*Adv Syst Sci Appl* 2022; **04**; 224-232
Published online at https://ijassa.ipu.ru.

# Cytokines Production in Test-Volunteers During 120-day Confinement in a Hermetically Sealed Chamber

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**Abstract:** The aim of the current study was to estimate the effect of 120-day confinement in a hermetically sealed chamber with an artificial environment on the ability of cells of innate immunity with pattern-recognition receptors and T-cells of adaptive immunity to synthesize cytokines. The experiment reproducing the conditions of a real spaceflight to the Moon was conducted in the ground-based analog facility called Nazemnyy Eksperimental'nyy Kompleks, or NEK, within the Institute of Biomedical Problems of the Russian Academy of Sciences in Moscow. The study presents the results of the analyses of cytokines production by immunocompetent cells from peripheral blood of six test-volunteers in response to *in vitro* antigen stimulation. We managed to demonstrate that the confinement led to the decreased LPS-induced cytokines and chemokines production. Also, increased Th1/Th2, Th1/Th9, and Th1/Th17 cytokines ratios in response to PHA were revealed.

***Keywords****:* immune system, cytokines, isolation

## 1. INTRODUCTION

Extreme conditions, which people have to inhabit in increasing frequency, cause an extension of functional load (both physical and mental) expressed in the overstrain of almost all physiological systems of the body that ensure adaptation to the extreme environmental factors. Unfortunately, the issue of the potential danger of activity in such kind of conditions for a healthy human organism has been poorly studied to date by the traditional medicine, as it focuses mostly on disease states. Only in recent decades specialists in aerospace medicine have begun to work on this problem. Not coincidently, for this is the spaceflight that causes the most pronounced effects of environmental conditions on human body. In this regard, the data from comprehensive systemic studies of all vital processes that take place in a healthy human organism during space missions and ground-based experiments, enrich the science with the knowledge of the responses to various environmental stimuli. Such investigations allow to bring the issues both of normal physiological body characteristics and physiological responses to certain stimuli, and of prediction of alterations in physiological state of organisms' systems and their early-stage symptoms diagnosis to a close.

Space flight factors which pose a serious threat on astronauts' health include increased radioactivity, neuro-psychological stress, intensive physical loads during extravehicular activity, artificial environment and microclimate, overloads (during launch and landing), lack of physical activity (hypodynamia), and, in fact, microgravity. Immune system is the first one to be exposed to the extreme conditions of environment.

From abundant experience of the studies on astronauts' immunity functioning, the adaptation to a complex of space flight factors leads to both quantitative and qualitative shifts in the innate immune system and in the adaptive one. Among them, the most important

are the following negative alterations: decrease in peripheral blood monocytes and granulocytes expressing pattern recognition receptors (TLR2, TLR4, and TLR6), decline in the functional activities of natural cytotoxicity system and T-cells of adaptive immunity, reduced synthesis of A- and G-immunoglobulins (dysgammaglobulinemia) [1–5].

Unfortunately, there exist a problem of laboratory of clinical immunology equipment onboard the space station. Such a laboratory would not only provide an opportunity to estimate the immune state of astronauts during both pre- and post-flight periods, but also allow to continuously monitor the state of immunological resistance during the whole mission. Undoubtedly, the results of the study of the immune system reactions after orbital flights may form the basis for the development of prospective countermeasures for prevention and correction of immune homeostasis disbalance. However, during the interpretation of these results one should consider the final stage of a space mission as not only a crucial, arduous, and emotionally stressful one, but also as the first trial faced by an astronaut after prolonged stay in the conditions of microgravity. In this regard, the investigation of the principles of immune system adaptation in ground-based model experiments simulating the conditions of long-term manned space missions and of orbital and planetary stations operation is of particular importance. The experiments on long-term confinement in the hermetically sealed chamber held in the ground-based analog facility (NEK) within the Institute of Biomedical Problems in Moscow are one of the kinds of simulations of basic characteristics of real interplanetary missions.

It is worth to note that the obligatory or voluntary lock-down regimes for citizens implemented in the first half of 2020 by the governments of many countries because of the risk of COVID-19 expansion became an unexampled social event. Many people put themselves in quarantine of their own free will to cease the infection transmission rates. Interestingly, in this situation this were ill or potentially ill individuals but the healthy ones, who were in quarantine. Undoubtedly, the countermeasures like social distancing and remote working help to stop or at least to decrease the circulation of the virus. However, there increases the risk of immune homeostasis disfunction.

