Chapter 2

Lymphocyte Signaling and Function in Altered Physiological Environments

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Abstract

The immune system is the body's defense against infectious organisms and other invaders. It is our immune system that keeps us healthy as we drift through a sea of pathogens. Healthy immune function depends on meticulous regulation of lymphocyte activation. Previous studies have shown unfavorable effects of μg on several physiological systems, including a significant reduction of the adaptive immune response. Lymphocyte movement through interstitium is critically important for the immune response. Thus, the activation of lymphocytes depends on various factors such as cell-to-cell contact due to temporary contact, permanent aggregation or by the uptake of soluble factors such as interleukin 1. Microgravity induced loss of lymphocyte locomotory activity, along with diminished lymphocyte activation, can be counteracted by nutritional supplements such as nucleotides. A study conducted by Andreazzoli et al., proposes that the knowledge of cellular and molecular mechanisms of gravity and its influence on T cells is required for creating the provision of therapeutic and possible preventive targets to keep the bone and immune systems of astronauts fully functional during long-term space missions, in addition to aiding regular people with immune deficiencies. When an immune system is compromised it can lead to various infections as well as cancerous growths. Discovering the ins and outs of the lymphocyte regulatory pathways can account for controlling and studying medicinal treatments for all forms or immune disorders. Therefore, studying both the long-term and short-term effects of microgravity is of great significance, as it has an invalidation nature that affects how the regulators of the immune system are readily able to function.

Keywords: lymphocyte, immunity, inflammation

1. Introduction

The study of lymphocytes in microgravity has been conducted by various researchers to investigate how the immune system is altered due to the change in the gravitational pull [1–3]. Immune system adaptation during spaceflight is a concern in space medicine. Decreased circulating leukocytes observed during and after space flight infer suppressed immune responses and susceptibility to infection. Research has provided sound studies that outline the correlation between extended exposure to microgravity and inhibited activation of lymphocytes. One of the major concerns from the effects of microgravity is the potential for astronauts to be afflicted with opportunistic infections that can only be acquired through a seriously compromised immune system [4]. The microgravity aspect of the space environment has been simulated on Earth to study adverse biological effects in astronauts. It is well known that immune cell function is severely suppressed in microgravity, which renders the cells of the immune system an ideal model organism to investigate the influence of gravity on the cellular and molecular level. In lymphocytes, microgravity affected the protein kinase C [5]. The various factors in the space environment that contribute to immune dysregulation during and post-spaceflight include exposure to microgravity, stress, deconditioning (the reduced physical activity and shift of fluids), and radiation exposure. The body's primary immune defense heavily relies on immune cell distribution and function, which can clearly be influenced by a combination or synergy of any of the factors described above that exist in the space environment. Lymphocytes are a type of white blood cell critical for adaptive immune responses. Of the blood cell types, lymphocytes are the most sensitive to ionizing radiation exposure.

During flight, the activation of cultured human lymphocytes is depressed to less than 3% of the ground controls when exposed to Concanavalin A [4]. When human lymphocytes are exposed to microgravity in a rapidly rotating clinostat, Concanavalin A stimulated T cell activation is depressed [6]. The functionality and activation of lymphocytes is altered and compromised due to space flight. Microgravity and radiation exposure in space suppress human immune systems but, some countermeasures to rescue immune systems in microgravity have been discovered. With the emphasis of space travel being centered on finding various areas to support substantial human life outside of earth, it is imperative that we find ways to supplement a safe journey to another planet.

2. Lymphocyte activation

Earthbound living organisms, including cells, are affected by two new environmental conditions known as microgravity and cosmic radiations when introduced to space. In several experiments dedicated to space missions and simulated microgravity, evidence has shown that microgravity causes a dramatic depression of the mitogenic in vitro activation of T lymphocytes [7]. Lymphocyte activation is dependent upon cell-to-cell contact established in three ways, temporary contacts, permanent aggregation or the uptake of soluble factors such as interleukin 1. Granted, in microgravity, it has been suggested that cell-to-cell interactions are less probable or even impossible.

