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NEW FORMYL-PEPTIDES, AS STIMULATOR OF NON-SPECIFIC ORGANISM RESISTANCE AGAINST MYCOBACTERIA

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Intoduction

Mandatory in 64 countries BCG vaccination certainly reduces the incidence of primary infection, morbidity, prevents the development of acute-progressive forms of tuberculosis, as well as the mortality rate [1]. According to statistical data in various European countries the index of epidemiological effectiveness of BCG is ranges from 2 to 2,4 (from 37 to 102 cases per 100 thousand population), that is beyond the epidemic threshold set by WHO for tuberculosis (50 cases per 100 thousand population) [2, 3]. If a primary infection is an issue of Epidemiology, the frequency of relapse - is a matter of immunology. So for the conversion of latent TB to the active form, which is observed in about 10% of patients, to a large extent it corresponds to a history of patients with various endocrine, nervous and immune pathologies. It is known, that tuberculosis accompanied by leukopenia, particularly by reducing the number of T- and B-lymphocytes, especially Th, and is characterized by decreased functional activity of phagocytes and by increased IgM level in blood serum [4]. As we know, the key element in the formation of TB infection is the inability of alveolar macrophage to complete phagocytosis of mycobacteria absorbed by them, that caused by both features of the pathogens biology and the tissue macrophages. [5, 6]. It is known that M. tuberculosis is capable long-term persistence and proliferation in alveolar macrophage cytoplasm because of high stability of them cell walls to the lysosomal enzymes action. Reduced microbicidal potency of alveolar macrophages is associated in particular with their localization in a high oxygen content. Mainly, the phenomenon of granulomatous reaction, inherent of TB, reflects the inadequacy in elimination of tuberculosis pathogen by alveolar macrophages. Some stages of granulomatous reactions development involving inflammatory mediators, as a pathogenetic basis for TB therapy, deserve a separate detailed study.

Thus, the inclusion of agents that can activate completed phagocytosis of mycobacteria by alveolar macrophages, in the base of anti-TB therapy is a promising direction in the prevention of latent tuberculosis reactivation.

Aim of the study is the investigation of immunomodulatory ability of new semi-synthetic formyl-peptides to activate the phagocytosis completion.

Materials and Methods

The ability of formyl-peptides activate the completeness of mycobacteria phagocytosis by alveolar macrophages absorbed by them were evaluated in vitro. For reaching the aim of the study we had used a broncho-alveolar lavage obtained by white laboratory male mice 2 months of age, weighing 22 ± 2 g. Total of 36 animals, 12 mice in 2 experimental and 1 control groups. The animals were kept in a vivarium of "Mechnikov institute of Microbiology and Immunology of NAMS of Ukraine" on a standard diet with specified conditions of animal management. Work with laboratory animals was performed according to the rules [7]. For broncho-alveolar lavage was performed 4-times washing the lungs through the intratracheal catheter 1 ml of sterile phosphate buffer. Lavage was centrifuged at 1000 rev / min for 4 min. The cell pellet was resuspended in 3 ml RPMI 1640 medium and the number of macrophages was determined in a Goryaev chamber to bring the cell concentration in the medium to 1×10^6 / ml of [8]. In setting up the reaction to the isolated from broncho-pulmonary washout of laboratory mice alveolar macrophages were added 0.1 ml of a 10% solution formyl-peptides and carried preincubated at 37 ° C for 30 minutes. Next was added 0.1 ml of BCG solution (0.25 ml saline solution 1 vaccine vial) and incubated in a thermostat at 37 ° C for 30 min and 60 min. Consideration of the reactions results performed by the light microscope under oil immersion increases after specific staining by Ziehl-Nielsen [9] with calculating of index of phagocytosis completion. As a comparison drug we used the officinal preparation "Liasten", that have the peptidoglycan's nature and is derived from the cell walls of Lactobacillus Delbrueckii.

To determine the lysosomal activity by the presence of peroxidase was treated with acridine orange causing selective staining red lysosomes. Consideration of the results was performed by fluorescent microscope. For detection peroxidase smears were fixed for 1 min in formalin alcohol, then they were applied for 4 min incubation mixture having the following composition: 40 mg of benzidine, 10 ml of 40% alcohol, and 0.02 ml of a 3% H_2O_2 solution. In places of peroxidase localization in the cells were observed yellow-brown or dark brown granules. Acid phosphatase activity was studied using azocoupling reaction for staining granules in the cytoplasm blue or purple. Results are expressed as mean cytochemical coefficient (LZC).

Statistical analysis of the data was performed using the software package "Statistica 6". The data were presented as mean and linear deviations.

