

ROLE OF β -DEFENSINS IN IMMUNE RESPONSE IN TUBERCULOSIS PATIENTS

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Abstract

Expanding of tuberculosis drug-resistance makes host-directed treatment an important part of tuberculosis treatment. Host-directed treatment is aimed at stimulating the production of antimicrobial peptides by the patient's immune cells. The use of β -defensins is very interesting in this field because of their pronounced bactericidal and bacteriostatic effects, as well as the ability to stimulate the chemotaxis of immune cells. The article presents a review on the immunological properties of the defensin family and the possibility of their use in practice. To complete the review 114 articles from "PubMed" resource were analyzed, 34 of them were chosen to review immunomodulatory and antimicrobial action of β -defensins.

The original research results on Human-beta-defensin-1 use as tuberculosis severity marker were also added to the research. To obtain our own research results, 100 TB patients and 20 healthy persons were included in the study. Human-beta-defensin-1 level in serum was investigated in all the patients at the treatment onset and in healthy persons. Mann-Whitney U test (for comparison of 2 independent groups) and Spearman's rank correlation coefficient were used for statistical data processing. It was found that Human-beta-defensin-1 level was significantly higher in TB patients than in healthy persons. A correlation of medium strength ($r=+0.53$, $p<0.05$) between Human-beta-defensin-1 and tuberculosis lesion volume was revealed. The data obtained allows using human β -defensin-1 as a diagnostic marker of tuberculosis.

Key words: *tuberculosis, β -defensins, immunity, prognostic marker.*

1. Introduction

According to the World Health Organization (WHO), tuberculosis is among the 10 most common causes of death in the world. In 2019, about 1,600,000 deaths from tuberculosis were recorded and about 10,000,000 new cases were detected, which corresponds to an incidence rate of 133 per 100,000 population [1].

According to the WHO objectives within the "End TB" program, it is necessary to reduce the incidence of tuberculosis by 90% and reduce the mortality rate by 95% to overcome the tuberculosis epidemic. For this, it is necessary to increase the susceptible tuberculosis treatment

effectiveness up to 85% and to increase multidrug-resistant tuberculosis treatment effectiveness up to 75% [2].

One of the main factors affecting the course of tuberculous process and the treatment effectiveness is severity of tuberculous lesions and the ability of the immune system to provide an adequate response. In this section, it is interesting to study the cationic peptides of the immune system, which have multi-profile functioning in the immune response: immunomodulating activity, direct antimicrobial action, selective inflammatory and anti-inflammatory properties and additional reparative activity [3].

Among the cationic peptides of the immune system, a family of defensins is distinguished. Defensins are cysteine-containing peptides consisting of 29–35 amino acid residues. They are able to form 3 invariant disulfide bonds, which determine the distribution of defensins into 3 subgroups – α , β and θ [4, 30].

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2. Purposes, subjects and methods:

2.1. The aim of the study was to review the available data and find out the immunological role of β -defensins and their possible practical role in the diagnosis, prediction and treatment of tuberculosis.

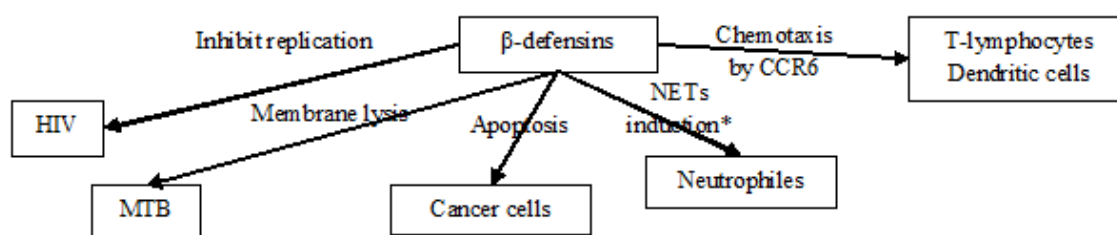
2.2. Subjects & Methods

114 articles from "PubMed" resource were analyzed, 34 of them were chosen to review immunomodulatory and antimicrobial action of β -defensins. To obtain our own research results, 100 TB patients and 20 healthy persons were

production of β -defensins by some cells triggers its production by others [8–9].

In addition, β -defensins exhibit direct antimicrobial activity by damaging the cell membranes of bacteria that have been captured by phagocyte or persist in extracellular matrix [10]. After penetration into the microbial cell, β -defensins can bind polyanionic DNA molecules [11]. The main immune role of β -defensins is shown in *Fig. 1*.

Some studies have already determined the role of β -defensins as markers of tuberculous lesion severity [12, 22], as well as markers of the



* Neutrophilic extracellular traps (NETs) are networks of extracellular fibers, primarily composed of DNA from neutrophils, which bind pathogens

Fig. 1. Immune action of β -defensins

included in the study. Human-beta-defensin-1 level in serum was investigated in all the patients at the treatment onset and in healthy persons. Mann-Whitney U test (for comparison of 2 independent groups) and Spearman's rank correlation coefficient were used for statistical data processing.

Conflict of interests. Authors have no conflict of interests.

3. Results

The defensins, namely β -defensins, were first isolated from human blood by Lehrer et al. in 1985 [17]. However, β -defensins subgroup is the most interesting among defensins, as they are produced in majority of the organs and body systems, including bronchial epithelial cells, neutrophils (azurophilic neutrophil granules), NK-cells and certain types T-lymphocytes [5, 25, 28]. Their expression is stimulated primarily by the influence of $\text{TNF-}\alpha$, IL-1 , in response to the recognition of bacteria by Toll-like receptor-mediated pathways, as well as by the direct action of bacterial agents. It makes them potentially sensitive markers of the disease severity [6, 29].

