Experimental Study of the Effects of Permafrost Microorganisms on the Morphofunctional Activity of the Immune System

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The immunobiological potential of a new microorganism species isolated from permafrost specimens (PMO strain 3M) collected from the Mammoth Mountain in Yakutia was studied in laboratory mice. PMO injected intraperitoneally in doses of 2500 to 50×10^6 microbial bodies caused characteristic dose-dependent effects on the structure and functions of the immune system (thymus and spleen indexes, functional activity of splenic macrophages, cellular and humoral immunity). Doses of PMO stimulating functional activities of both cellular and humoral immunity were detected.

Key Words: *permafrost microorganisms; cellular and humoral immunity*

According to modern concepts, Earth crust microorganisms participate in the regulation of substance and energy flow by maintaining the planet "homeostasis". Viable microorganisms have been detected in specimens of the ice and soil collected at different depths, even as deep as 4 km. The age of some of them seems to reach millions of years [1,3,6,8].

Vital activity of the biosphere, including microorganisms, largely determines human health. Degradation of the cryolithozone by natural reasons (objective natural events – cyclic changes in the climate on the planet) and man-created causes (increasing anthropogenic loading and economic activity) can lead to the release of microbiological substances, including those with modified antigenic properties, into the environment and hence, into biological turnover. It is impossible to evaluate their potential role for humans, because the mechanisms of their effects on ecosystems, including humans and modern living organisms, are unknown.

Numerous examples of the effects of extreme physical conditions of the environment on living organisms indicate that the habitat induces the development of characteristics adapting the organisms to these conditions. In the course of evolution under most unfavorable environmental conditions the microorganisms developed numerous adaptation reactions aimed at maintenance of their viability. These reactions included modification of intracellular structures and appearance of many specific defense reactions [2]. The effects of a complex of harsh environmental conditions (low temperatures, metabolic exhaustion, absence of light) of the permafrost on the biological potential of microorganisms are particularly interesting. It seemed to be interesting to transfer the adaptive potential of the permafrost microorganisms to the main adaptive systems of modern mammals, for example, to the immune system.

We evaluated the effects of viable microorganisms isolated from permafrost specimens on the immune

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system of modern mammals, specifically, laboratory mice.

MATERIALS AND METHODS

The study was carried out on a microorganism strain isolated from specimens of permafrost soil collected at the Mammoth mountain in Central Yakutia. Foci of permafrost there belong to the most ancient permafrost exposure in Eurasia. The specimens were collected from soil which did not defrost over more than 2 million years. The isolated microorganism strain was identified as the *Bacillus* genus, presumably a new species. The 16S rRNA nucleotide sequence of the bacillus was deposited in the DDBJ/EMBL/GeneBank, No. AB178889, identification No. 20040510203204.24251. The isolated strain exhibited the greatest similarity to *B. simplex* and *B. macroides*, the homology with their 16S rRNA reaching 96-97%. The strain was used in the first stage of studies of the biological characteristics of permafrost microorganisms (PMO strain 3M) as best comparable to the modern microorganisms.

The results of strain 3M PMO acute toxicity studies completely coincided with the results of studies of the probiotic *B. cereus* (strain IP5832) from Bactisubtil drug [5]. The adaptation reaction can be initiated by cells exposed to extreme factors, and hence we carried out an additional study of acute toxicity of strains 3M PMO and IP5832 after their preincubation at -5°C (permafrost temperature) for 72 h. Preincubation at low temperature reduced acute toxicity of 3M PMO by 34.7% in comparison with IP5832 strain. Therefore, the frosting-defrosting cycle was carried out before injection of the microorganisms to animals for reactivation of the bacterium adaptation potential.

Experiments were carried out on 126 F_1(CBA/Bl6) mice (18-20 g). PMO were injected to mice in single intraperitoneal doses of 2.5×10^3, 5×10^3, 10×10^3, 20×10^3, 50×10^3, 500×10^3, 5×10^6, and 50×10^6 microbial bodies (m.b.) in 100 μl saline. Controls were injected with saline. Microorganism concentrations were evaluated by the calibration curve on a photoelectrocolorimeter and verified by counting in a Goryaev chamber under a microscope.