Apparently, the negative shifts in the functioning of immunity may cause the development not only of allergies, infections and autoimmune disease, but also of malfunctioning in bone, nervous, and cardiac systems, in particular, during the irrational or partial use of countermeasures. Thus, even the very small chance of the threatening states and diseases development claims the most rapt attention from the point of view of early diagnostics and treatment. In connection with this, the significance of the studies on the human immunity adaptation to modelled space flight conditions increases.

To date, it is generally accepted that the interactions between the cells of immune system and those of different organs and tissues, as well as intercellular cooperation, positive and negative immune regulations are executed by cytokines. The latter are synthesized by immunocompetent cells in response to exogenous stimuli, such as pathogen-associated molecular patterns nearly of all types of microorganisms, chemical and physical damaging agents, as well as to a number of endogenous molecules which concentration rises in many disease states [6-9]. In this case cytokines take part in pathological processes including immunopathology, carcinogenesis, cardiac system disfunction, etc.

Taking into account the regulatory properties of cytokines the interest to the issue of space flight factors impact on them is of no surprise. Cytokine-producing ability is known to inhere in all types of leukocytes, as well as thrombocytes, mast cells, epithelial cells, endotheliocytes, dendric cells, fibroblasts, and cells of nervous system. At the same time, activated monocytes/macrophages and T-lymphocytes being the main producers synthesize and secrete the basic spectrum of cytokines and are by right called their “professional” producers. These very cells play the key role in the maintenance of blood “cytokine background”. In view of aforesaid, the aim of the current study was to estimate the effect of 120-day confinement in the hermetically sealed chamber with an artificial environment on

 

the ability of cells of pattern-recognition system of innate immunity and of T-cells of adaptive immunity to synthesize cytokines.

## 2. MATERIALS AND METHODS

### *2.1 General experimental conditions*

The experiment of 120-day confinement in the hermetically sealed chamber with an artificial environment was conducted in the IBMP ground-based analog facility (NEK) with three female and three male participants aged 24-45 years, who received a permission to take part in the experiment from a medical expert commission and signed an informed consent in accordance with the Declaration of Helsinki. The research program was approved at a meeting of the Academic Council and verified by the Commission on Biomedical Ethics of SSC RF-IBMP RAS (protocol No. 506 dated April 3, 2019).

The experiment simulated the conditions of a real space flight to the Moon including the following stages: the flight to the satellite and subsequent fly round to search for a landing site; the landing of the four of the crew members for lunar surface operations; the lunar orbital flight and remote control of lunar rover for the preparation of a moon base; the return to the Earth.

### *2.2 Sampling*

The immunological studies were conducted on the fasting samples of venous blood of the six almost healthy test-volunteers, i.e., the crew members of the 120-day confinement experiment "SIRIUS-19". The blood was sampled in background period (7 days before the experiment), during the experiment (on the $7^{th}$, $29^{th}$, $54^{th}$,$71^{st}$, $96^{th}$, and $120^{th}$ days of the confinement), and during the recovery period (7 days after the completion of the experiment). The blood was sampled from the cubital vein according to the standard method in aseptic conditions into the Greiner Bio-One vacuum tubes (Austria) containing standard amount of sodium-heparin anticoagulant.

### *2.3 Cytokines analyses*

Cytokine production was determined using the cultured whole blood cells stimulated by the following ligands: lipopolysaccharides (LPS) from Escherichia coli O127 (Sigma, USA) for Toll-like receptors (TLR) stimulation (0,1 μg/ml); phytohemagglutinin (PHA) fort T-lymphocytes stimulation (10 μg/ml). Heparinized (20 u/ml) venous blood was dissolved by a factor of five with RPMI-1640 (Sigma-Aldrich, USA) supplied with L-glutamine (0,3 mg/ml), 5mM HEPES, and gentamicin (100 μg/ml), and cultured for 24h or 48h in sterile round-bottom tubes at 37 °C and 5% $CO_2$. Following cultivation, supernatants were collected and stored at -80 °C until the analyses. In the 24h cultures stimulated with LPS the following cytokines were assessed: IFNa2, IL-10, IL-1b, IL4, IL6, IL8, TNFα, G-CSF, MDC, MCP-1, MCP-3, MIP-1α, MIP-1β, GRO, RANTES, IP-10; in the 48h cultures stimulated with PHA the following cytokines were assessed: IFNγ, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-13, IL-17A, TNFα, TNFβ, RANTES, IP-10, MCP-1, MCP-3, GM-CSF, MDC. The cytokines were analyzed using the commercial kit Human Cytokine/Chemokine Panel I (Millipore, Germany) for immunology multiplex assay according to manufacturer's instructions.

