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Could spaceflight-associated immune system weakening preclude the expansion of human presence beyond Earth's orbit?

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ABSTRACT

This year, we celebrate the 40th birthday of the first landing of humans on the moon. By 2020, astronauts should return to the lunar surface and establish an outpost there that will provide a technical basis for future manned missions to Mars. This paper summarizes major constraints associated with a trip to Mars, presents immunological hazards associated with this type of mission, and shows that our current understanding of the immunosuppressive effects of spaceflight is limited. Weakening of the immune system associated with spaceflight is therefore an area that should be considered more thoroughly before we undertake prolonged space voyages. *J. Leukoc. Biol.* 86: 000–000; 2009.

Introduction

In 1961, Yuri Gagarin became the first human to leave the confines of Earth. Since then, over 450 people have traveled into space, but so far, only 24 astronauts (those of the Apollo missions) have traveled beyond the first 400–500 km of the low-Earth orbit, in which the magnetic field of the Earth deflects a significant fraction of radiation. Beyond the Van Allen radiation belt, where charged particles are trapped in the magnetic field of the Earth, astronauts are exposed to solar and cosmic radiation.

On July 20, 1969, Neil Armstrong and Edwin Aldrin became the first humans to land on the moon. This summer, we celebrated the 40th birthday of this historic event. A few years ago, President George W. Bush proposed a manned return to the moon, with the moon to become the staging post for manned missions to Mars [1]. President Barack H. Obama's 2010 budget request, released on February 26, 2009, confirmed that NASA will stay on track to return to the moon by 2020. A mission to Mars and back will take a minimum of 520 days, of which roughly 1 month will be spent on the martian surface, and the rest will be spent in transit. At its furthest, the crew will be some 360 million km away from home. Consequently, astronauts will have to exercise an unprecedented level of autonomy and teamwork [2]. During the mission, they will experience not only microgravity but also various forms of stress, such as confinement, high expectations of performance, and risks of equipment failure or fatal mishaps. The enormous distance and long travel time to Mars will also probably affect the astronauts psychologically. The crew will therefore endure increased stress levels, radiation, as neither the moon nor Mars has magnetic fields or dense atmospheres that could attenuate them, and microgravity-induced changes, such as alterations in body fluid distribution, which could influence their immune system. As gravity has shaped the architecture of all biological systems on our planet, it is reasonable to observe aberrations in normal functioning of life in weightlessness. A long-term spaceflight will also pose a multitude of health risks, not only those associated with spaceflight, such as bone demineralization, skeletal muscle atrophy, and immune system suppression (**Fig. 1**), but also from common diseases that might cause specific problems under these circumstances. Another risk may be the development of pathogens in a closed environment, where air, food, waste, and water are recycled. Confinement of the crew during flight can and has resulted in the transfer of microorganisms among crew members [4, 5]. Finally, specific health risks might also be encountered on the lunar or martian surface, such as dust or chemicals that could irritate the respiratory tract, for example, or even new organisms. Indeed, 3 days on the moon during the final Apollo mission in 1972 left astronaut Eugene Cernan weary and filthy with rock dust. A trip to Mars will certainly multiply the hazards of space travel.

Humans are ready to accept great risks to go where no one has gone before, but do we have sufficient and sound biologi-

Abbreviations: AHCC=active hexose correlated compound, CNES=French National Space Center, ESA=European Space Agency, HDBR=head-down bed-rest, IML-2=International Microgravity Laboratory 2, ISS= International Space Station, PKA/PKC=protein kinase A/C, respectively, PMN=polymorphonuclear neutrophil, ROS=reactive oxygen species, SLS-1=Spacelab Life Sciences 1

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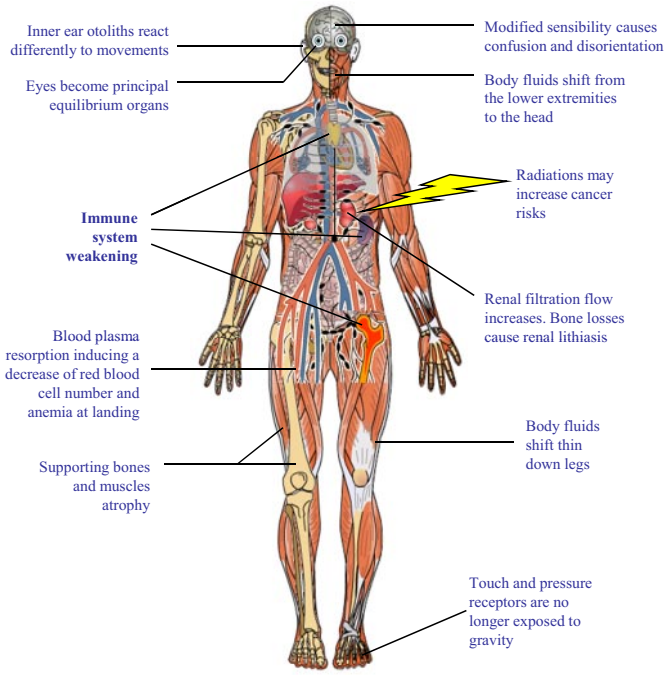

Figure 1. Spaceflight-induced alterations in the organism. During space missions, many factors combine to influence a variety of physiological functions. This schematic representation of the human body provides an overview of the most important of those functions [3].