Immunophysiological studies were carried out on day 14 after PMO injection. All studies were carried out in accordance with "Regulations for Studies on Experimental Animals" (Order No. 755 of the Ministry of Health of the USSR of August 12, 1977) and European Convention of Vertebrate Protection (March 18, 1986). The indexes of the thymus, spleen, and adrenals (percent proportion of organ to body weight) were estimated. The capacity of adherent (on the glass) splenic macrophages to phagocytosis (PC) of inactivated yeast cells and their metabolic activity in spontaneous NBT test were evaluated. Humoral immunity was evaluated by Cunningham's method [4] by the counts of nucleated cells in the spleen and the count of antibody-producing cells per 10^6 nuclears (APC/10^6) and per spleen (APC/spleen) on day 5 after intraperitoneal immunization of animals with sheep erythrocytes in a dose of 4×10^8 cells. Activity of cellular immunity was evaluated by delayed-type hypersensitivity (DTH) *in vivo* in response to sheep erythrocytes as described previously [7]. The animals were sensitized with 0.25% sheep erythrocyte suspension in 0.5 ml saline intraperitoneally. The resolving dose (50% sheep erythrocytes, 50 μl) was injected into the right hind paw; 50 μl saline was injected into the contralateral paw. The reaction was evaluated 24 h after injection of the resolving dose of sheep erythrocytes by measuring (with the slide gage) the edemas of the left and right paws and estimation of the increment in the volume of the right *vs.*

TABLE 1. Characteristics of the Cellular and Humoral Immunity (*M*±*m*)

Dose of PMO, m.b.	DTH, %	APC/10^6	Nuclears, $\times10^6$	APC/spleen
Control	26.0±1.78	754±33	105.0±5.3	79,170±3115
2.5×10^3	35.6±1.95**	829±47	138.0±8.8**	114,402±5354**
5×10^3	40.5±2.76**	1093±53**	152.0±7.9**	166,136±6747**
10×10^3	39.5±2.17**	957±48**	172.0±9.3**	164,604±6233**
20×10^3	43.4±3.94**	844±55	182.0±10.4**	153,426±5095**
50×10^3	29.1±2.48	1425±81**	219.0±11.6**	312,075±14,242**
500×10^3	30.2±2.55	1169±73**	228.0±12.5**	266,532±8516**
5×10^6	30.9±2.72	966±57**	269.0±15.7**	259,854±7448**
50×10^6	36.7±3.18**	814±52	95.0±8.4	77,330±5834

Note. **$p<0.01$ compared to the control.

left paw. The significance of differences between the groups was evaluated by Student's *t* test using SPSS 11.5 for Windows software.

RESULTS

Morphophysiological activity of the organs involved in immunogenesis and metabolism changed differently in response to the medium PMO doses (500×10^3 and 5×10^6 m.b.): the adrenal index increased, while the thymus index decreased (Fig. 1).

The changes in these parameters in response to low (below 20×10^3 m.b.) and high (50×10^6 m.b.) doses of PMO were universal: the thymus and adrenal indexes increased in comparison with the control. Lower doses of PMO (below 20×10^3 m.b.) promoted a simultaneous increase in the thymus and spleen indexes compared to the control. Increasing PMO dose above 50×10^3 m.b. changed activity vector: first towards elevation of the splenic index and then (in response to 50×10^6 m.b.) elevation of the thymus index.

PMO in all the studied doses stimulated functional activity of splenic macrophages in comparison with the control (Fig. 2). Phagocytic (PC) and metabolic (NBT) activities of macrophages increased in a wave-like mode with peaks at doses of 5×10^3 and 50×10^6

