Chapter

Spaceflight-Associated Immune System Modifications

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Abstract

Spaceflight is an adverse environment characterized by a unique combination of stressors affecting almost all physiological systems, including the immune system. Indeed, several studies have shown that about 50% of the astronauts have faced immunological troubles. Here, we will review how spaceflight affects immune cell development, innate as well as adaptive immunity, required to ensure an efficient protection of the host, with a particular focus on T and B cells. Indeed, to better appreciate the risks associated to future long-duration space missions and to develop pharmacologic or nutritional countermeasures allowing immune system protection, it is mandatory to fully understand how these cell types are affected by space conditions. Finally, we will compare immune changes observed in astronauts with those encountered in the elderly, thereby illustrating the societal interest of space research.

Keywords: space exploration, immunity, lymphocytes, stress, aging

1. Introduction

Since Yuri Gagarin became the first human to leave the Earth's confines in 1961, more and more humans have traveled into space, and manned space stations have been built. During spaceflights, the organism is subjected to a variety of chronic and acute stressors. The first category comprises factors such as microgravity, confinement, isolation, radiation, and disturbed circadian rhythm. The second category covers periods of intense activity, such as spacewalks, but also hypergravity exposure during takeoff and landing. While acute stressors have been described as beneficial to the host as they can mobilize individual's defense capacities, several studies have shown that chronic stress has deleterious effects, as it contributes to the weakening of the immune system and the development of pathologies such as inflammatory disease, infections, and cancers [1–5]. In that context, it is interesting to note that 15 of the 29 astronauts involved in the Apollo missions developed bacterial or viral infections during, immediately after, or within a week of landing [6]. In addition, the very first epidemiological study based on medical data collected from 46 astronauts who spent 6 months on the ISS showed that 46% of them exhibited significant immunological problems [7]. Among notable events, 40% were classified as rashes/hypersensitivities and 27% as infectious diseases. Taken together,

these data show that spaceflight-associated stressors affect, on average, the immune system of one out of two astronauts. Furthermore, these data demonstrate that immune system dysregulation occurs not only after landing but also during the flight [7].

In parallel of this immunological weakening, it is important to keep in mind that changes in microbial growth and pathogenicity have been observed [8, 9]. Depending on the bacteria studied, increased or decreased virulence [10, 11], altered sensitivity to antibiotics [12, 13], and/or increased biofilm formation [11, 13, 14] have been described as a result of the modulation of gene expression [9–11, 15, 16]. Moreover, there is some evidence to suggest that antibiotics may be less effective in space [12, 17, 18].

These microbial changes, combined with dysregulation of the immune system, certainly contribute to explain the increased susceptibility to infections observed in astronauts [19] (**Figure 1**). It is also noteworthy to keep in mind that, as the duration of space missions will increase, the potential for infectious diseases to arise during flight may become a critical issue because the probability of

