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CHARACTERIZATION OF THE BLOOD PROTEOME OF HEALTHY VOLUNTEERS REFLECTING ADAPTATION PROCESSES TO PROLONGED ISOLATION

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Introduction. Due to exposure to a set of extreme factors, prolonged ground-based isolation simulating a space mission induces various adaptive responses in the body. The study of such organismal responses at the molecular level is important for the development of high-quality medical support plans for subsequent space missions. Proteomic methods have proven effective for assessing the molecular mechanisms of adaptation.
Objective. To study the profile of proteomic physiologically active components in the blood of test subjects as criteria for adaptive strategies of physiological systems in response to exposure to extreme factors of prolonged isolation during ground-based simulation of spaceflight factors.
Materials and methods. The characteristics of proteomic physiologically active blood components were studied in six healthy volunteers aged 27–38 years (mean age 32.5 ± 5.5 years) under the conditions of a 12-month isolation (SIRIUS-23) in a hermetically sealed facility with limited living and working space and restricted resources for life support. Mass spectrometry methods were used to analyze dried blood spot samples. Statistical analysis was performed using the Statistica 12 software with non-parametric Mann–Whitney *U* test ($p < 0.05$). The biological processes involving the identified proteins were determined using the STRING database.
Results. Following one month of isolation, a significant increase in the expression of three proteins involved in the biological process of phagocytosis suppression was observed. Following three months of isolation, the concentration level of 22 extracellular matrix and exosome proteins significantly increased. Following six months of isolation, the concentration of 33 proteins involved in either cellular component processes or regulation of extracellular signaling significantly increased.
Conclusions. The data obtained allowed us to compile a preliminary version of biological processes, which involve proteins with reliably changing concentrations, during prolonged isolation in a specialized facility. The conducted study elucidates the proteomic mechanisms regulating biological processes in healthy individuals during the simulation of spaceflight factors.

Keywords: long-term ground-based isolation; proteome; proteins; “dried blood spots”; bioinformatics methods; adaptation; sealed facility; simulation of spaceflight factors

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ХАРАКТЕРИСТИКА ПРОТЕОМА КРОВИ ЗДОРОВЫХ ДОБРОВОЛЬЦЕВ, ОТРАЖАЮЩАЯ ПРОЦЕССЫ АДАПТАЦИИ К ДЛИТЕЛЬНОЙ ИЗОЛЯЦИИ

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Введение. Длительная наземная изоляция с имитацией космической миссии вызывает различные адаптационные реакции организма в связи с воздействием комплекса экстремальных факторов на человека. Изучение данных реакций организма на молекулярном уровне позволит разработать наиболее качественный план медицинского обеспечения последующих космических миссий. Для оценки молекулярных механизмов адаптации хорошо себя зарекомендовали методы протеомики.
Цель. Исследование профиля протеомных физиологически активных компонентов крови у испытателей как критериев адаптационных стратегий физиологических систем организма в ответ на воздействие экстремальных факторов длительной изоляции при наземном моделировании факторов космического полета.

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Материалы и методы. Изучение характеристик протеомных физиологически активных компонентов крови проведено у 6 здоровых испытателей-добровольцев в возрасте 27–38 лет (средний возраст 32,5 ± 5,5 года) в условиях 12-месячной изоляции («SIRIUS-23») в герметично замкнутом объекте с ограниченным объемом жилой и рабочей площади и лимитированными ресурсами для обеспечения жизнедеятельности. Методами масс-спектрометрии были исследованы образцы экстрактов сухих пятен крови. Статистический анализ проводился в программе Statistica 12 с применением непараметрического теста Манна – Уитни ($p < 0,05$). Биологические процессы, в которых участвуют выявленные белки, определены с помощью базы данных STRING.
Результаты. Через 1 месяц изоляции достоверно увеличивается экспрессия 3 белков, которые участвуют в биологическом процессе «подавление фагоцитоза». Через 3 месяца изоляции достоверно увеличивается уровень концентрации 22 белков внеклеточного матрикса и экзосом. Через 6 месяцев изоляции достоверно увеличивается концентрация 33 белков, участвующих в процессах клеточного компонента или вовлеченных в регуляцию внеклеточной сигнализации.
Выводы. Полученные данные позволили составить предварительную версию происходящих в организме биологических процессов, в которых участвуют белки с достоверно изменяющимся уровнем концентрации в условиях длительной изоляции в гермообъекте. Проведенное исследование расширяет представления о протеомных механизмах регуляции биологических процессов у здоровых лиц при моделировании факторов космического полета.

Ключевые слова: длительная наземная изоляция; протеом; белки; сухие пятна крови; биоинформационные методы; адаптация; гермообъект; моделирование факторов космического полета

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INTRODUCTION

The study of molecular-level responses of the body to various extreme factors acting in confined environments is a relevant research direction. Such conditions are associated with prolonged isolation under altered but controlled atmospheric conditions, work in small groups, occurrence of emergency situations, or specific patterns of physical activity. The research relevance is determined by the need to ensure the medical safety of both astronauts during long-duration missions and workers in certain Earth-based industries, as well as military personnel.