cal information to support prolonged space habitation, knowing that no humans can exist in space for a long time without science to support them? Returning astronauts have experienced altered immune function and increased vulnerability to infections during spaceflights dating back to the Apollo and Skylab missions [6]. Consequently, one may wonder if the weakening of the immune system could be a limiting factor for the expansion of human presence beyond Earth's orbit. This question is important to ensure survival in space stations and sustain habitation on the moon and Mars. Most of our knowledge about the effects of spaceflight on the immune system results from the analysis of samples subjected to short spaceflights or ground-based simulations of spaceflight conditions. These studies revealed that spaceflights affect a variety of immune parameters in humans and in animals. In this paper, we review the effects of spaceflight on microbial growth and virulence, lymphoid organs and cell populations, and innate and adaptive immunity, and we present countermeasures that have been developed to prevent immune compromise resulting from exposure to spaceflight conditions. Taken together, these data demonstrate that the weakening of the immune system associated with spaceflight is an area that should be considered more before we undertake prolonged space voyages.

MICROBIAL GROWTH, RESISTANCE, AND VIRULENCE

Analysis of medical events among astronauts aboard the Mir space station, over a period from March 1995 to June 1998, revealed a significant number of episodes of microbial infections, including conjunctivitis and acute respiratory and dental infections [7]. Evidence for in-flight cross-contamination with opportunistic pathogens such as *Staphylococcus aureus* has been reported [8, 9]. Moreover, a study of the microflora of cosmonauts from five spaceflights indicated that the crew exchanged intestinal flora [10]. As the duration and frequency of space missions increase, the potential for infectious diseases to arise during flight may become a critical issue, as microbial contamination will increase with time and require continued surveillance. Crew members are the predominant sources of bacteria, with other sources arriving with ground-supplied materials. Data from previous spaceflights have demonstrated that microorganisms are ubiquitous throughout the habitable modules of spacecrafts [9, 11, 12]. For example, a total of 234 species of bacteria and microscopic fungi were identified in the Mir environment, and bacteria consisted primarily of commensal human integuments, i.e., *Staphylocci*, *Micrococci*, and *Coryneform* bacteria [13]. Extremophilic and extremotolerant bacteria were isolated in the spacecraft assembly and the test and launch preparation facilities [14]. As a result of their proximity to space-faring objects, these bacteria pose a considerable risk for forward contamination of extraterrestrial sites. Microbes can also affect the integrity of the spacecraft. Indeed, most of the fungal species found on structural materials includes biodegraders of polymers [13].

Changes in cell growth characteristics during spaceflight were observed a long time ago for several microorganisms such as *Salmonella enterica*, *Escherichia coli*, and *Bacillus subtilis* [15]. More recently, studies have demonstrated a key role for microgravity in microbial physiology, regulation of gene expression, and pathogenesis [16, 17]. Bacteria can proliferate more readily in space, which suggests that this environment is better able to initiate growth that could lead to contamination, colonization, and infection. Furthermore, it has been shown that the growth of several bacterial strains, including *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *S. aureus*, is enhanced by catecholamines [18–20], and elevations of catecholamines and cortisol/corticosterones in the plasma are observed frequently in individuals who have flown [21]. Thus, opportunities for microbes to establish foci of infection are enhanced, especially as space travel stimulates their growth and has a negative impact on immune function.

In vitro experiments conducted in the 1980s and 1990s indicated that greater concentrations of antibiotics were generally required to inhibit microbial growth in space [22]. Antibiotics were typically less effective against suspension cultures in space, but these traits appeared to be transient, as attempts to reproduce the resistance after return to Earth have been unsuccessful [23, 24]. Various physiological, pharmacological, and pharmacodynamic changes during spaceflight can affect drug efficacy in vivo [25]. For example, a reduction in the diversity of the bacterial flora in the gastrointestinal tract may give rise to an increase in the size of the drug-resistance gene pool. It may also be facilitated by mutations induced by radiation. Indeed, high mutation frequencies in indicator genes carried by yeast have been reported after long-term spaceflight [26].

Direct evidence for a role of microgravity in microbial virulence has been provided by simulated microgravity experiments, in which increased production of heat-labile *E. coli* enterotoxin was shown [27]. Recently, Altenburg et al. [28] demonstrated that the growth of Saccharomyces *cerevisiae* and *Candida albicans* is increased under simulated microgravity and results in a morphogenic switch that is consistent with enhanced pathogenicity. These data are in agreement with a previous observation indicating that *S. cerevisiae* retrieved from Apollo 16 were better able to survive in intradermal lesions of artificially infected mice than *S. cerevisiae* grown on Earth [29]. Nickerson's groups [30, 31] performed several studies about the virulence of *Salmonella typhimurium*. They reported that *S. typhimurium* grown under spaceflight or simulated microgravity exhibited enhanced virulence, resistance to environmental stress, and survival in macrophages [30]. Studies performed on hindlimb-unloaded mice, a model used frequently to simulate some aspects of spaceflight conditions, reached the same conclusion [27]. Recently, global microarray and proteomic analyses indicated that the expression of 167 transcripts and 73 proteins of *S. typhimurium* is changed under microgravity, and the conserved RNA-binding protein, Hfq, was identified as a likely global regulator involved in the response to this environment [32]. Interestingly, it has been shown that this protein is also involved in the virulence of another pathogen, *Francisella tularensis* [33]. It is therefore evident that changes in gene expression that occur in microorganisms during spaceflight can increase their virulence.