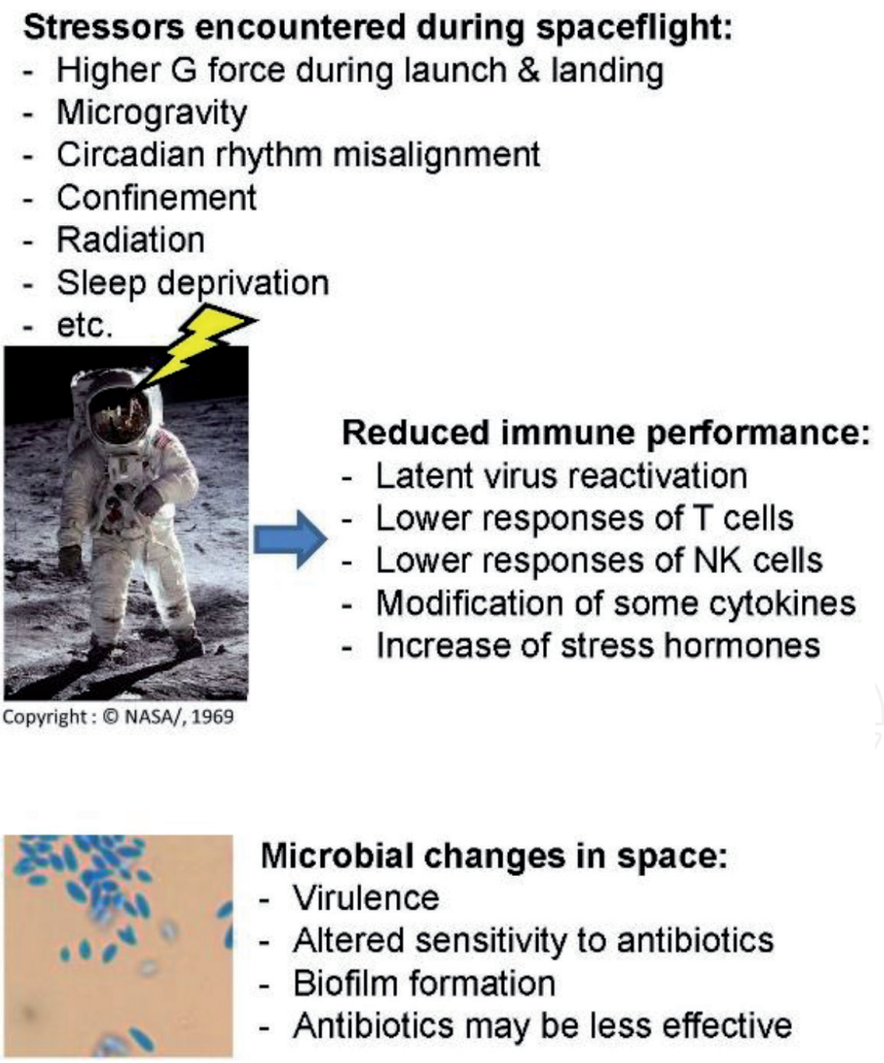


Figure 1.
Environmental changes associated with spaceflight (stressors) such as gravity change, the perturbation of the circadian rhythm (every day the residents of the ISS witness 16 sunrises and 16 sunsets), confinement, increased radiation, sleep deprivation, and nutritional factors weaken the immune system of about 50% of the astronauts. Most frequent immune changes consist in viral reactivations and lower responses of T and natural killer (NK) cells. These changes could be due to changes in cytokine expression and increased levels of stress hormones. In parallel, spaceflight environment might increase the virulence, resistance, and proliferation of some pathogens.

cross-contamination between crewmembers will increase. Additionally, microbial mutation rates may increase. Solar and cosmic radiation met during space missions, with a cumulative dose obviously increasing with mission duration, could contribute to the appearance of mutations potentially associated to resistance or diseases during such long and stressful endeavor as interplanetary missions.

Thus, caution should be paid to precisely understand how the immune system adapts, is modified/hampered, by unique environmental changes encountered during spaceflight. This knowledge is mandatory to allow the development of efficient biomedical strategies to preserve astronauts' heath during prolonged deep space exploration missions. In this chapter, we will review how spatial conditions affect the maturation of immune cells as well as the functions of mature immune cells required for the effective protection of the individual.

2. Effects on the maturation of immune cells

Cells that make up our immune system are derived from hematopoietic stem cells (HSC). These HSC will give rise to common myeloid progenitors (CMPs) and common lymphoid progenitors (CLPs). After several differentiation steps, CMPs will give raise to myeloid cells (granulocytes, monocytes, macrophages, and dendritic cells) and CLPs to lymphoid cells (B and T lymphocytes and NK cells). All of these cells are involved in natural and/or specific immunity.