Studies finding the reduction of lymphocyte activation during exposure to microgravity corroborates with the observations of Soviet and U.S. astronauts during the pre-Shuttle era [5]. The data also reflects the more recent data collected from 41 crew members of shuttle flights (Taylor et al., 1986) who each had declining immune functions to activate lymphocytes upon their return to earth. Additionally, recent studies are deriving similar results of a functional association between the cytoskeletal protein spectrin and PKCβ in the cytoplasm of lymphocytes [8]. PKCβ has a critical role in lymphocyte activation-related signaling, which could be disturbed by disorganization in the cytoskeleton due to gravity, resulting in a disturbed localization of signaling molecules. Results of experiments done by Dr. Chang and her colleagues indicate that microgravity was the causative factor for impaired T cell activation during spaceflight by inhibiting transactivation of key immediate early genes. [9].

Microgravity plays a major role in activating the self-limiting gene expression through miR-21. In microgravity the miR-21 will maturate and accumulates causing the inhibition of translation of the target genes, thus suppressing normal immune responses that would occur at ground level [10]. The heat map found in Hughes-Fulford et al. study shows the expression of 17 significant gene targets of miR-21 from three donors that regulated differently after 1.5 h of activation. They followed the activation under normal gravity and microgravity conditions on board the ISS ($P \leq 0.05$) (**Figure 1**). For some genes there were multiple gene probe sets targeted to different

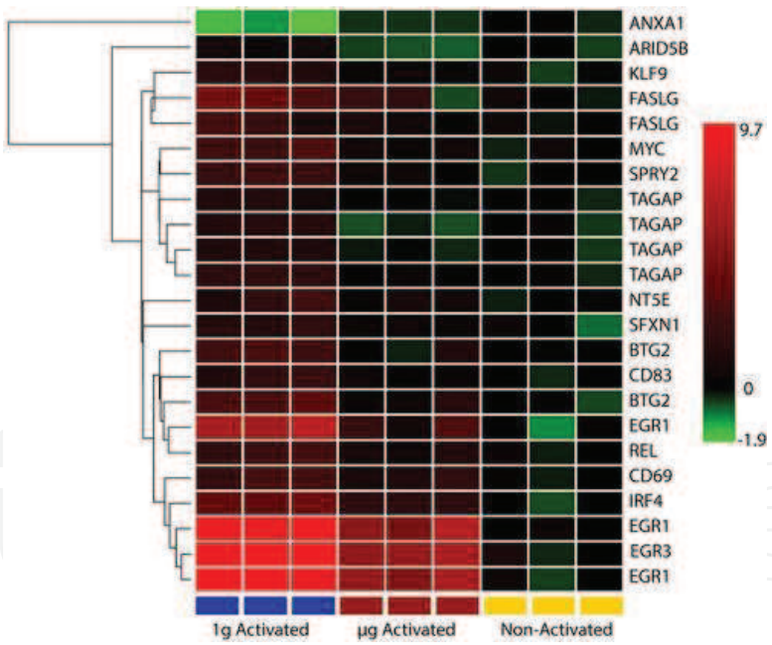

Figure 1. Heat map of 17 significant genes. Courtesy: [10]. Heat map of predicted miR-21 target genes showing differential regulation of T-cell activation after 1.5 h in microgravity (mg) and normal gravity (1 g). The Pearson's centered metric with centroid linkage was used to enforce the grouping of the genes. Rows are comprised of the gene probes while the columns indicate the expression profiles of the donors. Using log2 normalized expression values, the scale of the heat map ranges from green (indicating downregulation) to red (indicating upregulation). The blue-labeled columns illustrate normal gravity-activated samples, in the red labeled columns microgravity-activated samples are illustrated, and yellow-labeled columns illustrate microgravity-nonactivated T cells. Seventeen unique miR-21 gene targets are depicted with differential expression in normal gravity and microgravity condition (P # 0.05; FASLG P # 0.06). Even with considerable variability in nonactivated samples and some variability in microgravity activated donors the gene expression in normal gravity-activated samples become significantly more uniform after 1.5 h of activation.

