β Defensins as a novel treatment against tuberculosis

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Introduction

Tuberculosis (TB) remains one of the most lethal and ancient infectious diseases causing 2 million deaths and 8-10 million new cases of active tuberculosis worldwide annually. More over, the incidence of multidrug-resistant strains is increasing and there is not an efficient treatment.

A good alternative against conventional antibiotics are **Antimicrobial Peptides (AMPs)** as they are produced by most living organisms and they exhibit potent cytotoxic activity against bacteria, fungi, viruses, and parasites. AMPs are relatively short (<40), quite cationic and adopt amphipathic structures that present discrete hydrophobic and hydrophilic regions when they are associated with anionic interfaces.

Human β-defensins (HBD) are one of the most important natural occurring antimicrobial peptides in TB infection. HBD-2 is transported into mycobacteria-containing macrophage phagosomes to exert mycobactericidal and mycobacteristatic activity. HBD-3 and HBD-4 participate in *Mycobacterium tuberculosis* clearance by bacterial lysis and they are associated with long-term control of mycobacterial proliferation.

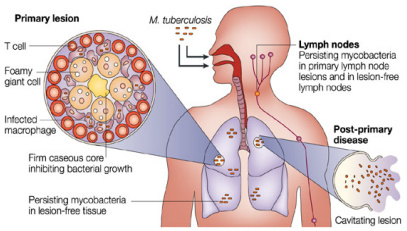

Figure 1. Mycobacterium tuberculosis infection. *TB infection starts with the inhalation of bacilli, followed by penetration into the alveoli of lung where they are engulfed by macrophages. After initial bacterial multiplication in alveolar macrophages, the bacteria are taken up by dendritic cells (DCs), which carry M. tuberculosis to the draining thoracic lymph nodes, which strengthen antibacterial responses by the recruitment and activation of additional macrophages. In most individuals, this interplay between the innate and adaptive arms of the immune response serves to control the infection. The site of the infection can be recognized as a granulomatous lesion or tubercle, which comprises a central area of mycobacterial-infected cells surrounded by other, non-infected phagocytic cells and foamy giant cells, with lymphocytes found at the periphery. The lesion is sealed off from surrounding tissue by a fibrotic capsule. Figure taken from (Graham et al., 2003).*

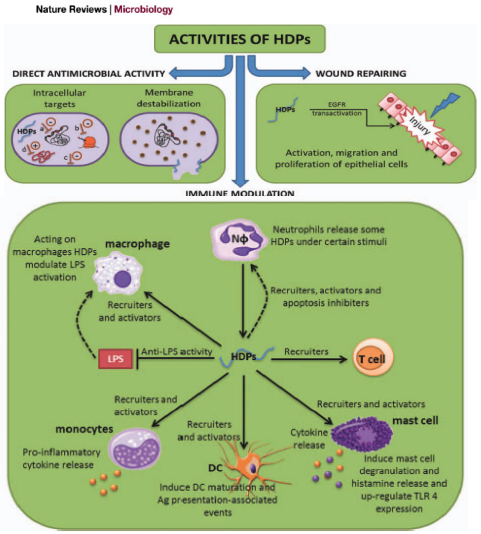

Figure 2. Functional properties of Host Defense Peptides (HDPs). *HDPs can interact directly with microbes leading to cellular death through membrane destabilization and cytoplasm leak out or by penetrating into the cell and acting toward cytoplasmatic targets. Among the intracellular lethal effects of HDPs figures (a) the peptides binding to DNA and the inhibition of DNA metabolism, (b) the inhibition of proteins syntesis, (c) the inhibition of cell wall syntesis, and (d) the activation of autolytic enzimes. HDPs are also important immunomodulators and wound healing and angiogenesis promoters contributing to the resolution of the infection or damage and maintaining the homeostasis. Figure taken from (Alba et al., 2012).*

Initial hypothesis and aims of the project

HBD-2, HBD-3 and HBD-4 participate in the immunopathogenesis of experimental tuberculosis and their synergy with antituberculous drugs suggests that they may be a promising complement to antituberculous chemotherapy.

Aims:

- Optimize HBD-2, HBD-3 and HBD-4 to get a higher antimicrobial activity which will help to reduce the localised infected area by *Mycobacterium tuberculosis*.
- The optimized peptides must have the same proprieties and structure as natural HBDs that enhance the interaction with the bacil cell wall.

