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NEW FORMYL PEPTIDE HAS THE STIMULATING PROPERTIES IN POINT OF IMMUNE SYSTEM CELLS

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Introduction

Tuberculosis infection (TBI) is still one of the major health problems, despite of global intensive medical and pharmaceutical efforts [1]. At nowadays TBI is responsible for 1,3 million death all over the world. As it is known, in the majority of immunocompetent individuals TBI is repressed by immune system, and as a result we can observe the latent TBI. The main danger hides in unpredictable activation process of latent TBI determining the spreading of infection among population [2]. But recent study gives a good chance for restriction of TBI distribution, which suggested that early infection may be eradicated in a part of individuals [3].Only fewer than 10 % of infected individuals shows the clinically evident of primary TBI, and the majority of them are able to contain the primary infection with the granulomas formation, which consist of lymphocytes and *M. tuberculosis* infected macrophages [4]. Few weeks after, as we know, granulomas undergo necrosis resulting in the death of the majority of M. tuberculosis. But the small proportion of bacilli that survived has been postulated to result in latent TBI [5].

So, our study is focused on finding new medicinal preparations and combining them into aerosol pharmaceutical form which has the aim to complete the phagocytosis of absorbed *M. tuberculosis* by macrophages, that can prevent the activation of latent TBI.

Now we investigate the ability of new formyl peptide to stimulate phagocytosis completion in vivo. This strategy is explained by the key role of the phagocytosis completion in preventing long-term persistence of *M. tuberculosis* in macrophage.

Formyl peptides are released by microbes and damaged tissues that are perceived as danger signals and is recognized by the innate immune system by formyl peptides receptors expressed on neutrophil granulocytes [6, 7]. Of course, the outcome of the continuously ongoing with invading microorganisms is possible due to the chemotactic potency of neutrophils, which can migrate in response to gradients of chemoattractants [8]. The formyl peptides potency as neutrophil chemoattractants was discovered in the middle of 1970's. Recent studies show that activated formyl peptide receptors (FPR1 and FPR2) trigger a variety of functions, including chemotaxis, degranulation, ROS (reactive oxygen) production and phagocytosis [9]

Aim To assess the exposure to new formyl peptides on blood cells count and imunocompetent cells functional activity in immunodepressed mice with BCG injection.

Materials and Methods

Animals

The ability of new formyl peptide to activate the completeness of phagocytosis by peritoneal macrophages absorbed by them was evaluated *in vivo*. For reaching the aim of the study we have used peritoneal macrophages obtained from white laboratory male mice 2 months of age, and weight - 22 ± 2 g. Total of 36 animals were randomized on 4 groups:

1 Group – (Control) - mice with NaCl solution (0,9 %) injection, (n=11),
2 Group – mice with dexamethasone injection, (n=11),
3 Group - mice with dexamethasone and BCG injection, (n=11),
4 Group - mice with dexamethasone, BCG and formyl peptide injection, (n=11).

Animals were kept in 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 [10].

Assessment of immune cells function activity

Staphylococcus phagocytosis test

This test relies on the uptake of heat-killed *Staphylococcus aureus* by macrophages over a brief period of time. The cell pellet was resuspended in 3 ml RPMI 1640 medium and the number of macrophages was determined in a Goryaev's chamber to bring the cell concentration in the medium to 1×10^6 / mL [11].

Heat-killed *S. aureus* suspended in phosphate-buffered saline was adjusted to $\times 10^6$ cells/mL and stored at –20°C. A 0,1mL of Hank's balanced salt solution, a 0,1mL of pooled serum, and a 0,1 mL of heat-killed *S. aureus* were added to 0,2 mL of buffy coat and then centrifuged for 5 min at 15000 rpm. The supernatant was removed. Then the sediment was smeared, fixed with methanol, stained with Romanowsky-Gimza stain and viewed under microscope with immersion (100×). 100 macrophages were counted. Number of *S. aureus*-engulfed macrophages were counted as positive cells. Phagocytic Index = Number of positive cells/100 cells. Lytic index = Total number of *S. aureus*/100.