A number of studies have analyzed the impact of spaceflight on the development of cells belonging to the myeloid lineage (or myelopoiesis). A decrease in the number of granulocyte and monocyte progenitors in rodents that have been in space or subjected to anti-orthostatic suspension (a model commonly used in the laboratory to reproduce many of the physiological changes observed in flight) has been demonstrated [20, 21]. The culture of human $CD34^+$ progenitors in flight has confirmed the inhibitory effect of microgravity on erythropoiesis (red blood cell production) [22]. Other studies have shown that the stressors encountered during spaceflight impact lymphocyte development (or lymphopoiesis). Diverse animal models have been used to address this question, such as mouse or the Iberian ribbed newt (*Pleurodeles waltl*, a urodele amphibian). The latter lends itself well to the constraints associated with space experiments and has all the cardinal elements of the mammalian immune system [23]. It has notably been observed that *P. waltl* larvae developed on board the ISS exhibit changes in the expression of IgM heavy-chain transcripts as well as a disruption in the expression of the Ikaros gene encoding transcription factors required for lymphopoiesis, suggesting that the latter could be weakened under spatial conditions [24]. This hypothesis was then confirmed in mice subjected to 21 days of anti-orthostatic suspension, which corresponds to a long-term mission at the human scale. It has been shown that this model induces a decrease in the number of CLPs and cells at the pro-B, pre-B, immature B, and mature B stages in the femoral bone marrow of suspended mice compared to control mice [25]. Furthermore, various causes of this weakening have been identified, such as a decrease in signal transduction by the interleukin-7 receptor and a decrease in the expression of transcription factors essential for B-cell development within the bone marrow. It has also been noted that this decrease in B lymphopoiesis is coupled with the remodeling of the bone tissue induced by the suspension, thereby reminding that all physiological systems interact within an organism and that these interactions have to be taken into account when analyzing the impact of stressors such as modeled microgravity. Finally, this sensitivity of hematopoiesis and the link with bone remodeling was confirmed in mice embarked on board the BION-M1 satellite for 30 days [26]. A decrease by a factor of two in the number of

B lymphocytes present in the bone marrow and a statistically significant decrease in the expression of factors required for the development of immune and bone cells were observed 7 days after returning to Earth but not on landing (**Figure 2**). This time delay can be explained by the fact that bone loss worsens after landing [27].

Note that in addition to the explanations presented above, glucocorticoids produced in response to chronic stress may contribute to altering B lymphopoiesis. Indeed, it has been demonstrated that continuous administration of corticosterone, via a subcutaneous implant, induces reprogramming of lymphopoiesis in mice, with a reduction of 30–70% of pro-B, pre-B, and immature B cells after 24 hours and a drop of 70–80% of pro-B and pre-B cells after 36 hours of treatment [29].

T lymphopoiesis (T-cell development in the thymus) is also affected by microgravity, as a decrease in T cells was observed in double-positive ($CD4^+CD8^+$) and single-positive ($CD4^+$ or $CD8^+$) maturation stages, when murine fetal thymuses were cultivated under simulated microgravity or spatial conditions [30]. This observation can be explained, at least in part, by the high sensitivity of thymocytes to stress [31]. Indeed, significant changes in mRNA expression from genes known to regulate stress and glucocorticoid receptors were observed in the thymus of mice subjected to a 13-day flight [32]. Another study did analyze the impact on murine T-cell antigen receptor (TCR) of being conceived and born under increased G force (2 G). This study revealed a disruption in TCR signaling and in the diversity of these receptor binding sites [33] (**Figure 3**) required for an individual to be able to specifically recognize peptides derived from the numerous antigens present in the