The use of proteomic methods for investigating the blood plasma protein composition offers significant opportunities for assessing molecular adaptation mechanisms. The blood proteome reflects both the state of homeostasis (at the level of constitutive proteins) and the body's overall responsiveness to a diverse set of extreme factors. Bioinformatics methods enable a detailed mapping of protein–protein interaction networks, allowing for the evaluation of pathways and biological processes that underlie adaptation to demanding living conditions. Previous proteomic studies under conditions of 120-day isolation have identified changes in the structural proteins of connective tissue and proteins involved in the autonomic regulation of the cardiovascular and immune systems [1–3].

It is known that the majority of blood protein fractions are not components of regulatory circuits at the systemic level, with the exception of hormones and the albumin-globulin fraction of proteins that maintain plasma osmolality. On the contrary, the blood proteome is highly variable, with its qualitative and quantitative modulation in a healthy individual being influenced by genotype, lifestyle, physical activity, diet, and the process of healthy aging [4]. Studying the parameters of various human physiological systems under controlled conditions allows external environmental conditions to be standardized and individual variability in physiological indicators to be reduced, since such studies involve practically healthy test subjects who have undergone medical selection. This approach permits identification of group patterns, which is particularly important when examining small samples of healthy individuals.

In this work, we aim to study the profile of proteomic physiologically active components in the blood of test subjects as criteria for adaptive strategies of the body's physiological systems in response to the impact of extreme factors during prolonged isolation in ground-based simulation of spaceflight conditions. The results of this study, through the proteomic characteristics of blood, will extend the current understanding of the role of physiological systems in adaptive responses of humans under conditions of daily life, performing work tasks, and overcoming emergency situations when living in isolated and confined environments during spaceflight simulation.

MATERIALS AND METHODS

The study was conducted within the framework of the SIRIUS-23 international project at the Ground-Based

Experimental Facility (GBEF) of the Institute of Biomedical Problems (IBMP RAS). This hermetically sealed facility provides an analog platform for simulating spaceflight research with multifunctional experimental and habitation modules. The facility is equipped with autonomous life support systems, as well as an externally controlled executive system that ensures the formation of an experimentally defined habitat with specific parameters.

The characteristics of proteomic physiologically active components of blood were studied by involving six healthy test volunteers aged 27–38 years (mean age 32.5 ± 5.5 years) under conditions of a 12-month isolation period in a hermetically sealed facility with a limited volume of living and working space and restricted resources for life support and accomplishing the tasks set during the experiment.

Extracts from dried blood spots of the subjects were subjected to analysis. Capillary blood samples in the amount of 80 μL were collected from each participant at five time points: prior to the experiment in the sealed facility — 60 days ("baseline"); during isolation — following 1, 3, and 6 months of isolation; and after its completion — on day 7 of the recovery period ("exit from isolation"). According to the timeline of the experiment, dried blood spot samples were divided into groups: group 1 — baseline, group 2 — 1 month of isolation, group 3 — 3 months of isolation, group 4 — 6 months of isolation, and group 5 — exit from isolation.

Capillary blood was obtained by puncturing the phalanx of the ring finger. A drop of blood was placed on filter paper, dried for 2–3 h, and stored at –20°C before gas chromatographic–mass spectrometric analysis.

For the purposes of analysis, the dried spot was cut out and extracted in 400 μL of a solution containing 50 mM ammonium bicarbonate and 2% sodium deoxycholate (BioChemica, AppliChem) at 37°C on a Thermomixer comfort shaker (Eppendorf) with shaking at 1400 rpm for 45 min. To reduce disulfide bonds, 10 μL of 0.5 M TCEP (pH 8), tris(2-carboxyethyl)phosphine hydrochloride, was added, and the sample was incubated for 15 min at 95°C and 950 rpm on a thermoshaker. Alkylation was then performed using 0.5 M iodoacetate, incubating for 30 min in the dark at room temperature.

The sample preparation procedure was completed with tryptic digestion in 50 mM ammonium bicarbonate buffer with the addition of 5 μL of trypsin at a concentration of 1 mg/mL. Incubation was carried out for 17 h at 37°C on a Thermomixer comfort shaker with shaking at 700 rpm. Then, 45 μL of a 10% aqueous formic acid solution was added to inactivate trypsin and precipitate sodium deoxycholate. The sample was centrifuged in an MR 23i centrifuge (Thermo Electron Corporation) at 14,000 g for 10 min, after which 95 μL of the supernatant was transferred to a new tube. At this stage, the sample was ready for mass spectrometric analysis.

The resulting mixture of tryptic peptides was analyzed by liquid chromatography-mass spectrometry using a Dionex Ultimate3000 nano-LC system (Thermo Fisher Scientific, USA) coupled to a TimsTOF Pro mass spectrometer (Bruker Daltonics, USA). Peptide separation was performed on a column (C18, 25 cm × 75 μm, 1.6 μm) (IonOpticks, Parkville, Australia). Measurements were conducted in the *m/z* range of 100–1700. Mass spectrometric analysis was performed using the parallel accumulation–serial fragmentation (PASEF) data acquisition method.

The obtained data were analyzed using PEAKS Studio 8.5 and MaxQuant version 1.6.7.0. Protein identification based on the detected peptides was performed using the SwissProt database with a set false discovery rate (FDR) threshold of 0.01.

