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ВЛИЯНИЕ МЕТАБОЛИТОВ МИКРООРГАНИЗМОВ МНОГОЛЕТНЕМЕРЗЛЫХ ПОРОД НА СИНТЕЗ ЦИТОКИНОВ МОНОНУКЛЕАРНЫМИ КЛЕТКАМИ ПЕРИФЕРИЧЕСКОЙ КРОВИ ЧЕЛОВЕКА *IN VITRO*

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Резюме. Вечная мерзлота является уникальной экосистемой, характеризующейся стабильно отрицательными температурами и в которой микроорганизмы могут находиться в состоянии гипометаболизма или анабиоза на протяжении геологического времени. Известно, что микроорганизмы занимают широкий ареал обитания благодаря наличию полифункциональных систем адаптации и коммуникации. Одним из проявлений данных систем является выработка вторичных метаболитов (МБ), в состав которых входят сигнальные молекулы, не имеющие строгой видовой специфичности. Биологическая активность сигнальных молекул в значительной степени зависит от количества бактериальных клеток и температуры их культивирования.

В данной работе использованы вторичные метаболиты *Bacillus sp.* из вечной мерзлоты, полученные при различных температурах культивирования микроорганизмов (при -5 °С – «холодовые» МБ и при 37 °С – «тепловые» МБ) в дозах $0{,}05 \times 10^6$ (малая доза) микробных клеток (м.кл.) в мл физиологического раствора или 500×10^6 (высокая доза) м.кл./мл. Оценено влияние МБ *Bacillus sp.* на продукцию TNFα, IL-1β, IL-8, IL-2, IFNγ, IL-4, IL-10 мононуклеарными клетками периферической крови (МНК) человека в супернатантах 24-часовых клеточных культур методом ИФА с использованием тест-системы «ВекторБЕСТ» (Россия) на спектрофотометре LUCY-2 (ANTHOS) (Австрия) согласно рекомендациям производителя.

Установлено, что по сравнению с контролем под влиянием МБ *Bacillus sp.* вне зависимости от температуры их получения и дозы бактерий активность синтеза МНК человека основного спектра цитокинов достоверно возросла ($p < 0{,}01$ для всех показателей), за исключением IL-8, уровень которого не отличался от контрольного под влиянием высокой дозы «тепловых» МБ. По сравнению с ФГА синтез цитокинов МНК зависел от дозы и температуры получения МБ. Так, под влиянием «тепловых» МБ уровень TNFα был достоверно ниже его уровня под влиянием ФГА не зависимо от дозы. Уровень IL-8 был снижен относительно ФГА под влиянием метаболитов от 500×10^6 м.кл. вне зави-

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симости от температуры их получения. Сравнение между собой влияния «тепловых» и «холодовых» МБ *Bacillus sp.* показало, что малые дозы «холодовых» метаболитов в большей степени стимулируют синтез провоспалительных цитокинов (TNFα, IL-1β, IL-8, IFNγ). Высокие дозы «тепловых» метаболитов *Bacillus sp.* в большей степени активируют МНК человека на синтез противовоспалительных цитокинов (IL-4 и IL-10). Учитывая, что TNFα, IL-1β и IL-10 являются цитокинами системного действия, ответственными не только за активацию системы иммунитета, а также мобилизацию других регуляторных систем организма, можно в перспективе рассматривать возможность использования вторичных метаболитов микроорганизмов многолетнемерзлых пород в качестве субстрата для разработки новых иммуномодуляторов и адаптогенов.

Ключевые слова: провоспалительные цитокины, противовоспалительные цитокины, мононуклеарные клетки крови, вторичные метаболиты бактерий многолетнемерзлых пород, антиген-индуцированная реакция бластной трансформации лимфоцитов

INFLUENCE OF METABOLITES OF MICROORGANISMS FROM PERMAFROST ON THE SYNTHESIS CYTOKINES BY HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS *IN VITRO*

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Abstract. Permafrost is a unique ecosystem characterized by consistently negative temperatures. It has been shown that microorganisms can be there in a state of hypometabolism or anabiosis during geological time. It is known that microorganisms occupy a wide habitat due to the presence of multifunctional systems of adaptation and communication. One of the manifestations of these systems is the production of secondary metabolites (MBs), which include signaling molecules that do not have strict species specificity. The biological activity of signaling molecules largely depends on the number of bacterial cells and the temperature of their cultivation.