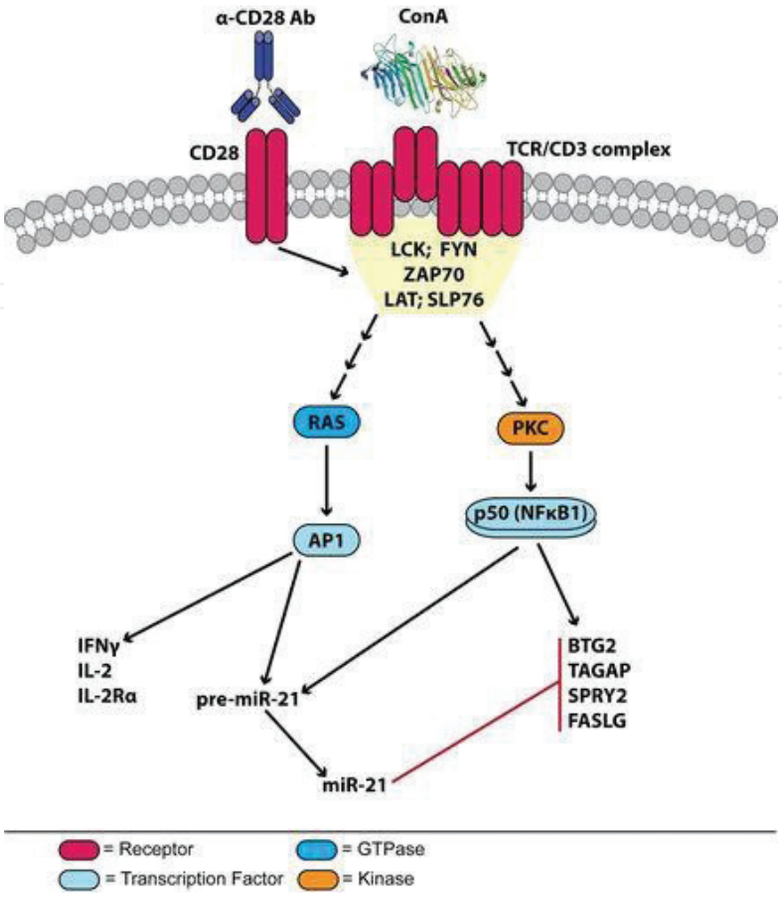

Figure 2. Hypothesized pathways of expression and downregulation. Courtesy: [10]. Postulated actions of miR-21 and gene expression of early T-cell activation. miR-21 is known to downregulate AP1 (28), additionally the presence of its seed sequence in 39-UTR of target genes, it will also downregulate BTG2, TAGAP, SPRY2, and FASLG as miR-21 expression increases after 24 h of activation. These results suggest that miR-21 projects a self-limiting mechanism to regulate T-cell activation. It is possible that miR-21 provides a mechanism of self-limited induction regulating T-cell activation.

regions. Three specific genes with multiple probe sets include FASLG, TAGAP, and EGR1, each showing the same trends of significant inhibition of gene expression under different gravities. Due to their uniformity in their activation profile of normal gravity, there is evidence that after activation with concanavalin A/anti-CD28 all three donors were stimulated in similar genes and pathways. In the microgravity flown samples gene expression was low and less uniformity than the activated normal gravity samples. Consequently, some cases produced a microgravity profile almost equivalent to nonactivated samples, illustrating the suppression of gene expression in all three donors. The onboard normal gravity-activated, microgravity-activated, and non-treated control conditions varied distinctly in their profiles across the 17 predicted miR-21 gene targets, due to this, early activation gene expression could be pinpointed for each condition.

In the study by Martinez et al., they compared the conditions of microgravity spaceflight, random positioning machine (RPM) and rotating wall vessel (RWV) during gene activation of iI2 and iI2r in the genes of mouse splenocyte. They were able to confirm two early activated T-cells, ligp1 and Slamf1 in which they confirmed activation of these genes for the ground samples and suppression of the mouse's immune function in the other conditions **Figures 2** and **3**.