The reliability of the liquid chromatography–mass spectrometry results was evaluated. In the mass spectrometric analysis of the samples, a total of 289,273 peptide-spectrum matches (PSMs) were identified with an FDR error of 1% at the maximum confidence level, enabling the identification of 632 distinct proteins. In each sample, between 281 and 427 distinct proteins were detected.

Analysis of the distribution of weighted peptide scores (-10lgP) for all PSMs indicated the statistical significance of the peptide-spectrum match. A histogram showing the number of target and false matches within each score interval allowed for the conclusion that false matches occur only in the low-score interval and do not exceed the significance threshold.

The distribution of detected peptide features was also analyzed. Figure 1 shows all detectable features, distributed by feature abundance measured as the area under the mass spectral peak curve. These features represent various values of mass-to-charge ratio, *m/z* (indicated in blue). The number of detectable features with results from the canonical database is highlighted in orange, while the number of detectable features with *de novo* results in addition to database results is highlighted in light blue.

The obtained LC–MS/MS data were semi-quantitatively analyzed using DIA-NN (version 1.8.1) based on

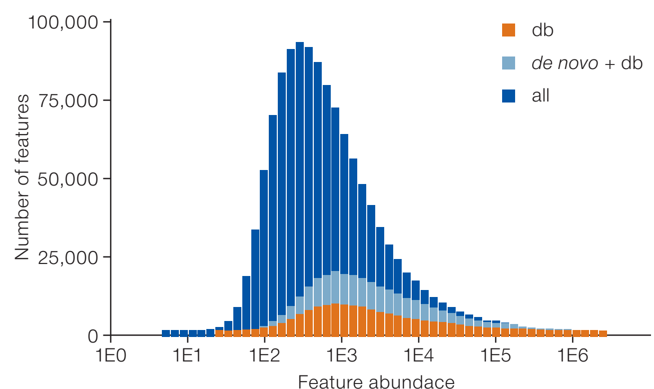

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Fig. 1. Distribution of identified peptide features of proteins: db — number of features from the canonical database; *de novo* + db — total number of features from the database; all — number of all identified features

normalized MS peak intensities, which reflect protein levels relative to values in baseline samples. The structural and functional grouping of proteins with their international name, gene, and abbreviation is provided according to the international UniProt KB and QuickGO databases within the text.

Statistical analysis was performed in Statistica 12 using the non-parametric Mann–Whitney *U* test ($p < 0.05$). The biological processes involving the identified proteins were determined using the STRING database.[1]

RESULTS AND DISCUSSION

The results of the semi-quantitative determination of proteins in dried blood spot extracts showed the following. Among the total of 632 distinct proteins identified in the biological samples, approximately 98 proteins had a concentration that reliably differed depending on the time point of sample collection.

As part of the work, a heat map of proteins with reliably changed concentrations in the blood was initially constructed. This map illustrates clusters of protein groups with similar expression trends across all samples, where the color of the cells represents the $\log_2$(ratio) relative to the mean protein abundance in different samples (Fig. 2).

The Volcano plot (Fig. 3) shows the proteins that demonstrated the greatest (including statistically significant) concentration changes in the subjects' blood depending on the experimental observation period. These proteins are located in the upper part of the graph and are distanced from the center.

Using the Mann–Whitney *U* test, a comparison of protein concentrations in the blood of subjects at different time points of isolation with baseline values was conducted, with a significance level for differences set at $p < 0.05$. The complete list of proteins with a reliably increasing concentration level in the blood at 1, 3, and 6 months of isolation is presented as a flowchart (Fig. 4).

Proteomic studies of healthy individuals under conditions of a one-year isolation in the SIRIUS-23 experiment revealed the following. Following one month of the