In this work we used secondary MBs of *Bacillus sp.* from Permafrost obtained at different temperatures of microorganism cultivation (at -5 °C – "cold" MBs and at 37 °C – "warm" MBs) in doses of $0{,}05 \times 10^6$ (small dose) of microbial cells (m.cl.) in ml of saline or 500×10^6 (high dose) m.cl./ml. The influence of MB of *Bacillus sp.* for the TNFα, IL-1β, IL-8, IL-2, IFNγ, IL-4 and IL-10 production by human peripheral blood mononuclear cells (MNC) in supernatants of 24-hour cell cultures was estimated by ELISA whith using the "VectorBEST" test system (Russia) on a LUCY-2 (ANTHOS) spectrophotometer (Austria).

It was found that the activity of synthesis by human MNC of the main spectrum of cytokines significantly increased ($p < 0.01$ for all indicators) under the influence of MB *Bacillus sp.* regardless of the temperature of their cultivation and the dose of bacteria. The exception was IL-8, the level of which under the influence of a high dose of "warm" MBs didn't differ from the control. Compared to PHA the cytokines synthesis by MNC depended on the dose and the temperature of obtaining of MBs. Thus, under the influence of "warm" MBs the level of TNFα was significantly lower than its level under the influence of PHA regardless of the dose. Regardless of the temperature of obtaining metabolites the level of IL-8 under the influence of metabolites from a dose of 500×10^6 m.cl. was reduced relative to the PHA group. Comparison of the influence of "warm" and "cold" MBs of *Bacillus sp.* showed that small doses of "cold" metabolites to a greater extent stimulate the synthesis of pro-inflammatory cytokines (TNFα, IL-1β, IL-8, IFNγ). High doses of "heat" metabolites of *Bacillus sp.* to a greater extent they activate human MNCs for the synthesis of anti-inflammatory cytokines (IL-4 and IL-10). Considering that TNFα, IL-1β and IL-10 are cytokines of systemic action and are responsible not only for the activation of the immune system, but also for the mobilization of other regulatory systems of the organism,

it can be assumed that the secondary metabolites of microorganisms from Permafrost will be efficient as a substrate for the development of new immunomodulators and adaptogens in the future.

Keywords: pro-inflammatory cytokines, anti-inflammatory cytokines, blood mononuclear cells, secondary metabolites of bacteria from permafrost, antigen-induced reaction of blast-cell transformation of lymphocytes

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Introduction

Permafrost is one of the unique ecosystems characterized by negative temperatures over geological time [13]. Viable microorganisms of various genera and species were found in permafrost rocks. The ones may be there in a state of hypometabolism or suspended animation. [4, 9, 14]. It is known that microorganisms occupy a fairly wide habitat due to the presence of multifunctional adaptation and communication systems. One of the manifestations of the work of these systems is the production of secondary metabolites (MB), which include signaling molecules that don't have strict species specificity [5, 11, 12]. The biological activity of signaling molecules largely depend on the number of bacterial cells and the temperature of their cultivation [12]. An experimental study of microorganisms (MO) of strain M3 *Bacillus sp.* isolated from permafrost of the late Neogene showed that a change in the temperature conditions of cultivation has a significant effect on the biological properties of bacteria *in vitro* and *in vivo*, in particular, it changes their enzymatic, immunotropic and reparative activity [6, 7, 8, 10]. It seems relevant to study the effect of MB of MO from Permafrost on the functional activity of immunocompetent human cells *in vitro* for development, in the future, immunotropic drugs based on them. It is known that cytokines as mediators of intercellular "communication" provide the regulation of immune responses and the coordinated interaction of cells of the immune system [2].

Purpose of the study: to assess the effect of the secondary metabolites of permafrost microorganisms on the spectrum and level of cytokine secretion by human peripheral blood mononuclear cells *in vitro* depending on the temperature of cultivation and the dose of bacterial cells.