LYMPHOID ORGANS AND CELL POPULATIONS

Several independent pieces of work have shown that spaceflight conditions affect lymphoid organs. Durnova et al. [34] observed hypoplasia of lymphoid organs (spleen, lymph nodes, and thymus) in rats that underwent a 22-day spaceflight. They demonstrated that spleen hypoplasia was a result of a reduction in lymphocytes and erythroid cell numbers and that lymph node and thymus hypoplasia were a result of a decrease in lymphocyte number. Similarly, Gridley et al. [35] noticed that after a 12-day space shuttle mission (STS-108), flown mice had smaller spleen masses in comparison with animal enclosure and vivarium controls. These observations were confirmed by a recent study [36] showing that C57BL/6 mice from a 13-day space shuttle mission (STS-118) had lower spleen and thymus masses. However, the results about the thymus mass appear to be more variable, as the thymus mass has been reported to be decreased [34, 36, 37], increased [38, 39], or similar to controls in other studies [40–42].

Many studies reported variations in the PBL populations of astronauts and animals and in the lymphoid organs of animals after spaceflights. These modifications could be mediated by changes in adhesion molecules [43, 44] and the redistribution of body fluids in the microgravity environment [45]. **Table 1** summarizes the data about leukocyte populations and shows a consistent increase in the number of neutrophils in the peripheral blood of humans and animals at the time of landing. The stress of landing could be responsible for this observation by mobilizing bone marrow PMNs into the circulation. Variable results were obtained for the other populations. Multiple reasons can explain these different results. First, markers used to quantify cells can differ between studies. For example, Gridley et al. [56] showed a decrease in B cell counts in spleens from C57BL/6 mice flown on STS-118, whereas another study [52] revealed an increase in B cell counts in spleens from C57BL/6 mice flown on STS-108. For STS-108 samples, B220 was used as a marker to identify B cells, whereas CD19 was used to quantify B cells in STS-118 samples [56]. As B220 is also found on NK cells, the increase in $B220^+$ cells noted by Pecaut et al. [52] could be a result of an increase in NK cells. On the other hand, Allebban et al. [48] and Ichiki et al. [49] observed a decrease in the number of peripheral blood B cells but no changes in spleen B cells from rats that were flown using IgG and OX33 as markers, respectively.

Another reason for these apparently contradictory results is that immunological changes are likely to depend on the duration of the spaceflight. For example, Stowe et al. [53] analyzed blood and urinary samples from astronauts who flew during 9 or 16 days (Table 1). Their study showed that the numbers of $CD4^+$ T cells and monocytes in the peripheral blood were increased after the 9-day spaceflight, which was contrary to the 16-day spaceflight, where $CD4^+$ T cell numbers were unchanged, and the monocyte numbers were lower. In parallel, they observed that the concentration of cortisol in the plasma was lower after the 9-day flight but increased after the 16-day flight. In contrast, the urinary epinephrine and norepinephrine levels were greater after the 9-day flight than after the 16-day flight, suggesting that sympathetic nervous system responses predominate after short spaceflights, and long flights are characterized by glucocorticoid-mediated changes, thus affecting the immune system differently. Another study revealed a correlation between increased epinephrine and norepinephrine levels and the number of white blood cells, monocytes, and B cells in astronauts' peripheral blood samples collected after five 4- to 16-day space shuttle missions [51].

These differences may also be a result of the fact that the number of flown individuals is reduced in some studies (see Table 1). Thus, individual variability can affect observations. For example, Chapes et al. [40] obtained different results when they studied two groups of six rats that flew for 8 days in the same animal enclosures. In particular, macrophage TNF-α secretion, blood cell distribution, bone structure, and bone mass showed inconsistent changes. These differences may be attributable to individual variability but also to variations in the flight profiles. Although the two missions were 8-day flights, the launch and landing times were different, and the animals were not dissected at the same time. One group was dissected in the evening and the other one at mid-morning. These times are close to the antithetical picks for daily circadian rhythms and could explain some differences between the missions' results, especially for stress hormones whose production depends on circadian rhythms.

Finally, the choice of species with which to perform space experiments and the rearing conditions of the animals can also affect the results. Thus, there are many variables that complicate the analysis of spaceflight-induced modifications. Fur-

TABLE 1. Spaceflight-Associated Changes in Leukocyte Populations Observed in Different Studies