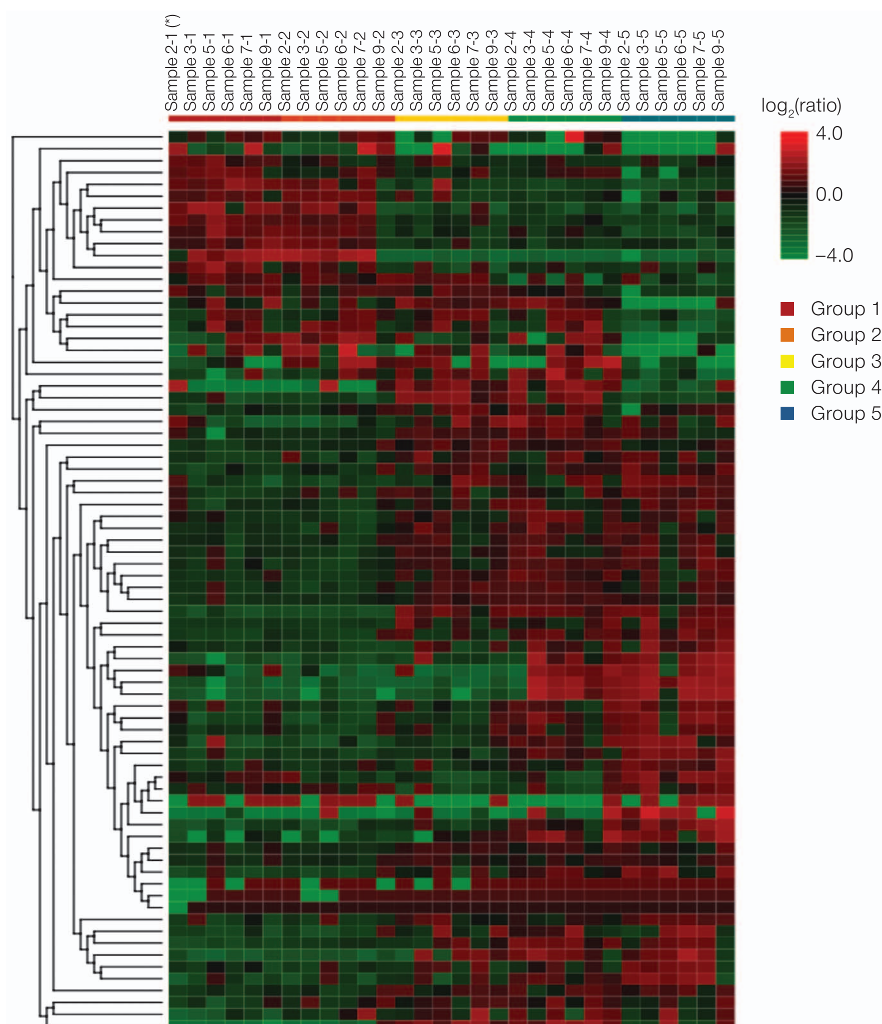

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Fig. 2. Heat map of the protein profile

[1] STRING. URL: https://string-db.org (access date 10.01.2026)

experiment, the blood concentration of three proteins significantly increased, including immunoglobulin lambda constant 3 (gene *IGLC3*), myeloblastin (gene *PRTN3*), and adiponectin (gene *ADIPOQ*). The use of the STRING database revealed that they were not connected in a single network, thus most likely not being co-activated. At the same time, all the aforementioned proteins were involved in regulating the biological process of GO:0050765, i.e., negative regulation of phagocytosis.

The contextual annotation of proteins with a reliably increased level in the blood after one month of the experiment, performed using the UniProt software, revealed the involvement of myeloblastin (gene *PRTN3*) in the metabolism of elastin, fibronectin, laminin, vitronectin, and collagen types I, III, and IV [5–7]. By cleaving and activating the F2RL1/PAR-2 receptor, myeloblastin enhances the barrier function of endothelial cells and maintains vascular integrity during the transendothelial migration of neutrophils [8, 9]. This protein also plays a role in the transendothelial migration of neutrophils, likely through binding to CD177 [9]. Myeloblastin induces inflammatory processes in neutrophils by interacting with the adenylate cyclase-associated receptor G3 (ADGRG3). It was previously shown that effective binding and activation of mPR3 by GPR97 requires a macromolecular complex CD177/GPR97/PAR2/CD16b, which induces the activation of PAR2 — a G-protein-coupled receptor known for its role in inflammation. Activation of PAR2 by this complex triggers an inflammatory response, stimulating antimicrobial activity at the same time as inducing endothelial dysfunction [10].

Adiponectin (*ADIPOQ* gene) is involved in the regulation of lipid metabolism and insulin sensitivity, exhibiting direct anti-atherogenic, anti-diabetic, and anti-inflammatory activity. It stimulates the phosphorylation and activation of adenosine monophosphate-activated protein kinase (AMPK) in the liver and skeletal muscles, enhancing glucose utilization and fatty acid oxidation [11]. Adiponectin counteracts TNF-alpha by negatively regulating its expression in the liver and macrophages. However, under certain circumstances, adiponectin is capable of inducing the expression of inflammatory

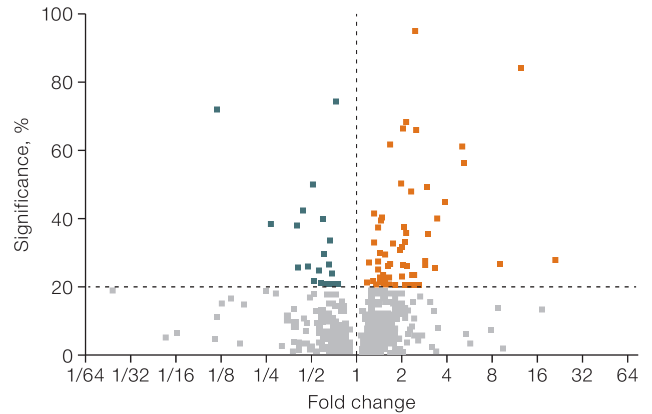

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Fig. 3. Volcano plot of proteins with the greatest concentration changes in the blood: ■ — proteins with a concentration change level above 1; ■ — proteins with a concentration change level from 0 to 1

