bacteria documented by alpha defensins [13]

- **Antiviral Activity:**

Antiviral activity of alpha defensins has been discovered a time ago but the antiviral activity has two aspects one is inhibition of viral invasion and the other is promotion of viral entry into host cells. For example, in case of HIV1 virus activity of HNP1-3 have shown non chemokine response. As these are capable of either direct viral inactivation or inhibition of adhesive and binding proteins gp120 and gp41 respectively on viral envelop, or the next step of viral fusion with cell membrane by down regulation co receptor CXCR4, direct membrane fusion of non-enveloped viruses like human papilloma virus are also inhibited by HNP1-3 and further viral RNA import and transcription is also inhibited, these features are quite promising in terms of viral prevention to prosecute infection in human cells but on contrary to that HNP 1 and Hd5 and 6 enhances a viral adhesion to epithelial cells and their entrance through tight junctions of trans epithelial cells. Thus, having quite, a contradictory and debatable effect against viruses in Figure 4.[14] "Opposing effects of human α-defensins on Inhibiting HIV-1 infection. (1) direct in activation of the virus (2) blockade of gp120-CD4 interactions (3) coreceptor downregulation, (4) Inhibition of gp41- and Env-mediated viral fusion (5) inhibition of nuclear import of viral RNA, (6) suppression of HIV transcription. Infection promoting mechanisms include: (7) enhancing viral adhesion/attachment, (8, 9) disrupting tight junction to promote trans-epithelial transmission of HIV"[14]

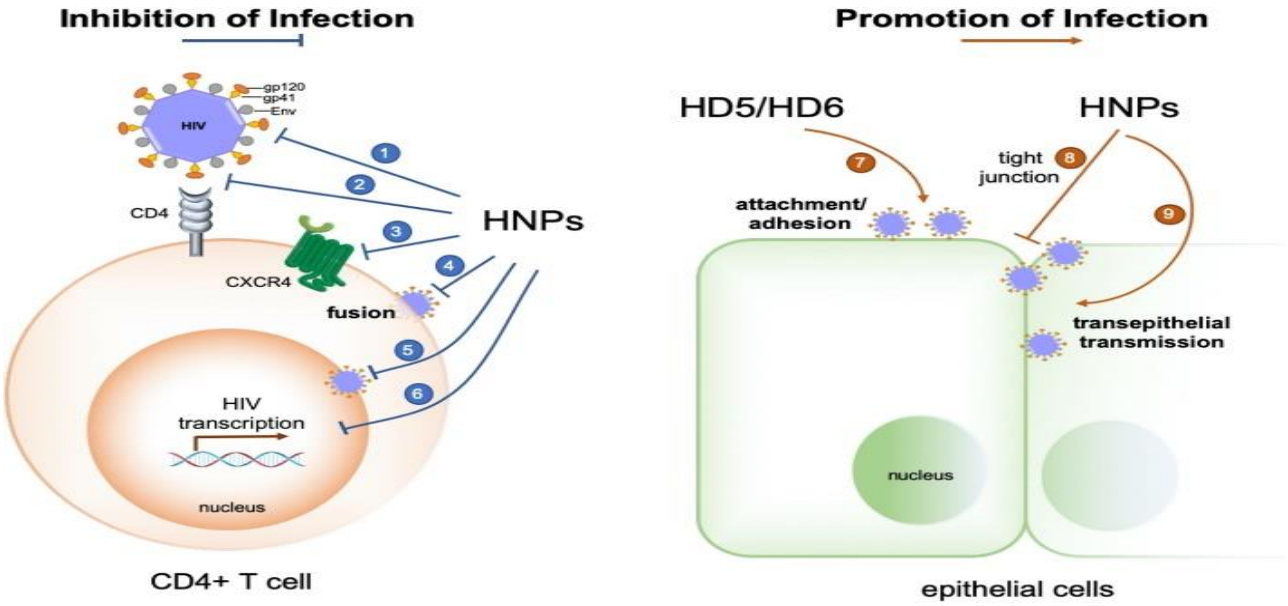

Figure 4: Direct viral inactivation or inhibition of adhesive and binding proteins gp120 and gp41 respectively on viral envelop [14]