Materials and methods

The object of study is *Bacillus sp.* M3 strain isolated from Late Neogene permafrost (Mamontova Mountain, Central Yakutia, age of permafrost 2.5-3 million years [1]. To obtain secondary MB, the MO suspension was prepared in aliquots of 0.05×10^6 (low dose) or 500×10^6 (high dose) microbial cells in 1 ml (m.cl./ml) of physiological saline and incubated at temperatures of -5 °C ("cold" metabolites – MB-C) and 37 °C ("thermal" metabolites – MB-T) for 72 hours. Every 24 hours, the samples were kept for 30 minutes at 22 °C. The MBs was separated through passing a suspension of MOs through bacterial filters with a pore diameter of 0.22 μm (Millipore, USA). The purity of the MBs was confirmed by control sowing on culture media.

The study was conducted on the culture of peripheral blood mononuclear cells (PBMC) of 3 independent donors (men aged 24-26 years). Mononuclear cells (MNC) were isolated on a Ficoll-Paque density gradient (= 1.077). The wells of 96-well immunological plates were loaded with 0.2×10^6 MNC in 180 μl of RPMI-1640 medium with the addition of 2 mmol glutamine and 80 μg/ml gentamicin. Several options for the reaction of blast-cell transformation of lymphocytes were posed. Negative control is the addition of 20 μl of RPMI-1640 medium (control group). A positive or mitogen-induced control is the addition of 20 μl of the polyclonal T-mitogen phytohemagglutinin (20 pg/ml, "Serva") (PHA group). And 4 variants of the experimental groups: adding 20 μl of "cold" or "thermal" metabolites obtained from 0.05×10^6 m.cl./ml (groups MB-C/0.05; MB-T/0.05) or 500×10^6 m.cl./ml (MB-C/500; MB-T/500). The reaction was set up in triplets for 24 hours. The levels of pro-inflammatory (TNFα, IL-1β, IL-8, IL-2, IFNγ) and anti-inflammatory (IL-4 and IL-10) cytokines were determined in cell culture supernatants by ELISA ("VectorBEST") on a LUCY-2 spectrophotometer (ANTHOS) (Austria) and expressed in pg/ml. The significance of differences between the groups was assessed by Student's t-test in the SPSS "11.5 for Windows" program. Differences were considered significant at $p < 0.05$.

Results

The results of the study are presented in the Table 1. Minimal levels of cytokines without stimulation with

TABLE 1. CYTOKINE LEVEL (pg/ml), M±m

Cytokines	TNFα	IL-1β	IL-8	IL-2	IFNγ	IL-4	IL-10
Control group	4.4±0.6	24.9±3.3	25.4±4.6	0.2±0.1	1.4±0.1	1.1±0.2	2.3±0.4
PHA group	264.0±6.0	157.2±10.3	249.0±11.3	0.9±0.2	17.3±1.6	1.8±0.1	5.2±0.5
Metabolites from 0.05 × 10^6 m.cl./ml							
MB-C	424.0±43.3 **, ^^	263.0±31.0 **, ^^	287.0±34.0 **	3.8±1.6 **, ^^	26.3±3.2 **, ^^	2.7±0.3 **, ^	8.4±0.3 **, ^
MB-T	63.5±4.3 **, ^^	261.0±33.0 **, ^^	355.0±42.1 **, ^^	1.8±0.1 **, ^^	13.6±2.4 **	3.8±0.5 **, ^	9.2±0.7 **, ^
Metabolites from 500 × 10^6 m.cl./ml							
MB-C	285.1±15.3 **	270.5±21.2 **, ^^	78.5±6.1 **, ^^	11.2±3.9 **, ^^	21.8±3.1 **	2.4±0.3 *, ^	8.7±2.9 **, ^
MB-T	54.9±2.7 **, ^^	247.8±18.6 **, ^^	26.4±7.9 ^^	0.9±0.1 **	9.1±2.2 **, ^^	19.4±4.3 **, ^^	44.8±5.5 **, ^^

Note. The difference from the control: *, p < 0.05; **, p < 0.01; difference from the PHA: ^, p < 0.05; ^^, p < 0.01.

antigens (control group) and their high indices under the influence of a polyclonal T-mitogen (PHA group) testify to the qualitative immunological reactivity of PBMC of donors (Table 1).