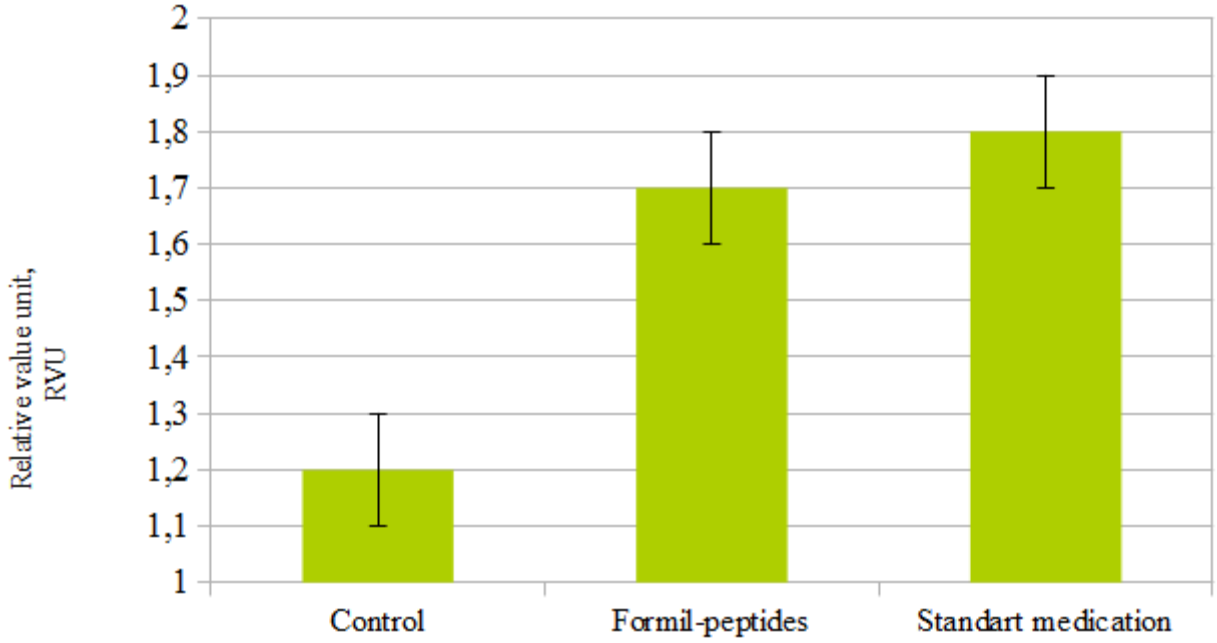

Fig. 1 - Index of phagocytosis completion of alveolar macrophages by in vitro incubation with formyl-peptides and standard medication.

Results and discussion

Incubation of alveolar macrophages and formyl-peptides leads to a significant increase the index of mycobacteria phagocytosis completeness for vaccine strains - (1,70 ± 0,31) and (1,20 ± 0,22), respectively, ($p < 0.05$). The standard medication - Liasten - also increased the "killing" ability of tissue macrophages compared to control: (1,8 ± 0,25) and (1,20 ± 0,22), respectively, ($p < 0.05$). Thus, new forms of peptides have the ability to increase the digestive capacity of phagocytes in relation to mycobacteria vaccine strains, with index of phagocytosis completion under the influence of formil-peptides at the level of the comparison officinal drug. Thus, new formil-peptides have the ability to increase the phagocytes digestive capacity for mycobacteria vaccine strains. However, the index of phagocytosis completion under formil-peptides influence was at the same level, as after the administration of standard medication.

The most important criteria for an effective functional activity of macrophages is the state of its lysosomal apparatus. Microbicidal potential of this organelle can be characterized by the content of hydrolytic enzymes peroxidase and acid phosphatase.

Lysosomal activity of alveolar macrophages exposed formyl-peptides significantly increased in comparison with the control - (97,80 ± 5,1) and (80,9 ± 4,3) acridine orange-positive cells, respectively, ($p < 0.05$). However, the effect of formyl-peptides on lysosomal activity of macrophages did not exceed the reference drug action - (97,80 ± 5,1) and (95,72 ± 5,3) acridine orange-positive cells, respectively, without significant differences. Under the influence of formyl-peptides also changed the level of acid phosphatase in the alveolar macrophages of mice, witch was expressed in the average cytochemical coefficient. Incubation with formyl-peptides resulted in a significant increase of LZC of acid phosphatase in macrophages - (2,08 ± 0,20) and (1,59 ± 0,14), respectively, ($p < 0.05$). Significant differences between the content of this enzyme in macrophages when exposed formyl-peptides and the reference drug were not detected.