- **Inactivation or neutralization of microbial products:**

Activity of HNP1-3 and θ defensins have been documented in mice against the lethal toxin of Bacillus anthracis it is a substrate-specific zinc-metalloprotease that can disrupt intracellular signaling making the cells weak and allowing the entry of bacteria unchecked. The alpha and sigma defensins have the ability to neutralize this exotoxin and the later also prevent the further germination of anthracs in host.[15] Moreover, HNP1, HNP 2 and BD2, 3 have recently shown binding activity with bacterial LPS [16] preventing its cytokine production through macrophage activation and preventing the LPS induced gene activation involved in sepsis, thus preventing this in mice.[17]

- **Mobilization and activation of phagocytes and mast cells:**

In innate immunity phagocytic activity of immune cells plays a very important role and for this the cells need to move out by four step mechanism including; rolling on endothelial surface, adhesion by cytokine receptors, diapedesis through chemotactic effect and migration to site of action as shown in Figure 5

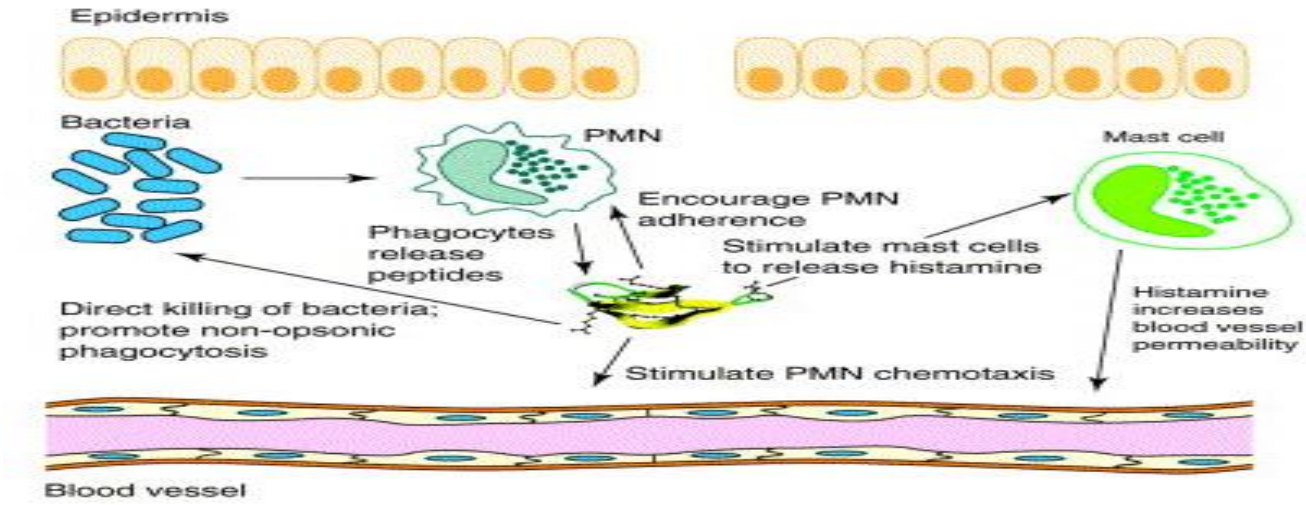

Figure 5: Mobilization and activation of phagocytes and mast cells in innate immunity [21]

- **Mast Cell activation and degranulation through defensins[18]**

The alpha defensins HNP 1- 3 and beta defensins HBD 3 and 4 act as chemoattractant for monocytes and macrophages.[18] HBD2 not only act as chemoattractant but has the ability to activate mast cells and thus production of inflammatory mediator prostaglandin D2. HNP1-3 and HBD 2 & 4 are not only involved as a chemotactic property but also increase the expression of production of cytokines receptors for strong binding of phagocytic cells and thus efficient migration to the site of action.[19]

