scientific reports

OPEN

Reactivation of latent human intracellular infections during a months-long expedition at the Antarctic Vostok station

Sofiya M. Shulgina[1]✉, Nikolay Y. Osetsky[1], Marina P. Rykova[1], Evgeniya N. Antropova[1], Tatyana V. Zhuravleva[1], Vacheslav A. Shmarov[1], Olga V. Kutko[1], Dariya D. Vlasova[1], Anastasiya A. Kotikova[1,2], Kseniya D. Orlova[1], Elina A. Zhirova[1] & Sergey A. Ponomarev[1]

The complex of spaceflight factors has a significant impact on the immunity functional activity. This influence is probably caused by factors such as isolation, monotony, physical inactivity, sensory deprivation, and increased psycho-emotional tension. Long-term wintering at polar stations can be the ground-based model that is most similar to space flight conditions. This research focused on examining the reactivation of latent human pathogens among 11 participants of the 64th Russian Antarctic expedition to Vostok station. Plasma, saliva and urine samples collected before arriving at the station and also during the wintering were studied. Even at the pre-expedition period, DNA of at least one of the studied pathogens was detected in the saliva and plasma of 10 out of 11 (90%) expedition members. During the wintering all the participants showed changes in the Epstein-Barr virus DNA concentration in body fluids. Moreover, 9 out of 11 (80%) participants showed the human herpes virus type 6 shedding in the saliva and/or plasma. Herpes simplex virus types 1 and 2 shedding was observed in 18% of subjects as well. Additionally, there was an assumption made about the relationship between dynamics of latent pathogen shedding, geomagnetic activity, and the psychological state of expedition participants.

Keywords Latent pathogens, Herpesviruses, Reactivation, Antarctica, Immunity

Currently, more than 90% of the world's population is infected with at least one latent intracellular pathogen of viral or bacterial nature[1]. The most common latent infectious agents include viruses of the *Herpesviridae* family, as well as bacteria from the *Chlamydiaceae* and *Mycoplasmataceae* families[2,3]. At the same time, most people are asymptomatic carriers without clinical manifestations of the disease[4,5]. However, under the influence of extreme environmental factors, which can lead to a decrease in the reserve capabilities of the organism and, as a consequence, suppression of the protective functions of the immune system, reactivation of latent infections can occur. The risk group includes elderly people, people with immunodeficiency states, as well as people exposed to long-term influence to unfavorable environmental factors, such as sailors, astronauts, and polar explorers[6–8].

The human organism, staying for a long time in the polar latitudes, is exposed to a complex of unfavorable environmental factors, similar to the conditions. The harsh Antarctic climate, frequent changes in weather conditions, extremely low temperatures for most of the year, alternation of polar day and polar night, increased radiation and chronic hypobaric hypoxia have a significant impact on the functional activity of the immune system[9,10]. At the same time, long-term isolation in a limited residential space of the station, monotony, hypokinesia, sensory and information deprivation contribute to increased psychoemotional tension and, as a consequence, a decrease in protective capabilities and asthenisation of the organism[11–13]. In addition, by 7th – 8th months of wintering at Vostok station, almost all polar explorers show signs of asthenia[9]. In this case the concept of asthenia is to describe a general depressed state of the body that is characterized by increased fatigue, hypoactivity, emotional lability, irritability, psychosomatic symptoms, sleep and appetite disorders[9,14]. Similar symptoms of asthenisation were also manifested in astronauts after the completion of primary adaptation period of long-term spaceflight[15]. It is important to take into account the influence of the psychoemotional factor and

[1]Institute of Biomedical Problems, Russian Academy of Sciences, Moscow 123007, Russian Federation. [2]Pirogov Russian National Research Medical University (Pirogov Medical University), Moscow 117997, Russian Federation. ✉email: sofiya.kayunova@mail.ru

sleep quality on the functional activity of the immune system, including cytokine profile, T-cell immunity and the development of allergic reactions[16–18]. The significant role of the isolation factor is also evidenced by the results of terrestrial isolation experiments with an artificial habitat, which revealed significant changes in the cellular and humoral immunity during both short-term and long-term isolation and hypokinesia[19,20].

Another significant risk factor for the health of polar explorers at Vostok station is chronic hypobaric and hypoxic stress, since the station is located on the glacial dome at an altitude of 3488 m above sea level and the average atmospheric pressure is 460 mm Hg. During early adaptation to hypoxia, significant changes in the endocrine system lead to an increase in the number of blood granulocytes and a decrease in their functional activity, as well as an increase in the level of proinflammatory cytokines, including Il-6 and Il-8 [21,22]. The functional activity of T cells is also affected in hypoxia conditions[23]. Additionally, NK cells and plasmacytoid dendritic cells involved in antiviral immune response show significant sensitivity to hypoxia factor[24,25].

In addition, increasing geomagnetic activity can be an additional stress factor for the human body in high latitude conditions. Geomagnetic perturbations are a consequence of changes in the magnetospheric and ionospheric current systems under the influence of solar wind stream[26].

The state of the magnetosphere is described by a number of different indices calculated using ground-based measurements of the magnetic field. Thus, the most frequently used Kp-index is calculated based on 3-hour measurements of observation stations in a wide range of latitudes and takes into account the oscillations amplitude of the magnetic field vector horizontal component minus the average variation of the geomagnetic activity indicator of quiet days[27,28].

Presently, the improvement of space weather monitoring technologies has resulted in the accumulation of a significant amount of data that indicates the complex impact of the Earth's magnetosphere oscillations on the human body. Although the physiological mechanisms of this process have not yet been fully studied, it is obvious that changes in geomagnetic activity seem to affect many body systems (especially the cardiovascular and nervous systems), as well as the mental state and cognitive capabilities of a person[29,30]. Furthermore, there is evidence that indicates the impact of increased geomagnetic activity on the innate immunity. Thus, the absolute number of neutrophils and basophils in the blood decreased significantly during geomagnetic storms, which, in combination with the suppression of the functional activity of lymphocytes, can contribute to a decrease in the immune system protective capabilities against internal and external pathogens[31,32].

Furthermore, cytokine profiles, stress hormone levels, and pro-inflammatory markers expression can change significantly in Antarctic station personnel during the expedition[9,13,21,33,34]. For example, during the Japanese and Australian Antarctic expeditions, some participants showed significant decreases in pro-inflammatory cytokines (TNF-α, IL-1Ra, IL-6 and IL-1β) during the early adaptation period that continued throughout the expedition[33,35]. Some members of the Australian expedition had decreased T-lymphocyte proliferative activity in response to phytohaemagglutinin stimulation and increased antibody titers to Epstein-Barr virus (EBV) lytic cycle proteins[36]. Moreover, the expansion of the EBV-infected cell population and increased viral DNA content in saliva may indicate the initiation of EBV reactivation[36,37].

Thus, the immune system's functional activity may be impaired due to a complex of factors associated with long-term wintering in the polar station, resulting in disordered immunological control of latent infections, initiation of their reactivation, as well as an increased risk of primary infection of seronegative members of the expedition[36,38].

The main danger of latent infections reactivation along with the suppression of the immune system functional activity is the development of a disease pattern. In conditions of prolonged isolation and autonomy of existence it poses a significant threat to the life, health and performance of polar expedition participants[39,40].

During expeditions to the Antarctic polar stations McMurdo, Palmer and Amundsen-Scott, 5 clinical cases of varicella-zoster virus (VZV) manifestations were reported among 204 polar explorers[38]. The average VZV incidence in the USA is 10 times lower than this[38]. It is important to note that 4 out of 5 cases were reported in the 30–39 age group, which is characterized by high immune activity[38]. Cases of clinical manifestation of latent infections along with the immune dysregulation were also observed in conditions of long-term space flight[39,40]. It may indicate a significant risk of developing diseases associated with latent pathogens in conditions of prolonged isolation, hypokinesia and increased psycho-emotional tension[40].

However, to study the effects of a complex of factors that are associated with space flight or polar wintering, it is worth to consider the small sample size of subjects and the high degree of individual variability in the parameters of both innate and adaptive immunity[6,41–44]. For example, during space flight, many cosmonauts showed multidirectional changes in their cytokine profile and gene expression associated with TLR signaling pathways[42,45]. Based on this, to prevent negative consequences of immune system imbalances, an individual approach is needed to assess the immune status of subjects as well as to monitoring subclinical reactivation of latent pathogens. In particular, personalized approach allows us to choose the most effective measures for preventing and treating changes in a person's immune status under the influence of negative environmental factors[45–48].