Conclusion

Thus, the co-incubation of alveolar macrophages and new formil-peptides activates BCG phagocytosis completeness. Also, there is the influence of the studied substances under significant lysosomal activity and increase the content of acid phosphatase in macrophages isolated from broncho-alveolar lavage in comparison with the control. The level of functional activity stimulation of phagocytes under the influence of formyl-peptide is the same, that we registered after the Liasten administration. It has indicating prospects of creation on their basis of the drug, which stimulates the body's non-specific resistance.

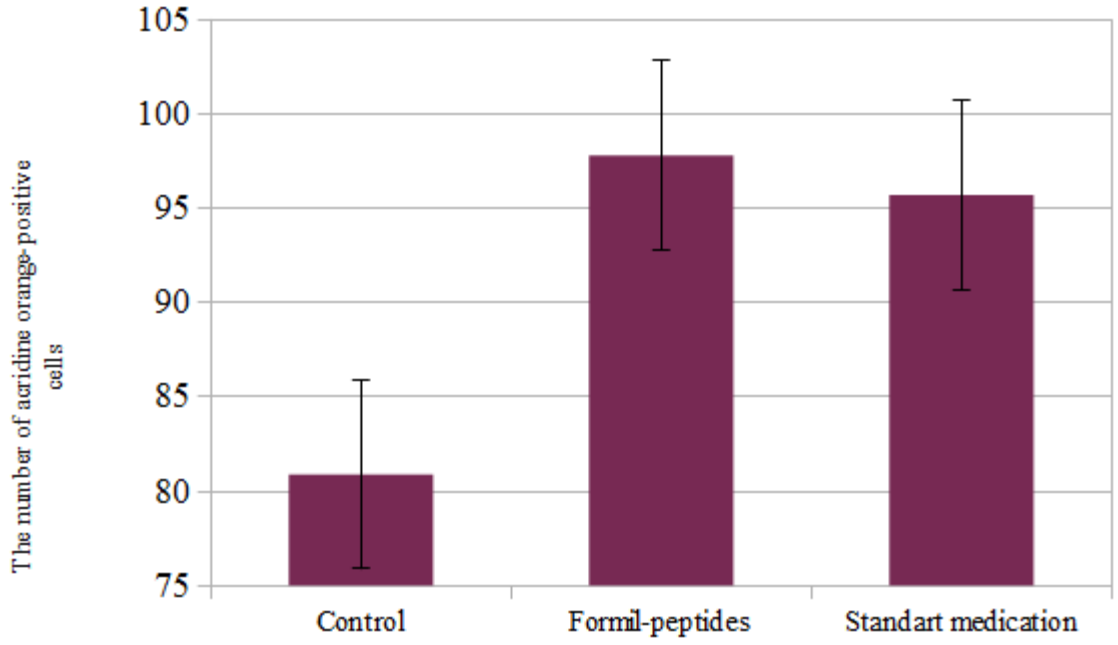

Fig. 2 - Lysosomal activity of alveolar macrophages exposed formyl-peptides and standard medication in vitro.