Reaction of lymphocyte transformation

Proliferative activity of lymphocytes was assessed by the level of their spontaneous and FGA-induced transformations in vitro (RBTL) [11]. The intensity of cell proliferation was assessed morphologically by the presence of blast forms appearing at the end of cultivation by using the light microscopy. The cells were cultured for 72 hours in RPMI-1640, with

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CO2 (5%). The FGA was introduced into the culture medium at a dose of 10 μg/mL.

Level of receptor's expression on lymphocytes

Level of receptor's expression on lymphocytes was examined in reaction of lymphocytes rosette formation with sheep red blood cells. Lymphocytes were obtained from the peripheral blood of mice by using ficoll velocity sedimentation gradient. 0,1 mL of isolated cells suspension (2×10^6/mL) was mixed with 0,1 mL of a 0,5% suspension of sheep red blood cells, incubated at T = 37°C for 10 min. Then the mixture was centrifuged for 5 min at 1000 rpm and incubated at T=4°C for an hour. Rosettes were fixed by adding to the each tube 0,05 mL of gluteraldehyde solution (3 %). After resuspension, they were left at room temperature for 20 min. At the end of this period, 5 mL of distilled water were added to the tubes, mixed gently and centrifuged at 1000 rpm for 10 min. The supernatant was removed, and slides were prepared from the sediment. After drying, the slides were fixed in 96% ethanol and stained by Romanowsky-Gimza. In each preparation 200 cells were counted, determining the percentage of lymphocytes that formed rosettes with 3 or more red blood cells [11].

Amount of blood cells

The number of different types of cariocytes in the leukogram was counted morphologically using the light microscope «Primo Star» (Carl Zeiss, Germany), taking into account not less than 200 cells in the preparation stained with Romanowsky-Gimza stain) [11].

Statistical analysis

The data were analyzed using SPSS software. Results of the numerical data are expressed as mean and standard deviation. Statistical significance was determined by using unpaired *t* test and one way ANOVA. $p< 0,05$ was taken as the level of significance) [12].

Results and Discussion

Our previous study, ex vivo experiments in mice, has revealed that the administration of dexamethasone before the introduction of BCG makes it possible to obtain a model for reducing the frequency of completion of phagocytosis by alveolar macrophages of adsorbed mycobacteria of the vaccinal strain [13]. In this paper, we used this approach to demonstrate the immunotrophy of a new synthetic formyl peptide in vivo.

The introduction of dexamethasone before the intraperitoneal administration of BCG led to decreased phagocytic index of peritoneum macrophages by 34 %, lytic index of peritoneum macrophages – by 76,8 %.

Tabl. 1 – **Exposure to new formyl peptide on imunocompetent cells functional activity in immunodepressed mice with BCG injection, (m±σ)**

Variables / Groups of animals	Phagocytic Index of peritoneum macrophages (PI), %	Lytic Index of peritoneum macrophages	LT stimulated FGA, %	LT spontaneous, %	Level of receptor's expression on lymphocytes, %
Control (NaCl 0,9 % solution)	68,7±2,1	7,3±0,5	67,41±3,18	18,34±1,52	27,9±1,72
BCG	65,2±3,0	6,9±0,42	69,82±4,06	30,40±2,7 [1)]	38,66±2,19 [1)]
BCG + Dexamethasone	43,18±2,6 [1)]	1,6±0,52 [1)]	36,51±3,42	8,04±0,52 [1)]	9,07±0,86 [1)]
BCG + Dexamethasone + Formyl peptide (16,7%) 1:10	62,9±2,9 [1), 2)]	5,7±0,50 [1), 2)]	52,34±3,77 [1), 2)]	15,46±1,22 [2)]	24,3±2,0

[1)] – compared with control, (n=11), (p<0,05). [2)] - compared with group of animals with BCG + Dexamethasone injections, (n=11), (p<0,05).

The introduction of the new formyl peptide to mice injected with BCG and dexamethasone promoted the increasing of the phagocytic activity of peritoneal macrophages. The phagocytic index in this group has no statistically difference with the control group. The lytic index has not reached the physiological norm - (5,7 ± 0,50) vs (7,3 ± 0,5), but was significantly higher (3,6 times) compared to the group of mice that have not obtained the formyl peptide after BCG and dexamethasone administration, ($p<0,05$).