In spite of the importance of the problem of health preservation of the participants of long-term polar expeditions, the influence of a complex of factors associated with the wintering at the polar stations, especially the inland stations, on the immune system and control of latent infections is poorly studied. Previous studies did not include the analysis of reactivation of mixt-latent pathogens of bacterial and viral nature during the prolonged (up to a year) Antarctic expedition. Thus, the aim of this study was to investigate the influence of a complex of factors associated with a long-duration human stay in the conditions of the Vostok polar station on the latent infections reactivation and the level of specific antibodies in the plasma of polar explorers. This study will help to assess the risks faced by people who have to work for a long time in conditions of sensory, information and motor deprivation caused by physical and social isolation of the micro-collective, including polar researchers and astronauts.

Materials and methods

Subjects and mission

The study was reviewed and approved by the Biomedicine Ethics Committee of the RF SRC-Institute of Biomedical Problems, Russian Academy of Sciences/Physiology Section of the Russian Bioethics Committee Russian Federation National Commission for UNESCO (protocol No487 from October 11, 2018). All participants provided their written informed consent to participate in this study in accordance with the Declaration of Helsinki. All methods were performed in accordance with the relevant guidelines and regulations.

The study was conducted during 11 months during the period of the 64th Russian Antarctic Expedition at Vostok station located 1253 km from the South Pole and 1260 km from the nearest coast at the ice dome at a height of 3488 m above sea level. The average atmospheric pressure in the station area is 460 mm Hg[9]. During the 64th Russian Antarctic Expedition, temperature fluctuated from −82.1°C to -23.2°C. The study involved 11 male subjects aged 35 to 67 years. The physical characteristics of the polar explorers are presented in the Supplementary Tab. S1.

Studied samples

Venous blood (5 ml), urine (10 ml) and saliva (1,5 ml) samples were taken in the morning on an empty stomach before (onboard the Research Vessel "Akademik Zubov" on the way to Antarctica) and during (2nd, 4th, 6th, 10th and 11th months of wintering) the subjects' stay at Vostok station. The collection of biomaterial was carried out on the same day for all subjects.

Blood was collected from the ulnar vein according to the standard method into vacuum tubes (Greiner Bio-One, Austria) with standard anticoagulant content (K3-EDTA). To obtain plasma and serum, blood was centrifuged for 10 min at 3000 rpm. Saliva was collected directly into cryotubes without the use of additional collection means before brushing and eating.

First morning urine samples were also collected.

The biosamples were frozen and stored at -20° C until the expedition returned.

Experimental parameters

The tested plasma, urine and saliva samples were used to determine the DNA concentration (IU/ml) of latent human pathogens of both viral and bacterial nature. The titers of specific antibodies were determined in the serum (Table 1).

DNA extraction

DNA extraction was performed from 1 ml of saliva, 1 ml of urine and 0.2 ml of plasma with K3-EDTA anticoagulant according to a standard protocol using a commercial DNA extraction kit (Vector-Best, Russia). The extraction process involved a threefold concentration of saliva and urine samples being precipitated and washed with DPBS. The extraction of DNA from the samples was done in parallel with the extraction of positive and negative controls from the DNA extraction kit.

Real-time polymerase chain reaction (RT-PCR)

Viral load (IU/ml) in samples extracted from urine, saliva and plasma was determined by RT-PCR according to a standard protocol using commercial kits (Vector-Best, Russia). The internal control included in the DNA extraction kit was used as a comparison DNA. All reactions were performed in 5 independent repeats. The amplification protocol consisted of 3 stages:

Pathogen	Accepted abbreviation	Specific antibodies
Herpes simplex virus types 1 and 2	HSV-1/2	HSV-1 IgG; HSV-2 IgG; HSV-1/2 IgG avidity
Varicella-zoster virus	VZV	IgG; IgG to surface glycoprotein gE
Epstein-Barr virus	EBV	IgG to VCA capsid protein; VCA IgG avidity; IgG to EBNA-1 nuclear antigen; IgG to EA early antigen
Cytomegalovirus	CMV	IgG; IgGtoIEAprotein; CMVIgG avidity
Human herpes virus type 6	HHV-6	IgG
Human herpes virus type 8	HHV-8	IgG
Mycoplasma hominis	*Mycoplasma hominis*	IgG; IgA
Ureaplasma urealyticum	*Ureaplasma urealyticum*	IgG; IgA
Chlamydia trachomatis	*Chlamydia trachomatis*	IgG; IgA; IgG to cHSP60 protein; IgG to the major outer membrane protein MOMP and *Chlamydia trachomatis* membrane-associated plasmid protein Pgp3

Table 1. Pathogens and types of specific antibodies studied in this investigation.

- Stage 1: 50 °C – 2 min;
- Stage 2: 95 °C – 2 min;
- Stage 3: 40 cycles (94 °C–10 s + 60 °C–20 s).

Fluorescence intensity was measured after the elongation stage. The detection was carried out on a Bio-Rad CFX-96 (Bio-Rad Laboratories, USA). Bio-Rad CFXManager software version 3.1 was used for data processing. The specificity and homogeneity of PCR products were confirmed by melting profile analysis in each reaction.

Enzyme-linked immunosorbent assay (ELISA)
The titers and avidity index of specific antibodies were determined by solid-phase ELISA using commercial kits according to the standard protocols (Vector-Best JSC, Russia) (Table 1). The measurement was carried out 5 times. Detection was performed using spectrophotometry.

Assessment of the psycho-emotional status of the subjects.

The data of psychological conversation with the expedition participants and assessment of their psychological state according to emotional uplift, melancholy, emotional tension and significant psychogenic stress was used to analyze the parameters of the psycho-emotional status of the group and individuals. Conflict situations and cases of medical intervention were also noted.

To quantify the psychoemotional state, each emotional state was assigned a numerical equivalent: emotional uplift - (+1), melancholy and emotional tension - (-0.5), stress, conflict, medical intervention - (-1). The individual psychological states of all 11 expedition participants were illustrated by graphs constructed based on these data.

Assessment of geomagnetic activity
During the Antarctic expedition, the K-index was used to evaluate the geomagnetic activity. The K-index is a parameter calculated as the maximum fluctuation of the horizontal component of the Earth's magnetic field during a three-hour interval expressed as an integer from 0 to 9, where 0 is a quiet magnetic field and 9 is an extreme geomagnetic storm. This parameter was monitored by the expedition members daily every 3 h using a digital magnetovariation complex MicroLog with a block of three-component sensors developed by N.V. Pushkov Institute of Earth Magnetism, Ionosphere and Radio Wave Propagation of the Russian Academy of Sciences. Data processing and K-index values calculation were performed using the "OPER" software.

Statistical analysis
Statistical data processing was performed using GraphPad Prism 8.0.2 software (GraphPad Software Inc., USA). Mann-Whitney test were employed to determine the significant differences in averaged values of all participants ($n = 11$) of viral DNA concentration between successive stages of experiments. One-way ANOVA with Tukey's multiple post hoc test was used to determine the significant differences in mean values of viral DNA concentration/specific antibody titers between time points within each participant. Each blood sample underwent 5 independent rounds of total DNA extraction, followed by RT-PCR to identify latent pathogens DNA concentrations. Additionally, 5 rounds of specific antibody titers measurements were carried out for each sample. Differences were considered reliable at a significance level of $p < 0.05$. In the data presented, one asterisk (*) indicates a $p \leq 0.05$ and two asterisks (**) indicates a $p \leq 0.01$ in the criterion used.

The correlation analysis was carried out using the nonparametric Spearman rank correlation test. The correlation coefficient was deemed reliable with a significance level of $p < 0.05$.

Results
During the 64th Russian Antarctic Expedition to Vostok station, all 11 polar explorers had EBV and HHV-6 DNA detected in their saliva and/or plasma. In addition in 2 (No 6 and 11) participants HSV-1/2 DNA was detected at least at one stage of the expedition. The urine samples were not positive for the DNA of the examined latent pathogens.

At the same time, before arriving at the station, 10 out of 11 expedition members (except for No 11) had EBV and/or HHV-6 DNA detected in their saliva. Additionally, EBV DNA was detected in the plasma of one of the explorers (No 4) (Fig. 1).