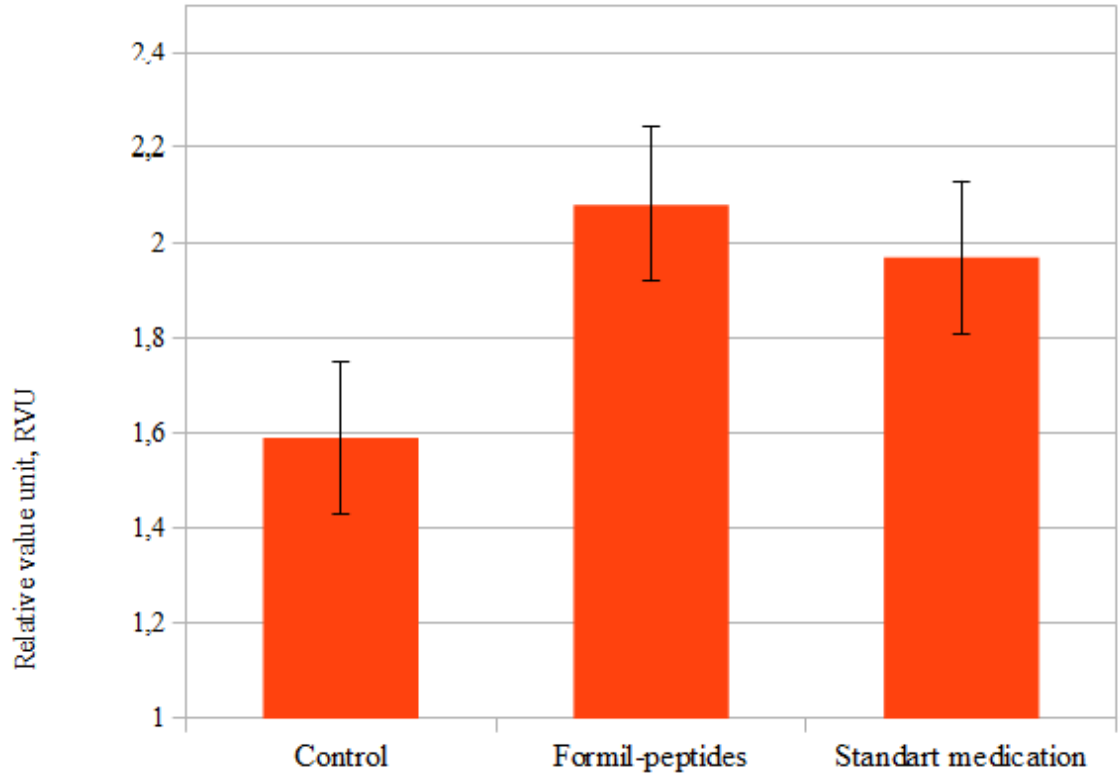

Fig. 3 — Cytochemical coefficient of acid phosphatase of alveolar macrophages exposed formyl-peptides and standard medication in vitro.

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Introduction. The key element in the formation of tuberculosis infection (TI) is the inability of alveolar macrophage to complete phagocytosis of mycobacteria absorbed by them, that caused by both features of the pathogens biology and the tissue macrophages. It is known, that M. tuberculosis is capable long-term persistence and proliferation in alveolar macrophage cytoplasm because of high stability of them cell walls to the lysosomal enzymes action. Mainly, the phenomenon of granulomatous reaction, inherent of tuberculosis (TB), reflects the inadequacy in elimination of tuberculosis pathogen of alveolar macrophages. Thus, the inclusion of agents that can activate completed phagocytosis of mycobacteria by alveolar macrophages, in the base of anti-TB therapy is a promising direction in the prevention of latent tuberculosis reactivation. **Materials and Method.** The ability of formyl-peptides activate the completeness of mycobacteria phagocytosis by alveolar macrophages absorbed by them were evaluated in vitro. For reaching the aim of the study we had used a broncho-alveolar lavage obtained by white laboratory male mice 2 months of age. As a comparison drug we used the officinal preparation "Liasten". To determine the lysosomal activity by the presence of peroxidase was treated with acridine orange causing selective staining red lysosomes. Acid phosphatase activity was studied using azocoupling reaction for staining granules in the cytoplasm blue or purple. Results are expressed as mean cytochemical coefficient (LZC). **Results and discussion.** Incubation of alveolar macrophages and formyl-peptides leads to a significant increase the index of mycobacteria phagocytosis completeness for vaccine strains - ($1,70 \pm 0,31$) and ($1,20 \pm 0,22$), respectively, ($p < 0.05$). The standard medication - Liasten - also increased the "killing" ability of tissue macrophages compared to control: ($1,8 \pm 0,25$) and ($1,20 \pm 0,22$), respectively, ($p < 0.05$). Lysosomal activity of alveolar macrophages exposed formyl-peptides significantly increased in comparison with the control - ($97,80 \pm 5,1$) and ($80,9 \pm 4,3$) acridine orange-positive cells, respectively, ($p < 0.05$). However, the effect of formyl-peptides on lysosomal activity of macrophages did not exceed the reference drug action - ($97,80 \pm 5,1$) and ($95,72 \pm 5,3$) acridine orange-positive cells, respectively, without significant differences. Incubation with formyl-peptides resulted in a significant increase of LZC of acid phosphatase in macrophages - ($2,08 \pm 0,20$) and ($1,59 \pm 0,14$), respectively, ($p < 0.05$). Significant differences between the content of this enzyme in macrophages when exposed formyl-peptides and the reference drug were not detected. **Conclusion.** As a result the co-incubation of alveolar macrophages and new formil-peptides activates BCG phagocytosis completeness. Also, there is the influence of the studied substances under significant lysosomal activity and increase the content of acid phosphatase in macrophages isolated from broncho-alveolar lavage in comparison with the control. The level of functional activity stimulation of phagocytes under the influence of formyl-peptide is the same, that we registered after the Liasten administration. It has indicating prospects of the medicinal preparation creation on the formyl-peptides basis, which stimulates the organism non-specific resistance.

Key words: Formil-peptides, non-specific resistance, lysosomal activity, in vitro.