Materials and Methods

HBDs Optimization

- **AMPA:** AMPA is an automated web server for prediction of protein antimicrobial region, which uses an antimicrobial propensity scale derived from high-throughput screening results that generate an antimicrobial profile and an antimicrobial index (AI), which can be calculated providing a fair assessment of the tendency for such amino acids to be present in an AMP sequence. As low half maximal inhibitory concentration (IC_{50}) values correspond to high activity, amino acids with a low index are the most favoured to be part of an AMP.

  AMPA server provided an interactive antimicrobial profile of the query sequences, they were analyzed and the single mutations were deliberately made always considering the conserved residues, the AI of each amino acid and the secondary structure of each AMP. Arginine was the residue chosen for all the single mutations because of its AI value and its cationic proprieties.

- **Collection of Anti-Microbial Peptides (CAMP):** CAMP is a very useful online database to compare different important proprieties of HBDs and the optimized peptides. This information will help to discriminate between the all the optimized peptides candidates.

- **Define Secondary Structure of Proteins (DSSP) software:** DSSP was used to confirm that the secondary structure of the optimized peptides were the same as the natural HBDs. As the secondary structure of AMPs contribute in the interaction with *M. tuberculosis* cell wall.

Results

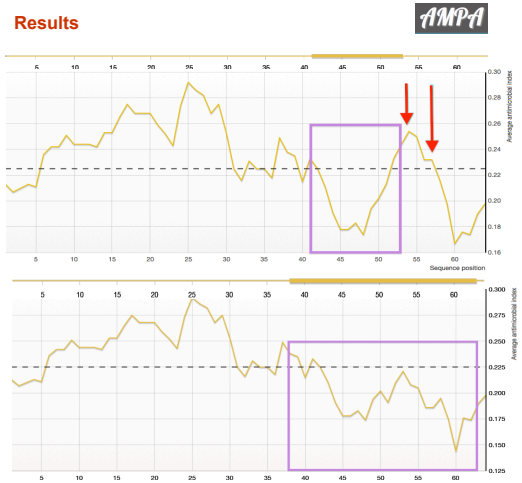

Figure 3. AMPA antimicrobial profiles of HBD-2 and Mut1HBD-2. *Two single mutations have enlarged the antimicrobial stretch two times HBD-2 size.*

Figure 4. Representation of HBD-2 and Mut1HBD-2 with PyMol program. *The single mutations are represented in green and circled with white.*

Name	Antimicrobial stretch	Propensity	Net charge	Hydropathy	Aliphatic index	Instability Index
HBD-2	41 – 53 (12)	0.221	+7	0.60	92.81	41.32
Mut1HBD-2 G54R/G57R	38 – 63 (24)	0.186	+9	0.48	92.81	41.46
Mut2HBD-2 S34R/G54R/G57R	39 – 63 (24)	0.176	+10	0.42	92.81	45.23
HBD-3	28 – 46 (18) 51 – 66 (12)	0.214 0.145	+12	0.00	95.97	55.16
Mut1HBD-3 T27R/L28R/G37R	25 – 46 (21) 51 – 66 (12)	0.197 0.145	+15	-0.24	90.15	69.69
Mut2HBD-3 T27/G37R/S56R	25 – 46 (21) 50 – 66 (13)	0.203 0.14	+15	-0.17	95.97	63.14
HBD-4	32 – 54 (22)	0.221	+7	-0.38	92.08	48.00
Mut1HBD-4 D27R/E45R/A56R/D63R	28 – 65 (25)	0.194	+14	-0.51	90.69	44.22
Mut2HBD-4 A56R/D63R/E64R	30 – 71 (41)	0.194	+12	-0.50	90.69	47.64

Table 1. Comparison of HBDs and optimized peptides proprieties. *Data taken from AMPA and CRAMP web servers.*

Conclusions

The optimized peptides have very similar properties to natural HBDs, but they have a lower propensity value, a higher net charge and a longer antimicrobial stretch, suggesting that they might have a higher activity thereby enhancing the reduction of the localised infected area by the bacil. *In vitro* activity assays should be done to confirm these computacional results.

References

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- **Klüver E, Adermann K, Schulz A.** (2006). Synthesis and structure-activity relationship of beta-defensins, multi-functional peptides of the immune system.
- **Torrent M, Di Tommaso P, Pulido D, Nogués MV, Notredame C, Boix E, Andreu D.** (2012). AMPA: an automated web server for prediction of protein antimicrobial regions.