Study	Flight duration	Species	*n*	Lymphocytes			Monocytes	Granulocytes	
				T cells	B cells	NK cells		Neutrophils	Eosinophiles
Taylor et al. [46]	3–18 days 11 missions	Human	41	↓ of total circulating lymphocytes at landing				↑ nb in PB at landing	% ↓ in PB at landing
	6 and 8 days	Human	11		↓ in PB at landing		↓ nb in PB at landing		
Sonnenfeld et al. [47]	14 days	Rats	5	% ↑ in BM and in S at landing		↓ nb in BM and in S at landing			
Allebban et al. [48]	9 days	Rats	29	↓ nb in PB at landing. No change in S				↓ nb in PB at landing	
Ichiki et al. [49]	14 days	Rats	15	↓ nb in PB at landing. No change in S			↓ nb in PB at landing	↑ nb in flight and at landing	
Stowe et al. [44]	8 days	Human	6	↑ of total circulating lymphocytes 2 days before launch			↓ nb in PB 2 days before launch and at landing	↑ nb in PB at landing	No changes during mission
	9 days	Human	5	↑ of total circulating lymphocytes 2 days before launch			Unusually low nb 10 days before flight	↑ nb in PB at landing	No changes during mission
	14 days	Human	5	No changes in total circulating lymphocytes during mission			↑ nb in PB at landing	↑ nb in PB at landing	No changes during mission
Chapes et al. [39]	10 days	Rats	6	nb and % ↓ in PB at landing			nb and % ↓ in PB at landing	% ↑ in PB. Neutrophilia at landing	
Chapes et al. [40]	8 days two missions	Rats	6	No change in nb and % in PB from mission 1					
				nb and % ↓ in PB at landing			nb and % ↓ in PB at landing	↑ % in PB at landing	
Crucian et al. [50]	10–18 days four missions	Human	27	No changes in total circulating lymphocytes at landing			No change in PB total nb; $CD14^+/CD16^+$ % ↓ in PB at landing	↑ nb in PB at landing	
				% ↓ in PB at landing	No significant change of PB % at landing				
Mills et al. [51]	4–7 days and 11–16 days	Human	11	↑ nb in PB at landing		↓ nb in PB at landing	↑ nb in PB at landing		No change during mission
Pecaut et al. [52]	12 days	Mice	12	↑ in BM %; ↓ in S %[a]	↓ in BM %; ↑ in S %[a]	No change in S %; ↑ in BM %[a]	No change in S % ↑ in BM %	↓ in S %; ↓ in BM %[a]	
				↑ of total lymphocytes % in S					
				No significant change in PB, S, and BM cell numbers; no significant change of PB %					
Stowe et al. [53]	9 days	Human	16	↑ $CD4^+$ nb in PB at landing	No change in PB at landing	↓ nb in PB at landing	↑ nb in PB at landing	↑ nb in PB at landing	
	16 days	Human	12	No change in PB at landing	No change in PB at landing	No change in PB at landing	↓ nb in PB at landing	↑ nb in PB at landing	
Rykova et al. [54]	125–195 days	Human	15	No change in PB at landing	No change in PB at landing	↓ % in PB at landing			

(continued on next page)

TABLE 1. ***(continued)***

Study	Flight duration	Species	*n*	Lymphocytes			Monocytes	Granulocytes	
				T cells	B cells	NK cells		Neutrophils	Eosinophiles
	8–10 days	Human	15	No change in PB at landing	↑ % in PB at landing	No change in PB at landing			
Crucian et al. [55]	12–14 days	Human	17	No change in PB at landing ↓ total lymphocytes % in PB at landing	↑ % in PB at landing	↓ % in PB at landing	No change in PB at landing	↑ % in PB at landing	
	179–215 days	Human	8	No change in PB at landing ↓ total lymphocytes % in PB at landing	↑ % in PB at landing	↓ % in PB at landing	No change in PB at landing	↑ % in PB at landing	
Gridley et al. [56]	13 days	Mice	12	↓ nb in S at landing	↓ nb in S at landing	↑ nb in S at landing			
Baqai et al. [36]	13 days	Mice	12	Number of total lymphocytes ↓ in S, no changes in %			Number ↓ in S, no changes in %	Number ↓ in S, no changes in %	

n, Number of flown individuals that was analyzed; nb, number; PB, peripheral blood; BM, bone marrow; S, spleen; ↓, decrease; ↑, increase. [a]Versus animal enclosure module ground control but not vivarium ground control. Results from long duration missions are written on a gray background.

thermore, these data indicate a high sensitivity of lymphoid organs and cell populations to differences in spaceflight conditions, as well as post-flight procedures, environment, and experimental designs.

INNATE IMMUNITY

The number, function, and development of cells involved in innate immunity are affected by spaceflight. As shown in Table 1, increases in the number of neutrophils were observed consistently after flights, which are likely a result of the stress associated with landing, and the number of peripheral blood monocytes and NK cells was lower most often. Changes in function were observed in neutrophils, monocytes, and NK cells. Neutrophil phagocytic and oxidative functions were affected by spaceflight [54, 57]. Astronauts' monocytes exhibited a reduced ability to engulf *E. coli*, elicit an oxidative burst, and degranulate [54, 58]. Moreover, it was shown that their response to gram-negative endotoxins (LPS), which they could encounter during infection, was modulated by spaceflight-associated factors [59]. This alteration in the responsiveness of the crew's monocytes could be a result of the decreased expression of CD14 and the increased expression of TLR4, as LPS responsiveness depends on the physical association of the LPS/CD14-TLR4-myeloid differentiation protein 2 complex [60, 61]. Another possible explanation is provided by the observation that the levels of the LPS-binding protein were increased in astronauts' plasma. NK cell cytotoxicity and delay in hypersensitivity skin test responses to common recall antigens were also depressed severely under spaceflight conditions [54, 62–67]. Decreased numbers of bone marrow-derived CFUs were reported for flown (CFU-monocytes and CFU-granulocytes) and hindlimb-unloaded rodents (CFU-macrophage) [47, 49, 68, 69]. In a recent study, Ortega et al. [70] studied bone marrow cells from the humerus of C57BL/6 mice after a 13-day flight (STS-118) to determine how spaceflight affects the differentiation of cells of the granulocytic lineage. Their study indicated differences among bone marrow subpopulations, increased macrophage development in the bone marrow of flown mice, and the activation of neutrophils in response to landing.