Human herpesvirus type 6
First of all, it is important to note that averaging the HHV-6 DNA concentrations value ($n = 11$) in saliva and plasma did not yield statistically significant differences between the consecutive time points (according to the Mann-Whitney criterion) (see Supplementary Fig. S2 and S3). Thus, this study requires a personalized data processing approach to avoid missing the dynamics of reactivation in all participants.

In all 11 polar explorers, HHV-6 DNA was detected in samples at least at one point during the expedition (Fig. 2). In this instance all expedition participants can be divided into 2 main groups. The first group (No 3, 6, 8 and 11) had shedding of HHV-6 in saliva during the expedition. The second group (No 2, 4, 5, 7, 9 and 10) had viral DNA detected in saliva prior to arriving at the station. Separately, it is possible to point out expedition participant No 1, who had HHV-6 DNA shedding in saliva during all phases of the expedition, but only in plasma during the 11th month of wintering (see Supplementary Fig. S4). During the 2nd month of wintering, viral DNA concentration in saliva of 2 participants (No 6 and 10) reached a high point, but the level of specific antibodies was consistently low (Fig. 2B, D). For 1 of the polar explorers (No 6), we can discuss subclinical reactivation of HHV-6 during this period, as viral DNA was not detected in saliva during the background period.

During the 4th – 6th months of wintering, there was a peak of HHV-6 DNA content in saliva for 5 other subjects (No 3, 4, 5, 7, 11) (Fig. 2B). In addition, just 1 of them (No 3) showed a decrease in the level of specific

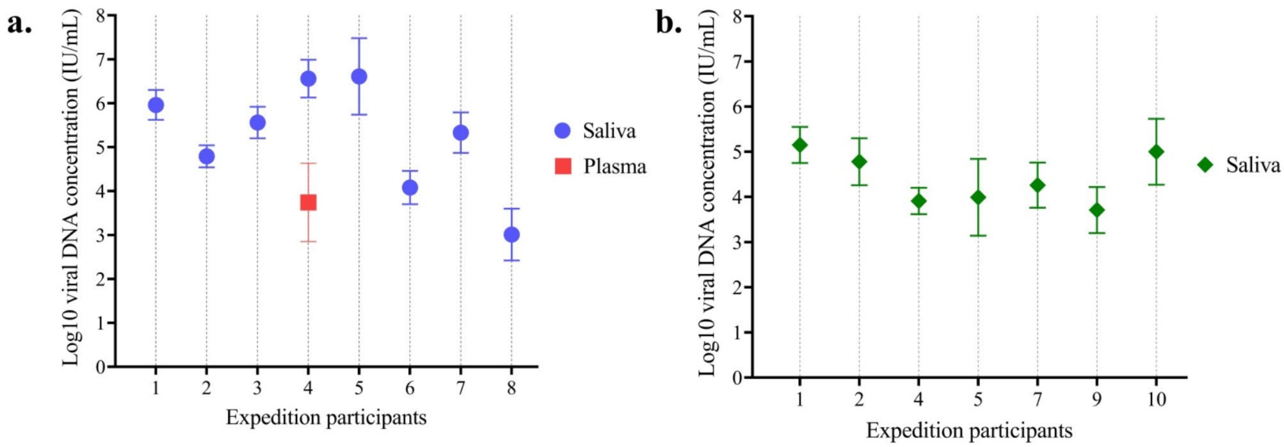

Fig. 1. Mean values (log10) of viral DNA concentration in the biomaterial before arriving at the station. **(a)** The baseline concentration (mean values (IU/ml) ± standard deviation) of EBV DNA in saliva and plasma of the expedition participants. **(b)** The baseline concentration (mean values (IU/ml) ± standard deviation) of HHV-6 DNA in saliva of the expedition participants.

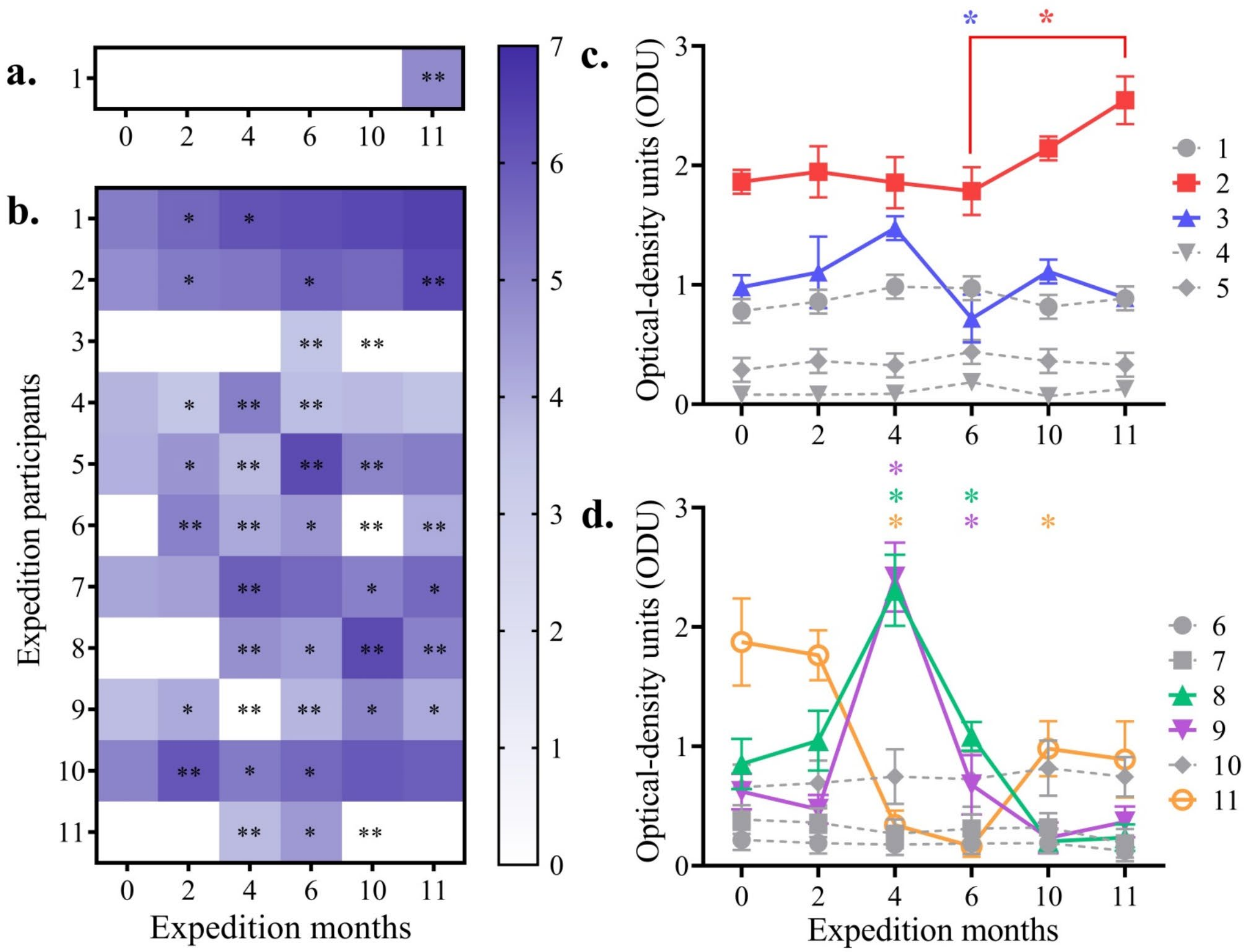

Fig. 2. Dynamics of the HHV-6 lytic cycle in the biomaterial of the expedition participants. **(a)** The mean values (log 10) of HHV-6 DNA concentration (IU/ml) in plasma. **(b)** The mean values (log 10) of HHV-6 DNA concentration (IU/ml) in saliva. Dynamics of IgG antibody to HHV-6 titers (optical density units ± standard deviation) in the serum of the expedition participants No 1–5 **(c)** and No 6–11 **(d)**. (*) - $p \le 0.05$; (**) - $p \le 0.01$ in one-way ANOVA with Tukey's aposteriori test for pairwise comparison.

antibodies at this time (Fig. 2C). At the same time for 3 expedition participants (No 3, 8, 11) we can discuss the subclinical reactivation of HHV-6 associated with the primary detection of viral DNA in biological samples during this period.

Furthermore, one polar explorer (No 9) demonstrated the presence of viral DNA during the background period and during the 2nd month of wintering. The virus reappeared in the 6th month of the expedition after being eliminated from saliva between the second and 6th months of wintering (Fig. 2B).