Table 2: Multiple biological activities of mammalian defensins

Biological Activity		α-Defensin	β-Defensin	θ-Defensin
Microbicidal activity		All	All	All
Antiviral activity		HNP1, 2, 3, 4, HD5, RMAD	HBD1, 2, 3,	All
Exotoxin-inactivating activity		HNP1~3	ND	RTD
Endotoxin-neutralizing activity		HNP1, HNP2	HBD23	ND
Chemotactic activity	Mast cell	ND	HBD2	ND
	Mo/Mϕ	HNP1~3	HBD3, 4	ND
	DC	HNP1, HNP2	HBD1, 2, 3, mBD2, 3, mBD29	ND
	T cell	HNP1, HNP2, HNP4	HBD1, 2, 3	ND
Cell-activating activity	Epithelial cell	HNP1~3	HBD2, 3, 4	ND
	Mast cell	ND	HBD2	ND
	Mo/Mϕ	HNP1~3	ND	ND
	DC	ND	HBD3, mBD2	ND
Adjuvant activity		HNP1~3	HBD1, 2, mBD2, 3	ND

Abbreviations used: Mo/Mϕ, monocyte/macrophage; HNP1~3, a mixture of human neutraphil peptide 1~3; RMAD, rhesus monkey myeloid α-defensin; ND, not documented; HBD, human β-defensin; mBD, mouse β-defensin; RTD, retrocyclin (a synthetic artificial human θ-defensin).

Conclusion

Although it has been evident that defensins have been playing an integral role in immune response against pathogens but still there are some unanswered questions about multiple genomes found that appear like defensins but the role is unclear [20], further the activation and production of cytokines. And cytokine and defensin meditated immune cells activation is quite overlapping phenomenon that needs to be opened up in detail. In addition to this complete knock out models for defensins are still not present for finding out all the intracellular and molecular pathways, all the work done so far in *in vitro* or *ex vivo*, which is limiting factor in further molecular research.