The results of a study of the effect of MB of MO from Permafrost on the synthesis of cytokines by human peripheral blood mononuclear cells showed the following. "Cold" metabolites obtained from 0.05 × 10^6 m.cl./ml at -5 °C (MB-C/0.05 group) stimulated human PBMCs to activate the synthesis of pro-inflammatory (TNFα, IL-1β, IL-8 , IL-2, IFNγ) and anti-inflammatory (IL-4 and IL-10) cytokines compared with the control ($p < 0.01$ for all cytokines). In this group the activity of the synthesis of cytokines TNFα, IL-1β, IL-2 and IFNγ was significantly ($p < 0.01$) higher, and the synthesis of IL-4 and IL-10 was moderately higher ($p < 0.05$) than in PHA group. There were no differences between these groups in level of IL-8 ($p > 0.05$).

The synthesis activity of the entire studied cytokine spectrum increased ($p < 0.01$ for all cytokines) under the influence of "thermal" metabolites obtained from 0.05 × 10^6 m.cl./ml at 37 °C (MB-T/0.05 group) compared with the control. In this experimental group the level of TNFα was decreased ($p < 0.01$), the levels of IL-1β, IL-8 and IL-2 were significantly increased ($p < 0.01$ in all cases), the levels of IL-4 and IL-10 were moderately increased ($p < 0.05$ in both cases) compared with the PHA group. The level of IFNγ was at the level of IFNγ in the PHA group ($p > 0.05$).

"Cold" metabolites obtained from 500 × 10^6 m.cl./ml at -5 °C (MB-C/500 group) significantly increased the synthesis activity of the entire studied spectrum of cytokines compared to the control. We noted that the synthesis activity of IL-1β ($p < 0.01$) and IL-2 ($p < 0.01$) increased significantly, the levels of IL-4 ($p < 0.05$) and IL-10 ($p < 0.05$) increased moderately compared with these indicators in the PHA group. No differences were found in the levels of TNFα and IFNγ ($p > 0.05$).

Under the influence of "thermal" metabolites obtained from 500 × 10^6 m.cl./ml at 37 °C (MB-T/500 group) the synthesis activity of the entire studied spectrum of cytokines was significantly higher ($p < 0.01$ for all cytokines) except for IL-8 ($p > 0.05$) compared with the control group. The levels of TNFα, IL-8 and IFNγ were reduced ($p < 0.01$ in all cases) and the secretion of IL-1β, IL-4 and IL-10 ($p < 0.01$ in all cases) were significantly increased compared with the PHA group. The level of anti-inflammatory IL-10 was 8.6 times higher than in the PHA group.

Thus, the analysis of the obtained data showed that the effect of the secondary metabolites of microorganisms from Permafrost on the cytokine response of immunocompetent cells of human peripheral blood *in vitro* to a certain extent depends on both the dose and the temperature of bacterial

cultivation at which these metabolites were obtained. Moreover, the synthesis of TNFα was predominantly stimulated by MB obtained from MO at a negative temperature of cultivation (MB-C/0.05 and MB-C/500 groups). The synthesis of IL-8 was largely stimulated by low doses of MB (MB-C/0.05 and MB-T/0.05 groups). The synthesis of IL-2 was largely stimulated by high doses of MB obtained from MO at a negative temperature of their cultivation (MB-C/500 group). The activity of the synthesis of IFNγ was maximum for MB obtained at a negative temperature (groups MB-C/0.05 and MB-C/500). The maximum levels of secretion of IL-4 and IL-10 were observed under the influence of a high dose of MB obtained from MO at a positive temperature of cultivation (MB-C/500 group). The exception was IL-1β, its level was significantly high in all experimental groups ($p < 0.01$ compared with control and PHA groups).

Summarizing the foregoing it can be concluded that low doses of "cold" metabolites of MOs from Permafrost (obtained from 0.05×10^6 m.cl./ml at -5 °C) show their immunobiological activity to a greater extent towards the activation of the synthesis of pro-inflammatory cytokines (TNFα, IL -1β, IL-8, IFNγ). High doses of "thermal" metabolites of MO MMP (from 500×10^6 m.cl./ml) obtained during the cultivation of bacteria at positive temperatures (37 °C) to a greater extent activate human PBMC for the synthesis of anti-inflammatory cytokines (IL-4 and IL-10).