As well as, we have observed the *ex vivo* enhancing of FGA-stimulated (by 1,4 times) and spontaneous lymphocyte transformation (by 1,8 times) after the formyl peptide's administration compared to the group without formyl peptide administration, ($p<0,05$).

Also, should be highlighted recent data, which suggested that new formyl peptide is able to promote the expression of receptors on lymphocytes. As we can see, the percent of receptors expression on lymphocytes have raised by 2,7 times after the formyl peptide's administration in mice with BCG and dexamethasone injections and was comparable with normal control, (Tabl. 1).

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Tabl. 2 – Exposure to new formyl peptide on blood cell's count in immunodepressed mice with BCG injection, (m±σ)

Variables / Group of animals	Total leucocyte count, 10^9/л	Eosinophil, %	Banded neutrophil,%	Segmented neutrophil,%	Monocyte, %	Lymphocyte, %
Control (NaCl 0,9% solution)	5,17±0,31	3,01±0,2	3,06±0,28	35,4±2,81	6,02±0,4	53,20±0,37
BCG	5,21±0,22	5,1±0,37[1)]	15,10±0,41[1)]	27,2±1,6[1)]	12,0±0,17[1)]	34,56±0,41[1)]
BCG + Dexamethasone	3,10±2,60[1)]	6,51±0,43[1)]	8,09±0,32[1)]	28,03±1,82[1)]	10,1±0,72[1)]	44,18±0,32[1)]
BCG + Dexamethasone + formyl peptide (16,7 %) 1:10	4,81±0,3[2)]	2,40±0,52[2)]	5,46±1,22[1),2]	30,5±2,21[2)]	6,20±0,62[2)]	49,87±0,26[2)]

*[1)] – compared with control, (n=11), (*p*<0,05). [2)] – compared with group of animals with BCG + Dexamethasone injections, (n=11), (*p*<0,05).*

The investigation of blood cell count showed that formyl peptide administration gave rise of total leucocyte count (by 1,5 times), which was decreased after dexamethasone applying. Percent of eosinophil in peripheral blood was significantly lower compared to the group with dexamethasone and BCG injections and reached the norm in this group.

BCG injection caused the significant growth in banded neutrophil's count - by 15 times - compared to the normal level. Dexamethasone administration with BCG has led to the similar effect, but in some lower degree – increase by 8 times. Formyl peptides exposure came out in banded neutrophils reliable count decreasing compared to the respective control – (5,46±1,22) % versus (8,09±0,32) %, (p<0,05).

BCG injection has led to the decline in reliable count of segmented neutrophils (by 1,3 times) comparing with control. There were no reliable difference in data between two groups of mice - with Dexamethasone and BCG administration and only BCG administration in count of segmented neutrophils. When under the influence of formyl peptide injections the reliable count of segmented neutrophils was commensurate with normal control.

The BCG and dexamethasone injections have caused increasing the monocyte level – (10,1±0,72) % vs (6,02±0,4) % and decreasing of lymphocyte level – (44,18±0,32) % vs (53,20±0,37) %, (p<0,05)б which was corrected by formyl peptide administration – (6,20±0,62) % and (49,87±0,26) % with no statistical differences with observed levels of monocytes and lymphocytes in normal control group.

Conclusions

The introduction of the new formyl peptide to mice injected with BCG and dexamethasone promoted increasing of the phagocytic activity of peritoneal macrophages. What is demonstrated by significantly growing of phagocytic and lytic indexes in this group compared to the group of mice that have not obtained the formyl peptide after BCG and dexamethasone administration, (p<0,05).

Also, was observed the *ex vivo* enhancing of FGA-stimulated (by 1,4 times) and spontaneous lymphocyte transformation (by 1,8 times) after the formyl peptide's administration compared to the appropriate control group, (p<0,05).