The results of the analyses were processed using Statistica software (v.10.0) for Microsoft Windows. The significance of the results was assessed using the Wilcoxon signed-rank test.

 

## 3. RESULTS AND DISCUSSION

During half of the century passed from the beginning of the space exploration by humanity the investigation of immunological reactivity in humans inhabiting and operating onboard a spacecraft has been the main task of space immunology. Cytokine profiling in Russian crew members of long-term space missions on the International Space Station demonstrated the pronounced changes in the ability of immunocompetent cells to produce cytokines in vitro upon the return to the Earth [1, 2]. For instance, the analysis of one of the key parameters characterizing the state of pattern-recognition receptors system of innate immunity, namely, the production of cytokines by peripheral blood cells in response to LPS (the ligand of TLR4), indicated the decrease in the ability of proinflammatory cytokines (IL-1β, IL-6, IL-8, TNFα) secretion by cultured monocytes obtained from cosmonauts during the period of early re-adaptation to Earth conditions. Even more significant changes were observed during the analysis of cytokine-producing ability of peripheral blood lymphocytes upon PHA stimulation. In particular, upon the completion of long-term space missions, the shifts towards both the increase and the decrease in cytokines including IFNγ, IL-2, TNFβ, IL-4, IL-5, IL-6, IL-10 were detected in the supernatants of cell cultures. It is worth to note that these in vitro investigations of immunocompetent cells' cytokine-producing ability were conducted using the cultured whole blood cells, since this eliminates the probability of monocytes and granulocytes unwanted activation or death. Besides, the cells were cultivated in conditions of natural microenvironment with the preservation of the balance of all humoral factors acting in vivo [10].

Based on the above said, we analyzed the ability of cytokine production by immunocompetent cells of test-volunteers in different stages of their stay in conditions of 120-day confinement using the induced cytokine synthesis in response to LPS or PHA in cultured whole blood cells.

During the experiment the significant changes in induced synthesis of some cytokines in response to LPS were noted (Table 1). Thus, we managed to demonstrate a significant decrease in the production of IL-1β (on the 54th, 71st, 96th, and 120th days), IL-6 (on the 120th day), as well as of TNFα (on the 71st and 120th days) and IL-10 (on the 71st and 120th days). Interestingly, the decline in LPS-induced synthesis of IL-1β, TNFα, and IL-10 was observed a week past the experiment as well. In addition to the changes described above, a significant decrease in the production of a number of chemokines was also detected: MCP-1 (on the 29th day), MCP-3 (on the 29th day), MIP-1α (on the 120th day), and IP-10 (on the 29th, 54th, 71th, and 120th days). At the same time, during the experiment the increase in the synthesis and secretion of IL-8 (on the 54th day) and MIP-1β (on the 29th day) was observed. The concentrations of other cytokines in the supernatants of cell cultures stimulated with LPS did not significantly change during the whole experiment, however, there were pronounced individual shifts toward both the increase and the decrease in their concentrations compared to background.

**Table 1**. LPS-induced cytokine production in whole blood cell cultures from test-volunteers participated in 120-day confinement in a hermetically sealed chamber with an artificial environment. (Me; q75-q25) (n=6)