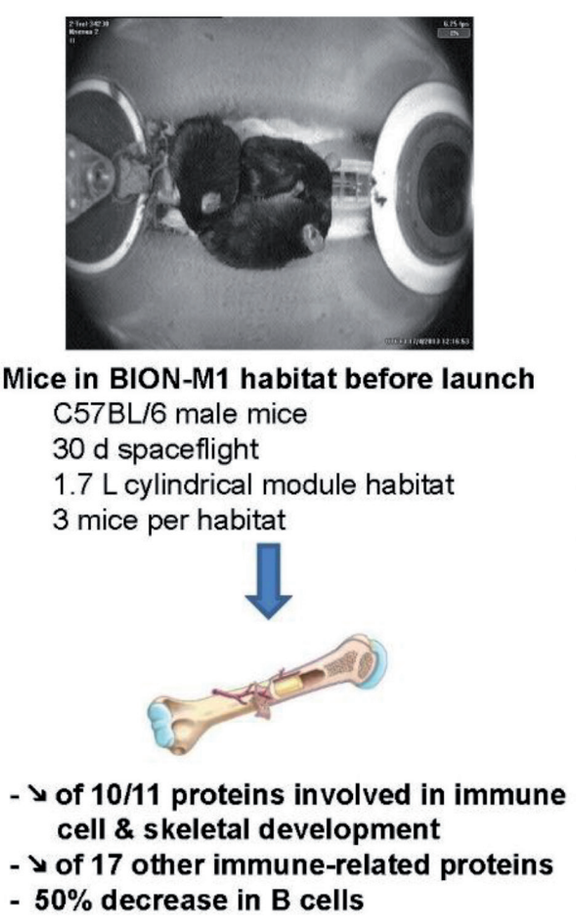


Figure 2.
Analysis of the femur from mice flown for 1 month on board the BION-M1 biosatellite revealed a decrease in the expression of 10 out of 11 proteins involved in immune cell and skeletal development, a decrease of the expression of 17 other immune-related proteins, and a 50% decrease in the number of B cells present in the bone marrow. Furthermore, this study showed that spaceflight effects were aggravated 1 week after landing [26]. Picture of mice in BION-M1 habitat from [28].