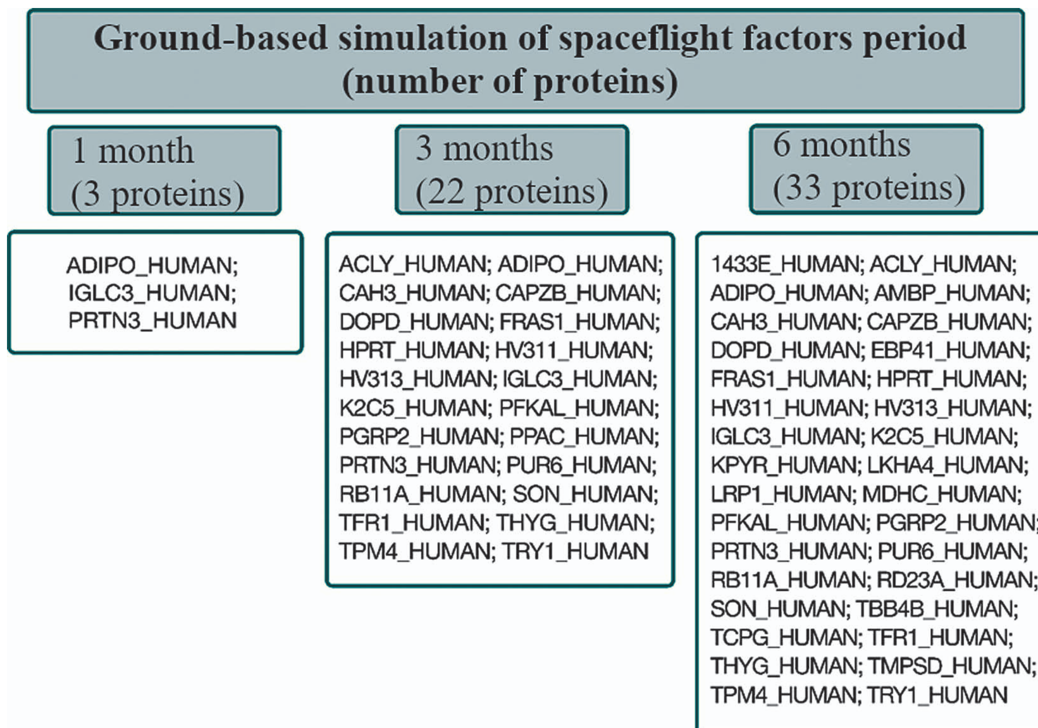

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Fig. 4. List of proteins with a reliably increasing concentration level in the blood of subjects at different isolation time points compared to baseline values

mediators in skeletal muscles, immune, and non-immune cells through various mechanisms. For instance, in macrophages, adiponectin can act as both anti-inflammatory (under prolonged exposure to elevated levels) and pro-inflammatory (under short-term elevation) factors [12].

Immunoglobulin lambda constant 3 (*IGLC3* gene) encodes the constant region of immunoglobulin light chains. Secreted immunoglobulins mediate the effector phase of humoral immunity, leading to the elimination of bound antigens [13]. The antigen-binding site is formed by the variable domain of one heavy chain together with the variable domain of its associated light chain. Variable domains are assembled through the process referred to as V-(D)-J recombination and can subsequently undergo somatic hypermutation, which, upon antigen exposure and selection, provides affinity for a specific antigen [14].

It should be noted that, on the basis of reliably increased protein levels in the blood, the main biological process activated in the volunteers after one month of stay in the sealed facility was that of suppressing phagocytic activity. It is worth noting that all the three proteins described above remained at reliably high levels both following 3 and 6 months of isolation. We believe this to be an adaptive response to changes in the external and internal environment of the body under conditions of a sealed facility. At the same time, none of the canonical "phagocytic code" proteins, i.e., proteins directly regulating phagocytic activity, with significantly altered concentrations were detected [15–18].

During the study, after three months of isolation, the concentrations of 22 proteins were reliably increased in the volunteers' blood (Fig. 4). These proteins are involved in regulating primarily intracellular functions, the antigen recognition system, and processes of reversible hydration of carbon dioxide.

The use of the STRING database revealed that the majority of proteins in this group (11 proteins and 2 proteins) were connected in unified networks, implying their co-activation through a single mechanism (Fig. 5).

These proteins belong to the extracellular space and are contained in exosomes: GO:0005615 Extracellular space (17 proteins) and Extracellular exosome (13 proteins), with eight of them being present in both compartments (Fig. 6). The data obtained characterize the involvement, in the response to prolonged stay in a sealed facility, of processes occurring both inside the cell and in the extracellular space.

A group of proteins whose levels were elevated after 3 and 6 months of isolation deserves particular attention. These are TPM4_HUMAN (tropomyosin-4), K2C5_HUMAN (keratin, type II cytoskeletal 5), DOPD_HUMAN (D-dopachrome decarboxylase), HV313_HUMAN (variable region of immunoglobulin heavy chains (IGHV3-13)), SON_HUMAN (RNA-binding protein, mRNA splicing cofactor), CAH3_HUMAN (carbonic anhydrase 3), HV311_HUMAN (immunoglobulin heavy variable 3-11),