Changes in cytokine expression were observed in astronauts and flown animals, thereby providing another explanation for the weakening of natural immunity under spaceflight conditions. Of interest is the alteration of IFN production, which is a first line of defense in the case of viral infection [50, 71, 72]. For example, decreased IFN secretion and low NK cell activity were observed for lymphocytes isolated from the peripheral blood of cosmonauts after returning from a 7-day spaceflight [73]. In another study, splenocytes from rats flown for 1 week exhibited decreased IFN-γ production in response to Con A, which is a T cell mitogen [42]. Furthermore, exposure to the spaceflight environment can increase anti-inflammatory mechanisms, as LPS-activated splenocytes of mice that were flown on STS-118 produced more IL-6 and IL-10 and less TNF-α than control mice [36]. The same study showed that many of the genes responsible for scavenging ROS were up-regulated after the flight, suggesting that the cells attempted to scavenge ROS produced during spaceflight. Indeed, an increase in the superoxide response by murine PMNs was reported even after short periods of microgravity [74].

Reactivation of herpes viruses was observed during spaceflight and has been used as an indicator of the immune status of astronauts [75–78]. Recently, the reactivation of the vari-

cella zoster virus has been observed in the saliva of astronauts [79], suggesting an asymptomatic reactivation as a result of spaceflight-associated stresses. In another study, Mehta et al. [75] demonstrated that the reactivation of latent virus occurred in astronauts before the flight and that cytomegalovirus may become reactivated further during spaceflight. A decreased resistance to viruses has also been observed in hindlimb-unloaded mice. Indeed, Gould and Sonnenfeld [80] reported that female Swiss/Webster mice, which are normally resistant to infection with the D variant of encephalomyocarditis virus, showed a marked increase in susceptibility to infection when suspended, and controls showed no increase in susceptibility to the virus. This correlated with a drop in IFN production. Elevated levels of stress hormones observed in astronauts and hindlimb-unloaded mice suggest that spaceflight- and hindlimb-induced stresses may be responsible for the reactivations of latent viruses [77, 78, 81–85]. Indeed, hormones such as catecholamine, which are released during stressful situations, regulate immune functions through adrenergic receptors located on immune cells, particularly β2-type receptors [86–89]. Additionally, glucocorticoids, which are overproduced in response to stress, can alter innate and acquired immunity [21].

T CELLS IN LOW-GRAVITY CONDITIONS

In the 1970s, several investigators reported that lymphocytes from crew members on United States and Soviet missions had decreased responses to mitogens at the time of landing [90]. Similar observations were reported in rats [91–93] and in mice [56]. Cogoli's group [94] performed many studies to understand why the activation of human T lymphocytes is depressed under low-gravity conditions. In their initial experiment, cultures of purified lymphocytes were activated in-flight with Con A, a T cell mitogen. Radioactive thymidine was added to the culture to evaluate the degree of activation. This experiment revealed that lymphocytes exposed to microgravity showed almost no activation in comparison with lymphocytes activated on Earth. This observation was confirmed by three separate experiments performed in Spacelab, SLS-1, and IML-2 [95–97]. Thus, there is a strong and consistent depression of T cell activation under space conditions.

Three signals are required for full T cell activation (**Fig. 2**). The first signal is delivered by the linkage of the TCR/CD3 complex with the antigenic peptide presented on a MHC molecule by anti-CD3 antibodies or by Con A. The second signal is delivered by IL-2 secreted by the T cell itself, which is recognized by the IL-2R. The third signal is a costimulatory signal delivered by the accessory cells (usually monocytes) via the B7/CD28 interaction by anti-CD28 antibodies or by IL-1, which is secreted by accessory cells upon interaction with T cells. Then, various protein kinases, GTPases, second messengers, and transcription factors are involved in the signaling cascade.

The first possible explanation for the depression of T cell activation is that the binding of the mitogen is altered under low G conditions. To study the binding of Con A and the formation of patches and caps of Con A receptors, Cogoli's

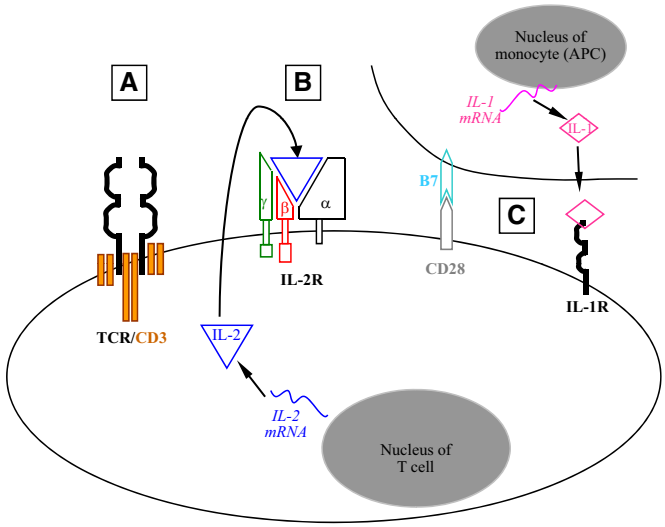

Figure 2. Signals required for full T cell activation. Three signals are required for full T cell activation. The first signal is delivered through the linkage between the TCR/CD3 complex to peptide-bound MHC molecules. The second signal is delivered by IL-2. The third signal is a costimulatory signal delivered by accessory cells or APC via the B7/CD28 interaction or by IL-1, which is secreted by accessory cells upon interaction with T cells.

groups [98, 99] performed experiments on sounding rockets. Fluorescent-labeled Con A was added to cells as soon as low-gravity conditions were established or a few minutes after the onset of microgravity. Two experiments were performed, one with lymphocytes purified from human peripheral blood [98] and one with the Jurkat T cell line [99]. All demonstrated that the influence of low gravity on the delivery of the first signal of activation is rather small and that rapid processes such as mitogen binding, patching, and probably capping are not involved in the depression of the in vitro activation of T lymphocytes [100].