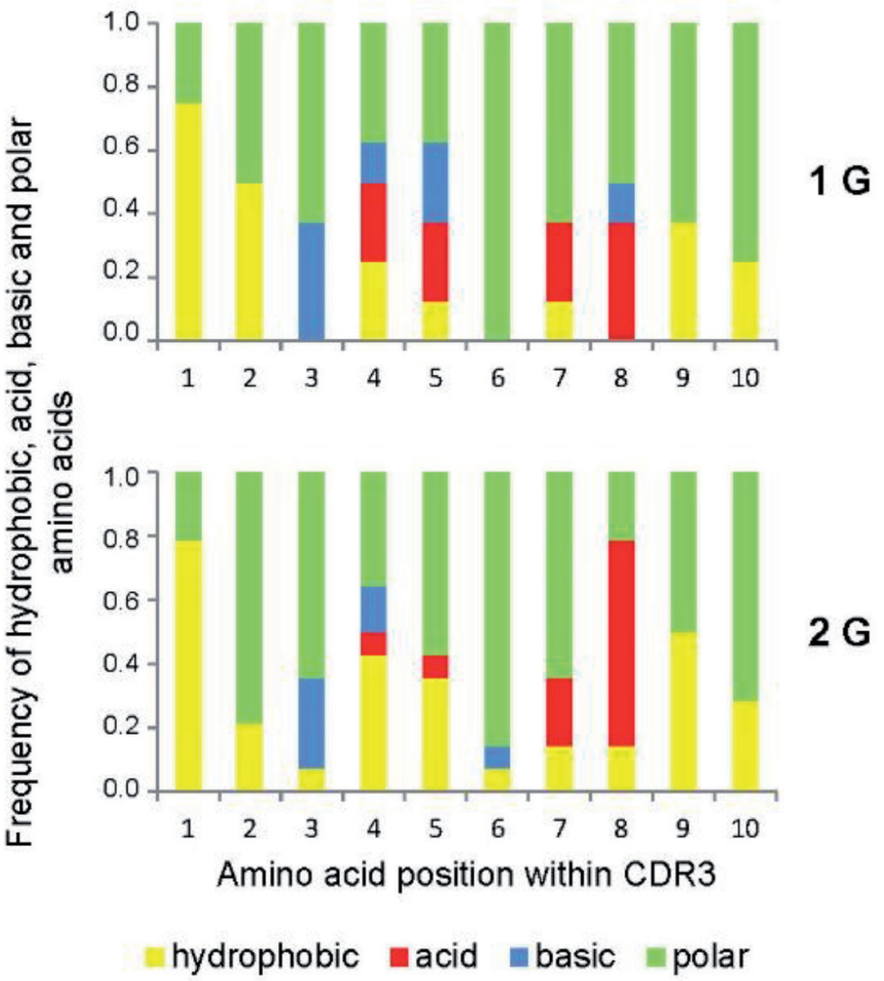


Figure 3.
Exposure to hypergravity during pregnancy affects TCR binding sites, thereby suggesting that the protection of the host might be affected [33]. T cells recognize an antigenic peptide on an MHC molecule at the surface of an antigen-presenting cell (APC) (dendritic cell, monocyte, macrophage, B cell). This recognition is ensured by the T-cell receptor (TCR) whose binding site is composed of six small polypeptide loops: two CDR1 loops, two CDR2 loops, and two CDR3 loops. CDR1 and CDR2 loops bind the MHC molecule. CDR3 loops bind the peptide. This figure presents the frequency of hydrophobic, acid, basic and polar amino acids at each position within TCR CDR3 loops from murine pups conceived and born at 1 or 2 G.

environment; 85% of the TCR repertoire was different in 2 G pups compared to control pups. Thus, the diversity of T-cell antigen receptor repertoire is significantly altered by 2 G exposure, which will likely affect host defense.

The impact of a model aiming at mimicking socio-environmental stresses experienced by astronauts [34] was then studied. This model involves the chronic exposure of mice to unpredictable socio-environmental stresses of various types (e.g., confinement, isolation, cage tilt, paired housing, perturbed circadian rhythm) and moderate intensity. It was demonstrated that this type of stressors only modifies 25% of the TCR repertoire [35]. Consequently, it appears that a change in the gravitational force has a much greater impact than socio-environmental stresses on the T-cell antigen receptor repertoire.

3. Effects on phagocytic and NK cells

Natural or innate immunity is the body's first line of defense against a pathogen after the skin and epithelial surfaces. It enables a non-specific response to be implemented, involving various types of immune cells such as neutrophils, monocytes,

and macrophages, to destroy pathogens by phagocytosis and the release of microbicide substances.

Several studies have been conducted to understand how the space environment affects this immunity. For instance, an increase in the level of blood neutrophils in both humans and animals has often been observed after landing and can be attributed to the stresses encountered during this phase. Indeed, stress can induce the mobilization of these cells stored in the bone marrow [36, 37]. However, other explanations are also possible, such as changes in the expression of adhesion molecules [38]. It has also been shown that spatial conditions decrease the phagocytic and oxidative functions of neutrophils [39, 40] and induce, in monocytes, dysregulation in cytokine production, a reduced capacity to engulf *Escherichia coli* as well as lower reactive oxygen species (ROS) production and degranulation [41, 42]. Lower cytotoxicity of natural killer cells that provide immunological resistance and defense against foreign microorganisms but also against cells transformed because of, for example, a viral infection was observed [43, 44]. In addition, the reactivation of latent herpes viruses has frequently been reported. For example, Varicella-zoster virus (VZV) DNA has been detected in the saliva of astronauts during and immediately after a flight, while no VZV DNA was detected before launch [45]. Additional studies have revealed the presence of VZV in the saliva of 50% of astronauts during short spaceflights [46] and have shown that this percentage can increase up to 65% during long-duration missions [47]. Significantly, a few cases resulted in the development of shingles [45]. These viral reactivations are frequently coupled with