| Cytokines pg/ml | Days of confinement | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **-7 d** | **7 d** | **29 d** | **54 d** | **71 d** | **96 d** | **120 d** | **+7 d** |
| **IL-1b** | 886,2; 486,4-1454,0 | 351,0; 282,4-686,2 | 498,8; 439,0-639,7 | **366,1*;** 232,1-402,5 | **180,8***; 113,7-311,8 | **339,9***; 239,3-398,6 | **190,5***; 181,7-215,6 | **520,8***; 336,6-596,8 |
| **IL-6** | 1974,0; 1610,5-2058,5 | 1481,5; 1218,4-1793,5 | 1878,0: 1353,0-2147,0 | 1794,0; 1389,0-1857,0 | 1442,0; 973,0-1611,3 | 2211,0; 1966,8-2702,8 | **809,9***; 604,8-839,9 | 2169,0; 1685,3-2281,5 |
| **IL-8** | 1897,0; 1654,3-4139,3 | 1863,0; 1488,5-2729,3 | 2342,0; 1847,0-5826,0 | **2077,0***; 2023,0-2878,0 | 3470,0; 2372,3-4927,8 | 3756,5; 2734,3-5363,8 | 2871,0; 1797,0-2954,0 | 3137,5; 2408,5-6085,0 |
| **TNFα** | 755,4; 681,0- | 615,6; 492,6- | 556,9; 479,8- | 599,7; 416,4- | **408,3***; 295,3- | 1385,5; 1129,5- | **506,1***; 335,5- | **573,2***; 428,6- |

 

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 952,1 | 737,4 | 713,6 | 619,4 | 579,8 | 1681,3 | 547,7 | 725,4 |
| **IL-4** | 155,6; 107,5-205,7 | 141,6; 119,6-199,4 | 261,7; 188,4-289,8 | 153,0; 118,3-202,7 | 186,3; 173,9-282,3 | 263,2; 227,8-304,5 | 202,7; 135,6-213,8 | 187,3; 167,8-309,0 |
| **IL-10** | 630,1; 480,8-789,9 | 291,2; 264,8-413,6 | 665,3; 403,6-1137,0 | 353,8; 253,8-430,6 | **189,3***; 137,0-255,9 | 1021,9; 662,5-1418,8 | **137,6***; 89,4-402,5 | **197,5***; 137,7-263,0 |
| **IFNa2** | 13,7; 12,5-22,3 | 19,4; 11,7-28,8 | 18,9; 16,9-20,1 | 17,8; 12,1-35,0 | 19,7; 11,2-28,9 | 24,6; 19,2-36,7 | 21,9; 21,2-38,9 | 22,6; 11,6-33,3 |
| **G-CSF** | 614,1; 406,0-987,3 | 320,3; 267,5-678,6 | 850,6; 517,8-1964,0 | 435,4; 355,9-713,0 | 329,1; 289,1-357,6 | 939,9; 451,4-1247,3 | 282,5; 190,8-313,6 | 352,5; 325,1-619,8 |
| **GRO** | 3363,0; 2613,0-6756,8 | 4212,5; 1564,8-7129,0 | 3581,0; 2865,0-7258,0 | 7438,0; 6616,0-8041,0 | 4334,5; 3785,0-9643,5 | 10436,0; 6867,0-11287,0 | 4891,0; 4266,0-8520,0 | 7254,0; 3779,3-11021,3 |
| **MCP-1** | 3088,5; 1911,3-3749,0 | 3987,0; 3561,8-4311,5 | **1857,0***; 1222,0-2765,0 | 3250,0; 2769,0-4052,0 | 4836,5; 3525,5-5808,5 | 4499,0; 3948,0-5983,0 | 3826,0; 3540,0-3839,0 | 3749,0; 2982,8-4900,8 |
| **MCP-3** | 447,6; 368,8-510,5 | 330,5; 217,7-546,6 | **245,9***; 210,6-268,9 | 344,9; 227,5-412,7 | 470,7; 272,8-736,9 | 607,4; 436,2-872,8 | 329,8; 307,2-357,4 | 482,7; 363,5-595,8 |
| **MIP-1a** | 5059,0; 4718,0-5223,0 | 3442,0; 3108,5-3917,0 | 2457,0; 2256,0-5624,0 | 5430,0; 3815,0-5997,0 | 2793,5; 1527,0-6358,8 | 7276,0; 3507,0-8239,3 | **2259,0***; 2155,0-2778,0 | 4867,0; 3558,8-6728,0 |
| **MIP-1b** | 3258,0; 957,6-4848,8 | 2841,5; 1680,8-4065,8 | **3765,0***; 1180,0-4422,0 | 2701,0; 1628,0-3618,0 | 2570,5; 2010,0-2687,8 | 3428,5; 1444,5-6012,5 | 2436,0; 1329,0-3994,0 | 3220,5; 1634,5-4070,8 |
| **MDC** | 279,5; 156,4-416,7 | 186,7; 154,7-261,1 | 240,6; 146,1-306,3 | 474,9; 235,0-557,3 | 248,9; 185,9-301,6 | 282,4; 238,4-484,4 | 205,4; 157,6-326,8 | 265,6; 145,2-394,3 |
| **RANTES** | 1325,0; 1053,5-1682,0 | 1328,1; 878,8-1797,5 | 983,8; 776,7-1893,0 | 1256,0; 1047,0-1475,0 | 1721,5; 1467,8-2291,0 | 2212,0; 1536,8-2903,8 | 1323,0; 1224,0-1929,0 | 2020,0; 1337,5-2248,8 |
| **IP-10** | 1084,5; 947,5-1323,8 | 465,9; 450,0-646,3 | **300,0***; 268,5-443,4 | **660,3***; 331,1-853,4 | **232,8***; 172,5-483,6 | 341,0; 269,8-672,9 | **392,0***; 391,5-405,9 | 616,3; 450,8-1121,6 |