An alteration in the IL-2/IL-2R function may be another cause of the depression in T cell activation, as IL-2 drives T cell proliferation and differentiation into armed effector cells [101]. Experiments performed with human PBL during SLS-1 and IML-2 demonstrated that the secretion of IL-2 and the amount of IL-2R are strongly depressed under microgravity [96, 97]. An independent study confirmed that IL-2R is not expressed on the surface of cells activated in low gravity [102]. Recently, Gridley et al. [56] also noted that the IL-2 expression was decreased in mice that flew on STS-118. Moreover, when low-gravity conditions were modeled in the random positioning machine, the genetic expression of IL-2 and IL-2Rα was inhibited in Con A-activated cells [103, 104], and IL-2Rβ was not affected. These data demonstrate that the nonresponsiveness of T lymphocytes under microgravity is a result of a perturbation of the IL-2 and IL-2R gene expression and that low gravity differentially affects gene expression. The selective effect of microgravity on gene expression was confirmed by a study of early gene expression in PBL activated by Con A under microgravity [105].

The third signal needed for T lymphocyte activation is provided by IL-1 and by the contact between T cells and accessory cells. Flight studies have measured reduced and normal levels of IL-1 for cells activated with Con A under microgravity [97, 106]. However, investigations performed under simulated microgravity supported the latest findings that IL-1 secretion is not affected adversely by microgravity [103, 107]. Cell-to-cell interactions and cell motility are important for cell communication and signal delivery. Cellular interactions occur under microgravity, as aggregates of human peripheral blood lymphocytes were observed in independent space experiments [94, 108]. Nonactivated human peripheral blood lymphocytes are able to move under low-gravity conditions [106], whereas lymphocytes cultivated at 1 G show this capability only when they are activated or in the presence of a chemoattractant. Furthermore, the mean velocity of cells activated with Con A under microgravity was significantly higher than that of cells cultivated at 1G. On the contrary, Meloni et al. [109] found that the motility of J-111 monocytes is reduced severely in the random positioning machine. Significant changes in the cytoskeletal structure of these cells, which plays an important role in cell motility [110], were also observed. As T lymphocytes were found to be highly motile under microgravity, even in the absence of a mitogen, it can be argued that an impaired motility of human monocytes could hinder the delivery of the costimulatory signal to activate the B7/CD28 pathway, thereby providing a second explanation for the loss of T cell activation in space.

Inhibition of T cell proliferation could also result from alterations in the downstream signaling events [102]. PKA and PKC are key regulators of T cell activation [111]. The cytoskeleton is involved in signal transduction [110] and has been described as the structure through which cells sense gravity [112]. An association between the cytoskeleton and PKC exists [113]. A disorganization of the cytoskeleton [99, 109, 114–116] could therefore result in a disturbed localization of signaling molecules. This hypothesis was confirmed by the analysis of the distribution of PKC isoforms, which was shown to be altered in monocytes and lymphocytes exposed to low gravity [117–119]. However, a recent study indicated that the phosphorylation of PKC was not down-regulated in T cells incubated with Con A and anti-CD28 under microgravity [104]. Furthermore, although some studies suggested that there may be a microgravity-induced PKC defect [117, 120, 121], Cooper and Pellis [107] were able to fully activate T cells to proliferate under simulated microgravity with PMA and ionomycin, suggesting that it is the signaling pathways upstream of PKC activation that are sensitive to simulated microgravity. Hughes-Fulford's group analyzed differential gene expression in Con A and anti-CD28 activated human T cells [104] and discovered that the impaired induction of early genes regulated primarily by transcription factors NF-κB, CREB, Ets (E26 transformation-specific) like protein, AP-1, and STAT1 contributes to T cell dysfunction under altered gravity. They also showed that the PKA pathway is down-regulated under microgravity. As NF-κB, AP-1, and CREB are regulated by PKA, these findings indicate that PKA is a key player in gravity-mediated modulation of T cell activation. As the absence of gravity has a negative impact on signaling pathways essential for early T cell activation [111], it is not surprising that the expression of downstream targets, such as genes involved in proliferation, apoptosis, biosynthesis, and secretion, is affected by microgravity.

Cytokine data were reported recently for crew members on short- and long-duration missions on the ISS [55]. Both groups had a low secreted IFN-γ:IL-10 ratio on the day of landing after activation of peripheral blood T cells with anti-CD3 and anti-CD28. Another study performed on PHA-stimulated splenocytes of mice flown on STS-108 revealed that IL-2 and IFN-γ were significantly lower after the flight [35]. IFN-γ depresses TH2 cell activity [122, 123], and a low IFN-γ:IL-10 ratio indicates a shift toward the TH2 subset [124]. Thus, a TH2 cytokine shift is associated with spaceflight. Another cytokine study reported that mice flown on STS-118 had a significantly higher IFN-γ:IL-10 ratio compared with control mice [56], suggesting a shift away from TH2 cells. However, cells other than TH1 and TH2 cells in the splenocyte mixture studied by these authors could have contributed to the levels for both cytokines. If a TH2 shift persists during long missions, it could represent a significant clinical risk for TH2-related autoimmune diseases, allergies, hypersensitivities, and disease susceptibility related to diminished cell-mediated immunity.

