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Chapter 7

Spaceflight: Immune Effects and Nutritional Countermeasure

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Abstract

Microgravity is predicted to be a significant challenge to immune system during space travel. Consequences of weakened immune responses range from increased disease susceptibility to neoplastic growth. Degree of immune dysfunction is considered proportional to duration of stay in spaceflights. As a result of these risks, there is major concern over potential health risk for space travels that ultimately result in serious and considerable loss of mission objectives. Therefore, here is a need to explore the immune effects of spaceflight and its countermeasures. Several attempts have been made to develop effective measure to alleviate or prevent immune dysfunction due to microgravity. Among them, immunonutritional model has been shown to effectively modulate and upregulate immune system. This is further supported by our experiments demonstrating that supplementation of nutritional substrates like nucleotide and mushroom extracts active hexose-correlated compound (AHCC) effective in maintaining or restoring immunity in microgravity analog models.

Keywords: space travel, immune system, countermeasures, nutrition

1. Introduction

Microgravity and stress of space travel affect many organ systems and their functions in the body. Exposure to microgravity may produce changes in the performance of the immune system at the cellular level and in the major physiological systems of the body. Consequently, abnormal immune responses observed in microgravity may pose serious consequences, especially in future long-term space missions. Existing evidence suggests that spaceflight

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environment impairs immune system function in space travelers. Spaceflight environment has a cumulative effect on the body due to inherent stressors such as microgravity, cosmic radiation, and increase in corticosteroids [1]. A weakened immune system increases susceptibility to diseases and infectious pathogenesis. Immunosuppression puts hosts at risk for adverse effects such as infection from previously innocuous microorganisms that they are harboring or from microorganisms in their surroundings. Level of immune dysfunction is considered proportional to duration of stay in spaceflights. Longer the durations, such as missions to Moon and Mars and other deep space exploration flights, the effects are likely to be irreversible [2]. Maintenance of a healthy immune system is vital for resistance to infection and is essential in the homeostasis required for resistance to neoplastic disease, for prevention of autoimmune disease, tissue repair, and wound healing.

Based on the evidence, NASA in its roadmap has documented the immunological risks and consequences in space travel and exploration [3]. Among the highlighted risks are carcinogenesis caused by immune system changes, immunodeficiency, infections, altered wound healing, allergies and hypersensitivities, altered host-microbial interactions. As a result of these risks, NASA is concerned of major impact on health and mission objectives and irreversible potential loss of life which ultimately will result in serious and considerable loss of mission objectives. Therefore, it is essential to consider and highlight the immune effects of spaceflight and its preventive measures.

2. Spaceflight: stress and immune response

Space travelers are subjected to myriad of stressors of psychosocial, physical and environmental origin like microgravity, increased radiation, sleep deprivation, persistent circadian misalignment, and nutritional factors [4]. A common clinical observation is often the adverse relationship between stress and human disease. Stress such as injury or physical and physiological stress can result in metabolic stress and can cause severe impact on host health. Metabolic response to stress almost always results in adverse effects on the host defense mechanisms. Stress-related events cause breakdown in physical barrier, disrupt phagocytic cell function, and decreased antigen presentation altering cellular immune reactions. Stress is also suspected to play a role in morbidity and mortality in other immune-based diseases such as cancer, inflammatory bowel diseases, and even aging [5–13]. Although such dysfunctions have been thought of primarily as immunosuppressive, recent data have suggested immunoregulatory dysfunctions may play a more central role in stress-induced immune alterations [14]. Sleep alterations are suggested to modulate the stress-health relationship [15, 16]. Poor sleep, in turn, is associated with subsequent decrements in mental health including symptom reporting, incident cases of mood and anxiety disorders, and immune function [17–20]. Recent research suggests that stress is also associated with increased latent viral reactivation, upper respiratory tract infections, and increased wound healing time [21–26].

For instance, the decrease in immune cell function has been observed after flights of varying duration in the Soyuz, Skylab, Salyut, and Space Shuttle programs [27, 28]; these studies have also reported a reduction in lymphocyte proliferation. Reduced interferon-γ (IFN-γ) production

in suspended mice has been correlated to increased susceptibility to viral infection similar to that observed in rats flown on the US Space Shuttle. Therefore, there is an increased risk of infections among crewmembers during spaceflight resulting from working and living in a crowded, closed environment with limited capabilities for air revitalization and disinfection. Consequently, alterations in the immune response during spaceflight, as well as stress, aerosols, and altered fluid distribution within the body could increase the incidence of infectious diseases during long-duration space missions. Importantly, bacterial infection can be a major cause of morbidity after any traumatic injury, but trauma during spaceflight may substantially increase the infectious risk. An increase in neutrophil counts has been reported in tail-suspended rats [29, 30]. It is well established that activation of large numbers of neutrophils is likely to result in excessive generation of free radicals and associated tissue damage. Neutrophils also produce nitric oxide (NO), another free radical that reacts with superoxide to form peroxynitrite, decomposing to the highly toxic hydroxyl radical.

Enclosed cabin of spacecraft and free-floating environment increase the potential for infections among the crewmembers. Like all the objects, microbes are also in free-floating state increasing the potential for inoculation by inhalation increasing the regional susceptibility of respiratory tract. Altered metabolism and virulence reported in *in vivo* models of simulatory microgravity [31–35]. Increased morbidity and slower rate of wound healing in *S. aureus* sepsis reported in experimental animals exposed to test environment. The evidence is significant as the possibility of sepsis increases in space cabin environment as well as after return to earth from space mission. Spaceflights has profound effect on ecologic control of the gastrointestinal tract as cosmonauts, upon returning to earth, were found to have their normal gut flora replaced by potential pathogenic microorganisms.

Oxidative stress is known to occur in disuse and in many pathological conditions, and is now widely considered a major trigger of the imbalance between protein synthesis and degradation leading to muscle atrophy [36]. Reactive oxygen species (ROS) and elevated proinflammatory cytokines, in particular, TNF-α, mediate muscle atrophy via the redox-sensitive transcription factor nuclear factor-κB (NF-κB). It has been suggested that the exposure of brain to simulated microgravity can induce expression of certain transcription factors, which are oxidative stress dependent [37]. We have reported that the regulation and production of free radicals, and the relationship between oxidative stress and production of inflammatory cytokines and their subsequent effects on the healing of traumatic skeletal injuries in animals as well as cells subjected to analog microgravity.

3. Space travel and immune response studies

US Apollo missions were the first to identify the immune dysfunction [38]. The study of spaceflight immunology is limited due to relative inaccessibility, difficulty of performing experiments in space and inadequate provisions in this area in the United States and Russian space programs [39]. Most of the immune studies performed in the early days of spaceflight era had astronauts and cosmonauts participated in such studies. These studies assessed the immune effects by in vitro analysis of blood samples that were obtained before and after spaceflights.