References

1. Prasad SV, Fiedoruk K, Daniluk T, Piktel E, Bucki R. Expression and Function of Host Defense Peptides at Inflammation Sites. Int J Mol Sci. 2019; 21(1):104. DOI: https://doi.org/10.3390/ijms21010104.
2. Machado LR, Ottolini B. An evolutionary history of defensins: a role for copy number variation in maximizing host innate and adaptive immune responses. Front Immunol. 2015; 6:115. DOI: https://doi.org/10.3389/fimmu.2015.00115
3. Ganz T, Lehrer RI. Defensins. Pharmacol Ther. 1995; 66(2):191-205. DOI: https://doi.org/10.1016/01637258(94)00076-F
4. Wilde CG, Griffith JE, Marra MN, Snable JL, Scott RW. Purification and characterization of human neutrophil peptide 4, a novel member of the defensin family. J Biol Chem. 1989; 264(19):11200-3. DOI: https://doi.org/10.1016/S00219258(18)60449-1
5. Selsted ME, Harwig SS. Determination of the disulfide array in the human defensin HNP-2: a covalently cyclized peptide. J Biol Chem. 1989; 264(7):4003-7. DOI: https://doi.org/10.1016/S00219258(19)84952-9
6. Hill CP, Yee J, Selsted ME, Eisenberg D. Crystal structure of defensin HNP-3, an amphiphilic dimer: mechanisms of membrane permeabilization. Science. 1991; 251(5000):1481-5. DOI: https://doi.org/10.1126/science.2006422
7. Ganz T. Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol. 2003; 3(9):710-20.
8. Bensch KW, Raida M, Mägert HJ, Schulz-Knappe P, Forssmann WG. hBD-1: a novel β-defensin from human plasma. FEBS letters. 1995; 368(2):331-5. DOI: https://doi.org/10.1016/0014-5793(95)00687-5
9. Yang D, Liu ZH, Tewary P, Chen Q, de la Rosa G, Oppenheim JJ. Defensin participation in innate and adaptive immunity. Curr Pharm Des. 2007; 13(30):3131-9. DOI: https://doi.org/10.2174/138161207782110453.
10. Cole AM, Hong T, Boo LM, Nguyen T, Zhao C, Bristol G, et al. Retrocyclin: a primate peptide that protects cells from infection by T-and M-tropic strains of HIV-1. Proc Natl Acad Sci. 2002; 99(4):1813-8. DOI: https://doi.org/10.1073/pnas.052706399
11. Yang D, Biragyn A, Hoover DM, Lubkowski J, Oppenheim JJ. Multiple roles of antimicrobial defensins, cathelicidins, and eosinophil-derived neurotoxin in host defense. Annu Rev Immunol. 2004; 22:181-215. DOI: https://doi.org/10.1146/annurev.immunol.22.012703.104603
12. Prasad SV, Fiedoruk K, Daniluk T, Piktel E, Bucki R. Expression and Function of Host Defense Peptides at Inflammation Sites. Int J Mol Sci. 2019; 21(1):104. DOI: https://doi.org/10.3390/ijms21010104.
13. Hazlett L, Wu M. Defensins in innate immunity. Cell Tissue Res. 2011; 343(1):175-88. DOI: https://doi.org/10.1007/s00441-010-1022-4.
14. Xu D, Lu W. Defensins: A Double-Edged Sword in Host Immunity. Front Immunol. 2020; 11:764. DOI: https://doi.org/10.3389/fimmu.2020.00764.
15. Kim C, Gajendran N, Mittrucker HW, Weiwad M, Song YH, Hurwitz R, et al. Human alpha-defensins neutralize anthrax lethal toxin and protect against its fatal consequences. Proc Natl Acad Sci USA 2005; 102: 4830-5. DOI: https://doi.org/10.1073/pnas.0500508102
16. Scott MG, Vreugdenhil AC, Buurman WA, Hancock REW. Cut- ting edge: cationic antimicrobial peptides block the binding of lipopolysaccharide (LPS) to LPS binding protein. J Immunol 2000; 164(2):549-53. DOI: https://doi.org/10.4049/jimmunol.164.2.549
17. Motzkus D, Schulz-Maronde S, Heitland A, Schulz A, Forssmann WG, Jübner M, et al. The novel β-defensin DEFB123 prevents lipopolysaccharide-mediated effects in vitro and in vivo. The FASEB J. 2006; 20(10):1701-2. DOI: https://doi.org/10.1096/fj.05-4970fje
18. Allaker RP. Host defence peptides—a bridge between the innate and adaptive immune responses. Trans R Soc Trop Med Hyg. 2008; 102(1):3-4. DOI: https://doi.org/10.1016/j.trstmh.2007.07.005.
19. van Wetering S, Mannesse-Lazeroms SP, van Sterkenburg MA, Hiemstra PS. Neutrophil defensins stimulate the release of cytokines by airway epithelial cells: modulation by dexamethasone. Inflamm Res 2002; 51: 8-15.
20. Yamaguchi Y, Nagase T, Mikita R, Fukuhara S, Tomita T, Tominaga T, et al. Identification of multiple novel epididymis-specific -defensin isoforms in humans and mice. J Immunol 2002; 169(5):2516-23. DOI: https://doi.org/10.4049/jimmunol.169.5.2516
21. Yang D, Liu ZH, Tewary P, Chen Q, De la Rosa G, Oppenheim JJ. Defensin participation in innate and adaptive immunity. Curr Pharm Des. 2007; 13(30):3131-9. DOI: https://doi.org/10.2174/138161207782110453
22. Sau S, Sun J, Lee CY. Molecular characterization and transcriptional analysis of type 8 capsule genes in Staphylococcus aureus. J Bacteriol. 1997; 179(5):1614-21. DOI: https://doi.org/10.1128/jb.179.5.1614-1621.1997.
23. Oppenheim JJ, Biragyn A, Kwak LW, Yang D. Roles of antimicrobial peptides such as defensins in innate and adaptive immunity. Ann Rheum Dis. 2003; 62(suppl 2):ii17-21. DOI: http://doi.org/10.1136/ard.62.suppl_2.ii17