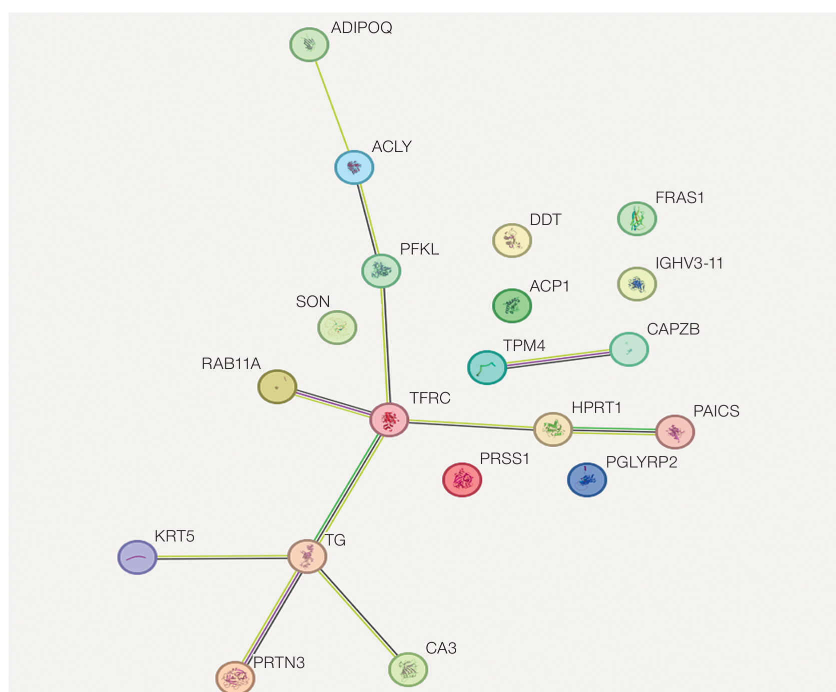

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Fig. 5. Protein–protein interaction network of proteins with a reliably increasing concentration in the blood of subjects following three months of isolation (using the STRING database); protein–protein interaction lines: lime green — co-mentioned in literature; crimson — protein interaction determined experimentally; black — protein co-expression; green — genomic proximity of the protein-encoding genes (node colors are generated arbitrarily by the software)

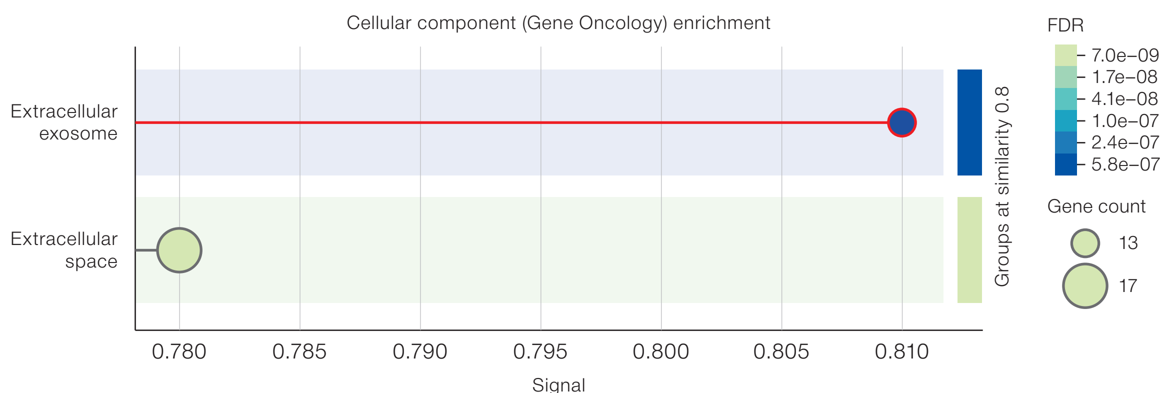

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Fig. 6. Graphical representation of the enrichment degree with proteins of significant cellular components (exosomes and extracellular space) in the blood of subjects following three months of exposure to ground-based spaceflight simulation conditions: FDR — false discovery rate

HPRT_HUMAN (hypoxanthine-guanine phosphoribosyltransferase playing a key role in purine nucleotide generation), RB11A_HUMAN (Ras-related protein, a small GTPase family member), PGRP2_HUMAN (peptidoglycan recognition protein 2), THYG_HUMAN (thyroglobulin; precursor of iodinated thyroid hormones). Most proteins in this group are involved in regulating intra- and extracellular functions. For instance, keratin (type II cytoskeletal 5) is essential for forming intermediate keratin filaments in the basal layer of the epidermis and maintaining the skin barrier in response to mechanical stress. Additionally, K2C5_HUMAN regulates the recruitment of Langerhans cells to the epidermis and participates in the formation of the skin's immune defense (UniProtKB reviewed).

The variable region of immunoglobulin heavy chains — a peptide from the V-region of immunoglobulins — participates in antigen recognition (UniProtKB reviewed). Immunoglobulin heavy variable chain 3-11 facilitates antigen binding. This protein is assumed to be involved in immunoglobulin-mediated immune responses (UniProtKB reviewed).

The increase in the levels of these proteins may reflect the involvement of antigen recognition components of the immune system in the adaptive process.

At the three-month time point, an elevated level of the RNA-binding protein was identified. This protein acts as an mRNA splicing cofactor, promoting the efficient excision of specific nucleotide sequences from RNA molecules and the ligation of transcript sequences with TUBG1 (tubulin gamma-1 chain), KATNB1 (katatinin p80 B1 subunit with WD40 repeats), TUBGCP2 (tubulin gamma complex component 2), AURKB (Aurora kinase B), PCNT (pericentrin), AKT1 (RAC-alpha serine/threonine-protein kinase), RAD23A (lysine-specific demethylase RAD23A), and FANCG (Fanconi anemia group G protein), which are associated with the cell cycle and DNA repair. SON_HUMAN (SON protein) facilitates interactions between SRSF2 (serine/arginine-rich splicing factor 2), RNA polymerase II, being capable of binding to DNA. The SON protein is critically important for neuronal migration and brain tissue remodeling, involving TUBG1, FLNA, PNKP, WDR62, PSMD3, PCK2, PFKL, IDH2, and ACY1 [19].

