Immunobiology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Changes in immune cell signalling, apoptosis and stress response functions in mice returned from the BION-M1 mission in space

E.G. Novoselova[a,*], S.M. Lunin[a], M.O. Khrenov[a], S.B. Parfenyuk[a], T.V. Novoselova[a], B.S. Shenkman[b], E.E. Fesenko[a]

[a] Institute of Cell Biophysics, Pushchino, Moscow Region, Russia
[b] Institute of Biomedical Problems, Russian Academy of Sciences, Moscow, Russia

ARTICLE INFO

Article history:
Received 7 May 2014
Received in revised form 22 October 2014
Accepted 22 October 2014
Available online xxx

Keywords:
Caspase-3
Hsp72
Hsp90
Mice
NF-kappaB
Space

ABSTRACT

To explore the effect of the spaceflight environment on immunity in animals, C57/BL6 mice flown on a 30-day space high-orbit satellite mission (BION-M1) were analyzed. Cytokine response in mice was measured in tandem with the following parameters: the synthesis of inducible forms of the heat shock proteins HSP72 and HSP90α; activity of the NF-κB, IFR3, and SAPK/JNK signalling pathways; and TLR4 expression. In addition, apoptosis in the thymus was measured by caspase-3 and ph-p53/p53 ratio testing. In response to flight environment exposure, mice had a reduction in spleen and thymus masses and decreased splenic and thymic lymphocyte counts. Plasma concentration of IL-6 and IFN-γ but not TNF-α was decreased in C57BL6 mice. The NF-κB activity in splenic lymphocytes through the canonical pathway involving IκB degradation was significantly increased at 12 h after landing. One week after landing, however, the activity of NF-κB was markedly decreased below even the control values. Non-canonical NF-κB activity increased during the whole observation period. The activities of SAPK/JNK and IRF-3 were invariable at 12 h but significantly increased 7 days after landing. The expression of Hsp72 and Hsp90α was somewhat increased 12 h (Hsp72) and 7 days (Hsp90α). TLR4 expression in splenic cells was significantly increased only at 12 h, returning to normal 7 days after landing. To assess the apoptosis in thymus lymphocytes, caspase-3 and levels of p53 protein along with its phosphorylated form were measured in thymic lymphocytes. The results indicated that the high-orbit spaceflight environment caused an increase in the level of p53 but more notably in the activated, phosphorylated form of the p53 protein. The calculated ratio of the active to inactive forms of the protein (ph-53/p53) 12 h after landing increased by more than twofold, indicating the apparent induction of apoptosis in thymus cells. Interestingly, 7 days after the landing, this ratio was not restored, but rather increased: the specified ratio was four times higher compared to the ground-based control. Measurements of caspase-3 in thymic cells indicated more expressive increase in apoptosis. Taken together, the results of the present study indicate that spaceflight induces an imbalance in the immunity of mice, showing variation in signalling, apoptosis and stress response that are not restored by 7 days after landing. These changes are distinguished from classic stress-related alterations usually caused by conventional stressors.

Introduction

There are numerous reports demonstrating that stressors in the spaceflight environment, e.g., microgravity, radiation, disrupted circadian rhythms, altered nutrition and the psychological stress of confinement can have a profound impact on immune system status in human and animal subjects (Chapes et al., 1999; Crucian et al., 2008; Grove et al., 1995; Konstantinova et al., 1993; Lesnyak et al., 1993). The attenuation of Earth's protective geomagnetic field during high orbital missions provides an additional stressor. We have demonstrated earlier that the 250-fold screening of the Earth's geomagnetic field on land induced a decrease in proliferative activity of embryonic cells and impairment of interactions between the trophoblast and endometrium (Fesenko et al., 2010).