In 4 polar explorers (No 1, 2, 8 and 9), the maximum level of viral DNA was observed at the tenth and/or 11th month of wintering (Fig. 2B). During the 11th month of wintering, viral DNA was detected in plasma in 1 of 3 (No 1) participants, but specific antibodies remained stable (Fig. 2A, C).

Epstein-Barr virus

During the investigation of the dynamics of EBV DNA concentrations mean values ($n = 11$) in saliva and plasma, as in the case of HHV-6, there were no statistically significant differences between successive time points (according to the Mann-Whitney criterion) (see Supplementary Fig. S5 and S6).

EBV DNA, as well as HHV-6, was detected in the saliva and/or plasma of all 11 polar explorers at least at one stage of the expedition (Fig. 3). All expedition participants can also be divided into 3 main groups. The first group (No 9, 10 and 11) had shedding of EBV only in saliva during the expedition. The second group (No 2, 3, 6, 7 and 8) had viral DNA detected only in saliva prior to arriving at the station. The third group (No 1 and 5) was distinguished by the presence of viral DNA in saliva at all stages of observation and its shedding in plasma only during the end of the wintering. Separately, it is possible to point out expedition participant No 4, who had EBV

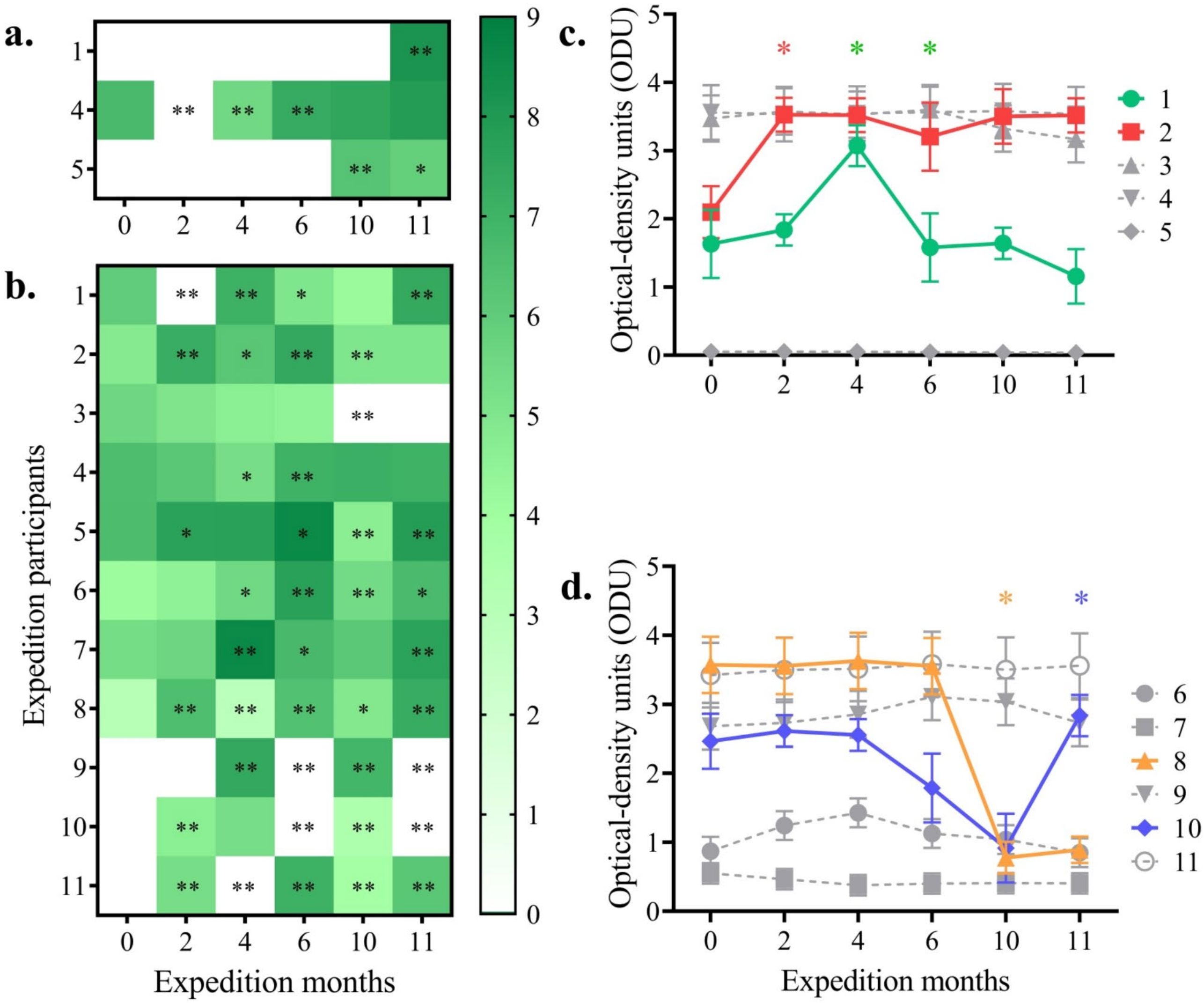

Fig. 3. Dynamics of the EBV lytic cycle in the biomaterial of the expedition participants. **(a)** The mean values (log 10) of EBV DNA concentration (IU/ml) in plasma. **(b)** The mean values (log 10) of EBV DNA concentration (IU/ml) in saliva. Dynamics of IgG antibody to EBV NA protein titers (optical density units ± standard deviation) in the serum of the expedition participants No 1–5 **(c)** and No 6–11 **(d)**. (*) – $p \leq 0.05$, (**) – $p \leq 0.01$ in one-way ANOVA with Tukey's aposteriori test for pairwise comparison.

DNA detected in both saliva and plasma before the expedition started (see Supplementary Fig. S7).The dynamics of viral DNA content in saliva usually had a two-peak character. Thus, the first peak of viral DNA concentration in saliva of 7 out of 11 participants (No 1, 5, 6, 7, 9, 10, 11) fell in the middle of the wintering, and the second peak occurs during its final stage. In 2 more subjects (No 3 and 4), the peak of EBV DNA concentration in saliva occurred in the pre- expedition period (Fig. 3B). In addition, in 1 person (No 3), viral DNA was no longer detectable by the 10th month of the expedition. In the second participant (No 4), the viral DNA content in saliva decreased gradually before increasing again during the 10th month of wintering. The dynamics of EBV DNA concentration in saliva of both subjects were observed against the background of stably high titers of antibodies to nuclear antigen (NA IgG) (Fig. 3C). At the same time, viral DNA was detected in plasma of 3 polar explorers (No 1, 4 and 5) (Fig. 3A). In 2 of them (No 1 and 5), EBV DNA was detected during the final stages of the expedition (at the tenth and 11th month).

In 1 participant (No 4) viral DNA was constitutively present in both saliva and plasma throughout the expedition except for the 2nd month of wintering, when viral DNA was detected in saliva but not in blood (Fig. 3A, B). It is also worth noting that during the expedition, the level of antibodies to EBV capsid antigen (VCA IgG) remained consistently high, but to early EBV antigen (EA IgG) remained consistently low for all subjects (see Supplementary Fig. S8 and S9).

Correlation analysis revealed a moderate positive correlation between EBV DNA concentration in saliva and the level of antibodies to EBV VCA antigen ($r = 0{,}324$; $p = 0{,}02$), as well as a weak negative correlation with the level of antibodies to HHV-6 ($r = -0{,}2621$; $p = 0{,}05$). In addition, the level of EBV antibodies to NA antigen had a weak positive correlation with the level of antibodies to HHV-6 ($r = 0{,}298$; $p = 0{,}027$) and a moderate negative correlation with the concentration of HHV-6 DNA in saliva ($r = -0{,}417$; $p = 0{,}002$) (see Supplementary Tab. S10).

Human simplex virus type 1 and 2

During the investigation of the dynamics of HSV-1/2 DNA concentrations mean values ($n = 11$) in saliva, as in the case of HHV-6 and EBV, there were no statistically significant differences between successive time points (according to the Mann-Whitney criterion) (see Supplementary Fig. S11).