Most of the experimental studies are of a pre- and postflight nature involving both humans and experimental animals and are divided into categories of short duration (< 2 weeks) and long duration (> 2 weeks) missions. In short duration flights, the majority of the outcomes are from postflight period analysis showing decreased cellular response to mitogens, decreased T cell counts and somewhat variable leukocyte counts [39]. Long-duration studies (1–12 months) that are performed by Russians, on board the Mir space station, have documented a 50% reduction in lymphocytic response to phytohemagglutinin (PHA) on the day after the mission, as compared with the preflight response. Levels returned to normal by day seven postlanding. Other studies showed decreased graft-versus-host response to xenoantigens and mitogen-induced IL-2 production [40]. The limited in-flight studies of delayed-type hypersensitivity (DTH) using commercial kits for the assessment of cell-mediated immunity showed significant suppression in half the subjects of 3–5 months in space and upon landing [41]. There have been several studies reported from space shuttle missions indicating alterations in lymphocyte response and decreased production of cytokines including interferons-α, β and γ and interleukin-2 [42].

Several studies have indicated that spaceflight can adversely affect tissue repair in muscle and bone. Mechanical unloading and physical deconditioning, which are thought to be central components in the effects of microgravity on the human body, have also broader clinical applications on Earth, for example, as it relates to prolonged bed rest or inactive geriatric patients. As a result, ground-based animal models have been used to mimic the mechanical unloading and physical deconditioning associated with microgravity and bed rest in humans. Because the phenotype of skeletal muscle is importantly dependent on mechanical loading, muscle plasticity is highlighted by the severe loss of mass (atrophy) after a few days of reduced weight-bearing activity such as bed rest or spaceflight. Hind limb unloaded (HU) of rats is an established model for atrophy which produces many of the muscular and systemic changes seen in humans as a consequence of muscle disuse [43, 44]. Consequently, results indicated that microgravity adversely affects the capacity of wounds to heal and that this may be related to a diminished cellular response to growth factors known to be present at sites of wounding [45, 46]. However, one such area of biomedical research where little is known concerns the effects of mechanical unloading and physical deconditioning on bone fracture repair and wound healing.

These research studies suggest that stress-induced changes in psychological, behavioral, and/or physiological functioning can be harmful and may result in negative health consequences. The clinical significance of these stressors and immune system changes must be defined, evaluated, and identified in space travelers.

4. Ground-based simulated microgravity studies

Due to high cost of spaceflight experiments and infrequency of flights, ground-based models that mimic the effect of microgravity have been extensively used. Among the models developed were human analogs model such as bed rest, physical stress, academic stress, and confinement, which allow some aspects of the spaceflight stressors [39]. Mechanical unloading and physical deconditioning, which are thought to be central components in the effects of microgravity on the human body, have also broader clinical applications on Earth, for example, as

it relates to prolonged bed rest or inactive geriatric patients. Exclusive use of human subjects for space research has severe limitations due to the ethical issues involved. Animal models provide more opportunities for research as it allows wider range of possible experiments. With variety of techniques available, rodents are preferred choice for space research studies. Several models were designed with specific effects to be studied in each individual model.

4.1. *In vivo* studies with rodent hind limb unloading model

Hind limb suspension of rodents was initially developed to study musculoskeletal system. In this system, the hind limbs of rodents are elevated to produce a 30° head-down tilt, which results in a cephalad-fluid shift and avoids weight-bearing by the hindquarters. When spaceflight effects were compared with ground-based weight unloading models, such as, bed rest studies and hind limb suspension model, there are many common features and effects [47–49]. These are shown in **Table 1**. Similar to many physiologic effects, the immune function and its dysfunction in both ground-based models is also very similar to spaceflight effects on the body. Many of the areas correlate with the spaceflight and its stress that have many consequential responses produced in body.

Antiorthostatic hind limb suspension of rodents, a ground-based model for simulation of microgravity, has summarized the physiologic and immunologic changes induced by antiorthostatic suspension and indicates a correlation with physiologic changes induced by spaceflight [50]. This position simulates the cephalad fluid and organ shift, a negative balance of water, nitrogen, and potassium; and increased metabolic turnover observed in astronauts during spaceflight. Studies using this model have shown interesting contradictory observations relative to organ-specific immunologic changes. Overall results of such antiorthostatic suspension models have shown a decrease in immunity.

	Spaceflight	Bed rest	Tail Suspension
Cephalic fluid shift	+	+	+
Redistribution of bones	+	+	+
Bone resorption	↓→	↓→	↓→
Calcium balance	↓	↓	↓
Fecal calcium	↑	↑	↑
Urine calcium	↑	↑	↑
Serum calcium	↑	↑→	↑→
PTH	↓	→	→
1, 25 (OH)2 D	↓	↓	↓
Serum osteocalcin	↓	→	↓
Bone strength	↓	ND	↓
Immune function	↓	↓→	↓

Table 1. Comparison of spaceflight to ground-based models of skeletal unloading.

4.2. *In vitro* studies in simulated microgravity using clinostat bioreactors

Numerous attempts to identify and separate the effects of microgravity and stress have met with difficult challenges, further raising the issue whether single cells are also affected by microgravity. Among the microgravity simulator models, an apparatus called a rotating wall vessel (RWV) developed by NASA is an ideal ground-based model system for examining the effects of microgravity on cells of the immune system without the presence of psychoneuroendocrine factors [51]. The RWV, based on clinostat technology, is a microgravity simulator Couette flow bioreactor. It consists of a zero-head space, aqueous filled culture vessel that suspends cells by rotating at low speed (10–60 rpm) around a horizontal axis. These conditions subject the cells to a randomized gravity field and low shear forces [52, 53]. Cells in the RWV are estimated to experience acceleration forces that simulate microgravity as low as 2 × 10–4 g. Using a Clinostat tissue culture apparatus, Cogoli [54] has shown that microgravity alters cell membrane permeability and thickness as well as cytoplasmic streaming. Several studies have reported the effect of microgravity on T lymphocyte activation. Clinostat culture studies showed that T cell responses to concanavalin-A (Con-A) were decreased by 50% [54]. Cooper and Pellis [55] have documented, using a clinostatic RWV bioreactor, that during polyclonal activation the signaling pathways leading to protein kinase C (PKC) activation are sensitive to simulated microgravity. Although several investigators used cell cultures subjected to analog microgravity to study potential impacts that space travel imposes on humans, the *ex vivo* has a serious lack of *in vivo* measurements of immune-physiological responses.

In the bioreactor microgravity cultured cells, there was a reproducibility of significantly suppressed PHA response when compared to static cultured cells as described by Cooper and Pellis [55]. In preliminary experiments, supplementation of the culture medium with nucleoside-nucleotide mixture or uridine (preferred nucleotides for solubility and bioavailability) significantly enhanced the PHA response in bioreactor microgravity. To our knowledge, this is the first observation documenting the reversal of decreased PHA response in simulated microgravity using the NASA bioreactor. Continuation of these studies using the biotechnology of *in vitro* modeled microgravity will provide additional data to support the hypothesis and prove the countermeasure effects of nucleotides.