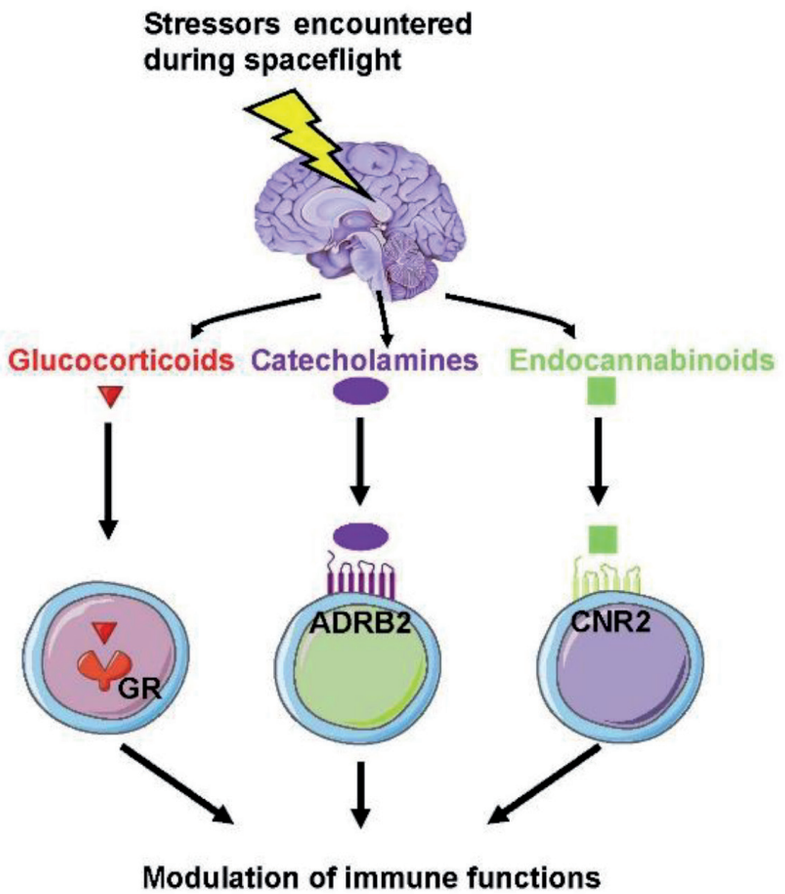


Figure 4.
Stressors encountered during space missions can induce the production of glucocorticoids, catecholamine, and endocannabinoids. Numerous immune cell types have receptors for these molecules. Their functions can therefore be directly affected by the binding of these molecules on these receptors. GR, glucocorticoid receptor; ADRB2, beta-2 adrenergic receptor; CNR2, cannabinoid receptor type 2.

a decrease in the production of interferons (cytokines constituting a first response in the event of viral infection) and to a higher level of stress hormones known to be able to regulate immune functions. Indeed, a variety of immune cells expresses glucocorticoid receptors, cannabinoid receptors, and adrenergic receptors (**Figure 4**). Thus, molecules produced in response to stressing events can directly affect immune cells and can be responsible for the reactivation of latent viruses [48–53].

Furthermore, virus reactivation could be a good biomarker of immunity weakening [54]. In support of this neuromodulation of the immune system, studies conducted on humans subjected to acute- (parabolic flight), medium- (1–2 weeks on board the ISS), or long-duration (4–7 months on board the ISS) gravitational stress demonstrated that there is a shift from an alert state of natural immune cells after acute gravitational stress to a decrease of their activity after spaceflight [55–57]. These changes were associated with changes in stress response, with a predominance of sympathetic nervous system responses after short flights, whereas long flights were characterized by glucocorticoid-induced changes. These data demonstrate that beside gravity change, stress responses are an important contributor to spaceflight-associated immune changes and once again highlight the importance of taking into account interconnections between physiological systems (here the nervous and immune systems).

4. Effects on antigen-presenting cells and lymphocytes

Specific or adaptive immunity is the second line of defense against the entry of foreign substances, particles, or cells into the organism. It involves natural and specific immune cells (antigen-presenting cells and lymphocytes) that will cooperate to develop a response specifically directed against the intruder.

APCs are a heterogeneous group that treat and present antigens in the form of peptides to $CD4^+$ T lymphocytes unable to recognize a native antigen via their TCR. These cells are crucial in triggering an immune response. This group includes dendritic cells, monocytes/macrophages, and B lymphocytes.