*Significant difference compared to background ($p<0,05$).

It is known that LPS are complex biopolymers composed of a glycolipid and a polysaccharide characteristic to bacteria; they are the essential structural components of the outer layer of the external membrane of gram-negative bacteria which induce the signal transduction through TLR4. The latter is mostly expressed by myeloid cells, namely, monocytes and macrophages. The activation of TLR4 by LPS is accompanied by the signaling via MyD88-dependent pathway and leads to the activation of NF-κB and cytokine (TNF-α, IL-1, IL-6, etc.) and chemokine (MCP-1, MCP-3, GM-CSF, etc.) release [11,12,13]. One could suppose that the decrease in the content of cytokines and chemokines such as IL-1β, IL-6, TNFα, MCP-1, MCP-3, MIP-1α, IP-10 in the supernatants from the LPS-stimulated cultures of whole blood cells observed in the experiment was mediated by the decrease in the level in the peripheral blood of monocytes and granulocytes expressing surface CD14/TLR4 complex. However, as it was earlier demonstrated [14], 120-day confinement in a hermetically sealed chamber with an artificial environment led to a significant increase in both absolute and relative counts of TLR4+-monocytes in the peripheral blood. The increase in their percentage was noted on the 29th, 57th, 63rd, 87th, 96th, and 120th days of the experiment. The absolute count of the monocytes rose on the 7th, 29th, and 96th days of the confinement, as well as on the 7th day upon the completion of the experiment. As for granulocytes, their content significantly increased on 57th, 87th, 96th, and 120th days of the experiment compared to background, with the significant changes demonstrated only for absolute counts. On the other hand, the decline in the LPS-induced proinflammatory cytokine production could be explained by the increased synthesis of the main regulatory proinflammatory cytokine, namely, IL-10, but the data from the experiment demonstrated the decrease in this parameter during the experiment. It is possible, that the

 

phenomenon observed indicates the changes in the TLR signaling pathways induced either by an inhibitory action of LIRLs or some similar regulatory receptors, or by the failure of MyD88-dependent or MyD88-independent intracellular signaling cascade.

In general, the data obtained evidence the wavy fluctuations in LPS-induced cytokine production in whole blood cell cultures in conditions of the confinement in the hermetically sealed chamber towards the decrease in the supernatant cytokine levels at the final stage of the experiment.

During the investigation of cytokine synthesis in response to PHA, a polyclonal T-lymphocytes activator, a number of peculiar changes was observed (Table 2). Firstly, a significant decrease in the contents of IFNγ (on the 7th day), IL-6 (on the 29th, 54th, and 71st days), IL-8 (on the 71st day), RANTES (on the 29th, 54th days), and MDC (on the 120th day) in the supernatants of PHA-stimulated cultures was demonstrated. Secondly, on the 29th day of the experiment we noted an increase in the production of IL-2, IL-4, IL-17A, and on the 96th day an increase in TNFβ and MCP-3 was detected.