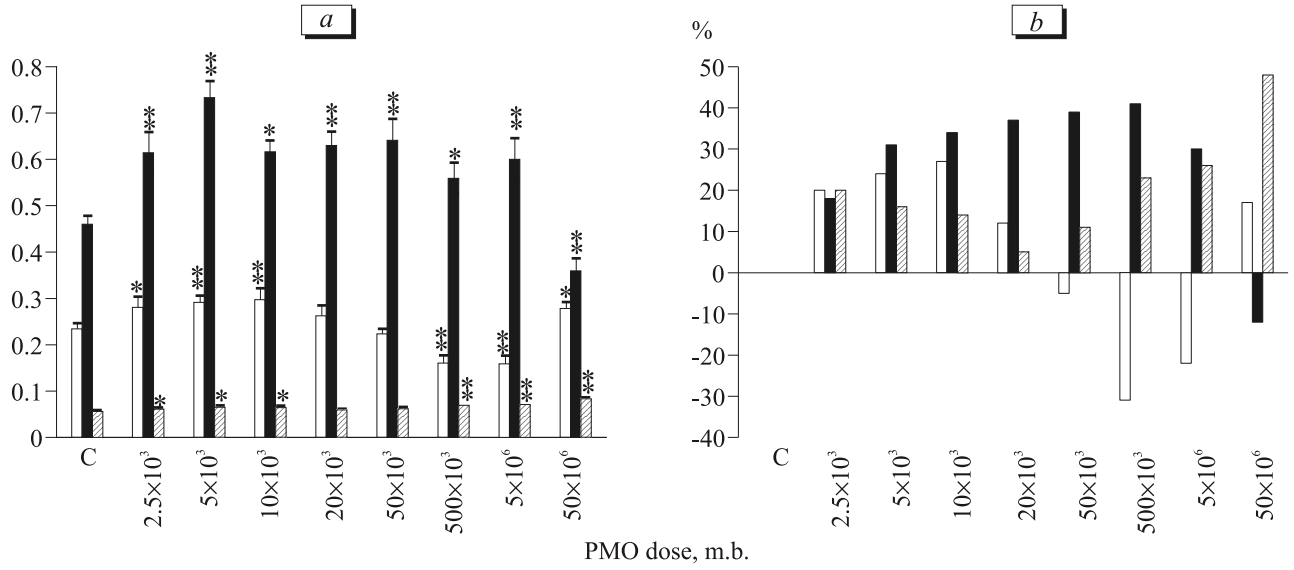

Fig. 1. Morphophysiological activity of the viscera in experimental groups (*a*) and its differences from the control (*b*). Light bars: thymus index; dark bars: splenic index; hatched bars: adrenal index. Here and in Fig. 2: **p*<0.05, ***p*<0.01 compared to the control (C).