In turn, carbonic anhydrase-3 is involved in the reversible hydration of carbon dioxide. Its increased levels in the blood may reflect the body's response to the specific atmospheric conditions within a sealed facility (UniProtKB reviewed).

In addition, at the three-month point of the experiment, the subjects exhibited elevated levels of hypoxanthine-guanine phosphoribosyltransferase, HPRT, which catalyzes the conversion of hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate, enabling the reutilization of purines as components for DNA and RNA restructuring. Importantly, hypoxanthine-guanine phosphoribosyltransferase positively regulates dopamine metabolism.

An increase in the level of the Ras-related protein, belonging to the small GTPase (Rab) superfamily, a known marker for protein transport, sorting, and recycling in the endosomal pathway, was detected. According to the UniProt database, this protein is involved in biological processes related to extracellular matrix organization.

At the same time, after three months of isolation, the participants exhibited an increase in the concentration of peptidoglycan recognition protein 2, which cleaves biologically active peptidoglycan (PGN) into biologically inactive fragments. This protein is involved in maintaining anti-inflammatory homeostasis [20], recognizing and tagging foreign agents, and inducing the development of innate immune responses to the presence of foreign peptidoglycans [21–23]. Thus, the involvement of peptidoglycan recognition protein 2 in the processes of homeostasis, apoptosis, inflammation, and pathogen clearance underpins its important role in maintaining the specific and non-specific resistance of the organism in response to prolonged stay within a sealed facility.

During the same period, the subjects showed an elevated level of thyroglobulin, which is a precursor of

iodinated thyroid hormones. In our opinion, the increase in the level of this protein reflects altered activity of the thyroid gland as one of the participants in the adaptation process to isolation conditions.

The sustained, consistently elevated level of the above proteins throughout the entire experimental period most likely represents a characteristic feature of the immune system response to isolation conditions.

At the six-month time point of ground-based spaceflight simulation, 33 proteins with a reliably increased concentration level were identified, predominantly involved in intracellular and extracellular interaction processes (Fig. 4). The STRING database revealed that most of these proteins are connected in a protein–protein interaction network (Fig. 7).

According to Fig. 7, 20 proteins are linked in a unified network, implying their co-expression and participation in regulation of biological processes primarily at the level of vesicles, cytosol, exosomes, and the extracellular space. The data presented in Fig. 8 expands the current understanding of involvement of this protein group; thus, 5 proteins were involved in regulating biological processes in the cytosol, 10 in vesicles, 15 operated in the extracellular matrix, and 25 — at the level of exosomes. Some proteins participated in biological processes across multiple compartments.

The use of the QuickGO software allowed us to establish that 24 proteins were involved in regulating biological processes in GO:0005615 “Extracellular space”; 19 and 20 proteins — in GO:0070062 “Extracellular exosome” and GO:0031982 “Vesicle”, respectively; 4 proteins — in GO:0072562 “Blood microparticle”; and 18 proteins — in GO:0005829 “Cytosol”.

The increased representation of proteins participating in intra- and intercellular signaling indicates an enhanced role of paracrine communication, which involves cells of different types.

CONCLUSION

The dynamics of protein concentrations in dried whole blood spot extracts from healthy volunteers has been studied using proteomic methods based on liquid chromatography–mass spectrometry. The study has clarified the course of adaptive strategies in the physiological systems of healthy individuals in response to prolonged isolation factors. The obtained results have clinical significance for developing means aimed at preventing changes in such specific immune system processes as phagocytosis function and antigen presentation.