**Table 2**. PHA-induced cytokine production in whole blood cell cultures from test-volunteers participated in 120-day confinement in a hermetically sealed chamber with an artificial environment. (Me; q75-q25) (n=6)

| Cytokines pg/ml | Days of confinement | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **-7 d** | **7 d** | **29 d** | **54 d** | **71 d** | **96 d** | **120 d** | **+7 d** |
| **IFNγ** | 2061,5; 833,2-3329,0 | **1105,6***; 621,9-1857,8 | 3161,5; 1160,8-5837,0 | 2702,0; 1168,0-4171,0 | 2277,5; 1425,8-4663,0 | 2793,5; 1402,5-4927,8 | 2570,5; 812,9-4101,0 | 4584,5; 2604,9-6316,3 |
| **IL-2** | 231,0; 175,6-281,5 | 197,7; 94,6-350,4 | **411,8***; 238,7-514,4 | 277,0; 269,9-330,4 | 239,7; 191,7-354,6 | 290,9; 223,1-344,9 | 149,4; 105,6-188,0 | 454,6; 247,6-868,5 |
| **TNFα** | 749,7; 459,3-1482,5 | 857,6; 511,2-1062,6 | 1236,2; 869,2-2443,5 | 1293,0; 721,2-2208,0 | 965,7; 582,9-2035,6 | 1257,5; 694,4-1795,8 | 1031,0; 417,8-1593,0 | 1644,5; 1264,0-2602,5 |
| **TNFβ** | 16,5; 11,7-20,3 | 14,7; 8,1-18,1 | 41,1; 16,4-77,5 | 25,5; 22,1-41,7 | 29,7; 15,5-39,1 | **18,0***; 13,6-40,3 | 13,1; 6,6-18,9 | 33,3; 23,0-71,8 |
| **IL-4** | 578,3; 428,9-675,1 | 608,3; 428,5-759,3 | **841,6***; 518,5-1577,3 | 795,5; 705,2-1075,0 | 617,9; 403,3-913,3 | 661,5; 490,7-835,8 | 452,1; 382,9-544,7 | 836,0; 435,6-1182,8 |
| **IL-5** | 38,0; 25,4-62,1 | 33,8; 22,7-74,7 | 85,8; 35,8-133,0 | 52,4; 49,5-91,8 | 69,0; 36,7-99,9 | 67,5; 46,9-70,7 | 36,7; 31,1-60,8 | 60,2; 41,0-85,9 |
| **IL-6** | 1574,5; 1506,3-1711,8 | 1699,0; 1596,0-2012,8 | **1133,0***; 794,5-1284,8 | **1317,0***; 583,7-1454,0 | **1174,5***; 859,1-1405,8 | 1458,5; 1008,2-1466,5 | 1521,0; 1047,7-1725,8 | 1281,9; 684,2-2105,8 |
| **IL-13** | 367,1; 189,7-558,9 | 232,6; 202,3-354,9 | 462,5; 351,2-502,7 | 383,3; 217,2-479,7 | 451,0; 327,2-517,4 | 234,9; 204,1-398,7 | 271,0; 150,1-360,1 | 281,0; 181,6-366,8 |
| **IL-10** | 1424,4; 438,4-2583,3 | 684,9; 523,8-926,7 | 1762,0; 1143,8-2970,5 | 1302,0; 1247,0-1777,0 | 799,3; 496,0-1319,2 | 1150,9; 555,2-1904,5 | 554,7; 157,1-1051,1 | 610,8; 474,0-1324,9 |
| **IL-9** | 40,4; 30,5-51,6 | 26,9; 14,1-38,4 | 53,6; 24,7-62,2 | 63,4; 36,5-69,8 | 28,9; 16,1-59,4 | 23,8; 15,1-37,4 | 14,8; 11,3-26,6 | 51,8; 40,6-63,1 |
| **IL-17A** | 292,1; 248,1-335,1 | 336,7; 235,6-429,7 | **380,4***; 306,3-435,3 | 336,6; 323,5-491,0 | 255,1; 154,4-353,7 | 315,0; 231,2-546,8 | 259,7; 247,7-279,9 | 237,5; 175,3-373,4 |
| **IL-8** | 5719,5; 5274,3-6348,5 | 5811,5; 4754,3-6730,8 | 4919,5; 4238,5-7701,3 | 5904,0; 5360,0-6854,0 | **5175,5***; 3845,3-6062,5 | 5739,5; 4249,8-8315,3 | 4487,0; 2689,3-8489,0 | 3832,5; 2585,0-5133,5 |
| **GM-CSF** | 195,4; 130,7-547,6 | 166,8; 128,1-226,2 | 457,1; 291,8-599,0 | 236,0; 205,3-357,8 | 358,6; 245,3-498,7 | 284,6; 224,8-363,5 | 259,5; 131,5-425,4 | 251,4; 183,6-778,6 |
| **MCP-1** | 4752,0; 2411,8-5792,5 | 4277,5; 2845,0-5665,8 | 4357,0; 2414,3-6177,5 | 4761,0; 3715,0-7281,0 | 4699,5; 2183,0-5160,3 | 4367,5; 2765,0-6045,8 | 3502,5; 2262,8-4330,5 | 3869,0; 2102,3-5050,3 |
| **MCP-3** | 1550,5; 847,8-2245,5 | 1421,0; 926,0-2375,8 | 1639,0; 890,6-1926,3 | 1489,0; 1345,0-6304,0 | 1297,5; 818,7-1563,8 | **2932,0***; 1533,8-9231,5 | 644,0; 566,7-988,1 | 1002,8; 558,8-1851,0 |
| **MDC** | 1058,4; 490,5- | 996,6; 380,6- | 3089,5; 1371,9- | 2105,0; 632,8- | 2218,5; 489,7- | 1435,5; 579,8- | **485,3***; 318,6- | 1746,5; 1645,0- |