Finally, we should not forget that space radiation affects T cells. For example, the thymuses from mice flown on STS-118 were used to evaluate the expression of cancer-related genes, as thymic lymphoma is a common finding after radiation exposure [125]. The expression of 30 of the 84 evaluated genes involved in cell transformation and tumorigenesis was modified significantly, shortly after return from the spaceflight environment [56].

HUMORAL IMMUNITY

By comparison with natural immunity and T cell responses, our knowledge of the spaceflight-induced alterations of the humoral immune system is less developed. Studies about the levels of Ig in the plasma did not reveal significant changes after short spaceflights [44, 54, 126]. Different results were reported after long-duration missions: Konstantinova et al. [127] reported increased levels of serum Ig, particularly total IgA and IgG, and Rykova et al. [54] indicated that the total amounts of serum IgA, IgG, and IgM were unchanged after prolonged missions.

Studies about the effects of spaceflight on the antibody responses to specific antigens are rare. The only pieces of work addressing this question have been published recently [54, 128, 129]. In 2008, Rykova et al. [54] quantified IgM and IgG antibodies to HSV, CMV, EBV, and herpes virus type 6 in plasma samples from 30 cosmonauts who flew in the ISS to determine the effects of spaceflight on viral-specific humoral responses. No significant changes were found in the antiviral antibody levels determined by ELISA after long and short spaceflights compared with the pre-launch values. Unfortunately, that study did not provide information about potential reactivation of these latent viruses in the analyzed individuals. To determine if the humoral response is affected by spaceflight conditions, our team performed the Genesis experiment

during the Perseus space mission. Adult *Pleurodeles waltl* (urodele amphibian) remained in the Mir space station for 5 months and were immunized with protein. These are the only animals to have been immunized in space and are among the few vertebrates that have lived onboard a space station for an extended period of time. The quantification of IgY (the physiological counterpart of human IgA) [130] and IgM heavy chain mRNA in the spleen of animals killed 10 days after landing showed that the IgY, but not the IgM, expression is increased in flown animals, which supports previous data published by Konstantinova et al. [127]. To better understand spaceflight-induced modifications of the humoral response, we determined how these animals used their families of VH genes to build specific antibodies in response to the antigenic challenge. We focused our attention on IgM, as this isotype represents 75% of the antibodies in *P. waltl* [130]. These studies indicated that genes belonging to the VHII and VHVI families encode variable domains of specific IgM heavy chains produced by immunized animals [128]. However, the VHII and VHVI families were found in 28% and 58% of IgM heavy chains from animals immunized on Earth and in 61% and 24% of IgM heavy chains from animals immunized onboard Mir, respectively. Then, we determined how these animals used their individual VHII and VHVI genes. The experiments revealed an increase in the expression of IgM heavy chain mRNAs encoded by the VHII and VHVI.C genes and a strong decrease in IgM heavy chain mRNAs encoded by the VHVI.A and VHVI.B genes in spaceflight animals. Thus, different heavy chain mRNAs were expressed by spaceflight animals, demonstrating that this environment affects the humoral immune response [129].

ELISA tests performed under microgravity, 0.38 G (the martian gravity), 1 G, and 1.8 G, indicated that antibody binding does not depend on gravity [131]. The same conclusion was reached when the linkage between Con A and the TCR was studied (see T Cells in Low-Gravity Conditions section). Thus, ligand-receptor interactions appear unaffected by spaceflight conditions and are not responsible for immune depression.

It should be noted that radiation encountered during space missions may also affect the humoral response, as significant decreases in IgG1, IgG2a, and IgG2b in mice exposed to chronic low-dose γ-irradiation have been reported [132]. Furthermore, it was shown that solar-equivalent proton radiation induced an acute and severe depression of B cells and specific antibody formation [133, 134].

Finally, a differential sensitivity of the cellular and humoral immune systems to spaceflight conditions seems to exist, as it was shown that the cellular, but not the humoral, responses are affected by short periods of time in flight [44, 54, 126, 135]. Modifications of the humoral responses were only observed after long-term flights [127–129]. A spaceflight-induced modification in the expression of cytokines may contribute to this differential sensitivity. Indeed, as indicated in T Cells in Low-Gravity section, a TH2 cytokine shift is associated with spaceflights, and it has been demonstrated that if naïve $CD4^+$ T cells react to type 1 signals, such as IFN-γ and IL-12, they differentiate into IFN-γ-secreting TH1 cells to fight intracellular pathogens, or alternatively, in the presence of IL-4, they differentiate into TH2 cells whose main function is to promote humoral and antihelminth immunity [136].

COUNTERMEASURES

Several countermeasures have been tested to prevent compromises in resistance to infection resulting from exposure to spaceflight conditions. The three most promising countermeasures are presented below.