It should also be noted that 2 polar researchers out of 11 (No 6 and 11) had a subclinical reactivation of HSV-1/2 during the wintering at Vostok station (Fig. 4A). At the same time, 2 rounds of subclinical reactivation of HSV were observed in 1 of the polar explorers (No 6). For the first time, viral DNA was detected during the period of early adaptation to polar station conditions against the background of increasing HSV-2 IgG antibody titers and some decrease in HSV-1 IgG titers. The second, more significant peak of subclinical reactivation was observed at the final stage of expedition against the background of a slight decrease in the titers of specific IgG antibodies to both HSV-1 and HSV-2 (Fig. 4B). In another expedition participant (No 11), subclinical reactivation was observed only at the 10th month of wintering on the background of stably high titers of HSV-1 specific antibodies and absence of HSV-2 specific antibodies (Fig. 4C).

Another latent pathogens

We also measured the shedding of other studied pathogens in saliva, urine and plasma, as well as the titers of specific antibodies (Table 1). In the same time DNA of CMV, VZV, *Mycoplasma hominis*, *Ureaplasma urealiticum* and *Chlamydia trachomatis* was not detected in the plasma, urine and saliva samples of polar explorers. However the titers of specific antibodies to these pathogens were changed in some of those subjects during the expedition. The maximum number of statistically significant changes in antibody titers to these pathogens was observed during the middle and second half of the period of stay at the station (see Supplementary Fig. S12 - S16).

Interrelation with geomagnetic activity and psychoemotional state

Although no statistically significant correlations were found between the DNA levels of the studied latent pathogens in saliva and plasma and the K-index of geomagnetic activity, it is worth noting that the increase of viral DNA concentration in saliva and its detection in plasma in some participants at the 10th month of expedition approximately coincides with the prolonged period of increased geomagnetic activity from the thirty first to the fortieth week of the expedition (Fig. 5; see Supplementary Tab. S10).

Thus, in the 10th month of the expedition, an increase in the HHV-6 DNA level was noted in the saliva of participants No 8 and 9, and the level of EBV DNA increased in the saliva of participants No 9 and 10 and in the plasma of participant No 5 (Figs. 2 and 3). At the same time, reactivation of HSV-1/2 was observed in saliva of participant No 11 during the 10th month of the expedition (Fig. 4).

If we assume a delayed effect of the increase in geomagnetic activity, then we can also consider the level of viral DNA in the 11th month of the expedition. Thus, during this period, an increase in the HHV-6 DNA was observed in the saliva of participants No 2, 6, and 7 and in the plasma of participant No 1 (Fig. 2). Moreover, the level of EBV DNA increased in the saliva of participants No 1, 5, 7, 8, and 11 and in the plasma of participant No 1 (Fig. 3). At the same time, in participant No 6, reactivation of HSV-1/2 in the saliva was noted in the 11th month of the expedition (Fig. 4).

During the analysis of the psychoemotional state, two major periods of its decrease were observed in most participants: from expedition week 15 to 17 (4th month of expedition) and from expedition week 38 to 40 (9th month of expedition) (Fig. 6). Although no reliable correlations between the level of the polar explorers' psychoemotional state and the level of latent pathogen DNA/specific antibody titers were found, some participants during this period showed a tendency towards an increase in the level of viral DNA in saliva and plasma (see Supplementary Tab. S17).

Thus, in the 4th month of the expedition, an increase in the HHV-6 DNA level was noted in the saliva of participants No 4, 7, 8 and 11, and the level of EBV DNA increased in the saliva of participants No 1, 7 and 9 and in the plasma of participant No 4 (Figs. 2 and 3).

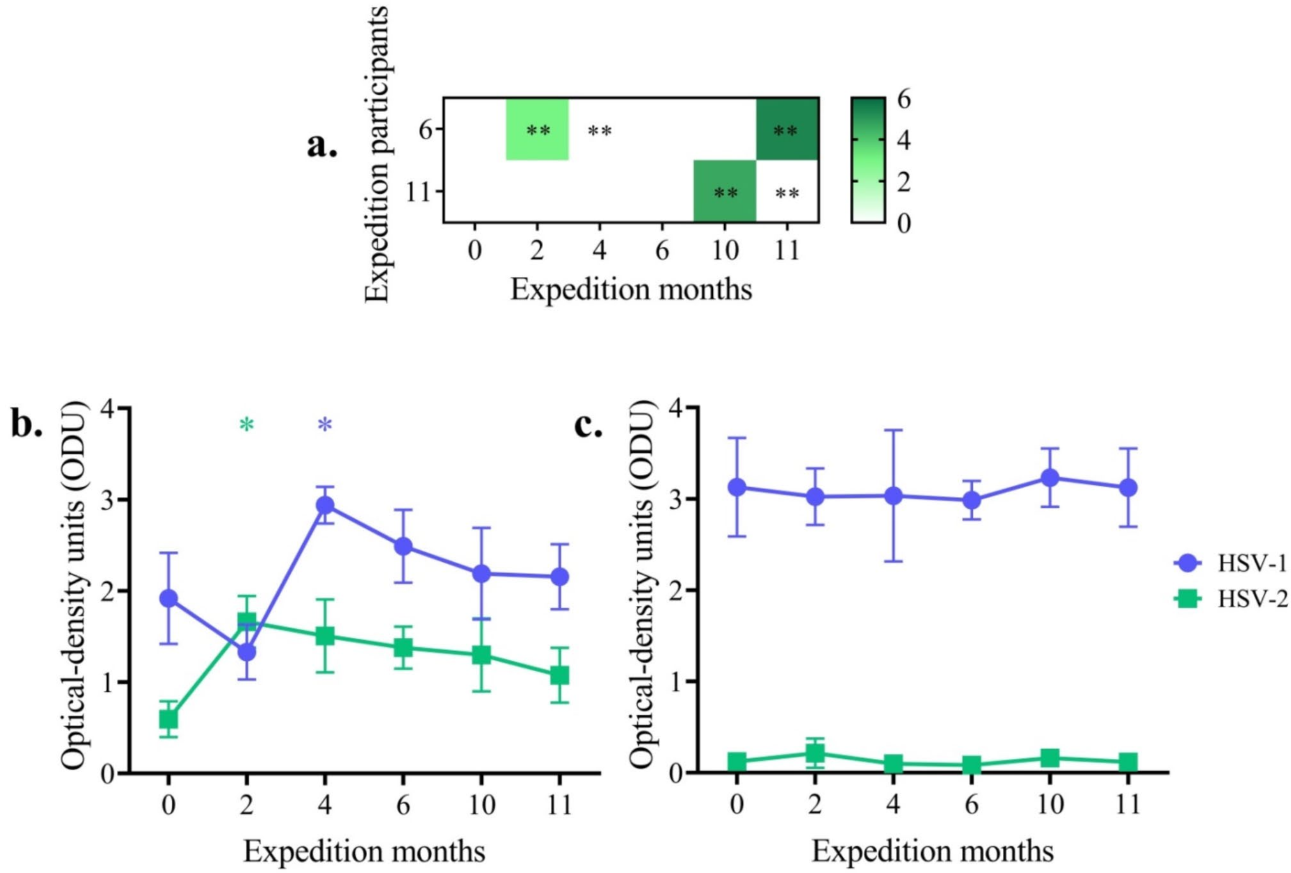

Fig. 4. Dynamics of the HSV-1/2 lytic cycle in the biomaterial of the expedition participants. **(a)** The mean values (log 10) of HSV-1/2 DNA concentration (IU/ml) in saliva. Dynamics of IgG antibody to HSV-1 and HSV-2 titers (optical density units ± standard deviation) in the serum of the expedition participant No 6 **(b)** and No 11 **(c)**. (*) – $p \le 0.05$, (**) – $p \le 0.01$ in one-way ANOVA with Tukey's aposteriori test for pairwise comparison.