During the countermeasure experiments, we studied the effects of housing environments on the production and expression of biologically and immunologically important molecules, namely cytokines, nitric oxide (NO), and inducible nitric oxide synthase (iNOS).

5. Countermeasure for prevention of immunosuppressive effects

Several attempts have been made to develop effective measure to alleviate or prevent immune dysfunction. There is definitely a need for countermeasures that will maintain normal immune system during spaceflight, especially when missions are prolonged. Almost all were found to be inadequate and presented adverse responses. For example, the use of immunomodulator agents and neurohormonal regulation was suggested to ameliorate the immune dysfunction in space [56]. However, the suggested methods of neurohormonal regulation by using agents that act upon the nervous system may have deleterious effects on systems besides the

immune system. The use of immunomodifying preparations such as LPS, MDP, and proteoglycans had no beneficial effects. In clinical practice, these compounds exhibited toxicity. Use of growth factors and interleukins was beneficial to small extent but had no influence on the increased corticosteroid (due to microgravity environment) levels [57, 58]. Most of the microgravity studies have documented and analyzed the immunosuppressive effects of true or simulated microgravity; however, there are scanty reports of efforts to modulate the immune system, host defense system, and its function. Especially, scarce are the studies that approach the issue of the maintenance and restoration of immunosurveillance.

Nutrition has played a critical role throughout the history of exploration, and space exploration is no exception. Environmental impacts like radiation, and spacecraft and spacesuit atmospheres can alter nutritional status and nutritional requirements of spaceflight. The physiological changes that occur during spaceflight influence spaceflight nutritional requirements. Therefore, understanding the nutritional requirements of space travelers and the role of nutrition in human adaptation to microgravity are as critical to crew safety and mission success. Many potential targets for nutritional countermeasures proposed to counteract or mitigate some of the negative effects of spaceflight on the human body. Recently, immunenutritional model has been shown to effectively modulate and upregulate immune system where a nutritional substrate has benefits beyond basic nutrition. Based on our extensive experience in R&D of nutritional immunomodulation, we evaluated two nutrients, which we have been studying for several years.

6. Dietary nucleotides

Dietary nucleotides are reported to restore innate and adaptive T-cell mediated immunity both at the peripheral mature immune compartment and stem cell level (**Figure 1**) [59, 60]. Nutritional upregulation of the global host defense mechanisms would have the great advantage of being technically feasible and applicable in people and it would be economical without the untoward effects. Laboratory findings and progress in multidisciplinary emerging field of nutritional immunology justify emphatically the proposed novel approach of nutritional modulation of host defense system in space travel. With the experimental evidence and information of nucleotide nutrition on immunity, it is plausible to provide both the prophylactic and therapeutic approach to the modulation of host defense mechanisms during spaceflights (**Figure 2**) [61].

6.1. Nucleotide supplementation in microgravity experiments

6.1.1. Hind limb unloading (HLU) in vivo model

We have documented that nucleotide supplementation significantly reversed the immunosuppression observed *in vivo* HLU model and *in vitro* BIO model [62]. The results were dramatic in the HLU group where the control chow group had significantly lower popliteal lymph node (PLN) response as compared to other housing groups. This effect of immunosuppression was reversed by dietary supplementation of nucleotides with Uracil effect reported the highest and significant as compared to the chow group in HU. Thus, the antiorthostatic HLU model of modeled microgravity can be used successfully to document nutritional immunomodulatory countermeasure. We also assessed the stress effect by measuring the serum levels

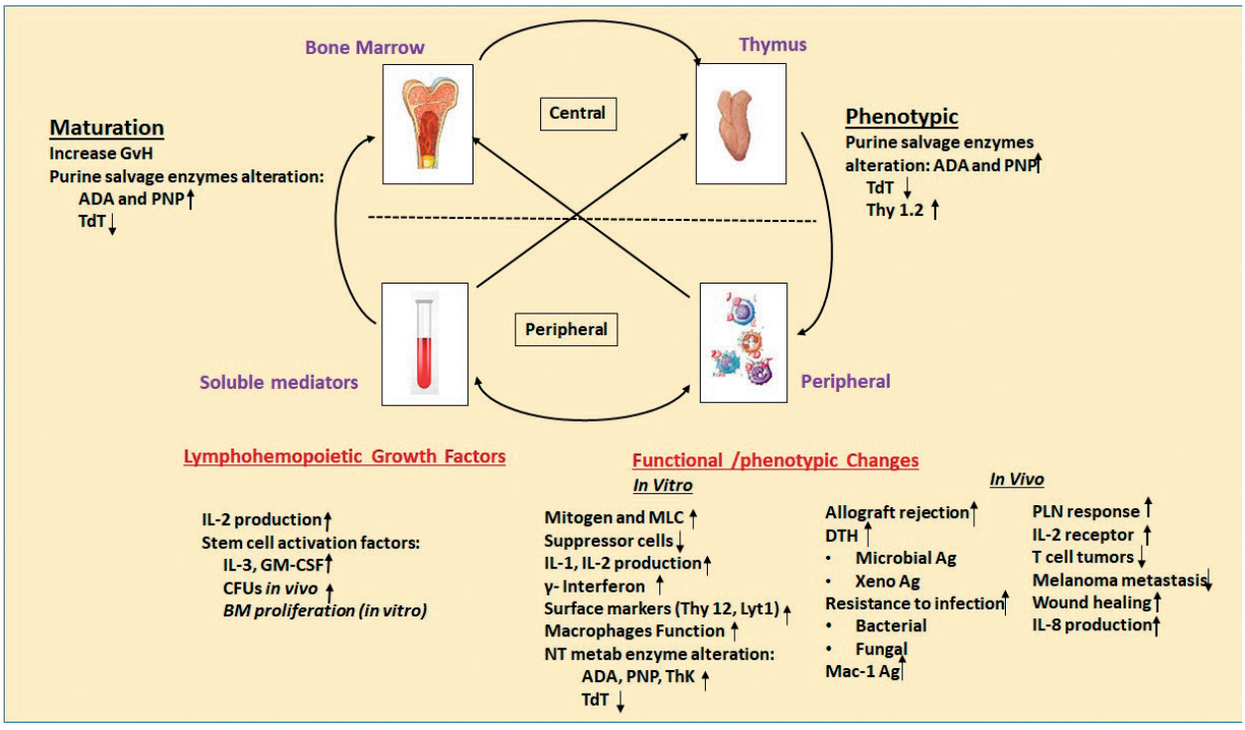


Figure 1. Pleiogenic immunologic responses by supplemental nucleotides.

of corticosterone (CORT) in experimental groups. The experimental evidence showed that in non-HLU animals, there was minimal effect of supplemented nucleotides (at the given dose) and did not encounter the immune depressive effects seen in HLU animals. Thus, nucleotide supplementation was beneficial for immune restoration in modeled microgravity environmental conditions. These results confirm our observations that RNA and Uracil are effective in maintaining or restoring immunity when the animals are under stressful situations (such as protein starvation, total starvation or dietary nucleotide deficiency, and HLU) or other trauma (such as sepsis, or inflammatory hypersensitivity stimulations). Our data also show the HU group had increased oxygen radicals (ROS) to 130% in the brain as compared to control mouse brain. This ROS increase was inversely proportional to glutathione levels (75%) in the brains. Therefore, our data confirm that oxidative stress is induced in animals subjected to hind limb unloaded.