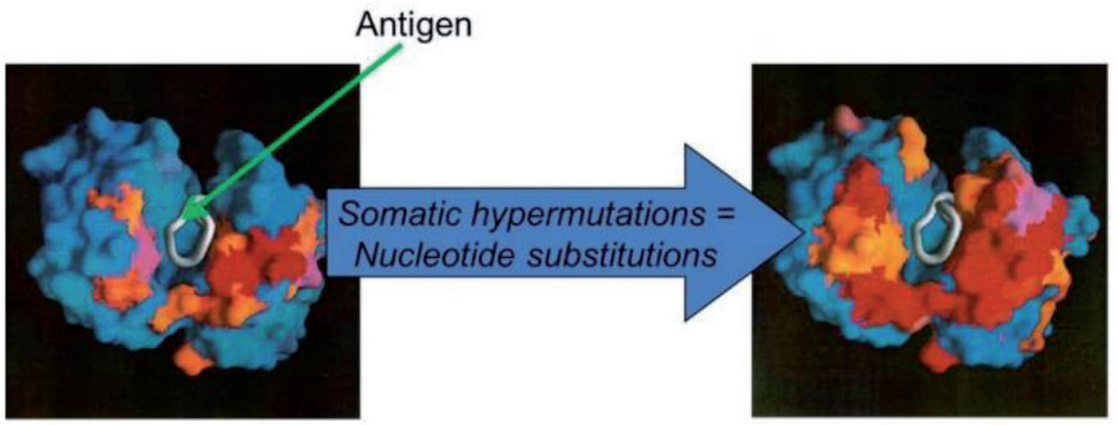
Even though the antigen presentation function is an essential immune process, very little information is available on the impact that environmental conditions encountered during spaceflights could have on this function. Only one study has been published on dendritic cells and revealed that microgravity reduces their production, their phagocytic capacities, and the surface expression of costimulatory/adhesion molecules involved in the presentation of antigenic peptides [58]. These data suggest that certain functions of antigen-presenting cells, required for the development of an effective immune response, may be disrupted in microgravity.

On the other hand, numerous studies have shown a significant reduction in T-cell activity under both real and simulated microgravity. This lower activity [59] results from spaceflight-induced modifications of the expression of genes essential for the proper functioning of T cells such as those encoding interleukin-2 and its receptor [60], translation of mRNAs [61], cell-cell interactions [62], alterations of the structure of the cytoskeleton [63–66], signal transduction enabling T-cell activation [67–69], and cell cycle regulation [70].

B lymphocytes are another cell type that acts in synergy with T lymphocytes to ensure optimal protection of the individual. These cells, at the maturation stage called plasmocyte, produce large quantities of antibodies, which, by binding specifically to the antigen, contribute to its elimination. Antibodies and B lymphocytes constitute humoral immunity whose modulation by spatial conditions has been much less studied than that of T lymphocytes. For many years, researchers have been satisfied with the quantification of antibodies present in the serum/

plasma of astronauts, but these studies generated conflicting results. For example, Konstantinova et al. [71] reported increased levels of serum IgA and IgM, while Rykova et al. [40] indicated that the amounts of serum IgA, IgG, and IgM were not affected after prolonged space missions. Subsequently, further studies were conducted to determine how changes in gravity affect humoral immunity and demonstrated that stresses encountered during spaceflight quantitatively and qualitatively affect the production of antibodies in response to antigenic stimulation. Changes in the expression of VH gene segments, encoding a large part of the antibody binding sites, have been observed in adult *P. waltl* immunized on board the Mir space station [72, 73] as well as a twofold decrease in the frequency of somatic hypermutations (SHM) that enable the diversification of antibody binding sites, in order to improve their affinity for the antigen [74] (**Figure 5**). Very recently, changes in the use of the gene segments required to create the antibody repertoire have also been observed in immunized mice subjected to anti-orthostatic suspension [75]. The antibody repertoire is therefore most likely modified under either real or simulated microgravity. In addition, a decrease in the expression of several effectors involved in immunity was observed 7 days after landing in mice that had been on board the BION-M1 biosatellite for 30 days [26]. This observation confirms the negative effect of spaceflight on the immune system and demonstrates that this impairment persists for at least 7 days after the return to Earth. This conclusion is in line with the studies that revealed disruptions in the production of antibodies in *P. waltl* still visible 10 days after landing [72–74].