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|---|---|---|---|---|---|---|---|---|
| | 2986,8 | 1931,8 | 4431,8 | 3199,0 | 4325,8 | 1873,3 | 1499,0 | 2280,3 |
| **RANTES** | 1921,5; 1382,3-3050,3 | 1484,5; 1353,8-2105,8 | **1490,5***; 1297,0-2250,3 | **1435,0***; 1235,0-2636,0 | 1768,0; 1132,9-2419,0 | 1758,0; 1238,5-2738,8 | 1264,5; 1034,0-2195,3 | 2357,5; 1941,4-2768,3 |
| **IP-10** | 4467,5; 3672,3-5837,3 | 4358,5; 3277,3-5682,8 | 4371,5; 2892,3-5610,8 | 5049,0; 3727,0-5407,0 | 3469,5; 3025,3-4582,0 | 5290,0; 3538,0-5714,5 | 3087,0; 2376,5-6222,3 | 3976,0; 2740,5-6241,0 |

* Significant difference compared to background (p<0,05).

During the last decade the researchers focus of the study of T-helper subpopulations (Th1, Th2, Th9, Th17). This line of investigation allowed to localize the particular subpopulations among the naïve T-helpers, so-called minor subsets in charge with the secretion of certain cytokines [15].

As we demonstrated in the current work, starting already from the 29th day of the confinement in the hermetically sealed chamber there occurred the disbalance in the production of cytokines by the four types of T-helpers (Table 3). These changes were characterized by the high ratios of Th1/Th2, Th1/Th9, and Th1/Th17 cytokines (INF-γ / IL-4, INF-γ / IL-10, INF-γ / IL-9, INF-γ / IL-17A) compared to background. At the same time, there were no statistically significant changes in Th2/Th9, Th2/Th17, and Th9/Th17 ratios (IL-4/ IL-9, IL-4/ IL-17A, IL-10/ IL-17A).

**Table 3**. The coefficients of the balance of Th1-/Th2-/Th9-/Th17-cytokines produced in PHA-stimulated cultures of whole blood cells from test-volunteers participated in 120-day confinement in a hermetically sealed chamber with an artificial environment. (Me; q75-q25) (n=6)