6.1.2. Bone density after hind limb suspension or spinal cord injury in a rat model of osteoporosis

Space travelers are subjected to significant bone loss due to increased resorption and altered remodeling of bone tissue. In spite of calcium supplementation, increased excretion of calcium, reduced absorption of calcium from intestine, and diminished vitamin D synthesis due to space suit ultimately result in bone loss. Bone loss is proportional to the length of time in space. The changes in bone during spaceflight are similar to those seen in osteoporosis. Dietary nucleotides have long been known to positively affect the immune system and more recently have been shown to have beneficial effects in rapidly proliferating tissues.

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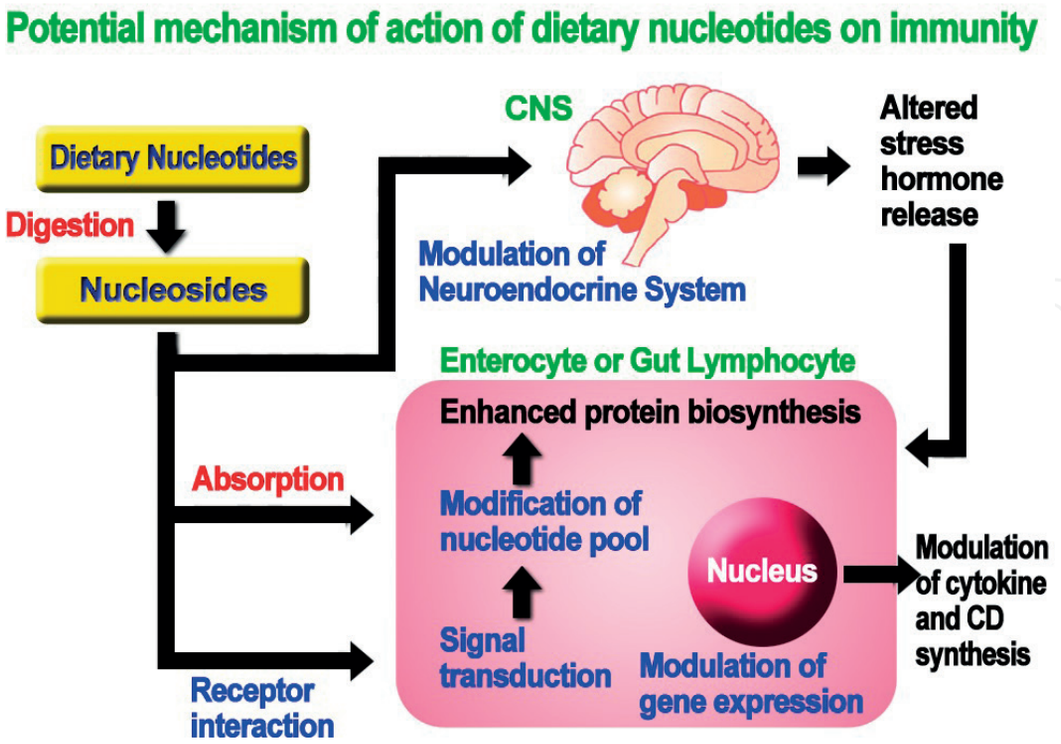


Figure 2. Potential mechanism of action of dietary nucleotides on immunity.

We have studied the effects of dietary nucleotides on bone loss after a disuse model of tail suspension in rats and have found that, in addition to exerting positive effects on the immune system, a nucleotide-enriched diet reduced the amount of bone loss seen in these animals. In a different model of bone demineralization, we have recently found that providing a diet of normal rat chow enriched with enhanced nucleotides reduces bone density loss in the femur when initiated immediately following spinal contusion injury [63].

6.1.3. Bioreactor in vitro modeled microgravity for T cell suppression and lymphocyte locomotion

In microgravity, immune suppression is a documented phenomenon in astronauts. It is also documented in *in vitro* and *in vivo* studies in modeled microgravity and the antiorthostatic rodent models of microgravity. In our earlier study, we reported that in the BIO microgravity cultured cells, there was a reproducibility of significantly suppressed phytohemaglutinin, a T cell mitogen response when compared to static cultured cells [1, 55]. We also reported that supplementation of the culture medium with nucleoside-nucleotide mixture or uridine (preferred nucleotides for solubility and bioavailability) significantly enhanced the PHA response in bioreactor microgravity.

Lymphocyte locomotion along the interstitium is integral to the immune response. Microgravity is a stressor that inhibits this phenomenon. The microgravity cell culture analog system also has the same effect on locomotion inhibition of lymphocytes [64]. Since locomotion is critical for an optimal immune response, countermeasure strategies for its restoration in lymphocytes

were sought. When lymphocytes were treated with 0.5 ng/ml phorbol myristate acetate (PMA) after exposure to microgravity culture, recovery of locomotion through type I collagen was 87%. However, in the human setting, PMA is a tumor promoter and cannot be administered. Studies with hind limb suspended mouse splenocytes displayed immune suppression, which was mitigated by the use of nucleotides and nucleosides (NS/NT). In lymphocytes cultured in modeled microgravity using the NASA BIO model of microgravity, it is shown that the NS/NT mixture used was able to orchestrate locomotion recovery by more than 87% documented with PMA in lymphocytes from three normal human donors.

7. Active hexose-correlated compound (AHCC)

AHCC is a nutritional substrate known for immune enhancing properties in humans and in laboratory studies. It is being widely used around the globe as a nutritional supplement for health and well-being in normal and patients with various afflictions to improve quality of life. AHCC is a compound obtained from enzyme-fermented extract of the mycelia of basidiomycetes mushrooms. AHCC consists of oligosaccharides, amino acids, lipids, and minerals [65]. The main components of AHCC are oligosaccharides (~74% of AHCC), and approximately 20% of AHCC are partially acetylated α-1, 4-glucans with a mean molecular weight of 5 kDa. These oligosaccharides including α-1, 4-glucans are believed to be the active components of AHCC [66, 67].