Stressors of anticipation factors can depress immune responsiveness and even destruct sensitive immune cells (Fong, 2004;

Abbreviations: NF-kappaB, nuclear factor kappa B; IKK, I-kappaB kinase; SAPK/JNK, stress activated protein kinase; JNK, Jun N-terminal kinase; IFN, interferon; Hsp, heat shock protein; IL, interleukin; TNF, tumour necrosis factor; IRF-3, interferon regulatory factor; GAPDH, glyceraldehude-3-phosphate dehydrogenase; TLR, toll like receptor.

* Corresponding author. Tel.: +7 4967 739 325; fax: +7 4967 33 05 09.
E-mail address: elenanov.06@mail.ru (E.G. Novoselova).

http://dx.doi.org/10.1016/j.imbio.2014.10.021
0171-2985/

Stowe et al., 2001). Whereas flight-related stressors result in higher mutation rates in bacteria (Moeller et al., 2012) as well as modulated gene expression patterns (Baqai et al., 2009; Guo et al., 2012), the possibility for malignant transformation could also be increased.

Spaceflight alters the functions of a variety of immune cells involved in the innate immune system, leading to a decreased ability to phagocytise bacteria and generate an oxidative burst (Kaur et al., 2004, 2005). Such abnormalities in immune function also led to a diminished responsiveness of lymphoid cells to appropriate stimuli. Indeed, microgravity was demonstrated to depress lipopolysaccharide-induced tumour necrosis factor in RAW 264.7 cells and primary mouse macrophages *in vitro* (Wang et al., 2014). Spaceflight-associated factors also modulate the response of monocytes to gram-negative endotoxins (Kaur et al., 2008). Previous studies documented a decrease in interactions among immune cells, resulting in the loss of regulatory events (Cooper et al., 2001; Sonnenfeld, 2005). Crucian et al. (2013) demonstrated that specific parameters such as a leucocyte distribution, T cell functions and cytokine production profiles were altered during spaceflight.

The thymus is a central organ of the immune system, and thymic cells are most susceptible to apoptosis. Indeed, many of these T cells naturally undergo intrathymic apoptosis during positive selection (De Bosscher et al., 2003; Vacchio et al., 1999). T cells are also very responsible to stressors, which obviously increase apoptosis in the thymus and lead to cell count loss and so-called age-independent involution. The thymus has been reported to serve as a source of T cell reconstitution after great loss of this leucocyte type (Mackall et al., 1995), which could certainly result from exposure to stressors during spaceflight. On the other hand, we were also interested in spleen cells due to the fact that the spleen is a major source of immune cells involved in humoral and cell-mediated immunity.

Although the post-flight status of the immune system is now sufficiently established, there is an obvious lack of data on the activities of the NF-κB, IRF3, SAPK/JNK and TLR4 signalling cascades. In addition, the expression of heat shock proteins and TLR4 has not yet been explored. The present study was designed to examine the effects of spaceflight on the cytokine response in tandem with examining the following: the synthesis of inducible forms of heat shock proteins HSP72 and HSP90α; activities of the NF-κB, IRF3 and SAPK/JNK signalling pathways; p53 phosphorylation associated with apoptosis in the thymus; and TLR4 expression in splenic lymphocytes.

The NF-κB pathway is important for the expression of genes that are involved in the control of the host inflammatory response. The constitutive activation of this pathway is associated with a wide variety of diseases, including inflammation. It has been reported that LPS interacts with LPS-binding protein and CD14, which presents LPS to TLR4, thereby activating inflammatory gene expression through NF-κB signalling (Surbatovic et al., 2013). We have recently shown that IKK Inhibitor XII, a selective inhibitor of NF-κB signalling that functions by decreasing NF-κB phosphorylation and thereby reducing the probability of translocation of the NF-κB into the nucleus, also prevented SAPK/JNK activation in LPS-treated cells (Novoselova et al., 2014). These results coincide with findings demonstrating that SAPK/JNK signalling is regulated by the NF-κB pathway (Papa et al., 2004, 2006). In addition, the role of toll-like receptor 4 (TLR4) and interferon regulated factor 3 (IRF-3) in cellular stress response involving NF-κB and heat shock proteins demonstrated in many reports (Walker et al., 2012; Takeda et al., 2003). Furthermore, the signalling pathways can be affected not only by inflammatory signals, but also by physical fields (Cherenkov et al., 2009; Khrenov et al., 2007) or physiological stressors (Coffey, 2014; Nazari et al., 2013). We hypothesised that spaceflight environment that enclosed many stressors of various origins can affect referred characteristics of immune cell activity.