β -defensins exhibit chemotactic activity and activate migration of T-lymphocytes, macrophages, dendritic cells [7, 34]. The expression of β -defensins is activated directly under the influence of *M. tuberculosis* (MTB). The

transition of latent tuberculosis infection to the active phase [23]. In addition, defensins can act as a marker of treatment efficacy, since their level probably progressively decreases to normal value while recovery and achieving a positive effect of treatment [13, 21].

A decrease in the level of β -defensins was also found in patients with severe comorbidities, such as diabetes mellitus, accompanied by a greater severity of tuberculosis lesions and slower healing [26]. In a study by Zhu et al., 2011, a correlation between the low level of defensins and the development of multidrug-resistant tuberculosis was suggested [27].

The listed properties of β -defensins also determine their promising role in improving the pathogenetic treatment of tuberculosis, the so-called host-directed therapy [31]. In a study by A. Kalita, 2004, a synergistic effect of β -defensins and anti-tuberculosis therapy was observed, since β -defensins damage membranes of *M. tuberculosis* and facilitate the penetration of anti-tuberculosis drugs into the cells [14], and also have a direct effect on pathogens located intracellularly [9]. In another study, the bactericidal activity of neutrophils that do not contain defensins and neutrophils that contain them was experimentally compared, and it turned out that the family of defensins plays a key role in the

destruction of mycobacteria [25]. The study by Rivas-Santiago et al., 2008, showed the bacteriostatic role of β -defensins and their ability to prevent the reactivation of latent mycobacteria by binding *M. tuberculosis* DNA [28]. The study by Sharma et al. found the minimum concentration of β -defensins-1 in vitro (2 mg/ml), which inhibits the intracellular growth of *M. tuberculosis*. Comparison of the action of β -defensins and Rifampicin in vitro showed a high inhibitory activity of the antimicrobial peptide [32].

Artificial administration of recombinant defensins is not reliable due to the high cost and short half-life of the peptides. This idea is under development, but recombinant β -defensins demonstrate bactericidal activity against chemoresistant *M. tuberculosis* strains in vitro [23]. However, a possible solution is to stimulate the production of defensins by the body's own cells. In 2011 and 2015, Rivas-Santiago et al. demonstrated in experimental animal models (research in mice) that the use of L-isoleucine stimulates the production of its own β -defensins and reduces bacterial excretion and the severity of infiltrative tuberculosis lesions [15–16]. Later in 2013, a similar study was performed on pigs and showed that the use of L-isoleucine in combination with zinc intensifies biosynthesis of β -defensins and stimulates the macrophage immune response [18]. The same results were obtained when studying the cell model [33].

The study of the mechanism of L-isoleucine action in the culture of epithelial cells suggested that the stimulation of β -defensins production occurs by an intracellular effect on the chiral receptor or enzyme by the activation of transcription factors of the NF- κ B family [20].

Increased immune response may be associated with an increase in the synthesis of β -defensins under the influence of L-isoleucine and by CCR6-mediated chemotaxis of CD4 + T-lymphocytes stimulated by influence of isoleucine and leucine in the N-terminal region of the α -helix of β -defensins [19].

The original research results

Human-beta-defensin-1 level was 21.48 ± 2.88 U/L in TB patients and 8.97 ± 2.56 U/L in healthy persons (Fig. 2). The differences between the groups were significant ($p < 0.05$). A correlation of medium strength ($r = +0.53$, $p < 0.05$) between

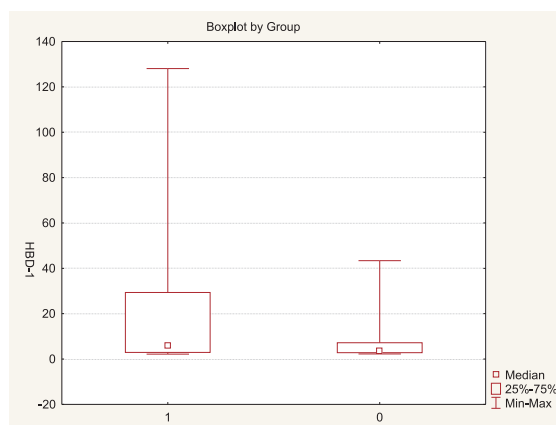


Fig. 2. Differences in Human-beta-defensin-1 level in TB patients (Group 1) and healthy controls (Group 0)

Human-beta-defensin-1 and tuberculosis lesion volume was revealed.

4. Conclusions

The determination of β -defensins level can predict severity of tuberculosis and the effectiveness of anti-tuberculosis therapy. Secondly, β -defensins, due to pronounced bactericidal and bacteriostatic effects, as well as the ability to stimulate the chemotaxis of immune cells, can be used to increase the effectiveness of anti-tuberculosis therapy. However, the use of recombinant β -defensins is associated with difficulties in their synthesis and the instability of peptides artificially introduced into the body. Therefore, the prospect of improving the pathogenetic therapy of tuberculosis is to stimulate the production of defensins by the human cells by the use of L-isoleucine. Study of human β -defensin-1 in tuberculosis patients of our hospital revealed positive correlation of medium strength between human β -defensin-1 level in serum and volume of tuberculosis lesions which allows using human β -defensin-1 as a diagnostic marker of tuberculosis.

List of abbreviations:

NK – Natural killers

TB – Tuberculosis

WHO – World Health Organization

Conflict of interests

The authors declare that they have no competing interests.

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