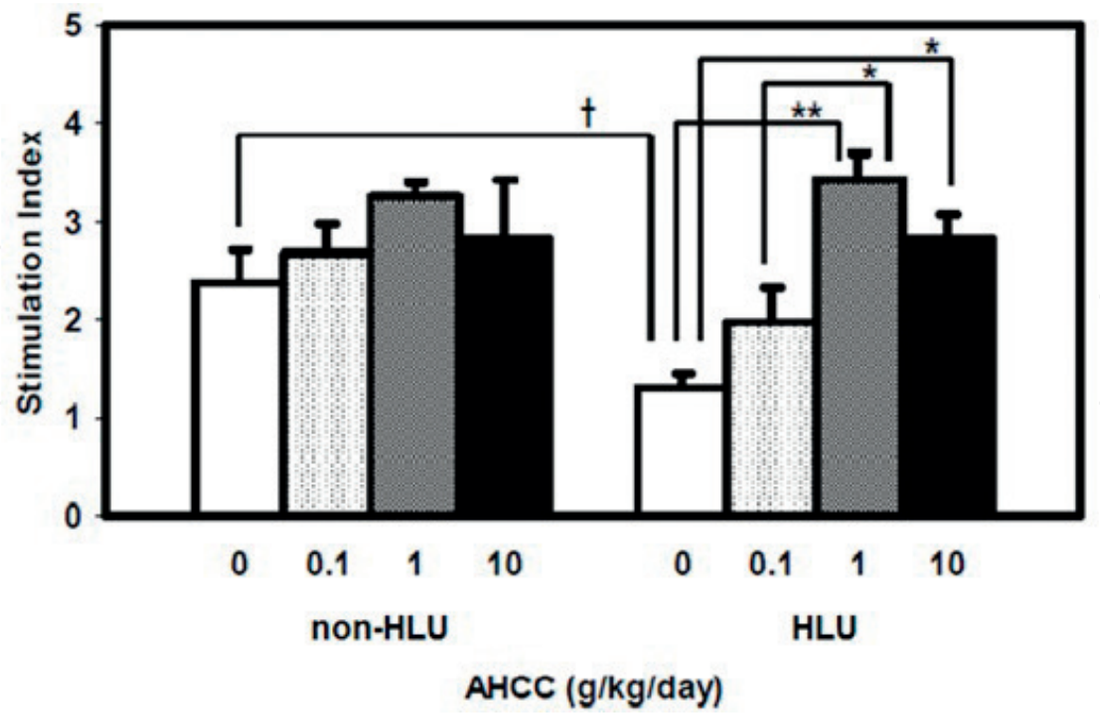


Figure 3. Stimulation index for *in vivo* PLN proliferative response vs. AHCC dose in control and HLU mice. means ± SEM; *p <0.05, **p =0.001, †P <0.05.

We examined the effect of AHCC on microgravity-induced immune changes by using a hind limb unloading (HLU) of mice as a microgravity analog [68]. A beneficial effect of AHCC on T cells has been reported in various models [69–71]. We induced immune changes by using a hind limb unloading (HLU) of mice as a microgravity analog and accessed the effect of AHCC supplementation on various immune functions. To access the immune function, Popliteal lymph node (PLN) response was analyzed as it involves all phases of immune response, e.g., antigen processing and presentation, followed by proliferative phase of immune response. PLN response was significantly decreased in mice in the HLU group compared to that in mice in the control group, and AHCC supplementation significantly reversed this response (**Figure 3**). AHCC reversed HLU-induced T cell dysfunction in PLNs. Since T cells play an important role in acquired immunity, a countermeasure for T cell dysfunction is imperative.

Spaceflight environment is one of the serious immune-compromised conditions due to closed space, and recycling of air and water may increase the risk of microbial load and reactivation of opportunistic pathogens, latent bacteria, and viruses. Neutrophils-, macrophages-, or monocytes-mediated innate immunity is the first step to exclude pathogens. To assess over-

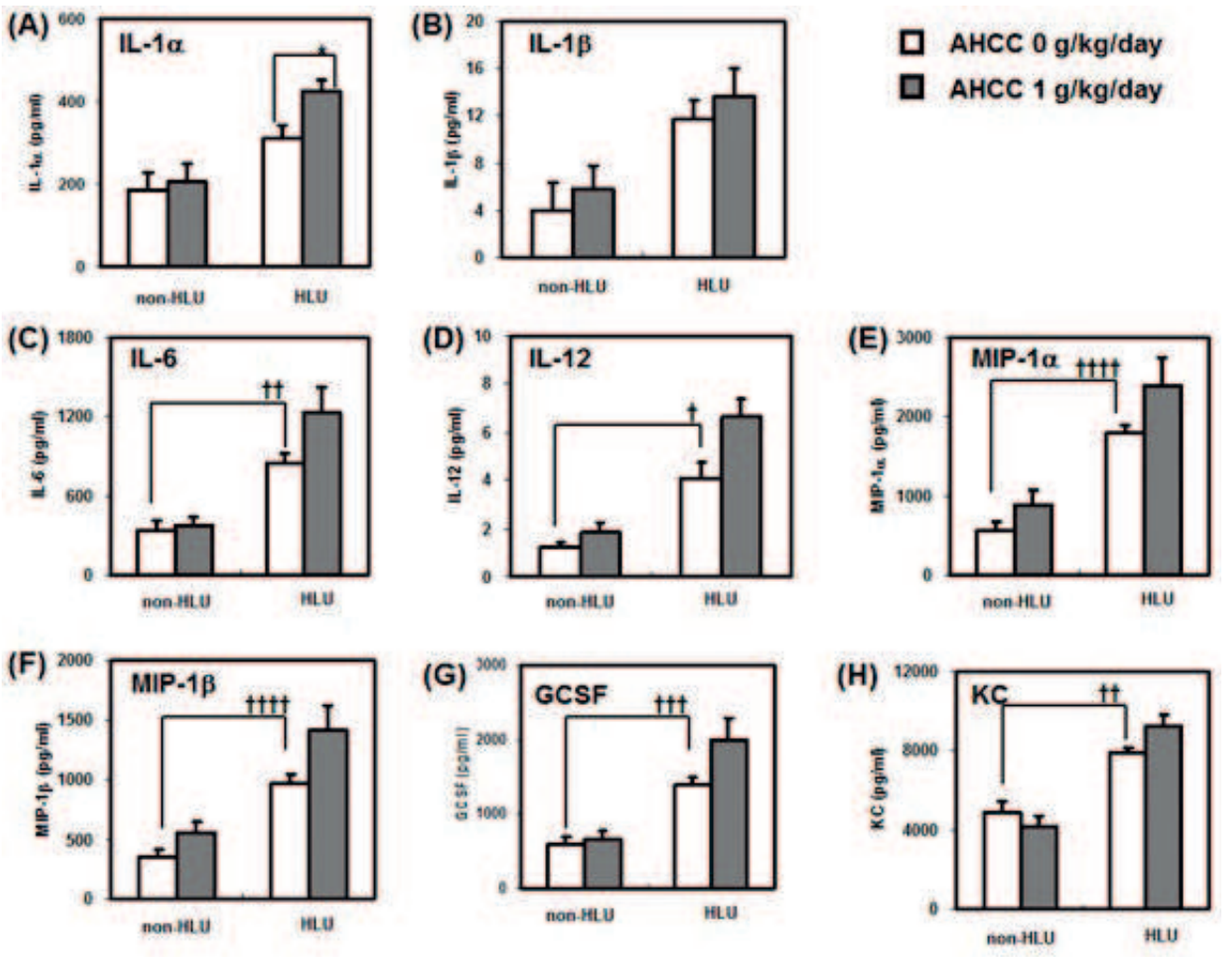


Figure 4. Production of cytokines and chemokines vs. AHCC dose in control and HLU mice.