Table 1
Food paste composition.

Parameter	Per 100 g of wet paste	Per 100 g of dry paste
Moisture, %	74.6	
Raw protein, %	11.3 ± 0.4	44.5
Carbohydrates, %	8.8 ± 0.7	34.6
Ash, %	2.4 ± 0.2	9.4
Ca, %	0.58 ± 0.006	2.28
Mg, mg/kg	707 ± 7.0	2783.4
K, mg/kg	256.8 ± 25.7	1011.0
Zn, mg/kg	0.93 ± 0.09	3.66
P, mg/kg	0.035	0.14
Fe, mg/kg	14.27	56.18
Vitamin A, mg/kg	0.205	0.81
Vitamin D, mg/kg	0.16	0.06
Vitamin E, mg/kg	1.18	4.65
Vitamin B_1, mg/kg	0.28	1.10
Vitamin B_2, mg/kg	0.8	3.15
Vitamin B_6, mg/kg	0.64	25.2
Vitamin K_3, mg/kg	1.42	5.59
Lysine, %	0.6	2.36
Methionine + cystine, %	0.37	1.46
Tryptophane, %	0.07	0.28
Energy value, kcal		361.4

After characterising these processes, we expect to obtain new information that could clarify the mechanisms of immune dysregulation during spaceflight. The present study was designed to evaluate immune cell activity in male C57/BL6 mice after a 30-day high-orbit spaceflight (550 km, higher than conventional manned spaceflights) on board the BION-M1 satellite (Roskosmos Program, Russia). For the present study, thymus, spleens and plasma samples were collected from mice both 12 h and 7 days after landing.

Materials and methods

Animals and conditions of the spaceflight

A satellite BION-M1 (Roskosmos Program, Russia) with animals on a board was launched from the launch site Baikonur (Kazakhstan) on April 19, 2013. Male 4- to 5-months-old C57/BL6 mice were maintained during the 30-day high-orbit (550 km) flight in special modules, with three animals per module. Food was supplied as a water-based paste on a scheduled basis. No additional water supply was provided. The composition of the food paste is shown in Table 1. Other details of this programme have been reported recently (Andreev-Andrievsky et al., 2014). Three animal groups were used. These were the spaceflight group and two ground-based control groups, which consisted of mice kept in a vivarium (vivarium control) and mice transported with the flight group by plane to the launch site and then returned to the vivarium after the spacecraft launch (traffic control). By using telemetry from the BION-M1, the traffic controls were exposed to environmental conditions comparable to those of the spaceflight group (i.e., temperature, humidity, CO_2) for 30 days. The flight and control groups each consisted of 5 mice. The comparisons of the spaceflight group with each of the control groups are indicated in illustrations.

Specimen collection

Animals were subjected in intervals to cervical dislocation at 12 h and 7 days after landing, and spleen and thymus were harvested and weighed. The interval between procedures was approximately 15–20 min. Half of the spleens, thymuses and blood plasma samples were used in our experimental assays. The rest of the tissues were distributed across a large team of investigators under the guidance and organisation of the Roskosmos BION-M1 Program.

Please cite this article in press as: Novoselova, E.G., et al., Changes in immune cell signalling, apoptosis and stress response functions in mice returned from the BION-M1 mission in space. Immunobiology (2014), http://dx.doi.org/10.1016/j.imbio.2014.10.021