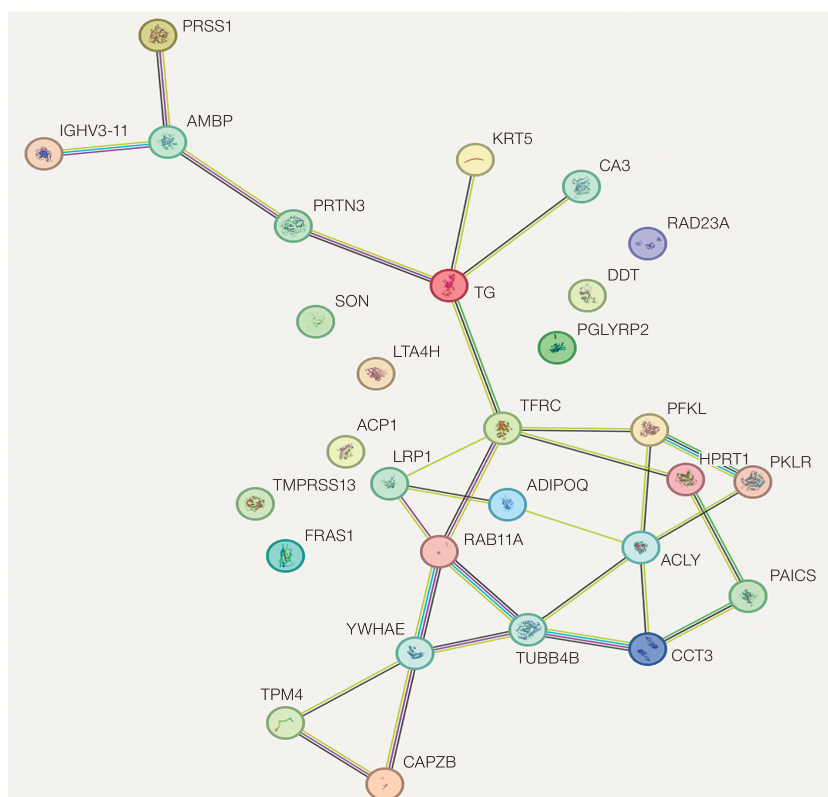

Figure prepared by the authors based on original data

Fig. 7. Protein–protein interaction network of proteins with a reliably increasing concentration level in the blood of subjects following six months (using the STRING database); protein–protein interaction lines: lime green — co-mentioned in literature; crimson — protein interaction determined experimentally; black — protein co-expression; blue — interaction indicated in curated databases; green — genomic proximity of the protein-encoding genes (node colors are generated arbitrarily by the software)

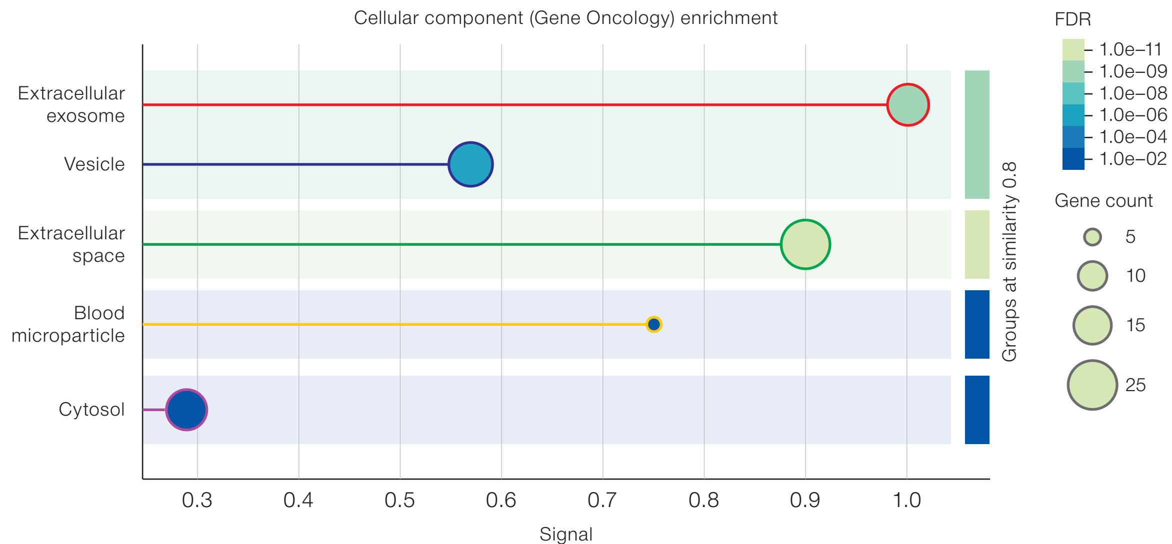

Figure prepared by the authors based on original data

Fig. 8. Graphical representation of the enrichment degree with proteins of significant cellular components (extracellular exosomes, extracellular space, blood microparticles, vesicles, cytosol) in the blood of subjects following six months of exposure to ground-based spaceflight simulation conditions: FDR — false discovery rate

Following 1, 3, and 6 months of isolation, the participants showed an increase in the activity of various physiological processes, both intracellular and those carried out in the extracellular space. These processes are capable of affecting systemic levels of adaptation. Thus, the main biological process activated after one month in a sealed facility, supported by reliably increased protein levels in the blood, was established to be the suppression of phagocytic activity. Furthermore, phagocytic activity remained suppressed both following 3 and 6 months of isolation.

At the same time, after three months of isolation, the reliably increased concentrations of 22 proteins, the majority of which were found to be connected in a unified network (implying their co-activation through a single mechanism), formed another adaptive state. This state is characterized by the involvement of both intracellular metabolic processes and extracellular mechanisms. These processes can be considered as various facets of the immune system response to the conditions of stay in a sealed facility.

Following six months of isolation, an even greater number of proteins with a reliably increased content (33 proteins) was detected in the blood. Bioinformatics resources have shown all of them, except for 8, to be connected in a unified protein–protein interaction network, revealing intermolecular connections of various types. During the experimental period, biological processes occurring in the extracellular space, including those involving paracrine regulation via exosomes and microvesicles (MPs), were significantly enriched with proteins (i.e., activated). The activated processes of intracellular localization did not involve organelle proteins, although possibly including protein complexes and receptors. It should be noted that the analysis of the proteome from dried blood spots of the test volunteers, collected under the conditions of the long-term SIRIUS-23 isolation, did not reveal a reliable increase in clinically significant proteins — commonly accepted markers of various diseases. This indicates a sufficiently successful level of adaptation of the participants to the experimental conditions and preservation of their health.

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