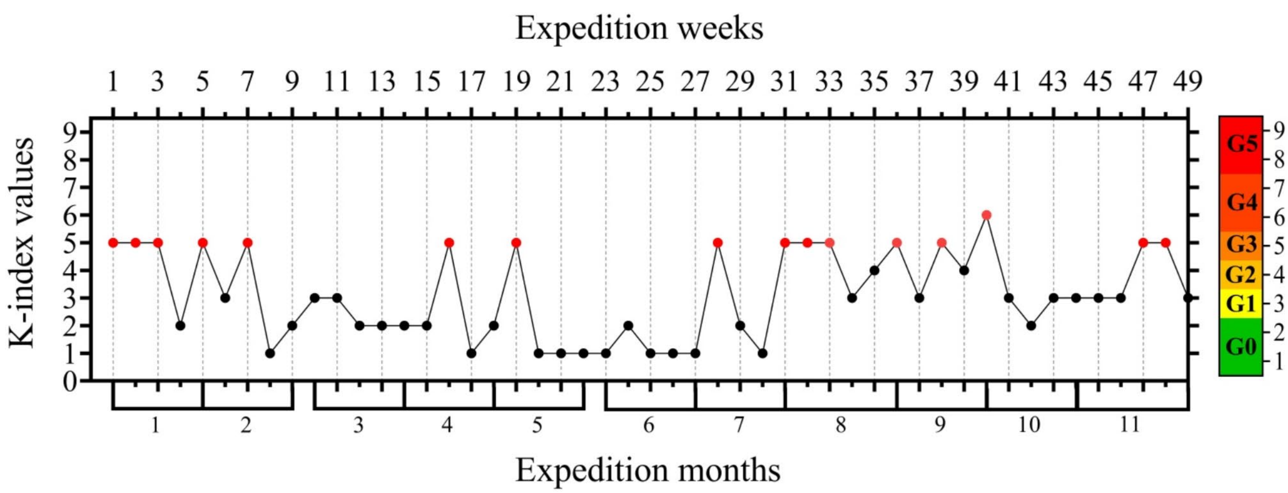

Fig. 5. Geomagnetic activity variation during the expedition. G0 - quiet geomagnetic background. G1 - intensified geomagnetic activity. G2 - active geomagnetic activity. G3 - weak geomagnetic activity. G4 - medium geomagnetic activity. G5 - strong geomagnetic storm.

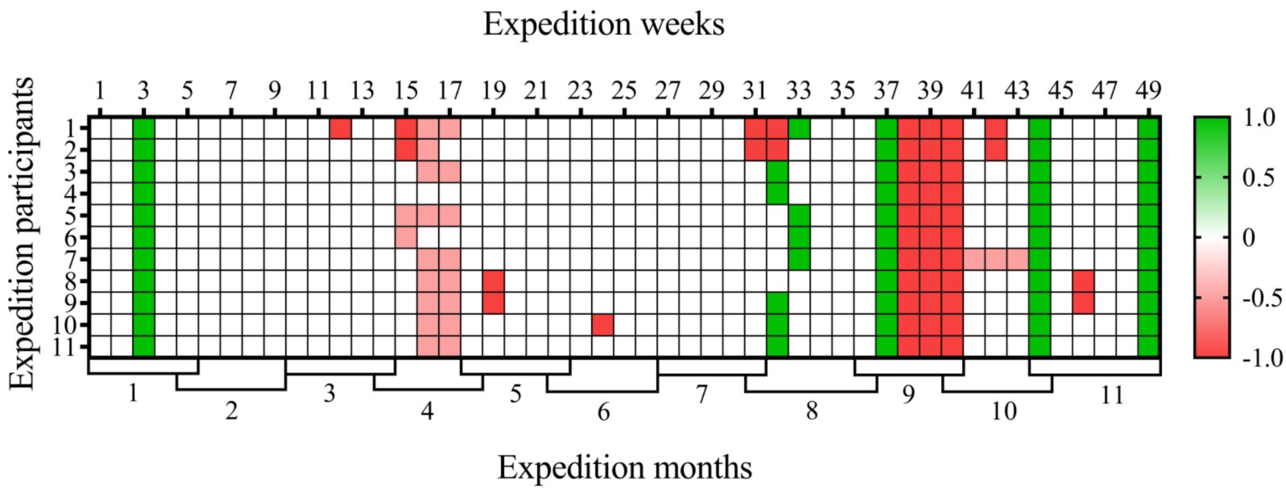

Fig. 6. Psychoemotional state of the expedition participants during the wintering at Vostok station. The months of the expedition are indicated at the bottom of the X-axis, and the weeks of the expedition are indicated at the top. Green – the most positive psychoemotional state (the maximum value of the emotional background parameter is + 1), red – the most negative emotional state (the minimum value of the psychoemotional background parameter is -1).

At the same time, the observed increase in the concentration of latent pathogens DNA in the biological fluids of some participants in the 10th month of the expedition may be associated with the delayed effect of prolonged psychoemotional stress in the 9th month.

Discussion

The uniqueness of a months-long expedition to Vostok polar station lies in the fact that, despite modern means of communication and transport, people for a long period of time find themselves almost completely cut off from the surrounding world in extreme conditions and are forced to solve problems without outside help. In this sense, polar winter represents a terrestrial model that is similar to the conditions of a long-term spaceflight.

For technical reasons it was only possible to conduct background biomaterial sampling on the way to Antarctica. During this period the organisms of the subjects were already exposed to a complex of stress factors related to the preparation for the expedition and the long journey, which could not but affect the state of the immune system. On this basis, the detection of latent pathogens DNA in the saliva and/or plasma of polar explorers during this period were quite expected. Furthermore, reactivation of herpesviruses has also been shown during the pre-flight period of spaceflight[49]. The results obtained appear to be logical due to the high level of psychoemotional stress and fatigue in both the pre-flight and pre-expedition periods, which significantly affect the immune system's protective capabilities.

During the Antarctic expedition, DNA of latent pathogens such as HHV-6 and EBV was detected in saliva and/or plasma of all subjects at least at one stage of the expedition. In addition to this, 2 of the subjects also had detectable HSV-1/2 DNA in saliva. In most cases, the peaks of viral DNA content occurred mainly during the middle and final stage of the expedition. One of the most probable explanations of the observed lytic infection dynamics may be the suppression of immune system functional activity caused by a complex of negative environmental factors. It is possible that it resulted in a weakening of the immune system's capacity to control latent infections. The absence of significant changes in the titers of specific antibodies to the latent pathogens and their avidity index against statistically significant changes in the concentration of viral DNA in saliva, and some cases subclinical reactivation of the pathogen are an indirect indication of this. Indeed, the absence of significant changes in the titers of specific antibodies to the studied pathogens in most of the subjects suggests that the complex of factors associated with a long-term stay at the Antarctic Vostok station influence the B-cell immune response. Therefore, it could be a contributor to the impaired immunological control of latent infections. It's clear that this hypothesis needs more confirmation.

The comparison of the DNA content of the studied latent pathogens in saliva and plasma with the change of geomagnetic activity during the expedition produced interesting results. Thus, the peaks of viral DNA concentration and the increase of specific antibody titers in the 10th month of the expedition are noted immediately after a prolonged period of increased geomagnetic activity.

The comparison of the DNA content of the studied latent pathogens in saliva and plasma with the change of geomagnetic activity during the expedition produced interesting results. Thus, the increase of viral DNA concentration and specific antibody titers in the 10th month of the expedition for some participants are noted immediately after a prolonged period of increased geomagnetic activity. In addition, if we assume a delayed effect of geomagnetic activity on the functional state of the immune system, this may explain the increase in the concentration of viral DNA in the saliva and plasma of some participants during the 11th month of the expedition. This is consistent with the literature data describing the interrelations of increased geomagnetic activity with the development of immunodeficiency states, including in healthy people. It can be one of the reasons for the decrease of immunological control of latent infections and the initiation of reactivation[50,51]. Additionally, geomagnetic fluctuations may cause changes in indicators of innate and adaptive immunity as a result of a decrease in melatonin level[52]. Thus, the increase in solar and geomagnetic activity resulted in a significant decrease in the total number of leukocytes, neutrophils and blood basophils, as well as a general decrease in lymphocytes' synthetic activity[32,51,53]. In addition, there are reasons to assume that the influence of increased solar activity may have an influence on the decrease of the total level of immunoglobulins IgG and IgM, as well as the increase in the level of IgA antibodies to β2-glycoprotein I - an autoimmune marker of antiphospholipid syndrome[50,54]. The issue of the association between human immune status and geomagnetic activity is relevant not only for residents of the northern regions and polar researchers. This problem can also be encountered by astronauts during the departure beyond the Van Allen radiation belts in the period of deep space exploration[55].

In this study, the small number of time points considered does not allow us to establish reliable correlations between the geomagnetic activity and the concentration of viral DNA in saliva. However, the presented data enable us to us to make an assumption about the trends of the relationship between the dynamics of latent pathogens' lytic cycle and the level of geomagnetic activity. Obviously, this hypothesis requires further confirmation.