all functions of these types of cells, we measured the levels of LPS-stimulated cytokine and chemokine production from splenocytes. Both inflammatory cytokines and chemokines were increased in mice in the HLU group and AHCC supplementation in HLU mice tended to further enhance the inflammatory cytokine and chemokine production (**Figure 4**). Increased interleukin like IL-6 is implicated in increased stimulated immune responses, e.g., during infection and after trauma, especially burns or other tissue damage leading to inflammation [72], while increased IL-12 (T cell stimulating factor) is known to be a stimulator of the TNF-α pathway and increases adaptive immunity. Enhancement of inflammatory cytokine and chemokine production by AHCC supplementation suggested to be effective for preventing infection [69, 73].

8. Summary and significance

Spaceflight observations thus far clearly document the adverse effects on the immune system, concomitant persistence of space environment stressors, and potential increase in virulence of infectious agents. It is imperative to design and develop effective countermeasures to secure health aspects of humans in space. The literature from experimental models and clinical human applications clearly documents that supplemental dietary nucleotides have beneficial effects on the immune system under stress conditions and environments. It is also known that supplemental dietary nucleotides had beneficial enhanced resistance to *Staphylococcus aureus* (SA), methicillin-resistant SA (MRSA), and *Candida albicans* infections in mice [74–77]. Similarly, use of a nucleotide containing formula in humans showed that there is a significant decrease in infectious complications in various patients. Immune enhancing nutritional supplements like AHCC have also found to be effective in restoring and maintaining immune system function in spaceflight analog animal model. These results emphasize the role of nucleotide nutrition and nutritional substrates like AHCC as a promising and plausible preventive measure to the immunologic consequences pre-/during /postspaceflight. A multipronged research will be an effective and safe countermeasure for spaceflight effects and to obviate stress and observed immune dysfunction. These studies should be of significant interest to NASA and other space agencies around the world by identifying profiles (immune, endocrine, and psychological) of individuals at risk for these immune dysfunctions with subsequent clinical manifestations and how nutritional countermeasures may impact such profiles. Such approaches should provide pragmatic clinical interventions for alleviation of stress and preservation of crew health particularly during long-term flights such as ISS, long-term interplanetary excursions, and deep space explorations.

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References

[1] Kulkarni AD, Kogiso M, Wakame K. AHCC Newsletter. 2006;**3-4**:3-4

[2] Lang T, Van Loon JJWA, Bloomfield S, Vico L, Chopard A, Rittweger J, Kyparos A, Blottner D, Vuori I, Gerzer R, Cavanagh PR. Towards human exploration of space: The THESEUS review series on muscle and bone research priorities. NPJ Microgravity. 2017;**3**:8

[3] Crucian B, Kunz H, Sams CF. Risk of Crew Adverse Health Event Due to Altered Immune Response. Human Research Program Human Health Countermeasures Element. Evidence Report—Human Research Roadmap—NASA. 2005

[4] Frippiat JP, Crucian BE, de Quervain DJ, Grimm D, Montano N, Praun S, Roozendaal B, Schelling G, Thiel M, Ullrich O, Choukèr A. Towards human exploration of space: The THESEUS review series on immunology research priorities. NPJ Microgravity. 2016;**2**:16040

[5] Bergsma J. Illness, the mind and the body: Cancer and immunology: An introduction. Theoretical Medicine. 1994;**15**:337-347

[6] Chorot P, Sandín B. Life events and stress reactivity as predictors of cancer, coronary heart disease and anxiety disorders. International Journal of Psychosomatics. 1994;**41**(1-4):34-40

[7] Kusnecov AW, Rabin BS. Stressor-induced alterations of immune function: Mechanisms and issues. International Archives of Allergy and Immunology. 1994;**105**(2):107-121

[8] Levenstein S, Prantera C, Varvo V, Scribano ML, Berto E, Andreoli A, Luzi C. Psychological stress and disease activity in ulcerative colitis: A multidimensional cross-sectional study. The American Journal of Gastroenterology. 1994;**89**(8):1219-1225

[9] Cohen S, Tyrrell DA, Smith AP. Negative life events, perceived stress, negative affect, and susceptibility to the common cold. Journal of Personality and Social Psychology. 1993; **64**(1):131-140

[10] McEwen BS, Stellar E. Stress and the individual. Mechanisms leading to disease. Archives of Internal Medicine. 1993;**153**(18):2093-2101

[11] Cohen S, Tyrrell DA, Smith AP. Psychological stress and susceptibility to the common cold. The New England Journal of Medicine. 1991;**325**(9):606-612

[12] Garrett VD, Brantley PJ, Jones GN, McKnight GT. The relation between daily stress and Crohn's disease. Journal of Behavioral Medicine. 1991;**14**(1):87-96

[13] Bonneau RH, Kiecolt-Glaser JK, Glaser R. Stress-induced modulation of the immune response. Annals of the New York Academy of Sciences. 1990;**594**:253-269

[14] Marshall GD Jr. The adverse effects of psychological stress on immunoregulatory balance: Applications to human inflammatory diseases. Immunology and Allergy Clinics of North America. 2011;**31**(1):133-140

[15] Hall M, Baum A, Buysse DJ, Prigerson HG, Kupfer DJ, Reynolds CF 3rd. Sleep as a mediator of the stress-immune relationship. Psychosomatic Medicine. 1998;**60**(1):48-51

[16] Hall M, Buysse DJ, Dew MA, Prigerson HG, Kupfer DJ, Reynolds CF 3rd. Intrusive thoughts and avoidance behaviors are associated with sleep disturbances in bereavement-related depression. Depression and Anxiety. 1997;**6**(3):106-112

[17] Gamaldo CE, Shaikh AK, McArthur JC. The sleep-immunity relationship. Neurologic Clinics. 2012;**30**:1313-1343

[18] Bollinger T, Bollinger A, Oster H, Solbach W. Sleep, immunity, and circadian clocks: A mechanistic model. Gerontology. 2010;**56**:574-580

[19] Ramsawh HJ, Stein MB, Belik SL, Jacobi F, Sareen. Relationship of anxiety disorders, sleep quality, and functional impairment in a community sample. Journal of Psychiatric Research. 2009;**43**(10):926-933