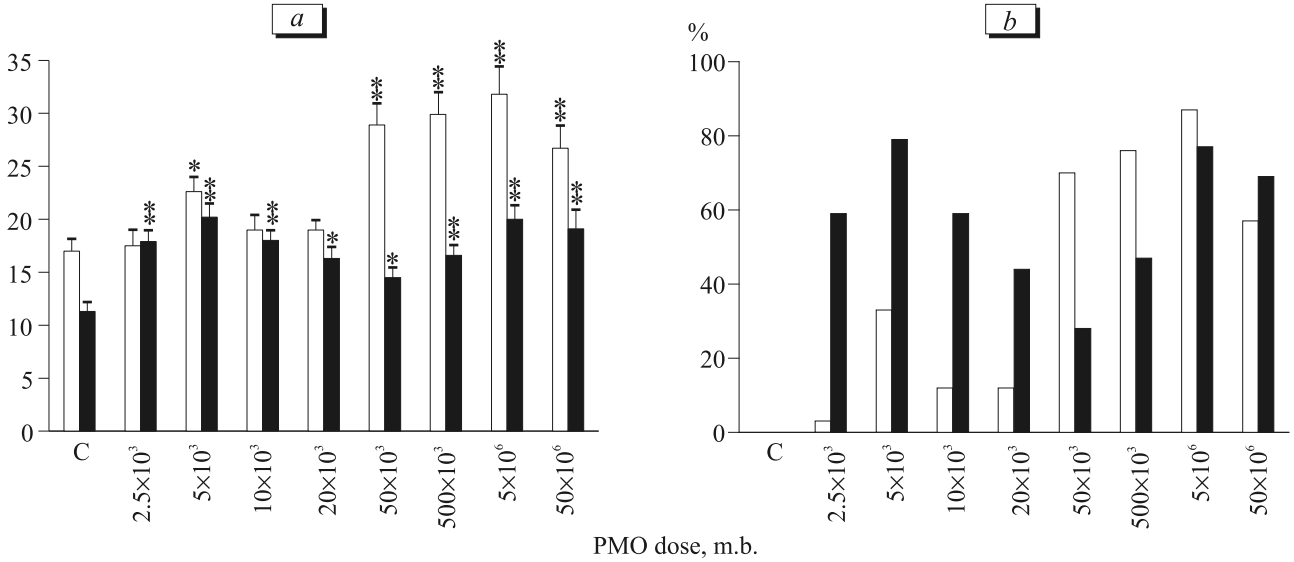

Fig. 2. Functional activity of splenic macrophages in experimental groups (*a*) and its differences from the control level (*b*). Light bars: phagocytic, dark bars: metabolic activity of macrophages.

m.b. PMO did not suppress humoral or cellular immunity in any of the studied doses (Table 1).

The functional constituent of humoral immunity in our study was presented by APC count per million splenocytes (APC/10^6), which changed in a wave-like mode with two peaks at doses of 5×10^3 and 50×10^3 m.b. The structural constituent of humoral immunity was presented by total splenocyte count (APC/spleen), which surpassed significantly the control level at all doses except the highest one. Activity of systemic immunity depended on the functional (count of APC/10^6) and structural (nuclear count) constituents, and hence, the count of APC in the spleen increased even more significantly in response to all doses except 50×10^6 m.b. Functional activity of cellular immunity (DTH reaction) also changed in a wave-like mode with peaks at doses of 20×10^3 and 50×10^6 m.b.

Comparative analysis showed that low PMO doses (5×10^3 and 10×10^3 m.b.) simultaneously increased functional activities of cellular (DTH reaction level) and humoral (count of APC/10^6) immunity in comparison with the control, while doses $>20\times10^3$ m.b. caused opposite changes in their activities. Medium doses (from 50×10^3 to 5×10^6 m.b.) stimulated mainly the humoral immunity, while the 50×10^6 m.b. dose mainly the cellular immunity.

Hence, the studied permafrost microorganism strain exhibited pronounced and mainly predictable effects on immunophysiological parameters of modern mammals. Stress exposure, including infection antigens, leads to reciprocal changes in the morphophysiological activities of the thymus and adrenals. In our study, activity of the thymus decreased in response to medium PMO doses, which should not be regarded as a stress reaction for some reasons: first, functional activities of cellular and humoral immunity in these groups persisted at an above-control level and, second, the thymus index and functional activity of cellular immunity increased in response to a higher dose (50×10^6 m.b.) of PMO. These reactions of the immune system in response to PMO also largely coincided with known reactions: predominant stimulation of the phagocytic and humoral components of the immune system under the effects of bacterial antigens; the dose-dependent effect and polarity of the Th1/Th2-dependent immune response. On the other hand, importantly that in low doses (below 50×10^3 m.b.) PMO promoted a simultaneous increase of the structural and functional potential of the cellular and humoral components of the immune system. Approaches to further studies of the immunobiological potential of PMO are outlined.

REFERENCES

1. S. S. Abyzov, N. E. Bobin, and V. V. Kudryashov, *Izv. Akad. Nauk SSSR*, ser. *Biology*, No. 6, 828-836 (1979).
2. M. E. Bekker, B. E. Damberg, and A. I. Rapoport, *Microorganism Anabiosis* [in Russian], Riga (1981).
3. A. V. Broushkov, V. P. Melnikov, Yu. G. Suhovei, *et al.*, *Uspekhi Gerontol.*, **22**, No. 2, 253-258 (2009).
4. *Methods of Investigation in Immunology*, Eds. I. Lefkovits and B. Pernis [in Russian], Moscow (1981).
5. I. B. Sorokulova, I. G. Osipova, N. V. Tereshkina, *et al.*, *Vestn. Rossiisk. Akad. Med. Nauk*, No. 1, 50-54 (2006).
6. A. N. Khimenkov and A. V. Broushkov, *Introduction in Structural Cryology* [in Russian], Moscow (2006).
7. A. J. Crowl, *Adv. Immunol.*, **20**, 197-264 (1975).
8. E. I. Friedmann, *Viable Microorganisms in Permafrost*, Russian Academy of Sciences, Pushchino (1994), pp. 21-26.