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Figure 3. Rotatory cell culture system. Courtesy: OIPL lab, Texas Southern University. The RCCS was originally developed in NASA's Johnson Space Center in order to simulate the microgravity conditions of space. It is structured is based on the principle of clinorotation, which is defined as the nullification of gravity's force by slowing the rotation around one or two axes. NASA's clinostat is a single axis device known as the rotating wall vessel (RWV), the RCCS is the commercial version of this device. The NASA rotating bioreactor simulates microgravity by gently moving the growth medium while growing cells are kept in suspension by a constant "free fall" effect.

In the study of Bradley et al., they investigated how T lymphocytes recognized the antigen presenting cells like the dendritic cells which invokes T cell proliferation driven by interleukin-2 (IL-2) and other cytokines. With this activation these cells are able to function in order to kill invaders and tumors, in retrospect, during spaceflight the production of cytokines is reduced along with reduced proliferations and effector functions, symbolizing the control that gravitational forces have over the immunity function. The researchers then propose that this may be the leading cause for the opportunity for cases of viral reactivation events and opportunistic infections associated with astronauts of numerous missions. In conclusion they found that over exposed culture of T cells in SMG resulted in increased expression of the inhibitory receptor, CTLA-4. Blockade of CTLA-4 interaction with DC ligands resulted in improved T cell IL-2 production [4].

In the study of the microgravity effects of embryotic growth, Shinde et al. found that their morphological changes in the EBs size and shape observed by clinorotation as compared to 1 g control EBs. The number of total EBs formed under clinorotation was less than 1 g indicating that there were no significant differences between proliferation in microgravity conditions and ground conditions. They were able to determine that the major effect of microgravity was the suppressive conditions of cardiomyogenic [11].

3. Lymphocyte locomotion

In order to foster efficient human exploration of space, various studies have been conducted to understand the fundamental role of gravity in development and function of biological organisms The effects of microgravity can be attributed to numerous physical phenomena relevant

to biological research; this included but is not limited to hydrostatic pressure in fluid-filled vesicles, sedimentation of organelles, and buoyancy-driven convection of flow and heat [12]. These physical phenomena can in turn, directly and indirectly, affect cellular morphology, metabolism, locomotion, secretion of extracellular matrix and soluble signals, and assembly into functional tissues [12]. In order to start locomotion on a surface or also transmigration through the epithelium, a resting lymphocyte changes its round shape into a polarized one. This involves a reorganization of the cytoskeletal network with a collapse of the vimentin system [13]. In long-term space travel, the crewmembers are exposed to various amounts of microgravity and radiation that invoke potential hazards to the immune system. The activation of T cells is a critical point in the immune responses. In both microgravity and modeled microgravity (MMG) the receptor-mediated signaling is inhibited which is followed by diminished DNA synthesis in the peripheral blood lymphocytes, which in turn diminish lymphocyte locomotion through type I collagen [14]. Lymphocyte motility through interstitium is critically important for the immune response. It is investigated *in vitro* using collagen as a model matrix. Motile morphology not only includes the constriction and longitudinal contraction shape changes, but also includes clustering [15]. Clustering is random and preceded the motile morphology, which in turn preceded locomotion. Two phases of locomotory activity have been investigated: (a) native motility in lymphoid cell populations, and (b) induced motility resulting from incubation of lymphocytes with matrix, polyclonal activators, and cytokines. Much of the characterization of lymphocyte translocation has been performed on the latter model.

Previous studies showed that modeled microgravity (MMG/RCCS) and Space Shuttle Missions STS-54 and STS-56) microgravity (MG) inhibit human lymphocyte locomotion [16]. Modeled microgravity also suppressed polyclonal and antigen-specific lymphocyte activation [17]. By applying video microscopy and digital scanning, we observed, both in lymphocyte samples from the RCCS and from the Shuttle missions, changes in cell shape, suggestive of a decreased ability to polarize.