[20] Strine TW, Chapman DP. Associations of frequent sleep insufficiency with health-related quality of life and health behaviors. Sleep Medicine. 2005;**6**(1):23-27

[21] Marucha PT, Kiecolt-Glaser JK, Favagehi M. Mucosal wound healing is impaired by examination stress. Psychosomatic Medicine. 1998;**60**(3):362-365

[22] Cohen S. Psychological stress and susceptibility to upper respiratory infections. American Journal of Respiratory and Critical Care Medicine. 1995;**152**(4 Pt 2):S53-S58

[23] Kiecolt-Glaser JK, Marucha PT, Malarkey WB, Mercado AM, Glaser R. Slowing of wound healing by psychological stress. Lancet. 1995;**346**(8984):1194-1196

[24] Krueger E, Krueger GR. How does the subjective experience of stress relate to the breakdown of the human immune system. In Vivo. 1991;**5**(3):207-215

[25] Kiecolt-Glaser JK, Glaser R, Shuttleworth EC, Dyer CS, Ogrocki P, Speicher CE. Chronic stress and immunity in family caregivers of Alzheimer's disease victims. Psychosomatic Medicine. 1987;**49**(5):523-535

[26] Glaser R, Kiecolt-Glaser JK, Speicher CE, Holliday JE. Stress, loneliness, and changes in herpes virus latency. Journal of Behavioral Medicine. 1985;**8**(3):249-260

[27] Levine DS, Greenleaf JE. Immunosuppression during spaceflight deconditioning. Aviation, Space, and Environmental Medicine. 1998;**69**(2):172-177

[28] Barger LK, Greenleaf JE, Baldini F, Huff D. Effects of space missions on the human immune system: A meta-analysis. Sports Medicine, Training and Rehabilitation. 1995; **5**:293-310

[29] Robinson RR, Doursout MF, Chelly JE, Powell MR, Little TM, Butler BD. Cardiovascular deconditioning and venous air embolism in simulated microgravity in the rat. Aviation, Space, and Environmental Medicine. 1996;**67**(9):835-840

[30] Tipton CM, Overton JM, Joyner MJ, Hargens AR. Local fluid shifts in humans and rats: Comparison of simulation models with actual weightlessness. Physiologist. 1987;**30**(1 Suppl):S117-S120

[31] Nickerson CA, Ott CM, Mister SJ, Morrow BJ, Burns-Keliher L, Pierson DL. Microgravity as a novel environmental signal affecting *Salmonella enterica* serovar *Typhimurium virulence*. Infection and Immunity. 2000;**68**(6):3147-3152

[32] Miller ES, Bates RA, Koebel DA, Sonnenfeld G. Antiorthostatic suspension stimulates profiles of macrophage activation in mice. Neuroimmunomodulation. 1999;**6**(3):160-167

[33] Fang A, Pierson DL, Koenig DW, Mishra SK, Demain AL. Effect of simulated microgravity and shear stress on microcin B17 production by *Escherichia coli* and on its excretion into the medium. Applied and Environmental Microbiology. 1997;**63**(10):4090-4092

[34] Fang A, Pierson DL, Mishra SK, Koenig DW, Demain AL. Gramicidin S production by *Bacillus brevis* in simulated microgravity. Current Microbiology. 1997;**34**(4):199-204

[35] Miller ES, Sonnenfeld G. Influence of antiorthostatic suspension on resistance to murine *Listeria monocytogenes* infection. Journal of Leukocyte Biology. 1994;**55**(3):371-378

[36] Jackson MJ. Redox regulation of adaptive responses in skeletal muscle to contractile activity. Free Radical Biology & Medicine. 2009;**47**(9):1267-1275

[37] Wise KC, Manna KS, Yamauchi K, Ramesh V, Wilson BL, Thomas RL, Sarkar S, Kulkarni AD, Pellis NR, Ramesh GT. Activation of nuclear transcription factor kappa-β in mouse brain induced by a simulated microgravity environment. In Vitro Cellular & Developmental Biology—Animal. 2005;**41**:118-123

[38] Kimzey SL, Fisher CL, Johnson PC, Ritzmann SE, Mengel CE. Hematology and immunology studies. In: Johnston RS, Dietlein LF, Berry CA, editors. Biomedical Results of Apollo. Washington DC: National Aeronautics and Space Administration; 1975 SP-368. 1975. pp. 197-226

[39] Taylor GR. Overview of spaceflight immunology studies. Journal of Leukocyte Biology. 1993;**54**(3):179-188

[40] Konstantinova IV, Rykova MP, Lesnyak AT, Antropova EA. Immune changes during long-duration missions. Journal of Leukocyte Biology. 1993;**54**:189-201

[41] Gmünder FK, Konstantinova I, Cogoli A, Lesnyak A, Bogomolov W, Grachov AW. Cellular immunity in cosmonauts during long duration spaceflight on board the orbital MIR station. Aviation, Space, and Environmental Medicine. 1994;**65**(5):419-423

[42] Lesnyak AT, Sonnenfeld G, Rykova MP, Meshkov DO, Mastro A, Konstantinova I. Immune changes in test animals during spaceflight. Journal of Leukocyte Biology. 1993; **54**(3):214-226

[43] Narici MV, de Boer MD. Disuse of the musculo-skeletal system in space and on earth. European Journal of Applied Physiology. 2011;**111**:403-420

[44] Däpp C, Schmutz S, Hoppeler H, Flück M. Transcriptional reprogramming and ultrastructure during atrophy and recovery of mouse soleus muscle. Physiological Genomics. 2004;**20**(1):97-107

[45] Delp MD. Unraveling the complex web of impaired wound healing with mechanical unloading and physical deconditioning. Journal of Applied Physiology (1985). 2008; **104**(5):1262-1263

[46] Radek KA, Baer LA, Eckhardt J, LA DP, Wade CE. Mechanical unloading impairs keratinocyte migration and angiogenesis during cutaneous wound healing. Journal of Applied Physiology (1985). 2008;**104**(5):1295-1303

[47] Carpenter RD, Lang TF, Bloomfield SA, Bloomberg JJ, Judex S, Keyak JH, et al. Effects of long-duration spaceflight, microgravity, and radiation on the neuromuscular, sensorimotor, and skeletal systems. Journal of Cosmology and Astroparticle Physics. 2010; **12**:3778-3780

[48] Morey-Holton E, Globus RK, Kaplansky A, Durnova G. The hindlimb unloading rat model: Literature overview, technique update and comparison with space flight data. Advances in Space Biology and Medicine. 2005;**10**:7-40

[49] Morey-Holton ER, Globus RK. Hindlimb unloading rodent model: Technical aspects. Journal of Applied Physiology. 2002;**92**:1367-1377

[50] Chapes SK, Woods KM, Armstrong JW. Ground-based experiments complement microgravity flight opportunities in the investigation of the effects of space flight on the immune response: Is protein kinase C gravity sensitive? Transactions of the Kansas Academy of Science. 1993;**96**(1-2):74-79