New formyl peptide is able to promote the expression of receptors on lymphocytes. The percent of receptors expression on lymphocytes has raised by 2,7 times after the formyl peptide's administration in mice with BCG and dexamethasone injections.

The formyl peptide administration normalizes the blood cells count compared to the appropriate control group, where the total count of leucocytes was decreased and ratio of neutrophils, lymphocytes and eosinophils were characterized by a disproportion.

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Introduction Tuberculosis infection (TBI) is still one of the major health problems, despite of global intensive medical and pharmaceutical efforts as it is known, in the majority of immunocompetent individuals TBI is repressed by immune system, and as a result we can observe the latent TBI. The main danger hides in unpredictable activation process of latent TBI determining the spreading of infection among population. Now we investigate the ability of new formyl peptide to stimulate phagocytosis completion in vivo. This strategy is explained by the key role of the phagocytosis completion in preventing long-term persistence of *M. tuberculosis* in macrophage.

Formyl peptides are released by microbes and damaged tissues that are perceived as danger signals and is recognized by the innate immune system by formyl peptides receptors expressed on neutrophil granulocytes. Recent studies show that activated formyl peptide receptors (FPR1 and FPR2) trigger a variety of functions, including chemotaxis, degranulation, ROS (reactive oxygen) production and phagocytosis.

Materials and methods The ability of new formyl peptide to activate the completeness of phagocytosis by peritoneal macrophages absorbed by them was evaluated *in vivo*. For reaching the aim of the study we have used peritoneal macrophages obtained from white laboratory male mice 2 months of age, and weight - 22 ± 2 g. Total of 36 animals were randomized on 4 groups:1 Group – (Control) - mice with NaCl solution (0,9 %) injection, (n=11), 2 Group – mice with dexamethasone injection, (n=11), 3 Group - mice with dexamethasone and BCG injection, (n=11), 4 Group - mice with dexamethasone, BCG and formyl peptide injection, (n=11). Animals were kept in 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. The peritoneum macrophages functional activity was assessed by using Staphylococcus phagocytosis test, proliferative activity of lymphocytes - by the level of their spontaneous and FGA-induced transformations in vitro (RBTL), level of receptor's expression on lymphocytes was examined in reaction of lymphocytes rosette formation with sheep red blood cells. The number of different types of cariocytes in the leukogram was counted morphologically using the light microscope «PrimoStar» (Carl Zeiss, Germany), taking into account not less than 200 cells in the preparation stained with Romanowsky-Gimza stain. Statistical significance was determined by using unpaired *t* test and one way ANOVA. $p< 0,05$ was taken as the level of significance. **Results and discussion** The introduction of the new formyl peptide to mice injected with BCG and dexamethasone promoted increasing of the phagocytic activity of peritoneal macrophages. What is demonstrated by significantly growing of phagocytic and lytic indexes in this group compared to the group of mice that has not obtained the formyl peptide after BCG and dexamethasone administration, ($p<0,05$). Also, was observed the *ex vivo* enhancing of FGA-stimulated (by 1,4 times) and spontaneous lymphocyte transformation (by 1,8 times) after the formyl peptide's administration compared to the appropriate control group, ($p<0,05$). New formyl peptide is able to promote the expression of receptors on lymphocytes. The percent of receptors expression on lymphocytes has raised by 2,7 times after the formyl peptide's administration in mice with BCG and dexamethasone injections. The formyl peptide

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administration has led to the normalization of blood cells count, when their depletion after dexamethasone and BCG injection has taken place. Conclusions The new formyl peptide administration to mice injected with BCG and dexamethasone promotes increasing of the phagocytic activity of peritoneal macrophages, enhance the FGA-stimulated and spontaneous lymphocyte transformation, enhances the level of receptors expression on lymphocytes compared with to the group of mice that has not obtained the formyl peptide after BCG and dexamethasone injection, (p<0,05). The formyl peptide administration normalizes the blood cells count compared to the appropriate control group, where the total count of leucocytes was decreased and ratio of neutrophils, lymphocytes and eosinophils were characterized by a disproportion.

Key words: tuberculosis infection, formyl-peptides, immune system cells, phagocytosis, immunosuppression, experiment.

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