The obtained data together with the previously obtained results [6, 7, 8, 10] are the basis for raising a number of questions about the action mechanisms of metabolites of microorganisms from Permafrost on human immunocompetent cells. Considering also that TNFα, IL-1β and IL-10 are systemic cytokines that are responsible not only for the activation of the immune system, but also for the mobilization of regulatory systems such as the nervous and endocrine [3, 14, 15], it is possible to consider the possibility to use secondary metabolites of Permafrost microorganisms as a substrate for the development of new immunomodulators and adaptogens.

References

1. Brushkov A.V., Melnikov V.P., Shchelchkova M.V., Griva G.I., Repin V.E., Brenner E.V., Tanaka M. Biogeochemistry of frozen rocks of Central Yakutia. *Earth's Cryosphere (Russia), 2011, Vol. 15, no. 4, pp. 90-100.* (In Russ.)

2. Budchanov Yu.I. Hormones and mediators of the immune system. Regulation of the immune response. Tver: Publishing house of Tver State Medical Academy, 2008. 10 p.

3. Chernykh E.R., Leplina O.Yu., Tikhonova M.A., Paltzev A.V., Ostanin A.A. Cytokine Balance in Systemic Inflammation: a new target for immunotherapy of sepsis. *Medical Immunology (Russia), 2001, Vol. 3, no. 3, pp. 415-429.* (In Russ.)

4. El-Registan G.I., Mulyukin A..L, Nikolaev Yu.A., Galchenko V.F., Suzina N.E., Duda V.I. Adaptogenic functions of extracellular autoregulators of microorganisms. *Microbiology, 2006, Vol. 75, no. 4, pp. 380-389.* (In Russ.)

5. Erin K., Shiner A., Kendra P. Interkingdom signaling: deciphering the language of acylhomoserine lactones. *Microbiol. Rev., 2005, Vol. 29, pp. 935-947.*

6. Kalenova L.F., Bazhin A.S., Novikova M.A. Effects of metabolites of bacteria *Bacillus sp.* from permafrost on speed repair of skin wound. *Journal of New Medical Technologies, 2014, Vol. 21, no. 4, pp. 53-61.* (In Russ.)

7. Kalenova L.F., Subbotin A.M., Bazhin A.S. Influence of bacteria from perennial frozen species of different geological age on the immune system. Journal of New Medical Technologies, eEdition, 2013, 1. Available at: http://elibrary.ru/download/39510323.pdf.

8. Kalyonova L.F., Novikova M.A., Subbotin A.M., Bazhin A.S. Effects of temperature on biological activity of permafrost microorganisms. *Bull. Exp. Biol. Med., 2015, Vol. 158, no. 6, pp. 772-775.*

9. Karaevskaya E.S., Demidov N.E., Shmelev D.G., Rivkina E.M., Bulat S.A. The study of the bacterial communities in the Antarctic Oases' permafrost by means of culturing. *Arctic and Antarctic Research, 2017, Vol. 2, no. 112, pp. 27-42.* (In Russ.)

10. Kolyvanova S.S., Solovyov V.S., Kalyonova L.F. The influence of bacterial strains of the genus Bacillus isolated from Permafrost of different geological age on the immunological parameters of laboratory animals. *Russian Journal of Immunology, 2015, Vol. 9 (18), no. 3 (1), pp. 105-107.* (In Russ.)]

11. Levin B.R., Rozen D.E. Non-nherited antibiotic resistance. *Nat. Rev. Microbiol., 2006, Vol. 4, pp. 556-562.*

12. Nikolaev Yu.A. Autoregulation of the stress response of microorganisms: DocSci. Dissertation Abstract, Biology. Moscow, 2011. 50 p. (In Russ.)

13. Shherbakova V.A. Anaerobic bacteria and archaea in permafrost deposits of the Arctic: DocSci. Dissertation Abstract, Biology. Moscow, 2018. 48 p. (In Russ.)

14. Surkova E.A., Kuzubova N.A., Ses T.P., Totolian Areg A. Features of cytokine-mediated regulation of focal and systemic inflammation in cOPD. *Medical Immunology (Russia), 2010, Vol. 12, no. 4-5, pp. 349-354.* (In Russ.) doi: 10.15789/1563-0625-2010-4-5-349-354.

15. Titova Zh.V., Bodienkova G.M. Cytokine net role in mechanisms of neuroimmune interaction (literature review). *Acta Biomedica Scientifica, 2013, Vol. 2, no. 90, pp. 171-175.* (In Russ.)

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