Finally, it has been shown that the proliferative responses of B and T lymphocytes are reduced when mice are subjected to gravity changes (anti-orthostatic



	SHM frequency (x 10^{-4}/pb)	P values (χ2 test)
Immunized on Earth (IE)	9.25	0.002 for IE/NI
Immunized in Space (IS)	4.65	0.031 for IS/NI

Figure 5.
Somatic hypermutations (SHM) are nucleotide substitutions whose purpose is to improve the affinity of antibody binding sites. The frequency of these mutations was determined in adult P. waltl immunized on board the Mir space station and in adult P. waltl immunized with the same antigen on Earth. This study showed that the frequency of these mutations is two times lower when animals are immunized on board the space station [74]. IE, P. waltl immunized on Earth; IS, P. waltl immunized in space; NI, not immunized.

suspension or 2 G hypergravity) for 3 weeks [76, 77]. However, the responses from these lymphocytes were not altered after 3 weeks of exposure to the model mimicking socio-environmental stressors encountered in flight [34]. These data suggest that the lower reactivity in lymphocytes induced by spaceflight is mainly due to gravity change.

Note that there is a break in the adaptation of mice at 3 G, which results in an increase in the serum corticosterone concentration and the level of anxiety [76]. These changes persist beyond 2 weeks after the return to normal gravity. This demonstrates that the hypergravity model should be used with caution if the effects of hypergravity are to be distinguished from those of a stress response. From 3 G, these two variables are cumulative.

5. Spaceflight as a model of accelerated immunosenescence

Certain immunological changes observed in astronauts or rodents on space missions can also be found in the elderly. For example, thymus involution, increased susceptibility to infections, and decreased response to vaccines may be correlated with impaired development of B- and T-lymphocyte function in the elderly [78, 79]. This thymus involution and changes in the development and response of immune cells are also observed when the gravitational force is altered, as illustrated by the reactivation of latent viruses in astronauts and the elderly. In addition, a recent study suggests that long-term spaceflight could induce an increase in inflammation as in the elderly (inflammaging), which could increase the risk of allergies or autoimmune diseases in astronauts [80]. Finally, aging is accompanied by changes in antibody production similar to those observed in flight. There is a decrease in antibody affinity [78] and a change in the use of antibody VH gene segments [81] as observed in *P. waltl* immunized in flight [73, 74], which affects the diversity of the antibody repertoire. It therefore appears that stresses encountered during space missions could lead to premature aging of the immune system.

6. Conclusion and perspectives

Studies conducted so far show that on average one out of two astronauts encounters immunological problems and that stressors encountered during spaceflights can affect all components of the immune system. It is therefore mandatory to understand in details how all immune cell types are affected by space conditions by unraveling the cellular and molecular mechanisms modified within these cells. Indeed, the impact of spatial conditions on certain cells and functions of the immune system have not yet been precisely determined. Furthermore, the impact of long-term missions is largely under-investigated. This is because, up to now, most scientific data are derived from space missions not exceeding 6 months in duration. In addition, the impact of spatial conditions on interconnections between the immune and other systems (such as the musculoskeletal, nervous, respiratory, and cardiovascular systems) should be studied using interdisciplinary approaches. All this knowledge is required (i) to gain a better understanding of the risks incurred during future long-duration space missions (such as planned mission to Mars), where the crew will be left to their own with no possibility of a rapid return to Earth, and (ii) to develop nutritional, psychosocial, and/or pharmacological countermeasures to reduce stress, preserve the immune system, and prevent the development or aggravation of diseases [82]. Another aspect that should be taken into account is in-flight monitoring of astronaut's health and diagnostic data using

miniature and autonomous biosensors to help establish personalized treatments. This corresponds to a new field of research, space biotechnology, which aims to use advanced techniques ("omics" techniques) for the quantitative detection of proteins, nucleic acids, and metabolites in situ [83–86]. Such biosensors capable of analyzing minimum quantities of body fluids and of generating semiquantitative or quantitative results in a few minutes and with minimal resource consumption in terms of weight, volume, reagent storage, and energy will be required to allow deep space exploration. These researches and technological developments could also improve health on Earth as they could led to new therapeutic strategies to treat age- and stress-related immunosuppression and could likely contribute to improve point-of-care diagnostics at a patient's bedside, in a doctor's office, or hospital.

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Conflict of interest

The authors declare no conflict of interest.

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