| Cytokine ratios | Days of confinement | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **-7 d** | **7 d** | **29 d** | **54 d** | **71 d** | **96 d** | **120 d** | **+7 d** |
| **IFNγ/IL-4** | 3,22; 1,41-4,31 | 1,97; 1,14-2,42 | 3,43; 2,65-3,80 | 3,45; 3,40-5,06 | **4,68***; 4,05-5,21 | 4,05; 3,35-6,00 | 3,93; 2,19-6,26 | 7,31; 2,16-16,42 |
| **IFNγ/IL-10** | 1,33; 1,22-1,54 | 1,70; 0,98-2,17 | 1,30; 1,24-1,77 | **1,59***; 1,52-2,27 | 3,17; 2,97-4,58 | 2,31; 2,07-2,83 | 4,42; 3,68-4,62 | 4,54; 3,02-6,59 |
| **IFNγ/IL-9** | 35,66; 30,72-48,00 | 48,24; 20,84-78,01 | 82,10; 42,46-100,60 | 61,31; 16,73-73,97 | **104,86***; 54,43-166,79 | **133,69***; 86,62-190,74 | 58,60; 42,57-220,40 | 69,61; 43,94-117,37 |
| **IFNγ/IL-17A** | 5,24; 2,84-10,20 | 2,80; 1,73-11,47 | **5,71***; 4,95-12,09 | 5,40; 3,47-9,88 | **12,01***; 7,43-21,54 | 6,20; 4,24-8,33 | 7,41; 3,13-13,76 | 15,37; 9,97-22,26 |
| **IL-4/IL-9** | 15,94; 13,27-18,35 | 26,23; 20,14-36,24 | 27,10; 22,96-33,07 | 17,75; 16,95-21,78 | 25,29; 21,43-33,39 | 26,88; 19,09-55,36 | 31,87; 21,56-43,01 | 13,50; 7,20-21,74 |
| **IL-4/IL-17A** | 2,50; 1,32-3,18 | 1,65; 1,52-3,47 | 2,87; 1,49-3,85 | 2,19; 0,91-2,91 | 2,52; 1,98-4,07 | 1,54; 1,40-1,63 | 1,60; 1,35-2,02 | 3,39; 2,24-4,66 |
| **IL-10/IL-17A** | 3,10; 1,61-8,31 | 2,14; 1,58-2,75 | 4,89; 4,27-5,34 | 3,85; 2,37-3,87 | 3,02; 2,02-6,50 | 2,26; 1,80-3,29 | 1,54; 0,65-3,76 | 3,49; 2,56-4,26 |

* Significant difference compared to background (p<0,05).

These changes indicate not only the alterations in the cytokine producing ability of T-lymphocytes but also the appearance of the signs of disbalance in the functioning of cytokines network which results from the increased Th1-cytokines secretion. The multidirectional action of the mediators secreted by Th1-, Th2-, Th9-, and Th17-cells is known to cause inter-suppression of these populations, due to which the dynamic balance in the functioning of Th1-, Th2-, Th9-, and Th17-cells is preserved. The disturbance of this balance can lead to the immune response suppression and the development of immunopathological state [16-18]. Stressful experimental conditions simulating the crew activity in “circumlunar orbit” and on the Moon surface could not but affect the reserve ability of T-cells to respond to an antigenic stimulus. The study of induced cytokine production by peripheral blood T-lymphocytes also revealed the multidirectional shifts in Th1-, Th2-, and Th17-cytokines secretion. The changes detected indicate the appearance of the sings of disbalance in Th1/Th2, Th1/Th9, and Th1/Th17 types of cytokines which posses both the local effects on monocyte-macrophage and lymphoid cells and the systemic action on distal organs and tissues [19,20].

 

Thus, the prolonged stay in the hermetically sealed chamber causes an organism reorganize to further function at a new level. Taking into consideration the cytokine-producing ability being one of the key parameters characterizing the state of the immune system, the disbalance of antigen-induced synthesis of cytokines can be referred to as a sign of stress in the immunity, and consequently the sign of risk of secondary immunodeficiency development.

## 4. DATA AVAILABILITY

All data used in this study can be obtained upon reasonable request.

## 5. FUNDING

This work was supported by the Russian Science Foundation, project no. 18-75-10086-P and theme 65.1 of the fundamental research of the IBMP RAS

## 6. CONCLUSION

The issue of the mechanisms in charge with the development of the defects in immunological reactivity in response to the effects of the extreme environmental factors on human body is of current importance, for the life of a human today is a continuous sequence of stress and charges. The results of the present study dedicated to immunocompetent cells' ability to produce cytokines in response to antigens conducted on the test-volunteers participated in the 120-day confinement in NEK at IBMP demonstrated that the prolonged exposure to the environmental factors of the hermetically sealed chamber had pronounced effect on the reserve abilities of cells of both innate and adaptive immunity. At this, the noteworthy variability of the results among the participants indicates the individual predisposition to the defects in the immune reactivity in the confinement conditions. The individual differences in the changes of immunocompetent cells activation potential point out that one should consider them when conducting preventive or corrective measures. Further accumulation of the data on the peculiarities of the human immune system functioning in conditions of a modelled spaceflight and the subsequent processing of these data and their comparison to those on the state of other systems of the organism will allow to proceed to the diagnosis of a pre-disease state, i.e., the stage when the disease development can be easily prevented without its aggravation in unfavorable environmental conditions.

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