Increased oxidative stress, which is harmful for cells and can induce many disorders, has been observed after radiation exposure and is associated with spaceflight [137, 138]. Indeed, it was shown that the urinary concentration of 8-hydroxy-2′-deoxyguanosine (a marker of oxidative damage to DNA) was higher and that RBC superoxide dismutase (an antioxidant enzyme) was lower after a long-duration spaceflight [139]. Many molecules have antioxidant properties. Among these molecules, two investigations (one in vitro and one in vivo) showed that *N*-acetyl cysteine, ascorbic acid, α-lipoic acid, L-selenomethionine, coenzyme Q10, and vitamin E succinate are effective in protecting against space radiation-induced oxidative stress, and a complete or nearly complete protection was achieved by treating cells or mice with a combination of these agents [140, 141]. The addition of D-selenomethionine in the food of rats also prevented the decrease in total antioxidants associated with high-energy charged-particle irradiation by regulating the expression of genes involved in the repair of radiation-induced DNA damage [142]. These data indicate that antioxidants, alone or in combination, are likely promising countermeasures for protection against space radiation-induced adverse biologic effects.

During spaceflight, crews have altered dietary intake [143]. In addition, nutrient absorption and metabolism appear altered under spaceflight conditions. A well-known example is calcium metabolism. A decrease of up to 50% in calcium absorption and an increase of up to 50% of its excretion were observed in three crew members, who spent 115 days onboard the Mir space station [144]. As it is clearly established that energy and nutrient intake have profound effects on immune function [145], dietary countermeasures have been developed. Several studies have analyzed the effects of supplemental dietary nucleotides on immune function using ground-based models of microgravity. Hales et al. [146] have shown that decreased proliferation of splenocytes in response to PHA under simulated microgravity can be restored by supplementation with uridine, but not inosine, indicating that pyrimidines are more effective. This in vitro study also revealed that splenocytes secreted more IL-1β, IL-2, and IFN-γ when cultured in the presence of a nucleoside-nucleotide mixture. In vivo studies confirmed these results by showing that RNA and uracil diets could restore IL-2 and IFN-γ production, in addition to the proliferation of PHA-stimulated splenocytes from hindlimb-unloaded mice [147, 148]. Thus, supplemental nucleotides, especially uracil/uridine, possess immunoprotective effects and enhance immune function; these supplements are potential countermeasures for the observed immune dysfunction associated with space travel.

Another interesting compound is AHCC, which is an extract prepared from cocultured mycelia of several species of *Basidiomycete* mushrooms that contain polysaccharides (74%), amino acids, and minerals. This product has been shown to have a positive effect on the immune system of humans and rodents [149, 150]. Consequently, it was tested on hindlimb-unloaded mice that have decreased resistance to bacterial infections [20, 151]. Aviles et al. [151] showed that oral administration of AHCC for 1 week before suspension and throughout the 10-day suspension period enhanced the resistance to *K. pneumoniae* infection. The same team demonstrated that AHCC increased the TH1 response significantly in Con A-stimulated splenocytes from hindlimb-unloaded mice [152]. This compound also restored the functions of peritoneal cells that are suppressed by hindlimb unloading and increased NO production in peritoneal cells isolated from hindlimb-unloaded mice. Other studies showed that AHCC enhanced resistance to infection in a mouse model of surgical wound infection and activated immune function to decrease the bacterial load in a murine model of intramuscular infection [153, 154]. Thus, it appears that AHCC restores innate immunity, which is greatly affected by hindlimb unloading, and represents another countermeasure with great potential.

Other tested strategies include, for example, the injection of pegylated IL-2, a molecule that has been shown to increase the absolute number and activity of NK cells, T cells, and monocytes in humans and animals. However, studies performed on rats subjected to two 8-day spaceflights did not produce any reliable conclusions about the effects of this molecule, as there were notable differences between some of the data obtained from each flight [40]. Recently, it has been shown that muscular exercise has a positive impact on antibody production. Indeed, Shearer et al. [155] reported that the rate of primary antibody production increased at a faster rate and to a higher level in women subjected to HDBR plus exercise than in ones subjected to HDBR-only or HDBR plus protein supplementation.

These data indicate that the addition of antioxidants, nucleotides, or AHCC in the food could prevent alterations of the immune system under spaceflight conditions but also under any other conditions where the function of the immune system is compromised on Earth.

CONCLUSION

Spaceflight is a unique stress model impacted consistently or intermittently by myriad stresses, including psychosocial and physical stresses, high G forces at the time of launch and landing, increased radiation, sleep deprivation, microgravity, and nutritional factors. This multitude of factors alters the immune system and could lead to compromised defenses against infections and tumors. Over the past two to three decades, there have been a large number of investigations of space effects on the immune system. Unfortunately, many variable results have been reported, which is likely a result of individuals who react differently to stressful situations, the low number of subjects, and the different characteristics of each space mission. Despite this variability, it is obvious that spaceflight has profound immunosuppressive effects on humans and animals. An 18-month trip to Mars will, without any doubt, multiply the hazards of space travel. However, our current knowledge about the effects of extended spaceflights on the immune system is restricted for the following reasons: Only few astronauts and animals have spent several months continuously in space (the current record holders are Valery Polyakov and Sunita Williams, who spent 438 and 195 days in space, respectively); the effects of solar and cosmic radiation on the immune system are unknown, and neither the moon nor Mars has magnetic fields or dense atmospheres that could attenuate them; and only 24 astronauts have been beyond the low-Earth orbit. It is therefore wise to foster research to advance scientific comprehension of the hazards that are present as humans explore space and to investigate the effectiveness of various potential countermeasures to combat the deleterious effects of spaceflight. Additionally, findings from these researches could have substantial applications on Earth to counter immune dysfunction.

AUTHORSHIP

Each author was in charge of one chapter of this review. Moreover, J.-P. F. coordinated and supervised the work.

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