The loss of locomotory activity in microgravity, along with decreased activation, suggests that tumor allografts may not be rejected in spaceflight, since the absence of locomotion portends poor invasion of the grafted tissue. Interestingly, activation of lymphocytes prior to exposure to analog microgravity partially or completely abrogated the inhibitory effect of microgravity on lymphocyte motility. Our data suggest that the loss of locomotory function and T cell activation is probably due to lesion(s) in transmembrane signaling possibly involving calcium independent PKC isoforms such as delta and epsilon [6, 14, 18, 19]. Gene expression data from our laboratory on normal and activated peripheral blood lymphocytes cultured in modeled microgravity indicate that selective genes involved in inflammatory processes are affected by changes in gravity. Locomotion can be recovered by nutritional supplements such as nucleotides [1, 2, 20] (Ward et al., 2007).

4. TH1 to TH2 transition in microgravity

Mature CD4+ helper T lymphocytes have been categorized into two major functional phenotypes, TH1 and TH2. Cytokine data from crew members on short- and long-duration

spaceflight showed a significant reduction of IFN-γ and IL-2 [21]. In the 45-day head down bed rest (HDBR) study designed to mimic a real space flight, they found similar decreases of IFN-γ after activation of peripheral blood T cells with anti-CD3 and anti-CD28. Additionally, there was a significant reduction of IL-17A suggesting a weakened T helper (Th1) along with Th17 types of responses. Defective T cell activation or cytokine expression, increased Th2 type cytokine production [22]. Various documentations of decreased Th-1 cytokines production have been assessed repeatedly in astronauts (Taylor et al., 1997). In most flight there is decrease in the IFNg:IL-10 ratio and a Th1:Th2 shift during space flight (Curcian et al., 2008). Th1:Th2 shift can most likely be seen in support of cell-mediated immunity during space flight (Taylor and Janney, 1992; [8]) yet unaltered humoral immunity during space flight (Fuchs and Medvedev, 1993; Stowe et al., 1993).

5. Countermeasures to rescue immune systems in microgravity

Astronauts are exposed to radiation and microgravity during space missions, which causes harm to their immune systems and other negative health effects. Previous studies report that, benzofuran-2-carboxylic acid and its derivatives (KMEG) may provide protection from radiation and restore normal immune function [23] **Tables 1** and **2**.

Since lymphocyte locomotion is inhibited due to gravity, direct activation of protein kinase C (PKC) bypassing cell surface events using the phorbol ester PMA rescues MMG-inhibited lymphocyte activation and locomotion. [14]. Microgravity exposure changed expression of 78 lymphocyte genes. Subsequent treatment with KMEG induced upregulation of six genes namely; CDCA 7, RAB 17, RPSA, YME1L-1, LY96, and AIF1 all involved in T cell early response, lymphocyte activation, growth and proliferation, and the downregulation of five genes namely; ANKHD1, AVP, TINAG L1, CDK5RA 2, and FYN all involved in tumor

Upregulated genes	Fold increase	Functions
RAB17	2.949	Plays an important role in the regulation of membrane trafficking
CDCA7	4.537	Early response gene mediating C-*myc*-related proliferation
RPSA	1.780	Required for the assembly and/or stability of the 40S ribosomal subunit
YME1L-1	1.559	Ensures cell proliferation, promotes anti-apoptotic activity and protects mitochondria from the accumulation of oxidatively damaged membrane proteins
LY96	1.482	Cooperates with TLR4 in the innate immune response to bacterial lipopolysaccharide (LPS)
AIF1	1.338	Promotes the proliferation of T-lymphocytes

The information on the functions of genes described was obtained from the Gene Card website (http://www.genecards.org/).

Table 1. Genomic analysis of significantly upregulated T cell proliferation genes in spaceflight KMEG-treated lymphocytes (μg LT) compared to ground KMEG-treated lymphocytes (1 ***g*** LT).

Downregulated genes	Fold decrease	Function
ANKHD1/ANKHD1-EIF4EBP3	−4.86	Isoform 2 may possess an anti-apoptotic effect and protect cells during normal cell survival through its regulation of caspases.
AVP	−3.016	Neurophysin 2 specifically binds vasopressin; acts as a negative regulator of innate immunity by inhibiting TLR2/TRL4 associated pattern recognition and pro-inflammatory cytokine production.
TINAGL1	−2.815	Maybe implicated in the adrenocortical zonation and in mechanisms for repressing the CYP11B1. This is a non-catalytic peptidase C1 family protein.
CDK5RAP2	−2.489	Potential regulator of CDK5 activity via its interaction with CDK5R1.
FYN	−2.442	Non-receptor tyrosine protein kinase that plays a role in many biological processes including regulation of cell growth, survival and cell adhesion.