Furthermore, it was interesting to observe the connection between the psychoemotional state of the polar explorers and the dynamics of the lytic cycle of latent infections. The reason for this interest was that many psychosocial stressors faced by polar expedition participants are very similar to those faced by astronauts during long-term spaceflight. These stressors include, first of all, a high degree of physical and psychosocial isolation, sensory monotony, shift of circadian rhythms during the polar day and night, and psychological stress associated with limited rescue opportunities in case of a threat to life and health in unfavorable environmental conditions[9,56,57]. Thus, Antarctica is an excellent environment for reproducing the conditions of isolation in the long-term space missions[48]. That is why the study of the influence of psychological changes occurring in the participants during a months-long expedition on the functional activity of the immune system is currently a highly relevant issue in the context of preparation for long-term spaceflight.

Analyzing the psychoemotional state of the expedition members during the period of taking biomaterial, it can be noticed that the primary detection of viral DNA in saliva or plasma, as well as changes in antibody titers in some participants at 4th and 10th months of wintering coincide with three-week periods of pronounced psychoemotional tension within the team (Fig. 6). Previously, the important role of the psychological factor in the regulation of the immune system functional activity was shown in an experiment involving male isolation for 10 days[58]. Changes in innate immunity parameters correlated well with an increase in the anxiety level calculated on the basis of psychological tests[58]. Consequently, it can be concluded that a prolonged period of psychoemotional tension can have a significant immunosuppressive effect on the some members of Antarctic expeditions. However, this statement is just an assumption and needs to be researched more thoroughly.

Thus, a general tendency of activation of the lytic cycle of herpesviruses at the background of no significant changes in the titers and the avidity of specific antiviral antibodies was observed in all participants of the expedition. The increase in viral DNA concentration in biological fluids of the subjects was primarily observed during the later stages of wintering. The study of reactivation of latent infections under spaceflight conditions revealed that herpesvirus reactivation was considerably more frequent in long-term International Space Station missions than in short-term Space Shuttle missions[41]. This can be explained by the development of immunosuppression caused by prolonged isolation, hypodynamia, and asthenia, which can result in impaired immunological control of latent infections[9,37].

In addition, the investigation discovered significant differences in the dynamics of DNA content of latent pathogens in the biological fluids of polar explorers during their stay at Vostok station for each of the detected viruses.

Additionally, the dynamics of the lytic cycle of the studied latent pathogens in saliva and plasma were significantly different. Thus, in saliva, EBV and HHV-6 DNA was detected more frequently than in plasma. Moreover, only saliva was found to have HSV-1/2 DNA. Presumably, this may be due to the fact that the blood of immunocompetent subjects constitutively contains a wide range of functional immune system elements. At the same time, secretory immunoglobulins of class A are the only antiviral immune system component in saliva. Consequently, viral particles are eliminated more effectively in the blood, which contributes to their preferential detection in saliva rather than in plasma. However, this phenomenon remains to be studied.

Furthermore, it was noted that the obtained results had a high degree of individual variability. The introduction of pre-flight quarantine can help reduce the risk of infectious diseases developing in astronauts and ground-based experimenters. However, controlling of the latent pathogens reactivation is very difficult due to their lifelong persistence in the host organism[2,59]. As a result, it becomes clear that a personalized approach is needed to study the immune system response of each individual subject. In the future, the personalized approach should be the basis for the development of monitoring, prevention and therapy of immune disorders, as well as cases of reactivation of latent pathogens in polar explorers, astronauts and volunteers in the ground-based experiment.

Based on the obtained data, it can be concluded that the conditions of a months-long expedition to the Antarctic Vostok station have a significant impact on the human immune system causing weakening of the immunological control of latent infections. It is supposed that the level of specific antibodies to latent pathogens may indicate the functional activity of the B-cell component of the immune system. And since immunity is a complex system with closely interconnected components, the functional activity of one of them may reflect the general state of the human immune system. It can be also concluded that geomagnetic activity fluctuations and negative psychoemotional state can have a negative effect on the immune system's functional activity and, as a consequence, reactivation of latent infections. However, it is yet to be determined which factor have the most impact on the dynamics of the lytic cycle of latent pathogens: psychoemotional status, geomagnetic activity, or any other. The obtained results are of great significance in understanding the peculiarities of human immune system adaptation to the conditions of high latitudes, as well as in assessing the risk of negative effects of latent infections reactivation in conditions of long-term isolation, including long-duration spaceflight.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Received: 31 July 2024; Accepted: 14 March 2025
Published online: 22 March 2025

References

1. James, C. et al. Herpes simplex virus: global infection prevalence and incidence estimates, 2016. *Bull. World Health Organ.* **98**, 315–329 (2020).
2. Cohen, J. I. Herpesvirus latency. *J. Clin. Invest.* **130**, 3361–3369 (2020).
3. Pirofski, L. A. & Casadevall, A. The state of latency in microbial pathogenesis. *J. Clin. Invest.* **130**, 4525–4531 (2020).
4. Jordan, M. C., Jordan, G. W., Stevens, J. G. & Miller, G. Latent herpesviruses of humans. *Ann. Intern. Med.* **100**, 866–880 (1984).
5. Zuhair, M. et al. Estimation of the worldwide seroprevalence of cytomegalovirus: A systematic review and meta-analysis. *Rev. Med. Virol.* **29**, e2034 (2019).
6. Crucian, B. E. et al. Immune system dysregulation occurs during short duration spaceflight on board the space shuttle. *J. Clin. Immunol.* **33**, 456–465 (2013).
7. Morukov, B. et al. Parameters of the innate and adaptive immunity in cosmonauts after long-term space flight on board the international space station. *Hum. Physiol.* **36**, 264–273 (2010).
8. Yu, Y. Z., Wang, Z. H., Zhang, W. & Wu, W. Effect of the environment in Antarctica on immune function and electroencephalogram. *Chin. J. Polar Res.* **2**, 45–52 (1994).
9. Ilyin, E. A. The psychological status of the polar explorers and its pharmacocorrection in conditions of annual isolation at 'Vostok' station in Antarctica. *Aviakosm. Ekol Med.* **51**, 5–14 (2017).
10. Iserson, K. V. Bioethical issues in Antarctica. *Camb. Q. Healthc. Ethics CQ Int. J. Healthc. Ethics Committees*. **30**, 136–145 (2021).
11. Kanas, N. Psychosocial value of space simulation for extended spaceflight. *Adv. Space Biol. Med.* **6**, 81–91 (1997).
12. Palinkas, L. A. & Suedfeld, P. Psychological effects of polar expeditions. *Lancet (London England)*. **371**, 153–163 (2008).
13. Strewe, C. et al. Sex differences in stress and immune responses during confinement in Antarctica. *Biol. Sex. Differ.* **10**, 20 (2019).
14. Young, P., Finn, B. C., Bruetman, J., Pellegrini, D. & Kremer, A. [The chronic asthenia syndrome: a clinical approach]. *Med. (B Aires)*. **70**, 284–292 (2010).
15. Kanas, N. Psychosocial support for cosmonauts. *Aviat. Space Environ. Med.* **62**, 353–355 (1991).
16. Vasenina, E. E., Gankina, O. A. & Levin, O. S. Stress, asthenia, and cognitive disorders. *Neurosci. Behav. Physiol.* **52**, 1341–1347 (2022).
17. Zachariae, R. et al. Monocyte chemotactic activity in Sera after hypnotically induced emotional States. *Scand. J. Immunol.* **34**, 71–79 (1991).
18. Zachariae, R., Jørgensen, M. M., Egekvist, H. & Bjerring, P. Skin reactions to Histamine of healthy subjects after hypnotically induced emotions of sadness, anger, and happiness. *Allergy* **56**, 734–740 (2001).
19. Ponomarev, S. A. et al. Changes in the cellular component of the human innate immunity system in short-term isolation. *Acta Astronaut.* **166**, (2019).
20. Ponomarev, S. A. et al. The impact of short-term confinement on human innate immunity. *Sci. Rep.* **12**, 8372 (2022).
21. Feuerecker, M. et al. Early adaption to the Antarctic environment at dome C: consequences on stress-sensitive innate immune functions. *High. Alt Med. Biol.* **15**, 341–348 (2014).
22. Hartmann, G. et al. High altitude increases circulating interleukin-6, interleukin-1 receptor antagonist and C-reactive protein. *Cytokine* **12**, 246–252 (2000).
23. Meehan, R. et al. Operation everest II: alterations in the immune system at high altitudes. *J. Clin. Immunol.* **8**, 397–406 (1988).
24. Klokker, M., Kharazmi, A., Galbo, H., Bygbjerg, I. & Pedersen, B. K. Influence of in vivo hypobaric hypoxia on function of lymphocytes, neutrocytes, natural killer cells, and cytokines. *J. Appl. Physiol.* **74**, 1100–1106 (1993).
25. Rohm, I. et al. Hypobaric hypoxia in 3000m altitude leads to a significant decrease in circulating plasmacytoid dendritic cells in humans. *Clin. Hemorheol Microcirc*. **63**, 257–265 (2016).
26. Akasofu Syun-Ichi, C. S. *Solar-Terrestrial Physics* (Clarendon, 1972).
27. Bartels, J. The standardized index Ks and the planetary index Kp. *IATME Bull.* **12b**, 97–120 (1949).
28. Takahashi, K., Toth, B. A. & Olson, J. V. An automated procedure for near-real-time Kp estimates. *J. Geophys. Res. Sp Phys.* **106**, (2001).
29. Samsonov, S. N. et al. The HELIO-geophysical storminess health effects in the cardio-vascular system of a human in the middle and high latitudes. *Wiad Lek*. **69**, 537–541 (2016).
30. Liddie, J. M. et al. Associations between solar and geomagnetic activity and cognitive function in the normative aging study. *Environ. Int.* **187**, 108666 (2024).
31. Karnaukhova, N. A. & Sergievich, L. A. [Correlation between change in solar activity and functional activity of animal blood lymphocytes in norm and pathology]. *Biofizika* **45**, 958–959 (2000).
32. Karnaukhova, N. A., Sergievich, L. A., Karnaukhov, A. V., Mit'kovskaia, L. I. & Karnaukhov, V. N. Quantity and quality of animal immunocompetent cells in relation with variations in solar activity. *Biofizika* **44**, 313–317 (1999).
33. Shirai, T. et al. TH1-biased immunity induced by exposure to Antarctic winter. *J. Allergy Clin. Immunol.* **111**, 1353–1360 (2003).
34. Williams, D. L., Climie, A., Muller, H. K. & Lugg, D. J. Cell-mediated immunity in healthy adults in Antarctica and the sub-Antarctic. *J. Clin. Lab. Immunol.* **20**, 43–49 (1986).
35. Tringali, G. et al. Circulating interleukin-1-beta levels after acute and prolonged exposure to low temperatures: human and rat studies. *Neuroimmunomodulation* **7**, 177–181 (2000).
36. Mehta, S. K., Pierson, D. L., Cooley, H., Dubow, R. & Lugg, D. Epstein-Barr virus reactivation associated with diminished cell-mediated immunity in Antarctic expeditioners. *J. Med. Virol.* **61**, 235–240 (2000).
37. Tingate, T. R., Lugg, D. J., Muller, H. K., Stowe, R. P. & Pierson, D. L. Antarctic isolation: immune and viral studies. *Immunol. Cell. Biol.* **75**, 275–283 (1997).
38. Reyes, D. P. et al. Clinical herpes Zoster in Antarctica as a model for spaceflight. *Aerosp. Med. Hum. Perform.* **88**, 784–788 (2017).
39. Crucian, B.E. et al. Incidence of clinical symptoms during long-duration orbital spaceflight. *Int. J. Gen. Med.* **9**, 383–391 (2016).
40. Mehta, S. K. et al. Dermatitis during spaceflight associated with HSV-1 Reactivation. *Viruses* 14, (2022).
41. Crucian, B. E., Stowe, R. P., Pierson, D. L. & Sams, C. F. Immune system dysregulation following short- vs long-duration spaceflight. *Aviat. Space Environ. Med.* **79**, 835–843 (2008).
42. Morukov, B. et al. T-cell immunity and cytokine production in cosmonauts after long-duration space flights. *Acta Astronaut.* **68**, 739–746 (2011).