[51] Schwarz RP, Goodwin TJ, Wolf DA. Cell culture for three-dimensional modeling in rotating-wall vessel: An application of simulated microgravity. Journal of Tissue Culture Methods. 1992;**14**:51-58

[52] Jessup JM, Goodwin TJ, Spaulding G. Prospects for use of microgravity-based bioreactors to study three-dimensional host-tumor interactions in human neoplasia. Journal of Cellular Biochemistry. 1993;**51**:290-300

[53] Tsao YD, Goodwin TJ, Wolf DA, Spaulding GF. Responses of gravity level variations on the NASA/JSC bioreactor system. Physiologist. 1992;**35**(s):s49-s50

[54] Cogoli A. The effect of hypogravity and hypergravity on cells of the immune system. Journal of Leukocyte Biology. 1993;**54**:259-268

[55] Cooper D, Pellis NR. Suppressed PHA activation of T lymphocytes in simulated microgravity is restored by direct activation of protein kinase C. Journal of Leukocyte Biology. 1998;**63**(5):550-562

[56] Fuchs SS, Medvedev AE. Countermeasures for ameliorating in-flight immune function. Journal of Leukocyte Biology. 1993;**54**(3):245-252

[57] Armstrong JW, Balch S, Chapes SK. Interleukin-2 therapy reverses some immuno-suppressive effects of skeletal unloading. Journal of Applied Physiology. 1994;**77**(2):584-589

[58] Armstrong JW, Kirby-Dobbles K, Chapes SK. The effects of rM-CSF and rIL-6 therapy on immunosuppressed antiorthostatically suspended mice. Journal of Applied Physiology. 1995;**768**(3):968-975

[59] Kulkarni AD, Yamauchi K, Hales NW, Ramesh V, Ramesh GT, Sundaresan A, Andrassy RJ, Pellis NR. Nutrition beyond nutrition: Plausibility of immunotrophic nutrition for space travel. Clinical Nutrition. 2002;**21**(3):231-238

[60] Kulkarni AD, Fanslow WC, Rudolph FB, Van Buren CT. Immunohemopoietic effects of dietary nucleotide restriction in mice. Transplantation. 1992;**53**(2):467-472

[61] Kulkarni AD, Rudolph FB, Van Buren CT. The role of dietary sources of nucleotides in immune function: A review. The Journal of Nutrition. 1994;**124**(8 Suppl):1442S-1446S

[62] Yamauchi K, Hales NW, Robinson SM, Niehoff ML, Ramesh V, Pellis NR, Kulkarni AD. Dietary nucleotides prevent decrease in cellular immunity in ground-based microgravity analog. Journal of Applied Physiology. 2002;**93**(1):161-166

[63] Kulkarni A, Richardson M, Ono K, Chandwani V, Johnson E, Kogiso M, Wakame K, Ambrose C, Martinez-Puig D. Nutritional nucleotides improve bone integrity in skeletal disuse. In: American Society for Parenteral and Enteral Nutrition 2008 Annual Conference; Chicago; USA. 2008

[64] Sundaresan A, Yamauchi K, Kulkarni AD, Pellis NR. Microgravity and modeled microgravity effects on lymphocyte signal transduction: Comparisons between human and mouse lymphocyte signaling. In: Proceedings of the 23rd ISTA Conference, Matsue, Japan. May 2002; p. 2

[65] Hales NW, Yamauchi K, Martinez AA, Sundaresan A, Pellis NR, Kulkarni AD. A countermeasure to ameliorate immune dysfunction in in vitro simulated microgravity

environment: Role of cellular nucleotide nutrition. In Vitro Cellular & Developmental Biology—Animal. 2002;**38**(4):213-217

[66] Kidd PM. The use of mushroom glucans and proteoglycans in cancer treatment. Alternative Medicine Review. 2000;**5**(1):4-27

[67] Ritz BW, Nogusa S, Ackerman EA, Gardner EM. Supplementation with active hexose correlated compound increases the innate immune response of young mice to primary influenza infection. The Journal of Nutrition. 2006;**136**(11):2868-2873

[68] Kogiso M, Wakame K, Sakai T, Yamamoto S, Sundaresan A, Kulkarni AD. Active hexose correlated compound and T cell response in hind limb—Unloaded BALB/c mice. International Journal of Surgical Research. 2015;**2**(5):32-38

[69] Aviles H, Belay T, Vance M, Sun B, Sonnenfeld G. Active hexose correlated compound enhances the immune function of mice in the hindlimb-unloading model of spaceflight conditions. Journal of Applied Physiology. 2004;**97**:1437-1444

[70] Gao Y, Zhang D, Sun B, Fujii H, Kosuna K, Yin Z. Active hexose correlated compound enhances tumor surveillance through regulating both innate and adaptive immune responses. Cancer Immunology, Immunotherapy. 2006;**55**:1258-1266

[71] Burikhanov RB, Wakame K, Igarashi Y, Wang S, Matsuzaki S. Suppressive effect of active hexose correlated compound (AHCC) on thymic apoptosis induced by dexamethasone in the rat. Endocrine Regulations. 2000;**34**:181-188

[72] van der Poll T, Keogh CV, Guirao X, Buurman WA, Kopf M, Lowry SF. Interleukin-6 gene-deficient mice show impaired defense against pneumococcal pneumonia. The Journal of Infectious Diseases. 1997;**176**(2):439-444

[73] Aviles H, Belay T, Fountain K, Vance M, Sun B, Sonnenfeld G. Active hexose correlated compound enhances resistance to *Klebsiella pneumoniae* infection in mice in the hindlimb-unloading model of spaceflight conditions. Journal of Applied Physiology. 2003;**95**:491-496

[74] Adjei AA, Takamine F, Yokoyama H, Shiokawa K, Matsumoto Y, Asato L, Shinjo S, Imamura T, Yamamoto S. The effects of oral RNA and intraperitoneal nucleoside-nucleotide administration on methicillin-resistant *Staphylococcus aureus* infection in mice. JPEN Journal of Parenteral and Enteral Nutrition. 1993;**17**(2):148-152

[75] Adjei AA, Yamamoto S. A dietary nucleoside-nucleotide mixture inhibits endotoxin-induced bacterial translocation in mice fed protein-free diet. The Journal of Nutrition. 1995;**125**(1):42-48

[76] Fanslow WC, Kulkarni AD, Van Buren CT, Rudolph FB. Effect of nucleotide restriction and supplementation on resistance to experimental murine candidiasis. JPEN Journal of Parenteral and Enteral Nutrition. 1988;**12**(1):49-52

[77] Kulkarni AD, Fanslow WC, Rudolph FB, Van Buren CT. Effect of dietary nucleotides on response to bacterial infections. JPEN Journal of Parenteral and Enteral Nutrition. 1986;**10**(2):169-171