The information on the functions of genes described was obtained from the Gene Card website (http://www.genecards.org/).

Table 2. Genomic analysis of significantly downregulated T cell proliferation genes in spaceflight KMEG-treated lymphocytes (μg LT) compared to ground KMEG-treated lymphocytes (1 g LT).

progression and metastasis. These findings suggest KMEG diminishes microgravity induced immune dysfunction, advancing development of countermeasures to lessen the risks faced by the crew members [18].

Various countermeasure studies have been conducted to find different ways to rehabilitate the compromised immune system. The compromised immune system can lead to various infections as well as cancerous growths. Also discovering the ins and outs of the lymphocyte regulatory pathways can account for controlling and studying medicinal treatments for all forms or immune disorders [3].

Tauber et al. [24] suggests that with all the viable information we have at our hands there is still an unanswered question as to how or by which mechanisms can the cell sense gravity to be affected. It is this question that causes them to propose that this mechanism is most important to explore because alleviating this unknown sensor may be the defying force to creating uninterrupted biological strife in space exploration. They introduce a popular model which enforces the idea that the cytoskeleton is the force that is affected and alarms the cell though unnatural tensions that there is a shift in the atmosphere, thus creating shock to the immune system.

The study done by Bradley et al. [4], further supports this notion to underlining the inhibitory pathways will be a viable countermeasure to restore T cell responsiveness in astronauts during long-term spaceflight or those living in microgravity environment following stimulations. If there is a way to predict the interruption of a pathway or even mimic the mechanism of a pathway to jump start healthy immune functions, test can immediately be conducted to indicate where and when these artificial mechanisms need to be administered to imitate the natural performance of the immune responses.

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6. Conclusion

To conclude, it is known that microgravity causes immune system modifications. Factors in the space environment contributing to immune dysregulation during and post-spaceflight include exposure to microgravity, stress, deconditioning and radiation. When human lymphocytes are exposed to microgravity in a rapidly rotating clinostat, concanavalin A stimulated T cell activation is depressed. Lymphocyte locomotion through type I collagen is diminished through the inhibition of receptor-mediated signaling in microgravity. Decreased production of Th-1 cytokines was documented repeatedly in astronauts and hence the defects in T cell activation or cytokine expression, increased Th2 type cytokine production. Although crew members and astronauts have a certain risk on their immune system due to their exposure to microgravity, some countermeasures to rescue their immune systems in microgravity have been discovered. Since lymphocyte locomotion is inhibited due to gravity, direct activation of protein kinase C bypassing cell surface events using the phorbol ester PMA rescues MMG-inhibited lymphocyte activation and locomotion. Previous studies also report that, benzofuran-2-carboxylic acid and its derivatives (KMEG) provide protection from radiation and restore normal immune function.

It is very important to study both the long-term and short-term effects of microgravity as it has a suppressant nature that affects how the regulators of the immune system are readily able to function. Throughout the various pieces of literature, it is having been found that the healthy immune function depends on precise regulation of lymphocyte activation [3]. The access that these studies provide will not only help astronauts who have long-term side effects from space travel, it will also amplify the creation for solutions to repairing the compromised immune systems of people who are already on Earth. Each piece of research looks into the how lymphocytes are activated and then potentially suppressed due to their exposure to the gravitational pull of microgravity. In a study done by Thiel et al. [25] they suggest that long-term in vitro studies should be taken into consideration to have supporting detailed and prolonged data analysis in hopes to identify and understand adaptation mechanisms of the immune system in altered gravity. Additionally, Andreazzoli et al. [19], proposes that extensive knowledge of cellular and molecular mechanisms of gravity and its influence on T cells is an invaluable requirement for the provision of therapeutic or preventive targets to keep the bone and immune systems of astronauts fully functional during long-term space missions as well as regular people with immune deficiencies.

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