43. Spielmann, G. et al. B cell homeostasis is maintained during long-duration spaceflight. *J. Appl. Physiol.* **126**, 469–476 (2019).
44. Stowe, R. P., Mehta, S. K., Ferrando, A. A., Feeback, D. L. & Pierson, D. L. Immune responses and latent herpesvirus reactivation in spaceflight. *Aviat. Space Environ. Med.* **72**, 884–891 (2001).
45. Berendeeva, T. A., Ponomarev, S. A., Antropova, E. N. & Rykova, M. P. Toll-like receptors in cosmonaut's peripheral blood cells after long-duration missions to the international space station. *Aviakosm. Ekolog. Med.* **49**, 49–54 (2015).
46. Dhabhar, F. S. Stress-induced augmentation of immune function–the role of stress hormones, leukocyte trafficking, and cytokines. *Brain Behav. Immun.* **16**, 785–798 (2002).
47. Crucian, B. E. et al. Countermeasures-based improvements in stress, immune system dysregulation and latent herpesvirus reactivation onboard the international space Station - Relevance for deep space missions and terrestrial medicine. *Neurosci. Biobehav Rev.* **115**, 68–76 (2020).
48. Diak, D. M. et al. Palmer station, Antarctica: A ground-based spaceflight analog suitable for validation of biomedical countermeasures for deep space missions. *Life Sci. Sp Res.* **40**, 151–157 (2024).
49. Mehta, S. K. et al. Multiple latent viruses reactivate in astronauts during space shuttle missions. *Brain Behav. Immun.* **41**, 210–217 (2014).
50. Stoupel, E. G., Abramson, E., Gabbay, U. & Pick A. I. Relationship between Immunoglobulin levels and extremes of solar activity. *Int. J. Biometeorol.* **38**, 89–91 (1995).
51. Tracy, S. M. et al. Associations between solar and geomagnetic activity and peripheral white blood cells in the normative aging study. *Environ. Res.* **204**, 112066 (2022).
52. Weydahl, A., Sothern, R. B., Cornélissen, G. & Wetterberg, L. Geomagnetic activity influences the melatonin secretion at latitude 70 degrees N. *Biomed. Pharmacother*. **55** (Suppl 1), 57s–62s (2001).
53. Karnaukhova, N. A. & Sergievich, L. A. Correlations between functional activity of animal blood lymphocytes and change in solar activity. *Biofizika* **44**, 762–763 (1999). at.
54. Stoupel, E., Monselise, Y. & Lahav, J. Changes in autoimmune markers of the anti-cardiolipin syndrome on days of extreme geomamagnetic activity. *J. Basic. Clin. Physiol. Pharmacol.* **17**, 269–278 (2006).
55. Nelson, G. A. Fundamental space radiobiology. *Gravitational Sp Biol. Bull. Publ Am. Soc. Gravitational Sp Biol.* **16**, 29–36 (2003).
56. Ponomarev, S. A. et al. Immunological aspects of isolation and confinement. *Front. Immunol.* **12**, 697435 (2021).
57. Salam, A. Exploration class missions on earth: lessons learnt from life in extreme antarctic isolation and confinement. in *Stress Challenges and Immunity in Space: From Mechanisms to Monitoring and Preventive Strategies* 425–439 (2012).
58. Shimamiya, T. et al. Effects of 10-day confinement on the immune system and psychological aspects in humans. *J. Appl. Physiol.* **97**, 920–924 (2004).
59. Mehta, S. K. et al. Latent virus reactivation in astronauts on the international space station. *NPJ Microgravity*. **3**, 11 (2017).

Acknowledgements
Authors expresses deep gratitude to the late Dr. Evgeny Aleksandrovich Ilyin, an honored scientist in the field of space biology, for his sensitive guidance at all stages of the organization, planning and implementation of the experiments, the results of which formed the basis of this article. The authors also express their gratitude to Andrey Alekseevich Shulgin, researcher at the Engelhardt Institute of Molecular Biology, for consultation on issues of bioinformatic data processing.

Author contributions
S.P., S.S. and N.O. conceived the project, S.P. and S.S. wrote the manuscript, M.R. and E.A. revised the manuscript, N.O., S.S., V.S., O.K., D.V., A.K., K.O. and E.Z. performed the experiments and data analysis. T.Z. performed the analysis and interpretation of the data on the polar explorers' psycho-emotional status. All authors contributed to the writing of this article. The final version of the manuscript was reviewed and approved by all authors.

Funding
The study was supported by the Ministry of Science and Higher Education of the Russian Federation under agreement No 075-15-2022-298 from 18 April 2022 about the grant in the form of subsidy from the federal budget to provide government support for the creation and development of a world-class research center, the "Pavlov Center for Integrative Physiology to Medicine, High-tech Healthcare and Stress Tolerance Technologies".

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-94539-4.

Correspondence and requests for materials should be addressed to S.M.S.

Reprints and permissions information is available at www.nature.com/reprints.